DETERMINATION OF THE PREVALENCE OF AFRICAN TRYPANOSOME SPECIES IN INDIGENOUS DOGS OF MAMBWE DISTRICT, EASTERN PROVINCE OF ZAMBIA

By

Malimba Lisulo

A dissertation submitted to the University of Zambia, School of Medicine in partial fulfilment of the requirements for the degree of Master of Science in Medical Parasitology

THE UNIVERSITY OF ZAMBIA

LUSAKA

OCTOBER, 2013

DECLARATION

I, **Malimba Lisulo**, do hereby declare that this dissertation is full representation of my own work and that its contents have only been submitted to the University of Zambia and not any other learning institution.

Date.....

Signed.....

CERTIFICATE OF APPROVAL

This dissertation of **Malimba Lisulo** is approved as fulfilling part of the requirements of the award of the Degree of Master of Science in Medical Parasitology of the University of Zambia.

Supervisor.....Sign.....Date.....

Examiner.....Sign.....Date.....

Examiner.....Sign.....Date.....

Examiner.....Sign.....Date.....

ABSTRACT

Throughout their long history of domestication, dogs have been sources of parasitic zoonoses, including gastrointestinal parasites and haemoparasites. As such, they have served as a link for parasite exchange, resulting in several emerging and re-emerging diseases. Among them is African trypanosomiasis, a re-emerging tsetse-transmitted disease which affects livestock and humans in sub-Saharan Africa, including Zambia. When infected with pathogenic trypanosome species such as *Trypanosoma congolense* and *T. brucei* subspecies, dogs become potential reservoirs of infection to livestock and humans, respectively. In this study, we determined the prevalence of trypanosome species in indigenous dogs of Mambwe district and investigated whether they serve as reservoirs of zoonotic *T. b. rhodesiense*.

A cross sectional survey of canine African trypanosomiasis (CAT) was conducted within Mambwe district, situated along the Luangwa valley which supports a high density of tsetse flies and is a historical human African trypanosomiasis (HAT) focus. Snow bowling technique was used to sample dogs from 5 chiefdoms within Mambwe. Microscopy and Loop mediated isothermal amplification (LAMP) were used as diagnostic techniques to detect trypanosome species.

A total of the 237 dogs were sampled and out of these, 14 (5.9%; 95% CI: 2.9 – 8.9%) were positive for CAT by microscopy. On the other hand, LAMP detected a total of 20 CAT cases (8.4%; 95% CI: 4.9 – 12.0%), including all the 14 cases detected by microscopy. According to LAMP, those infections were caused by *T. congolense* (4.2%; 10/237), *T. b. brucei* (2.5%; 6/237) and the human-infective *T. b. rhodesiense* (4.6%; 11/237) either as monolytic or mixed infections. CAT was detected in 3 (Munkanya, Nsefu and Malama) out of the 5 chiefdoms in Mambwe district. Detection of the Serum Resistance Associated (SRA) gene from trypanosomes isolated from dogs from the 3 chiefdoms is intriguing and suggests that the 3 chiefdoms were at risk of contracting HAT and that indigenous dogs could act as reservoirs of zoonotic *T. b. rhodesiense*.

These findings suggest that indigenous hunting dogs, most of which exhibited trypanotolerance, may be involved in the re-emergence of HAT in Mambwe district. Future studies should investigate (i) the influence of seasonal variation on vector burden and activity, and their impact on the prevalence of trypanosomiasis by use of more sensitive and specific molecular techniques such as LAMP, (ii) possible routes of infection with CAT in the hunting dogs.

DEDICATION

To the late Dr. Cecilia Jill Shinondo, who despite being ill, selflessly devoted her last days to lecture me in protozoology and co-supervise my research.

I dedicate this piece of writing to her. May her soul rest in eternal peace.

ACKNOWLEDGEMENTS

Thanks and praise to the Almighty God for the grace he gave me during my time of study.

I stand on the shoulders of many people whose efforts deserve acknowledging.

To Dr. M. Maimbolwa and the entire SACORE management, I would like to extend my gratitude for the financial assistance rendered to me during data collection.

Prof. B. Namangala my supervisor and mentor, I would not be in this position without your tireless and worthwhile contributions to my study.

Dr. S. Nzala, my co-supervisor I thank you for your contributions.

Special thanks also go to the Department of Veterinary and Livestock Development; Chilanga HQ, Chipata Provincial Office and Kakumbi Tsetse Research Station for all the necessary support.

Many thanks go to Mr. H. Ngalande, Dr. F. Mbayiyi, Dr. H. Tembo, Mr. M. Masuku, Mr. W. Hanamwanza, Mr. L. Phiri, Mr. F. Hang'undu, Ms. M. Phiri and Mr. M. Ngulube, who offered extensive support during sample collection. I would also like to acknowledge Mr. L. Moonga and Mr. J. Ndebe who repeatedly helped me to run the LAMP assays.

To Dr. P. Kelly, Dr. A. Knipes, Dr. M. Shehata, Dr. Y. Ahmed, Mr. C. Mweempwa and many others who took time to edit my research proposal, I say thank you.

To Dr. P. Musonda, Dr. B. Sikateyo, Dr. B. Andrews and Mr. P. Hangoma, I say thank you for helping with statistical analysis of my data.

Thanks also go to Mr. M. Mukumbwali for generating arc-view maps of study sites and Mr. M. Mushimbalume, the South Luangwa Area Management Unit regional manager for granting us permission to sample from their training centre (Nyamaluma).

Last but not least, I would like to appreciate my family and friends for their endless contributions. I particularly thank my wife Mpala and daughter Bupilo for their endurance and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAN	Animal African Trypanosomiasis
AIC	Akaike Information Criterion
BIC	Bayesian Information Criterion
CAT	Canine African Trypanosomiasis
CATT	Card Agglutination Test for Trypanosomiasis
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
GMA	Game Management Area
HAT	Human African Trypanososmiasis
HIV	Human Immunodeficiency Virus
LAMP	Loop Mediated Isothermal Amplification
NASBA	Nucleic Acid Sequence Based Amplification
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
RIME	Repetitive Insertion Mobile Element
ROC	Receiver Operator Characteristic Curve
SACORE	Southern Africa Consortium for Research Excellence

SLNP	South Luangwa National Park
SRA	Serum Resistance Associated gene
WBC	White Blood Cell
WHO	World Health Organisation
ZAWA	Zambia Wildlife Authority

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND:

Throughout their long history of domestication, dogs have been treasured amongst all animals as being man's closest companion. In almost all societies, dogs are widely utilized and offer several benefits to man with the main one being security (Wells, 2007). However, from the public health point of view, dogs have been sources of zoonotic parasites including gastrointestinal parasites and haemoparasites (Dantas-Torres, 2008; Nonaka *et al*., 2011). As such, dogs have served as a link for parasite exchange among livestock, wildlife and humans and hence remain an important source of emerging and re-emerging diseases in man (Cleaveland *et al*., 2001). There many infectious organisms trasmitted by arthropod vectors that affect dogs and these include protozoa (e.g. *Babesia*, *Leishmania* and *Trypanosome* species), bacteria (e.g. *Anaplasma* and *Ehrlichia* species) and helminths (e.g. *Dirofilaria* and *Dipylidium* species) (Dantas-Torres, 2008). One such important arthropod vector is Africa's tsetse fly (genus *Glossina*) which transmits pathogenic protozoan trypanosome species to a wide range of susceptible mammalian hosts (Namangala, 2011).

Various trypanosome species cause trypanosomiasis; a re-emerging disease that affects both livestock and humans in sub-Saharan African countries, including Zambia (WHO, 2010; Namangala, 2012; Peacock *et al*., 2012). Most trypanosome species cause animal African trypanosomiasis (AAT) or nagana in livestock, whilst only two species cause human African trypanosomiasis (HAT) or sleeping sickness in humans (Fèvre *et al*., 2008; Brun *et al*., 2010; WHO, 2010). Both AAT and HAT affect dogs resulting in canine African trypanosomiasis (CAT), which may range from asymptomatic, chronic to acute fatal forms depending on the breed of dogs and the causative parasites (Boyt, 1988). Specifically, CAT is caused by *Trypanosoma congolense*, *T. evansi* and *T. brucei* subspecies (Matete, 2003; Gow *et al*., 2007; Eloy and Lucheis, 2009). These species are also infective to other forms of livestock with

fatalities caused by *T. congolense* in cattle, *T. evansi* in camels and *T. brucei* subspecies in horses (Boyt, 1988; Uilenberg, 1998). However, of major public health interest is infection caused by the morphologically indistinguishable *T. brucei* subspecies (Boyt, 1988). This group encompasses one AAT causing species (*Trypanosoma brucei brucei*) and two zoonotic HAT causing species (Brun *et al*., 2010; Namangala, 2012). The latter are *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*, causing Rhodesian HAT in Eastern and Southern Africa and Gambian HAT in Western and Central Africa, respectively (Kennedy, 2008). When any *T. brucei* subspecies infects dogs, the impact of CAT is usually acute and fatal as compared to infection caused by the other species (Boyt, 1988; Ezeokonkwo *et al*., 2010). Furthermore, CAT is more acute and extremely fatal in exotic breeds of dogs than in the indigenous breeds which seem to have some trypanotolerance (Boyt, 1988; Abenga *et al*., 2005; Namangala *et al*., 2013). Although indigenous breeds of dogs get infected with trypanosomes, they either exhibit subclinical symptoms or may not exhibit any overt clinical signs of the disease at all. Regardless of how *T. b. rhodesiense* infection manifests in dogs, a high parasitaemia often exists (Namangala *et al*., 2012**b**), meaning tsetse flies have a high chance of picking up the disease during blood meals. Dogs are thus a potential source of HAT which may result when tsetse flies bite humans after taking a blood meal from a trypanosome-infected dog (Namangala *et al*., 2013).

In tsetse infested sub-Saharan African countries, both forms of HAT are predominantly acquired in remote rural areas where the disease is endemic (WHO, 2010). Tsetse flies pick pathogenic trypanosome species from infected wildlife or livestock or humans, and become infective (Lutumba *et al*., 2007; Kuepfer *et al*., 2011). Both wildlife and livestock are important reservoirs of Rhodesian HAT (Abenga *et al*., 2005; Brun *et al*., 2010, Namangala *et al*., 2012**a**). Humans will acquire HAT as they go about their daily routines of farming, hunting, fishing or washing, which expose them to infective tsetse fly bites (Brun *et al*., 2010; WHO, 2010). Since HAT occurs in remote areas, where health systems are either poor or do not exist, detecting and reporting such cases is a major problem (Cecchi *et al*.,

2009). HAT is a neglected tropical disease that causes patients to suffer a variety of debilitating symptoms and sequelae, leading to death if left untreated (Fèvre *et al*., 2008). In many instances HAT occurs in areas that are also endemic for other tropical diseases, the main one being malaria (Kagira *et al*., 2011). Complications arise when these two vector-transmitted diseases (malaria and HAT) overlap in a particular region, due to similar clinical signs that make diagnosis difficult (Bisser *et al*., 2006; Kagira *et al*., 2011). Other diseases with HAT-like clinical manifestations include tuberculosis, HIV/AIDS and enteric fever (Mwanakasale and Songolo, 2011; Wastling and Welburn, 2011). Therefore, correctly measuring HAT's incidence, morbidity and mortality becomes significantly impaired as a result of these confounding factors. These clinical similarities often cause unspecialized units within health care systems to misdiagnose HAT when it has been presented to them (Fèvre *et al*., 2008; Mwanakasale and Songolo, 2011). Misdiagnosis leads to administration of wrong drugs and ultimately death (Bisser *et al*., 2006). It is therefore critical that health personnel in HAT endemic regions correctly diagnose the disease early enough to avoid loss of lives (Kuepfer *et al*., 2011).

In Zambia, HAT is endemic in the Luangwa and Zambezi valley basins (Fig. 1; Namangala *et al*., 2012**a**; Munang'andu *et al*., 2012). Recent reports suggest an increase in the number of HAT cases within the old HAT foci especially in the northern part of the Luangwa valley (Namangala, 2009; Mwanakasale and Songolo, 2011), in addtiontoo several unpublished data from health centres (personal communication). Several old HAT foci exist along the valley basins whose current disease status remains unknown (Mwanakasale and Songolo, 2011). Among them is Mambwe district, situated in the central part of the Luangwa valley in the Eastern province (Anderson *et al*., 2011). The district commonly known as Mfuwe is a popular tourist destination with abundant wildlife species, and also highly infested with tsetse flies (Ministry of Livestock & Fisheries Development, 2010). Despite the presence of both the reservoir and vector, there has been very little active disease surveillance done, especially in humans (Mwanakasale and Songolo, 2011).

It has been postulated that during HAT epidemics, domesticated dogs are the first casualties rapidly succumbing to disease long before it can be noticed in humans (Matete, 2003). In Mambwe district, domesticated dogs are an integral part of most communities living within the Lupande Game Management Area (GMA). These dogs are not just used for security purposes, but much more for the purposes of illegal hunting (poaching). According to WHO (2010), hunting is listed among several other factors through which human infection is acquired. It is therefore very likely that these hunting dogs also get infected when they accompany their owners and harbour human-infective African trypanosomes (Samdi *et al*., 2006; Brun *et al*., 2010).

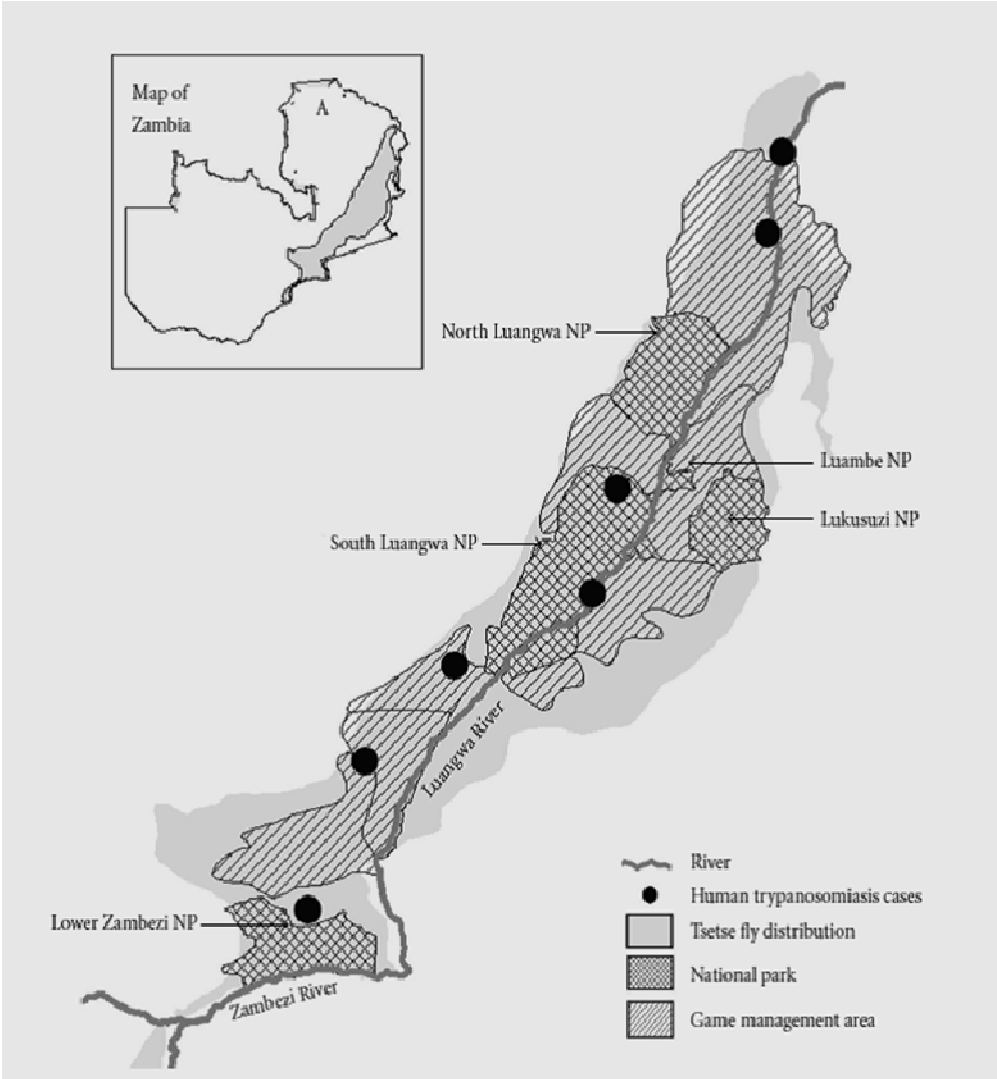


Figure 1: Distribution of the tsetse vector and presence of HAT along the Luangwa and Zambezi valley basins (adopted from Munang'andu *et al*., 2012)

This study therefore aimed at determining the prevalence of trypanosome species in indigenous breeds of dogs living along the central part of the Luangwa valley in Mambwe district and to establish whether they do serve as reservoirs of human infective trypanosomes.

1.2 STUDY JUSTIFICATION

Mambwe district occurs within the gazetted Lupande GMA (Fig 2) that shares borders with the South Luangwa National Park (SLNP); a haven for both the reservoir and vector of trypanosomes. Contact between reservoir/vector and human/livestock living in GMA's is a risk factor for contracting the disease as the latter offers alternative blood meals for tsetse flies (Ministry of Livestock & Fisheries Development, 2010; Munang'andu *et al*., 2012). Following recent influxes of humans and their livestock into this disease endemic GMA, it is certain that a new wildlife-livestock-human interface has developed, in which infective trypanosome species circulate (Anderson *et al*., 2011). As potential reservoirs of HAT, indigenous dogs used for hunting could play an important epidemiological role in this interface (Boyt, 1988; Samdi *et al*., 2006; Namangala *et al*., 2012**b**). Matete (2003) points out that sporadic and very low HAT prevalence in domesticated dogs has been closely reflected by disease occurrence in humans. However, very little is known about CAT in Zambia and its possible health implications on human communities living in tsetse infested areas. In this study, an attempt was made to screen indigenous dogs in Mambwe district for trypanosome infections, particularly the human infective *T. b. rhodesiense*. Knowledge gained from such a study may be a useful guide in understanding and controlling AAT and HAT (Cox *et al*., 2010).

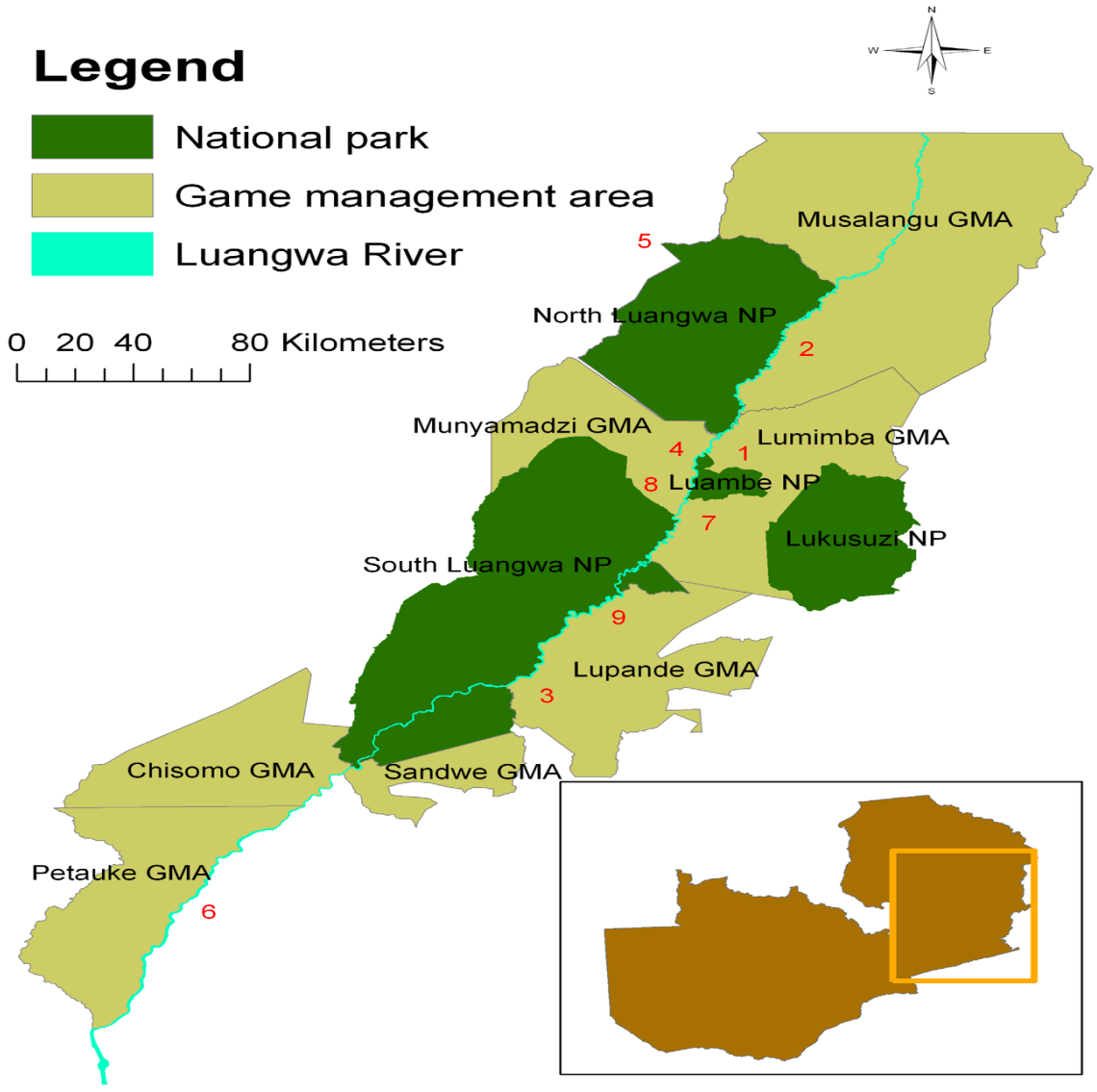


Figure 2: Map showing how Mambwe district (Lupande GMA) borders with South Luangwa National Park, the main source of tsetse flies and trypanosomiasis (adopted from Anderson *et al.*, 2011).

1.3 NULL HYPOTHESIS

Indigenous dogs in tsetse infested Mambwe district do not harbour human infective trypanosomes and therefore do not play an important role as reservoirs of HAT.

1.4 OBJECTIVES

1.4.1 GENERAL OBJECTIVE

To determiine the prevalence of CAT in indigenous breeds of dogs in the tsetse infested Mambwe district and to determine whether they serve as reservoirs of human infective trypanosome species.

1.4.2 SPECIFIC OBJECTIVES

1. To determine the prevalence, risk factors and clinical signs of CAT in the tsetse infested Mambwe district.
2. To identify the trypanosome species affecting indigenous dogs in Mambwe district.
3. To determine the prevalence of human infective *T. b. rhodesiense* in indigenous dogs of Mambwe district.
4. To compare the diagnostic accuracy of LAMP to microscopy in detecting CAT prevalence in dogs.

CHAPTER 2: LITERATURE REVIEW

2.1 AFRICAN TRYPANOSOMES

African trypanosomes are haemoflagellated unicellular organisms (PROTOZOA) that mainly exist as parasites in a wide range of mammalian hosts (Boyt, 1988). They were first discovered about a century ago and their existence linked to tsetse flies as vectors for their cyclic development and transmission (Sharma *et al*., 2009). Taxonomically, the African trypanosomes are classified as belonging to the following (Eloy and Lucheis, 2009; Brun *et al*., 2010; WHO, 2010):

Kingdom:	Protista
Subkingdom:	Protozoa
Phylum:	Sarcomastigophora
Class:	Zoomastogophora
Order:	Kinetoplastida
Suborder:	Trypanosomatina
Family:	Trypanosomatidae
Genus:	*Trypanosoma*
Section:	Salivaria

The presence and activity of one or more of these African trypanosomes within certain vertebrates causes a series of diseases collectively known as "Trypanosomiasis" (Boyt, 1988). This disease was originally enzootic and circulated within its natural reservoir hosts (wildlife) but later become zoonotic due to contact with non-reservoir hosts (livestock and humans) (Eloy and Lucheis, 2009).

As pathogenic parasites, African trypanosomes spend part of their life cycle in the tissues of both mammalian hosts and tsetse flies, in which definite stages of development occur before infective forms can be passed on to other mammalian hosts during the act of feeding (Boyt, 1988, Table 2.1). Thus, for disease to occur, African trypanosomes rely on the interaction between tsetse flies and mammalian

hosts to complete the transmission cycle (Fèvre *et al*., 2008; Grebaut *et al*., 2009). The dependency on tsetse-host interaction creates several opportunities in which disease transmission can be controlled (Butler, 2003; Docampo and Moreno, 2003). As parasites belonging to the salivaria section, most African trypanosomes are inoculated into mammalian hosts via infective mouthparts or saliva of tsetse flies as they take a blood meal (Barret, 2003; Eloy and Lucheis, 2009; Samdi *et al*., 2011, Table 2.1). Exceptions are seen in *T. evansi* and *T. equiperdum* which are transmitted mechanically and sexually, respectively (Boyt, 1988; Uilenberg, 1998; Namangala, 2012).

Table 2.1: Classification of the pathogenic African trypanosomes

Subgenus	**Species**	**Development in *Glossina***	**Hosts**	**Importance**
Duttonella	*Vivax* group:			
	T. vivax	Proboscis:	Wild & domestic (not pigs)	Major disease of cattle & ungulates.
	T. uniforme	"	"	Localised mild disease.
Nannomonas	*Congolense* group:			
	T. congolense	Proboscis & midgut:	Wild & domestic	Major disease of cattle & ungulates.
	T. simiae	"	"	Acute disease in domestic pigs
	T. godfreyi	"	"	Chronic disease in domestic pigs
Trypanozoon	*Brucei* group:			
	T. b. brucei	Salivary glands & midgut:	Wild & domestic & humans	Acute disease in dogs
	T. b. rhodesiense	"	"	Acute disease in humans
	T. b. gambiense	"	"	Chronic disease in humans
	T. evansi	No development:	Wild & domestic animals	Major disease of camels
	T. equiperdum	No development:	Wild & domestic equines	Major disease of horses
Pycnomonas	*T. suis*	Salivary glands & midgut	Wild & domestic pigs	Pathogenic only to pigs

Like other infections of animals transmissible to man, African trypanosomiasis is baffling in the diversity of forms in which it occurs (Anonymous, 1964). The African trypanosomes have an unusual characteristic amongst protozoa of never entering host cells, instead occupying a strictly extracellular niche i.e. in blood, lymph and cerebrospinal fluid (Awuoche, 2012). These parasites are capable of surviving and evading host immune killing by creating a thick immunogenic covering around their bodies known as the Variant Surface Glycoprotein which is constantly modified through the process of antigenic variations (Pays *et al*., 1983; Aitcheson *et al*., 2005; Namangala, 2012).

2.2 THE TSETSE VECTOR

It is said that "as long as tsetse flies are present, African trypanosomiasis remains a continuous problem preventing the full utilization of land resources in regions where development is critically needed" (Boyt, 1988). As shown in table 2.1 above, tsetse flies serve as vectors of various species of pathogenic trypanosomes in sub-Saharan Africa. Taxonomically, tsetse flies are classified as follows:

Kingdom:	Animalia
Phylum:	Arthropoda
Class:	Insecta
Order:	Diptera
Family:	Glossinidae
Genus:	*Glossina*

Tsetse flies belong to a single genus *Glossina*, comprising 3 extant subgenera, *Austenina* Townsend, *Nemorhina* Robineau-Desvoidy and *Glossina* Wiedemann that correspond to the *fusca, palpalis*, and *morsitans* groups respectively (Krafsur, 2009). Table 2.2 below lists these groups and their respective species. The distribution of the groups hinges on their affinities for specific habitats (Fèvre *et al.,* 2008). According to Leak (1999); the *morsitan* group prefer Savannah conditions, the *fusca* group are thicket or forest dwellers and lastly the *palpalis* group have an aquatic

affinity and are thus commonly known as riverine flies. Morphological differences in the flies' genitalia are also used as a basis for their classification (Despommier *et al*., 2005).

Table 2.2: Groupings of tsetse species and disease transmitted

Morsitans group: vectors of AAT & HAT	
Glossina logipalpis	*Glossina pallidepes*
Glossina morsitans morsitans	*Glossina swynertoni*
Glossina morsitans sub-morsitans	*Glossina austeni*
Glossina morsitans centralis	
Fusca group: vectors of AAT & HAT – less economically important	
Glossina nigrofusca nigrofusca	*Glossina tabaniformis*
Glossina nigrofusca hopkinsi	*Glossina nashi*
Glossina fusca fusca	*Glossina vanhoofi*
Glossina fusca congolensis	*Glossina medicorum*
Glossina fuscipleuris	*Glossina severini*
Glossina haningtoni	*Glossina brevipalpis*
Glossina shwetzi	*Glossina longipennis*
Palpalis group: vectors of AAT & HAT	
Glossina palpalis palpalis	*Glossina tachnoides*
Glossina palpalis gambiensis	*Glossina pallicera pallicera*
Glossina fuscipes fuscipes	*Glossina pallicera newsteadi*
Glossina fuscipes martini	*Glossina caliginea*
Glossina fuscipes quanzesis	

Unlike other dipteran disease causing vectors, both male and female tsetse flies feed on blood and transmit pathogenic African trypanosomes (Brun et al, 2010; Peacock et al, 2012). African trypanosomes, exploit the obligate blood feeding behaviour of tsetse flies (genus *Glossina*) in all endemic regions for their successful transmission (Van Den Abbeele et al, 2010). Consequently, African trypanosomiasis is geographically restricted to sub-Saharan Africa where environmental conditions favour the propagation of 30 species and subspecies of the tsetse fly vector (Brun et

al, 2010). Although not all *Glossina* species have been confirmed as disease vectors, 11 species are known to transmit African trypanosomiaisis (Despommier *et al*., 2005).

2.2.1 TSETSE FLY DISTRIBUTION IN ZAMBIA

Out of the 30 documented tsetse fly species and sub-species infesting African countries, only 4 species and 2 subspecies have been found in Zambia. These include two members of the *morsitans* group, (i) *Glossina morsitans* with two subspecies (*G. m. morsitans*; *G. m. centralis*) and (ii) *Glossina pallidipes.* The *fusca* and *palpalis* groups are each represented by *Glossina brevipalpis* and *Glossina fuscipes*, respectively. *G. morsitans* is the most widely distributed and commonly occurs in valley areas together with *G. pallidipes*, a species which is found in/or near areas of endemic sleeping sickness (Munang'andu *et al*., 2012). *G. brevipalpis* infests Zambia's Eastern and Northern provinces whilst *G. fuscipes* also formally known as *G. palpali* is confined to the shores of Lake Mweru and Tanganyika and the Luapula River (Evison and Kathuria, 1982). These flies' competence, like any other arthropod vector, is determined by their ability to acquire, maintain and transmit pathogens to another host (Goddard, 2003; Munang'andu *et al*., 2012).

2.2.2 DEVELOPMENT OF AFRICAN TRYPANOSOMES IN TSETSE FLIES

Teneral tsetse flies (newly hatched) are borne without infection (Brun *et al*., 2010). Infection is only acquired upon feeding on mammalian blood containing infective bloodstream trypomastigotes which when drawn into their alimentary canal, initiate a cycle of development and division that produces pathogenic metacyclic forms (Boyt, 1988). Depending on the infecting species of African trypanosomes, the parasites may successfully establish themselves in the midgut, the salivary glands or mouthparts (Geiger *et al*., 2005). Furthermore, the species of tsetse fly, strain of African trypanosome and ambient temperature, determine the duration when parasites appear in the mouthparts which is usually between 10-50 days post-infection. And once infected, tsetse flies remain infective for the rest of their lives

(Boyt, 1988; Despommier *et al*., 2005). A minimum dose of 300-500 African trypanosomes is sufficient to stimulate disease in a susceptible mammalian host, but an infected tsetse fly is capable of injecting over 40,000 mature metacyclic trypanosomes during the act of feeding (Despommier *et al*., 2005).

However, during this complex cycle of proliferation and development from the midgut to the salivary glands, the flies mount robust immune defences against African trypanosome infection at every step of the way, resulting in most flies failing to develop transmissible infection (Peacock *et al*., 2012). This explains the reason why only 0.1% of field captured flies carry mature infection; the parasites fail to complete their life cycle in the flies (Brun *et al*., 2010). The ability to develop infections in tsetse flies differs on the basis of individual host factors (Munang'andu *et al*., 2012). Variations are observed in infection rates from species to species with *T. vivax* ranking the highest and *T. brucei* species ranking lowest (Despommier *et al*., 2005). Amongst several factors that influence the susceptibility of tsetse flies to African trypanosome infection, sex is one of them (Peacock *et al*., 2012). The general observation is that, female tsetse flies have higher infection rates than males, partially because they live longer and therefore stand higher chances of acquiring infection (Despommier *et al*., 2005). On the contrary, the males of *Glossina m. morsitans, G. m. centralis, G. pallidipes and G. fuscipes fuscipes* show higher rates of salivary gland infection with *T. brucei* than their females (Peacock *et al*., 2012).

2.3 TRANSMISSION

AAT is mostly transmitted cyclically through the infective bite of tsetse flies (Uilenberg, 1998; CFSPH, 2009). However, infections of *T. vivax, T. evansi and T. simiae* are mechanically transmitted by other biting flies such as horse flies (Tabanids) and stable flies (Stomxys) (Uilenberg, 1998; Barret, 2003; Samdi *et al*., 2011; Namangala, 2012). During mechanical transmission, the insect passes the bloodstream trypanosomes from an infected animal to another in the course of interrupted feeding. However, the time between the two feeds is crucial for

effective transmission as trypanosomes normally die when the blood clots (Boyt, 1988). AAT can equally be spread by fomites such as surgical instruments, needles and syringes when used repeatedly on more than one animal at short intervals provided blood does not dry (Uilenberg, 1998; CFSPH, 2009). Sexual transmission is only observed in equines (horses, donkeys, mules), a disease known as dourine caused by *T. equiperdum*. The parasites are found in mucous exudates of the stallion's penis and sheath and the mare's vagina (Uilenberg, 1998; Gibson, 2007). Occasionally, all species of trypanosomes are transmitted congenitally from mother to offspring either through the placenta or when bleeding at birth (Uilenberg, 1998). Lastly, carnivores can become infected from their prey through abrasions in the oral mucosa, a phenomenon which has been demonstrated in artificial experiments (Uilenberg, 1998; Anderson *et al*, 2011).

It cannot be over emphasised that transmission through tsetse-bites is the commonest mode of acquiring infection even in HAT patients. As in AAT above, HAT can be acquired through fomites; accidental laboratory pricks or blood transfusion. Mechanical transmission via other blood sucking arthropods also occurs. Lastly mother-to-child infections where parasites are able to cross the placenta and infect the foetus have been reported (Ngoma *et al*., 2004; WHO, 2010). Vertical transmission from a pregnant mother to foetus does occur though authentic reports are rare. A few reports have shown congenital HAT infections diagnosed in newly born babies merely 5 days old and children of infected mothers who have never entered endemic countries (Lindner & Priotto, 2010). Most children suffering from HAT generally present a range of non-specific clinical signs which are either misdiagnosed or discovered too late (Ngoma *et al*., 2004). The consequences tragically end in brain damage, physical or mental sequelae or death (Lindner & Priotto, 2010). A more in-depth understanding of the types of local trypanosomes, animal reservoirs of *T. b. rhodesiense*, vectors and modes of transmission, might help to avoid future sleeping-sickness epidemics (Waiswa *et al*., 2003).

2.4 AFRICAN TRYPANOSOMES IN MAMMALIAN HOSTS

The ability to parasitize a broad spectrum of mammalian hosts makes African trypanosomes stand out as true multi-host parasites (Anderson *et al*., 2012; Munang'andu *et al*., 2012). Depending on the affected host species, trypanosomes may cause relatively mild infections or severe disease leading to death (Steverding, 2008).

2.4.1 INFECTION IN WILDLIFE

Wildlife carries a wide range of pathogenic African trypanosomes and is an important reservoir of infection to livestock and humans (Auty *et al*., 2012). In surveys conducted in wildlife along the Luangwa valley, human infective subspecies of African trypanosomes have so far been found in bushbuck, duiker, giraffe, impala, lion, warthog, waterbuck, zebra, leopard and buffalo, suggesting that the reservoir community is more diverse than previously anticipated (Anderson *et al*., 2011). Bushbucks are the most important reservoirs for *T. brucei* subspecies in the Luangwa valley and a high proportion of its blood is consumed by *G. pallidipes* (Anderson *et al*., 2011; Munang'andu *et al*., 2012). Hypothetically, this species of tsetse fly should be considered as occupational hazard especially to tourists, tour guides, game scouts and poachers as it is likely to carry mature human infection.

However, the presence of trypanosomes in wildlife does not necessarily make them susceptible to disease unless stressed; both host and parasite have evolved over time to establish a balanced relationship that does not produce disease (Aksoy *et al*., 2003). This enables wildlife to assume an epidemiological role as carriers or reservoir hosts from which tsetse flies acquire infection (Brun *et al*., 2010).

2.4.2 INFECTION IN DOMESTICATED ANIMALS

In livestock, the host-parasite relationship has not fully developed to prevent disease occurrence as observed in wildlife (d'Ieteren *et al*., 1998; Namangala *et al*., 2007). Only short breeds of cattle (N'dama, Muturu) and dwarf sheep and goats in

West Africa are known to exhibit such levels of tolerance to African trypanosomes as in wildlife (d'Ieteren *et al*., 1998; Abenga *et al*., 2005). However, most domesticated animals succumb to infection and show a variety of clinical signs (Boyt, 1988; Uilenberg, 1998; Abenga *et al*., 2005; Adeiza *et al*., 2008; Namangala, 2012). These infections usually result in severe disease in a lot of animal species (Morrison *et al*., 1981). The single most important cause of AAT in Eastern and Southern Africa is *T. congolense*, whilst in West Africa; *T. vivax* (together with *T. congolense*) is an important cause of cattle disease (Despommier *et al*., 2005).

A range of domestic animals which include cattle, sheep, pigs and dogs also carry trypanosomes with the human serum resistant-associated (SRA) gene making them potential reservoir hosts for HAT (Njiru *et al*., 2004). In East Africa, cattle have been documented to be important reservoirs for *T. b. rhodesiense* (Njiru *et al*., 2004; Fèvre *et al*., 2008, Brun *et al*., 2010). Available evidence also backs the rationale that dogs which are susceptible to a wide range of trypanosoma infections (Table 2.3) may be potential HAT reservoirs (Morrison *et al*., 1981; Boyt, 1988; Matete, 2003; Njiru *et al*., 2004; Namangala *et al*., 2013).

Table 2.3: The occurrence of African trypanosomes in domesticated animals

Trypanosome species	Domestic animals affected	Reservoir hosts	Experimental animals
T. congolense	Cattle, camels, horses, dogs, sheep, goats, pigs	Several species of wild animals	Rats, mice, guinea pigs, rabbits
T. simiae	Pigs	Wart hog, bush pig	Rabbits, monkeys
T. godfreyi	Pigs	Wart hog	None susceptible
T. vivax	Cattle, sheep, goats, domestic buffalo, horses	Several species of wild animals	Usually none susceptible
T.uniforme	Cattle , sheep, goats	Various wild ruminants	None susceptible
T. b. brucei	Horses, camels, dogs, sheep, goats, cattle, pigs	Several species of wild animals	Rats, mice, guinea pigs, rabbits
T. b. rhodesiense	As for *T. b. brucei*	As for *T. b. brucei*	As for *T. b. brucei*
T. b. gambiense	Pigs, sheep, goats, dogs	Humans	As for *T. b. brucei* (after initial adaptation where *T. b. gambiense* is concerned)
T. evansi	Camels, horses, dogs, domestic buffalo, cattle	Several wild animals in Latin America	As for *T. b. brucei*
T. equiperdum	Horses, donkeys, mules	None known	As for *T. b. brucei* (after initial adaptation)

The virulence of parasites, host response and distribution of the tsetse fly vector determine the disease epidemiology in animals. For instance, savannah and riverine groups of tsetse flies are considered the most important in spreading disease as they inhabit grazing and watering areas (Despommier *et al*., 2005). Thus tsetse-transmitted trypanosomiasis is an important constraint to livestock production in such areas with the impact being most pronounced on the economy (Uilenberg, 1998). The disease accounts for billions of dollars in annual losses (Simukoko *et al*., 2007). Other related losses include both direct livestock out-put (weight-loss, decrease in milk, decreased reproductive rate) as well as lost opportunity in terms of integration of livestock into crop production and the potential for crop-improvement (loss of draught power and manure) (von Wissmann *et al*., 2011).

2.4.2.1 CLINICAL SIGNS IN DOMESTICATED ANIMALS

Typically, AAT is a wasting disease in which there is a slow progressive loss of condition accompanied by weakness to the point of extreme emaciation, collapse and death (Boyt, 1988). When an infected tsetse fly bites, it injects infective metacyclic trypanosomes at the bite site (Brun *et al*., 2010). These undergo a cycle of proliferation resulting into swelling known as a chancre beneath the animal's skin before gaining access into the bloodstream (Uilenberg, 1998). Chancres are the first noticeable signs in African trypanosomiasis but in animals they are not very prominent due to their thick skins and hairy coats. Once in the bloodstream, African trypanosomes may either cause an acute or chronic (most common) disease, or remain asymptomatic (Boyt, 1988; Matete, 2003). When disease occurs, the affected animals generally exhibit hyperthermia, intermittent fever, progressive anaemia, lymphadenopathy, loss of condition, emaciation, decreased milk yield in dairy animals, neurological signs, oedema, cardiac lesions, diarrhoea, keratitis, lacrimation, loss of appetite, stress, abortions, premature births and perinatal losses, as well as testicular damage in males (Boyt, 1988; Uilenberg, 1998; CFSPH, 2009; Namangala, 2012).

A variety of non-specific clinical signs are observed in infected domesticated dogs. These include depression, sleepiness, pale mucous membranes, lethargy, anorexia, mass loss and a rough hair coat. Other signs are recumbence, enlarged superficial lymph nodes, dyspnoea and fever. Neurological observations include dullness and lack of coordination manifesting as paraplegia, bilateral hyperflexic patellar reflexes, lack of conscious proprioception in the pelvic limbs and marginally depressed hopping reflexes of the forelimb. More signs include corneal opacity, conjunctivitis with a mucopurulent discharge, uveitis, haemorrhage and turbidity in the anterior chamber and loss of vision. However, the predominant sign is the varying degree of bilateral cloudiness of the cornea due to the turbid aqueous humour (Boyt, 1988; Matete, 2003; Abenga, 2005; CFSPH, 2009; Ezeh *et al*., 2009; Namangala *et al*., 2013). There appears to be some level of resistance towards CAT in indigenous breeds of dogs compared to exotic breeds whose condition runs a lesser course with wasting and severe anaemia (Boyt, 1988). This was confirmed by Namangala *et al*., (2012**b**) who reported of CAT in Zambian exotic breeds from Chiawa GMA and SLNP. Those infected with *T. congolense* tended to exhibit chronic CAT with comparatively less severe clinical signs than the *T. b. rhodesiense* infected dogs that were more acute with fulminating parasitaemia, nervous symptoms, dyspnoea and died within a short period.

2.4.3 INFECTION IN HUMANS

Both wild and domesticated animals may serve as reservoirs of human-infective trypanosomes, posing a threat of spreading HAT, especially to livestock farmers in rural areas (WHO, 2010). This may not necessarily be of great importance for *T. b. gambiense* since humans are the main reservoirs. However, in the case of *T. b. rhodesiense*, domesticated (cattle, dogs) and wild animals provide this important role (Uilenberg, 1998; Njiru *et al*., 2004; Brun *et al*., 2010; Namangala *et al*., 2013). Interestingly, it has been observed that in many tsetse-infested regions, HAT is rarely diagnosed (WHO, 2010). In Zambia for instance, despite the existence of both the tsetse fly vector and AAT, HAT has so far only been reported in Isoka, Chama, Petauke, Nyimba, Rufunsa, Mpika, Kasama and Serenje districts in the Luangwa

valley basin and Chirundu, Solwezi and Kasempa in the Zambezi valley basin (Konnai *et al*., 2008; Namangala, 2009; Mwanakasale and Songolo, 2011). Though rarely reported, cases of HAT in travellers returning from Zambia do occur amongst tourists coming from the South Luangwa Valley in the Eastern Province (Moore *et al*., 2002; Health Protection Agency, 2010; MD travel Health, 2010; Richter *et al*., 2012).

Depending on the parasite species involved, HAT takes two different forms caused by either the anthroponotic *T. b. gambiense* or the zoonotic *T. b. rhodesiense* (Cecchi *et al*., 2009). The former causes Gambian HAT, a chronic disease that is fatal after several years of infection in its victims across Western and Central Africa (Brun *et al*., 2010). The latter causes Rhodesian HAT, an acute disease circulating amongst humans, wildlife and domesticated animals with fatalities experienced in less than six months in Eastern and Southern Africa (Wastling and Welburn, 2011). Thus the two forms of HAT, occurring in geographically distinct regions have distinct manifestations (Barrett *et al*., 2007). However, differences have also been reported within the Rhodesian HAT such that the Southern genotype tends to be much less acute than the Eastern genotype (Barrett *et al*., 2007; Brun *et al*., 2010; Kuepfer *et al*., 2011; Mwanakasale and Songolo, 2011).

Sporadic reports on non-human pathogenic African trypanosomes such as *T. b. brucei*, *T. congolense* and *T. evansi* have been documented to cause disease in humans (Truc *et al*., 1998; Joshi *et al*., 2005; Brun *et al*., 2010). Normally, humans and several other primates show resistance to such kinds of infections (Namangala, 2011). This is because humans possess trypanosome lytic factors called apolipoprotein L-1 (APOL-1) and possibly other high density lipoprotein fractions which prevent most species of African trypanosomes from establishing infection (Barrett *et al*., 2007; Namangala, 2011). Therefore, people who lack such factors will succumb to infection, hence the sporadic reports above (Barrett *et al*., 2007; Namangala, 2011). On the contrary, *T. b. rhodesiense* and *T. b. gambiense* cause conventional HAT because they are naturally resistant to APOL-1, rendering them infective to humans. The SRA gene in *T. b. rhodesiense* strongly and specifically

interacts with the C-terminal helix of APOL-1, thereby neutralising its lytic activity. This mechanism of natural resistance is still unclear in *T. b. gambiense* (Namangala, 2011; 2012).

It is claimed that 95% and 5% of HAT in Africa is caused by *T. b. gambiense* and *T. b. rhodesiense*, respectively (Fèvre *et al*., 2008; Kuepfer *et al*., 2011). Approximately 60 million people are feared to be at risk of contracting HAT in countries which are tsetse-infested (Fèvre *et al*., 2008; Kuepfer *et al*., 2011; Kagira *et al*., 2011). Unfortunately, less than 15% of these people are under active surveillance (Namangala, 2012), reflecting the degree of negligence in the disease (Kuepfer *et al*., 2011). In 2005, the annual reported cases of HAT were estimated between 50,000–70,000 (Lutumba *et al*., 2007; WHO, 2010; Brun *et al*., 2010). Current estimations stand at 30,000 cases per year with Zambia contributing less than 100 new cases annually (WHO, 2010). However, these published estimates by WHO don't capture the under-reported cases. It has been suggested that between 38% - 69% of Rhodesian HAT cases remain under-reported (Fèvre *et al*., 2008; Kagira *et al*., 2011; Mwanakasale and Songolo, 2011).

2.4.3.1 CLINICAL SIGNS IN HUMANS

As observed in AAT, both forms of HAT manifest when the infective metacyclic trypanosomes are intradermally injected into human tissue by tsetse flies. The inoculated parasites at the bite site rapidly transform by binary fission into blood trypomastigotes resulting into a painful or painless chancre (Brun *et al*., 2010; Richter *et al*., 2012; Kennedy, 2013). Chancres are rarely seen in Gambian HAT patients but occur in 19% of Rhodesian HAT patients. However, in travellers, both forms of disease present severely, with chancres and rash frequently witnessed (Brun *et al*., 2010; Richter *et al*., 2012). Both forms of HAT appear in two stages (Kagira *et al*., 2011; Kuepfer *et al*., 2011). The first stage is known as haemolymphatic stage (Brun *et al*., 2010; Namangala, 2012). When the infection has successfully established itself in the human host, the initial clinical signs exhibited by both diseases are intermittent fever, headache, pruritus, itching,

lymphadenopathy and to a lesser extent, hepatosplenomegaly (Namangala *et al*., 2012**a**). During this period, patients may show a number of symptoms such as tremors of the tongue and eyelids, loss of appetite, concurrent involvement of the muscular system (Chappuis *et al*., 2005; Namangala *et al*., 2012**a**). The second phase is known as meningoencephalitis stage, where neurological damage occurs resulting in disordered sleep patterns and neuropsychiatric symptoms (Brun *et al*., 2010; Kagira *et al*., 2011; Namangala, 2012). The meningoencephalitic stage is characterised by parasite invasion of the CNS which is an active process of crossing the blood-brain barrier, leading to coma and death if untreated (Brun *et al*., 2010; WHO, 2010; Kennedy, 2013). Sleep disorder is a dominant clinical sign and one that gives the disease its name (Namangala, 2012). Disturbance of the sleep cycle is evident in patients as the disease causes dysregulation of the circadian rhythm of the sleep/wake cycle and fragmentation of sleep pattern (Brun *et al*., 2010; WHO, 2010; Kennedy, 2013).

2.5 DIAGNOSIS

Diagnosis of both AAT and HAT relies on identification of the causative parasite(s) in blood, cerebral spinal fluid (CSF) and/or any other body fluid/tissues (Boyt, 1988; Wastling and Welburn, 2011). Early and accurate detection of these parasites is essential for successful interventions especially where HAT is concerned (Brun et al, 2010). Detection involves screening, diagnostic confirmation and staging of disease (Brun et al, 2010). Therefore, it is necessary that all suspected AAT/HAT cases are properly managed on the basis of accurate diagnosis and effective treatment (Boyt, 1988; Deborggraeve et al, 2008). Unfortunately, most of the current and available methods applied in AAT/HAT detection are considered less sensitive, unable to accurately identify species or are cumbersome to perform (Boyt, 1988; Matovu et al, 2010; Mwanakasale and Songolo, 2011).

2.5.1 CLINICAL SIGNS

Clinical signs provide indirect evidence of infections, but because symptoms are not pathognomonic, basing diagnosis entirely on manifestations is very unreliable and insufficient (Uilenberg, 1998; Wastling and Welburn, 2011). Furthermore, it is a challenge to clinically distinguish AAT/ HAT from other diseases. For instance it is difficult to distinguish anaemia and weight loss caused by AAT from that caused by other diseases such as babesia, theileriosis, malnutrition or helminth infections (Uilenberg, 1998; CFSPH, 2009). It is equally difficult to distinguish between Rhodesian and Gambian HAT in order to avoid wrong toxic treatment (Njiru *et al*., 2008). Furthermore, HAT symptoms can be mistaken for malaria, enteric fever, tubercular meningitis and HIV/AIDS or vice versa (Chappuis *et al*., 2005; Fèvre *et al*., 2008; Mwanakasale and Songolo, 2011; Wastling and Welburn, 2011).

2.5.2 MICROSCOPY

Since clinical evidence of the disease relies on seeing the actual parasites in the host's body fluids (CFSPH, 2009; Wastling and Welburn, 2011). Parasitological confirmation by microscopy has to be performed on lymph aspirate, blood or CSF (Boyt, 1988; Brun *et al*., 2010). Trypanosomes are identified directly by their movement between blood cells or indirectly when they cause cells to move (Uilenberg, 1998). Wet smears, thin and thick giemsa stained smears are useful in identifying parasites (Boyt, 1988). The advantage of using microscopy is that it is cheap and easy to use. It has the ability to diagnose diseases on the spot when parasites are found (Uilenberg, 1998). The disadvantage with microscopy is that it has very low sensitivity; detection is limited around 10^4 trypanosomes per ml of blood. Furthermore, speciation of trypanosomes is usually difficult and time spent between sampling and examination should be kept short to avoid lysis of parasites (Uilenberg, 1998; Brun *et al*., 2010).

In AAT diagnosis, care must be taken to differentiate pathogenic trypanosomes from avirulent species such as *T. theileri* (Uilenberg, 1998; CFSPH, 2009). In HAT

diagnosis, the two parasites cannot be morphologically distinguished. However, it is simpler to detect *T. b. rhodesiense* in giemsa stained thin and thick smears than *T. b. gambiense* due to the high densities of parasites circulating in blood in the case of the former (Brun *et al*., 2010; Wastling and Welburn, 2011). When parasites are found in blood, CSF is examined to stage the disease; patients are in stage 1 if White Blood Cell (WBC) count is $\leq 5\mu l^{-1}$ and trypanosomes are absent and in stage 2 if trypanosomes are present and/or WBC count of $\geq 20\mu l^{-1}$ (Brun *et al*, 2010; Wastling and Welburn, 2011). Patients whose WBC count falls between these values but don't have trypanosomes may or may not require stage 2 treatment (Wastling and Welburn, 2011).

In order to improve sensitivity, concentration methods such as quantitative buffy coat, miniature anion-exchange centrifugation technique and microhaematocrit centrifugation techniques are used (Chappuis *et al*., 2005; CFSPH, 2009; Brun *et al*., 2010; Wastling and Welburn, 2011). The significance is that parasites are concentrated in one area and this tremendously improves the sensitivity (Brun *et al*., 2010). However, the general cost of microscopy and material requirement for concentration methods limit its usage especially in routine diagnosis (Chappuis *et al*., 2005).

2.5.3 XENODIAGNOSIS

The use of experimental animals is a technique that is often employed to confirm trypanosome infections in both AAT and HAT (Matete 2003; Waiswa *et al*., 2003). This technique is quite useful for multiplying trypanosomes when the parasitaemia from the infected host is scanty (Uilenberg, 1998). The sensitivity of this technique is based on the parasite strain and susceptibility of the experimental animal (Uilenberg, 1998). Mice are frequently used because of their susceptibility to various trypanosome species including trypanozoons as shown in table 2.3 above (Boyt, 1988; Chappuis *et al*., 2005). However, this method's limitation is that not all trypanosome species, including some strains of *T. congolense* and *T. brucei,* become

established in experimental animals (Duleu *et al*., 2004) and it is very time consuming (CFSPH, 2009).

2.5.4 SEROLOGICAL METHODS

Several serological techniques have been developed to detect humoral responses in blood, serum and CSF (Wastling and Welburn, 2011).

In AAT, the indirect fluorescent antibody test (IFAT), complement fixation test (CFT) and enzyme-linked immunosorbent assays (ELISA) are routinely used to identify seropositive animals, especially in the diagnosis of Dourine and surra. Because reactions to previous infections can be detected, serology is useful only for a presumptive diagnosis. Furthermore, cross-reactions can occur as is the case of *T. evansi* which causes surra and the non pathogenic *T. theileri* (CFSPH, 2009).

Card agglutination test for trypanosomiasis (CATT) with 87-98% sensitivity and 93-95% specificity is used for diagnosis of Gambian HAT in endemic areas and is more sensitive than microscopy. It is fast and practical, allowing hundreds of people to be screened daily. However, no serological screening test is available for Rhodesian HAT (Brun *et al*., 2010).

The disadvantage with serology is that it is easy to get false positives and negatives; cure is not indicative as antibodies can persist three years after treatment; serology is not packaged for individual tests; production of antigen is complex and its use further requires skilled personnel (Wastling and Welburn, 2011)

2.5.5 MOLECULAR METHODS

Molecular diagnosis is the most sensitive and specific technique for trypanosome species detection. It is centred on the use of Polymerase Chain Reaction (PCR) and two Isothermal DNA amplification methods; Loop Mediated Isothermal Amplification (LAMP) and Nucleic Acid Sequence Based Amplification (NASBA). PCR is sensitive and specific for identifying and detecting both AAT and HAT (Anderson

et al., 2011; Wastling and Welburn, 2011). Advances in molecular methods of diagnosis have lead to the development of a multispecies PCR capable of distinguishing all the major pathogenic trypanosome species in a single test (Anderson *et al*., 2011). However, due to its demand for thermocycler and highly skilled manpower, PCR has limitations in resource-poor communities; hence Isothermal DNA amplification offers better alternatives in such situations.

LAMP is a novel gene amplification method that has the ability to detect all *T. brucei* subspecies, *T. congolense*, *T. vivax* and *T. evansi*. It has the following characteristics: 1) only one enzyme is required and the amplification reaction proceeds under isothermal condition; 2) It has extremely high specificity because of the use of 4 primers recognising 6 distinct regions on the target; 3) It has high amplification efficiency and enables amplification within a shorter time; 4) It produces tremendous amount of amplified products which makes simple detection by naked eye possible (Njiru *et al*., 2011; Wastling and Welburn, 2011; Njiru, 2012). NASBA on the other hand can detect *T. brucei* subspecies but does not discriminate between subspecies (Wastling and Welburn, 2011).

2.6 TREATMENT AND CONTROL OF AFRICAN TRYPANOSOMIASIS

In order to limit the impact of African trypanosomiasis, various strategies that focus on treatment and vector control are employed to break the cycle of transmission (Brun *et al*., 2010).

2.6.1 TREATMENT

The use of chemotherapeutic drugs is an effective intervention that kills and inhibits the development of trypanosomes (Uilenberg, 1998). The drugs used either block or disrupt vital processes that are essential to the development of invading trypanosomes (Boyt, 1988). Several drugs are used to prevent and cure AAT as shown in table 2.4 below. Depending on the type of infection, the drug of choice may be administered subcutaneously (SC), intramuscular (IM) or intravenously (IV) (Boyt, 1988). Amongst the available drugs, diminazene aceturate (Berenil) is

commonly used to treat cattle and other animal species. It is very effective against *T. congolense* and *T. vivax* but less effective with *Trypanozoon* species. Berenil also treats infections of other invading haemoparasites such as *Babesia* species (Uilenberg, 1998). Despite its many benefits, prolonged use of berenil as a routine drug has resulted in treatment failure in many animal species including dogs, due to development of parasite species that are resistant to the drug (Ezeh *et al*., 2009; Chitanga *et al*., 2011).

Table 2.4: List of drugs used in the treatment of AAT

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

In humans, the successful treatment of HAT depends on a handful of available active drugs (Table 2.5) that are stage-specific (Richter *et al*., 2012). Besides the limited choice, HAT drugs are associated with very toxic side effects (Brun *et al*., 2010). Worse off, these drugs are rarely available on the market and can only be sourced through WHO (Barret *et al*., 2007; Brun *et al*., 2010; WHO, 2010). The toxic levels in first (haemolymphatic) stage drugs are more bearable than second stage drugs (WHO, 2010). Pentamidine and suramin, are the only drugs used to treat first stage Gambian and Rhodesian HAT, respectively. The latter is more reactive of the two and causes toxic effects on bone marrow and kidneys. It can also treat Gambian

HAT but is avoided where *Onchocerca* species occur (Brun *et al*., 2010). Melarsoprol is the drug of choice in second (meningoencephalitic) stage HAT of both diseases and is highly toxic causing encephalopathy syndromes. Eflornithine, on the other hand, is only used in *T. b. gambiense*. It is less toxic than Melarsoprol but the regime is difficult to apply and may be used alone or in combination with Nifurtimox (WHO, 2010).

Table 2.5: List of drugs used in treatment of HAT

Drug	Stage	Route	Dosing	Side Effects
Trypanosoma brucei rhodesiense				
Suramin	First	IV	Test dose (4-5mg/kg) at day 1, then five injections of 20mg/kg every 7 days (3,10,17,24,31) at a dose of 1g per injection	Hypersensitivity reactions; albuminuria, cylinduria, haematuria, peripheral neuropathy
Melarsoprol	Second	IV	Three series of 3.6mg/kg spaced by intervals of 7 days; maximum dose of 180mg per day	Encephalopathic syndromes, skin reactions, peripheral motoric or sensorial neuropsthies, thrombophlebitis
Trypanosoma brucei gambiense				
Pentamidine	First	IM	4mg/kg at 24hrs intervals for 7 days	Hypoglycaemia, injection site pain, diarrhoea, nausea, vomiting
Eflornithine	Second	IM	100mg/kg at 6hrs intervals for 14 days	Diarrhoea, nausea, vomiting, convulsions; anaemia, leucopenia and thrombocytopenia
Melarsoprol	Second	IV	2.2mg/kg at 24hrs intervals for 10 days	Encephalopathic syndromes, skin reactions, peripheral motoric or sensorial neuropsthies, thrombophlebitis

2.6.2 VECTOR CONTROL

Vector control has long been thought to offer great hope for control of both AAT and HAT. Efforts to break the tsetse transmission cycle are focused on the fly biology (i.e. slow reproduction rates, mobility patterns, resting sites, reservoir hosts, susceptibility to available insecticides) (Schofield and Maudlin, 2001). In the colonial era, control efforts were based on mass destruction of wild mammals (reservoirs for *T. b. rhodesiense*), and indiscriminate chopping (deforestation) and use of dichlorodiphenyl-trichloroethane (DDT) on lower parts of trees (tsetse resting sites)

(Barret *et al*., 2003). More subtle approaches to tsetse control are now preferred to the former, which cause long-lasting damage to the environment. Insecticides are now applied at ultra low volumes through aerial spraying or impregnated on odour-baited tsetse traps and targets (Torr *et al*., 2006). The effectiveness of baits depends on ecological and behavioural patterns of the different species of tsetse flies. The flies are attracted to visual cues provided by large expanses of blue or black cloth and chemical clues such as acetone, a tsetse-attracting component of cow's breath (Barret *et al*., 2003). Pour-on application and oxen live-baits have also been successfully used to destroy tsetse flies (Cano *et al*., 2007). A much publicized approach to tsetse control involves release of sterile male flies, encouraging females to mate unproductively. This strategy contributed to the eradication of *Glossina austeni* from the small island of Unguja (Zanzibar) off the Tanzanian coast (Barret *et al*., 2003). However, an integrated application of all these methods is more preferred as it yields better results than using them individually (Krafsur, 2010).

CHAPTER 3: MATERIALS AND METHODS

3.1 STUDY DESIGN

A cross-sectional survey of CAT was carried out along the Central Luangwa valley in Mambwe district in the Eastern Province of Zambia.

3.2 STUDY AREA

The study area is located in Mambwe district in the Eastern Province of Zambia, lying between latitudes 10° and 15° South and longitudes 30° and 33° East (Simukoko *et al*., 2007). Mambwe district is typically savannah; it has a surface area of 4840 km² and an estimated human population of about 70,425. Its topography is made up of two major physical features; the Muchinga escarpment and the Luangwa valley, with the Luangwa river basin providing the main drainage system (Mambwe district council, 2008).

The climatic conditions in Mambwe are characterised by a long dry season from May to October and a wet season from November to April. Temperatures in the dry season range from 12°C to 40°C with a mean of 26°C. The district becomes hot and humid between August and October during which high evaporation and transpiration rates are attained. The valley ensures that a less pronounced cold season is experienced between May to July. (Mambwe district council, 2008). The average annual rainfall received is 895 mm, varying from 469mm to 1505 mm. During the rainy season, a lot of floods are experienced despite the limited amount of rain Mambwe receives. Water from surrounding districts in the plateau area drains down into the district, causing severe flooding that often results in the valley roads becoming impassable for many days (Mambwe district council, 2008).

Mambwe district has abundant wildlife and an increasing livestock population. The population of cattle and domestic dogs is estimated at over 5,000 and 8,000, respectively (Ministry of Agriculture & Livestock Development, personal communication). Three species of tsetse flies infest the district, namely *G. m.*

morsitans, G. pallidepis and *G. brevipalpis* (Ministry of Agriculture & Co-operatives, 2002). Depending on the levels of tsetse fly interaction with humans, domestic and wild animals; the distribution and impact of African trypanosomiasis is expected to vary from one location to another (Simokoko *et al*., 2007). With this in mind, 5 sites (Kakumbi, Malama, Msoro, Munkanya and Nsefu) were strategically marked for sampling as shown in figure 3.1 below. The chiefdoms are characterised as follows;

(i) Malama chiefdom is a wildlife zone where dogs are the only form of domesticated animals kept.

(ii) Nsefu and Kakumbi chiefdoms are wildlife zones where dogs and other domesticated animals are kept.

(iii) Munkanya and Msoro chiefdoms are at the edge of wildlife zones where dogs and other domesticated animals are kept.

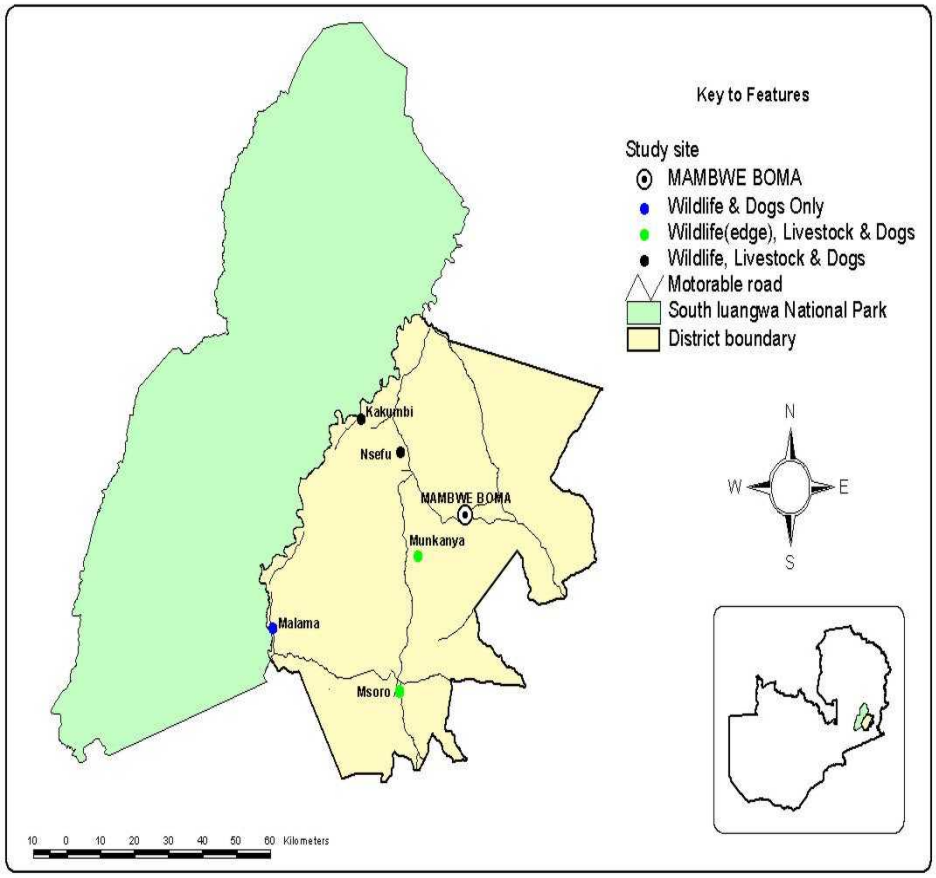


Figure 3.1: Map of Mambwe district showing the location of the chiefdoms sampled.

3.3 SAMPLE SIZE AND SAMPLING TECHNIQUE

The sample size was determined using the following formula with an estimated CAT prevalence of 5% at 95% confidence level.

$$N = \frac{Z^2 \times P(1-P)}{d^2}$$

Where;

N= required sample size

Z= Z statistic (usually 1.96)

P= expected prevalence (conservative 0.5)

d= P/2; acceptable accuracy range (+/- 0.025)

Therefore the calculated sample size was 292 dogs. However, this figure could not be obtained on the ground due to the hostility exhibited by several dog owners towards the study team. Local residents assumed that the study was working in collaboration with ZAWA to kill their dogs as a means of curbing poaching activities. They claimed that previous vaccination programs resulted in dogs dying, losing vision or becoming inefficient hunters, hence the resistance. Nonetheless, after the studies objective and expected benefits were fully explained, some of the dog owners responded positively and allowed sampling to take place. As a result, Snow bowling technique was used to collect samples from various household during the month of October, 2012. Any dog regardless of its age and sex was included in the study except those whose owners did not consent. All participating dogs received free vaccinations against rabies and those that were suspected to be AAT positive were treated with diminazene aceturate (Berenil).

3.4 BLOOD COLLECTION AND MICROSCOPY

Each participating dog was initially held by its owner before a skilled veterinary officer firmly held it to avoid unnecessary injuries during blood collection (Fig 3.2A). Anal temperature readings were recorded using digitized thermometers (Fig 3.2B).

After each reading, thermometers were sterilized with methylated spirit before inserting into the next dog. All dogs were physically examined by veterinarians for body condition score (Munang'andu *et al*., 2011; also see Appendix A), visible mucous membrane colour, superficial lymph nodes, corneal opacity and presence of ecto-parasites (ticks).

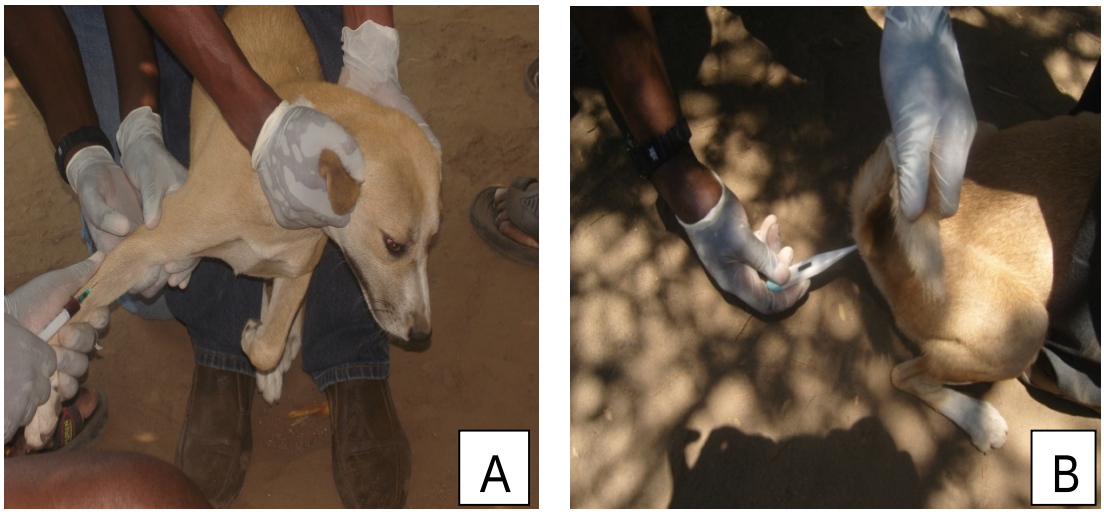


Figure 3.2: (A) Blood collection from the cephalic vein of a dog. (B). Anal body temperature reading using digitized thermometers.

A clean piece of cotton wool soaked in methylated spirit was used to sterilise the site marked for bleeding on the forelimb. About 2 ml of whole blood was collected from the cephalic vein using sterile 21G disposable needles (Fig. 3.2A) and transferred into labelled EDTA vacutainer tubes bearing the dogs' unique identifications (ID). Samples were held in tube racks in cooler boxes containing ice. Initial processing of samples was done the same day at Kakumbi Tsetse Research Station within Kakumbi chiefdom, Mambwe district. A small amount of each blood sample was drawn into a labelled heparinised capillary tube sealed with cristaseal on one end and centrifuged for 5 minutes at a speed of 2,500 rpm. Packed cell volume (PCV) values were determined using a haematocrit reader and recorded (Matete, 2003; Waiswa *et al*., 2003). In this study, in addition to pale mucous membranes, PCV values less that 37% were considered anaemic: mild (30-36%), moderate (18-29%) and severe (<18%) (Couto and Wellman, 1998). Thin giemsa stained smears were made (Fig 3.3A) and thoroughly examined by microscopy (Fig.

3.3B) for presence of haemoprotozoa including African trypanosomes at magnification x10 and x100 (immersion oil). The results by microscopy were recorded on individual dog record sheets bearing the exact ID.

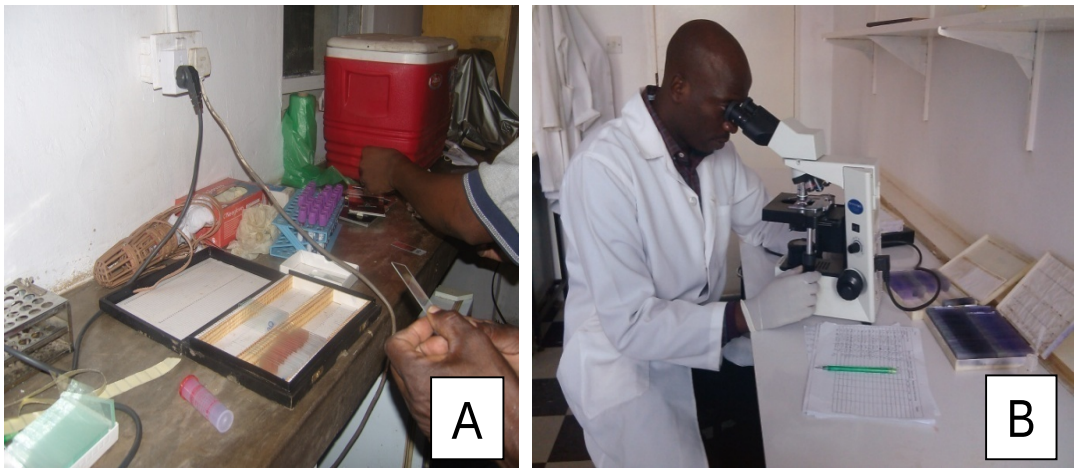


Figure 3.3: (A) Making of thin smears. (B) Microscopic examination of thin smears at Kakumbi Tsetse Research Station, Mambwe district.

3.4.1 DNA EXTRACTION

About 200 µl of blood sample was placed on a labelled FTA® Elute card (Whatmans FTA® Elute cards, Whatman, UK; Fig. 3.4A) and allowed to air dry for at least 30 minutes before being placed in sealed containers. Using Harris Uni-Core device, 3 sample discs (about 3 mm in diameter) were punched from the centre of each dried blood spot (Fig. 3.4B) and placed into labelled 0.5 ml eppendorf tubes. The discs were rinsed in 500 µl of double distilled water (DDW) and after removing all liquid, 30 µl of fresh DDW was added. The tubes were centrifuged to ensure that the discs were completely covered by the water, and the DNA was eluted by rapid boiling at 95°C for up to 30 minutes as described by Duscher *et al*., (2009). The resultant DNA was stored at -20°C until further use.

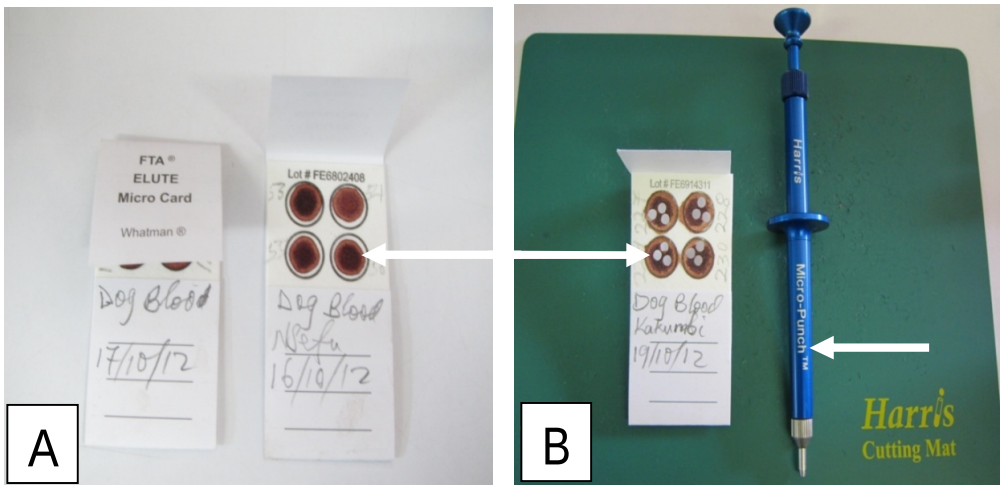


Figure 3.4: (A) Blood spots on Whatman FTA® Elute Cards. (B) Blood spots punctured using a Harris puncher as indicated by arrows.

3.4.2 LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

A LAMP reaction of 25 µl was performed using a Loopamp DNA Amplification Kit (Eiken Chemical, Tochigi, Japan) and the extracted parasite DNA as template, according to the manufacturer's instructions, with minor modifications. Briefly, 3 µl of template DNA was added to a 22 µl master mix containing 12.5 µl of reaction buffer (40 mM Trish-HCl (pH 8.8), 20 mM KCl, 16 mM $MgSO_4$, 20 mM $(NH_4)2SO_4$, 0.2% Tween 20, 1.6 M Betaine, 2.8 mM of each dNTP), 1 µl of *Bst* DNA polymerase enzyme (provided in the Loopamp DNA Amplification kit), 1 µl Loopamp fluorescent detection reagent (Eiken Chemical, Tochigi, Japan), 2 µl primer mix and 5.5 µl distilled water. This study made use of the recently designed primers specifically targeting the 18S rRNA gene of *T. congolense* (CON2-LAMP) (Thekisoe *et al*., 2007), the RIME gene of the *Trypanozoon* subgroup (RIME-LAMP) and the SRA gene of *T. b. rhodesiense* (SRA-LAMP) (Njiru *et al*., 2008), respectively (Table 3.1). The primer mixes were made as follows: (i) *T. congolense* amplification targeting the CON2 18s rRNA gene (BIP and FIP at 40 pmol each, F3 and B3 at 5pmol each); (ii) *T. brucei* amplification targeting the repetitive insertion mobile element (RIME) gene (FIP and BIP at 40 pmol each, Loop F and Loop B at 20 pmol each, F3 and B3 at 5pmol each); (iii) all RIME-LAMP positive samples were tested for *T. b.rhodesiense* targeting the SRA gene, (FIP and BIP at 40pmol each, Loop F and Loop B at 20 pmol each, F3 and

B3 at 5 pmol each). RIME-LAMP positive samples and SRA-LAMP negative samples were considered to be *T. b. brucei*. The reaction mixture was incubated at 64°C for 30 minutes in a heat block (Dry Thermounit DTU 1B, TAIEC Co., Saitama, Japan) and then at 95°C for 2 minutes to terminate the reaction. The LAMP products were visualized using a transilluminator (WD, H19, Good design award Co., Japan).

Table 3.1: List of Trypanosome species-specific LAMP Primer Sets

Target gene	Primer	Sequence	Specificity	Reference
RIME	FIP	5'-GGAATACAGCAGATGGGGCGAGGCCAATTGGCATCTTTGGGA-3'	*Trypanozoon*	Njiru *et al*., 2008
	BIP	5'-AAGGGAGACTCTGCCACAGTCGTCAGCCATCACCGTAGAGC-3'		
	F3	5'-CTGTCCGGTGATGTGGAAC-3'		
	B3	5'-CGTGCCTTCGTGAGAGTTTC-3'		
	LF	5'-GCCTCCCACCCTGGACTC-3'		
	LB	5'-AGACCGATAGCATCTCAG-3'		
SRA	FIP	5'-*GGACTGCGTTGAGTACGCATCCGCAAGCACAGACCACAG*-3'	*T.b.rhodesiense*	Njiru *et al*., 2008
	BIP	5'-*CGCTCTTACAAGTCTTGCGCCCTTCTGAGATGTGCCCACT*-3'		
	F3	5'-*GCGGAAGCAAGAATGACC*-3'		
	B3	5'-*TCTTACCTTGTGACGCCTG*-3'		
	LF	5'-*CGCGGCATAAAGCGCTGAG*-3'		
	LB	5'-*GCAGCGACCAACGGAGCC*-3'		
CON2 18s rRNA	FIP	5'-GCGCATGCGTCGGTGTTATTTTCGCGTGTGTGTTCATGTCA-3'	*T. congolense*	Thekisoe *et al*., 2007
	BIP	5'-ACTCTCCCCCCAAAATGGTTGTCCAAGCACGCAAATTCACAT-3'		
	F3	5'-TGTGTGTTTGTCGTGGAAGC-3'		
	B3	5'-ATTCGTGACCGCGTCAAA-3'		

3.5 DATA COLLECTION AND ANALYSIS

Data were collected and entered into a record sheet specific for each sampled dog (see Appendix B). The captured data were then entered, stored and statistically analysed using STATA version 11.0. The Fisher's exact test was used to determine whether there was an association between the outcome variable (microscopy and LAMP result) and categorical variables under consideration. Binary logistic regression was used to determine predictors of being positive to CAT by microscopy and LAMP. P values <0.05 were considered statistically significant; however, variables with P values ≤0.10 were still included in the multivariate regression model to take care of confounders. The Akaike information criterion (AIC) and Bayesian information criterion were used to ensure that the regression model fitted the data, and its predictive ability was determined through the generation of receiver operator characteristics (ROC) curve.

3.6 ETHICAL CONSIDERATIONS

Approval to conduct this study was sought on 17th July 2012 from the University of Zambia Biomedical Research Ethics Committee and was granted a 1 year approval on 24th July 2012 under Ref: 020-07-12 (see Appendix C). All ethical guidelines were followed and any information deemed sensitive was kept confidential. No names of participating dogs or their owners have been used in this study.

A total of 237 indigenous dogs (mongrel breed) were sampled from 47 villages within 5 chiefdoms in Mambwe district. Of these, 225 (94.9%; 95% CI: 92.1 – 97.7%) were recorded as hunting dogs. Gender wise, 128 (54.0%; 95% CI: 47.6 - 60.4%) were males and 109 (45.9%; 95% CI: 39.6 – 52.4%) were females, with ages ranging from 3 months to 16 years. Age was categorised as either young (<1 year) or adult (>1 year). Accordingly, 85 dogs (35.9%; 95% CI: 29.7 – 40.0%) were young and 152 dogs (64.1%; 95% CI: 58.0 – 70.2%) were adults. Their coat colour was clustered into 3 groups depending on the dominant colour; those that had black and any other colour (black +) were 20 (8.4%; 95% CI: 4.9 – 12.0%); brown and any other colour (brown +) were 207 (87.3%; 95% CI: 83.1 – 91.6%) and those with white and any other colour (white +) were 10 (4.2%; 95% CI: 1.6 – 6.8%).

4.1 CLINICAL APPEARANCE OF THE EXAMINED DOGS

Physical examinations revealed a total of 31 (13.1%; 95% CI: 8.8 – 17.4%) emaciated dogs (Fig. 4.1A) as described by Munang'andu *et al*., (2011). It was observed that 55 dogs (23.2%; 95% CI: 17.8 – 28.6%) were infested with ecto-parasites, mainly ticks of *Rhipicephalus* species (as described by Soulsby, 1982), found predominantly in the ears (Fig. 4.1B) and inter digital spaces.

Figure 4.1: Clinical examination of a dog showing (A) an emaciated adult and (B) heavy tick infestation (indicated by arrow) of the ears.

As observed by Bwalya *et al*., (2011), most of the examined dogs in this study were also seen passing tape and round worms in their stool during sampling. Anaemia, evidenced by pale mucous membranes (Fig. 4.2A), was recorded in 163 dogs (68.8%; 95% CI: 62.8 – 74.7%), while about 24 dogs (10.1%; 95% CI: 6.3 – 14.0%) had enlarged superficial lymph nodes. Furthermore, a total of 5 dogs (2.1%; 95% CI: 0.3 – 4.0%) exhibited corneal opacity (Fig. 4.2B) while 1 adult male dog exhibited scrotal oedema (Fig. 4.3)

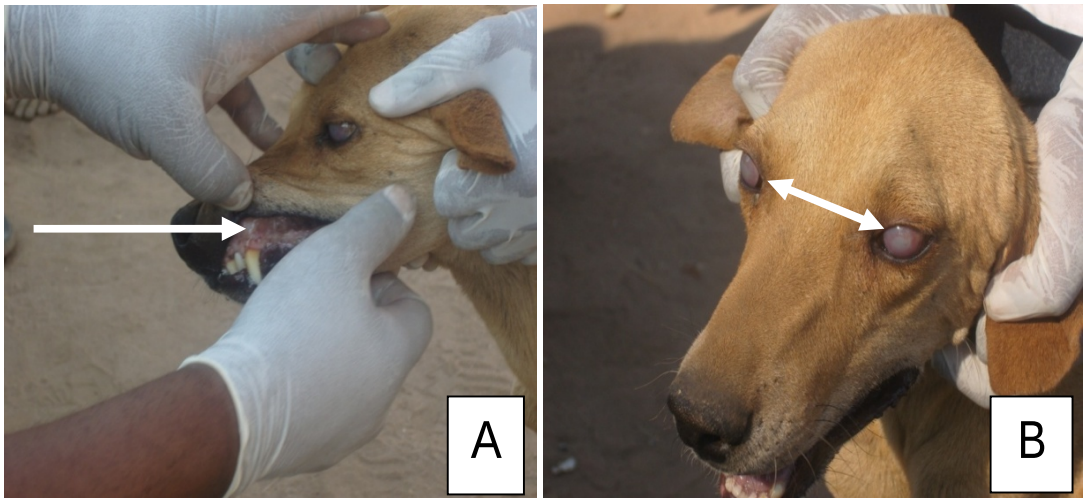


Figure 4.2: Clinical examination of a dog showing (A) pale mucus membranes of the mouth and (B) evidence of bilateral corneal opacity, indicated by arrows.

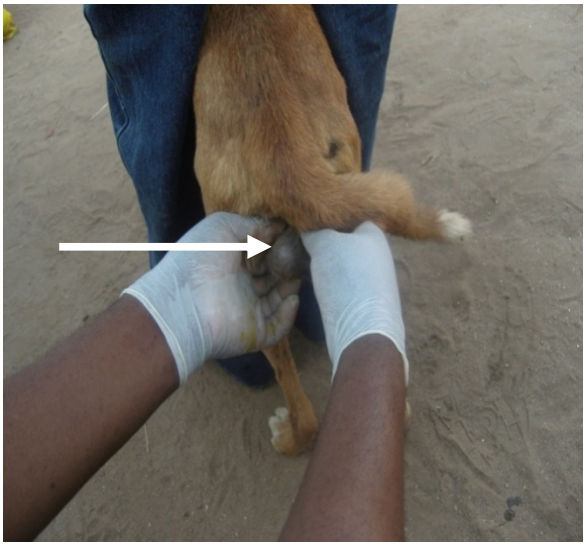


Figure 4.3: Clinical examination of an adult male dog showing scrotal oedema as indicated by arrow.

4.2 DETECTION OF AFRICAN TRYPANOSOMES IN DOG BLOOD BY MICROSCOPY

Out of the 237 sampled dogs, 14 tested positive for CAT by microscopy (5.9%; 95% CI: 2.9 – 8.9%). Of those, 11 were monolytic infections (4.6%; 95% CI: 2.1 – 8.1%), 10 caused by *T. congolense* (Fig. 4.4A) and 1 by *T. brucei* subspecies (Figure 4.4B), respectively. The other 3 were co-infections of both parasites (1.3%; 95% CI: 0.2 – 2.7%). Identification and speciation of trypanosomes was achieved by comparing the general body size and shape, location and size of kinetoplast, shape of posterior end, presence or absence of free flagellum and undulating membrane (Boyt, 1988).

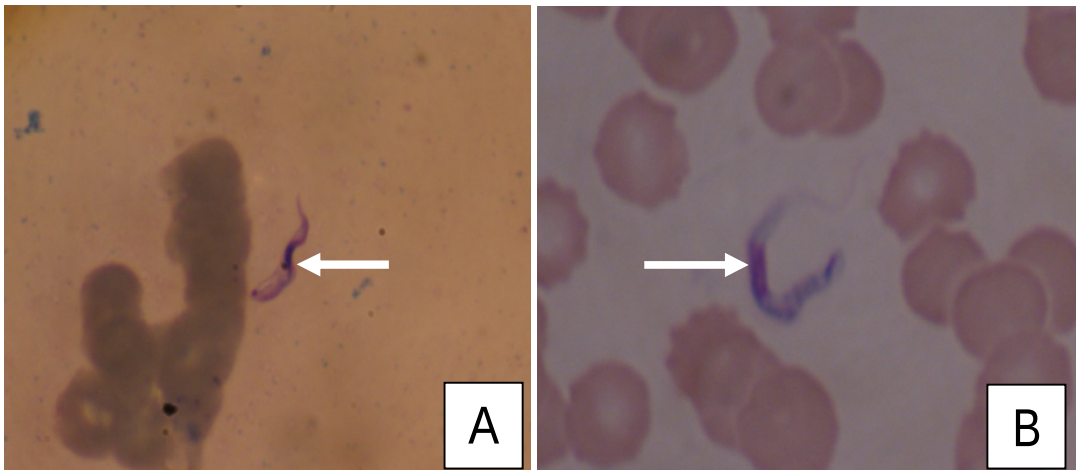


Figure 4.4: Representative Giemsa stained thin blood smear of a dog infected with (A) *T. congolense* and (B) *T. brucei* subspecies, indicated by arrows. Original magnification x1000

Interestingly, in all the CAT cases that involved bilateral corneal opacity (Fig. 4.2B) and that of scrotal oedema (Fig. 4.3), only *T. brucei* subspecies was identified in the blood smears.

The association between the outcome of interest (CAT by microscopy) and various independent variables (risk factors & clinical signs) was measured using Fisher's exact test and the results are presented in table 4.1 below.

Table 4.1: Measure of CAT (by microscopy) against several independent variables

MICROSCOPY					
VARIABLE	**TYPE**	**TOTAL (%) (n =237)**	**+VE (%) (n = 14)**	**95% CI**	**P-VALUE**
A. RISK FACTORS					
1. Chiefdom	Kakumbi	36 (15.2)	0 (0.0)	0.0 – 0.0	**0.003***
	Nsefu	68 (28.7)	3 (21.4)	4.2 – 46.0	
	Msoro	53 (22.4)	0 (0.0)	0.0 – 0.0	
	Malama	30 (12.6)	4 (28.6)	6.5 – 55.6	
	Munkanya	50 (21.1)	7 (50.0)	20.0 – 79.9	
2. Sex	Male	128 (54.0)	9 (64.3)	35.6 – 92.9	0.582
	Female	109 (46.0)	5 (35.7)	7.0 – 64.4	
3. Age	Young	85 (35.9)	1 (7.1)	1.2 – 22.6	**0.021***
	Adult	152 (64.1)	13 (92.9)	77.4 – 99.8	
4. Coat colour	Black+	20 (8.4)	1 (7.1)	1.2 – 23.7	0.653
	Brown+	207 (87.4)	12 (85.8)	64.7 – 98.7	
	White+	10 (4.2)	1 (7.1)	1.2 – 23.7	
5. Used for hunting	No	12 (5.1)	1 (7.1)	1.2 – 22.6	0.527
	Yes	225 (94.9)	13 (92.9)	77.4 – 99.8	
B. CLINICAL SIGNS					
1. Body condition	Normal	206 (86.9)	11 (78.6)	53.9 – 99.2	0.404
	Emaciated	31 (13.1)	3 (21. 4)	4.2 – 46.0	
2. Ecto-parasites	No ticks	182 (76.8)	12 (85.7)	64.7 – 98.7	0.531
	Ticks present	55 (23.2)	2 (14.3)	3.7 – 35.3	
3. Vision	Normal	232 (97.9)	11 (78.6)	53.9 – 99.2	**0.002***
	Loss of vision	5 (2.1)	3 (21.4)	4.2 – 46.0	
4. Superficial lymph nodes	Normal	213 (89.9)	11 (78.6)	53.9 – 99.2	0.157
	Swollen	24 (10.1)	3 (21.4)	4.2 – 46.0	
5. Mucous membranes colour	Normal	215 (90.7)	8 (57.1)	27.5 – 86.6	**0.001***
	Pale	22 (9.3)	6 (42.9)	13.2 – 72.5	
6. PCV	Normal	74 (31.2)	4 (28.6)	6.5 – 55.6	0.459
	Mild	65 (27.4)	3 (21.4)	4.2 – 46.0	
	Moderate	85 (35.9)	5 (35.7)	7.0 – 64.0	
	Severe	13 (5.5)	2 (14.3)	3.7 – 35.3	

***Indicates significant p-values at $p < 0.05$.

Based on microscopy, these data suggest that the prevalence of CAT seems to be associated with 4 variables including chiefdom, age, vision and mucous membranes.

Using binary logistic regression, both univariable and multivariable analysis (Tables 4.2 and 4.3, respctively) were used to determine true predictors of being CAT positive by microscopy, as well as sort for confounding factors.

Table 4.2: Univariable analysis of factors associated with CAT by microscopy

VARIABLE	SUB-VARIABLE	ODDS RATIO (OR)	OR (95% CI)	P-VALUE
A. RISK FACTORS				
1. Chiefdom	Kakumbi, Msoro & Nsefu	1		
	Malama	7.9	1.7 – 37.3	**0.009***
	Munkanya	8.4	2.1 – 33.7	**0.003***
2. Sex	Female	1		
	Male	1.6	0.5 – 4.8	0.430
3. Age	Young	1		
	Adults	7.9	1.0 – 61.1	**0.049***
4. Coat Colour	White+	1		
	Black+	0.5	0.0 – 8.5	0.611
	Brown+	0.6	0.6 – 4.7	0.590
5. Used for hunting	Non hunter	1		
	Hunter	0.7	0.1 – 5.6	0.716
B. CLINICAL SIGNS				
1. Body condition	Normal	1		
	Emaciated	1.9	0.5 – 7.2	0.347
2. Ecto-parasites	No Ticks	1		
	Ticks	0.5	0.1 – 2.5	0.422
3. Vision	Normal	1		
	Loss of vision	30.1	4.6 – 199.3	**<0.001***
4. Superficial lymph nodes	Normal	1		
	Enlarged	2.6	0.7 – 10.2	0.162
5. Mucous membrane colour	Normal	1		
	Pale	9.7	3.0 – 31.4	**<0.001***
6. PCV	Normal	1		
	Mild	0.8	0.2 – 4.0	0.832
	Moderate	1.1	0.3 – 4.2	0.897
	Severe	3.2	0.5 – 19.5	0.211

*Indicates significant p-values at $p < 0.05$.

Note: Zero CAT prevalence in Kakumbi and Msoro causes perfect predictive failure of chiefdom as a variable. Hence these two subvariables were merged with one of the three subvariables with CAT to ensure perfect analysis.

Only significant factors were considered for entry into a multivariable logistic regression model as shown in table 4.3 below.

Table 4.3: Multivariable logistic regression model of factors associated with CAT by microscopy

VARIABLE	SUB-VARIABLE	ODDS RATIO (OR)	OR (95% CI)	P-VALUE
A. RISK FACTORS				
1. Chiefdom	Kakumbi, Msoro & Nsefu	1		
	Malama	18.3	2.4 – 138.6	**0.005***
	Munkanya	10.4	2.0 – 54.7	**0.006***
2. Age	Young	1		
	Adults	12.6	1.4 – 114.5	**0.025***
B. CLINICAL SIGNS				
1. Vision	Normal	1		
	Loss of vision	17.8	1.2 – 267.3	**0.037***
2. Mucous membrane colour	Normal	1		
	Pale	3.3	0.6 – 17.4	0.159

* Indicates significant p-values at $p < 0.05$.

From the logistic regression analysis, CAT was predicted by Chiefdom, age, vision and colour of mucous membranes. Calculations of the area under the ROC curve (0.89; Fig 4.5) and model fittiness (df = 6, AIC = 82.9; BIC = 103.7) both show an acceptable predictive ability of the model.

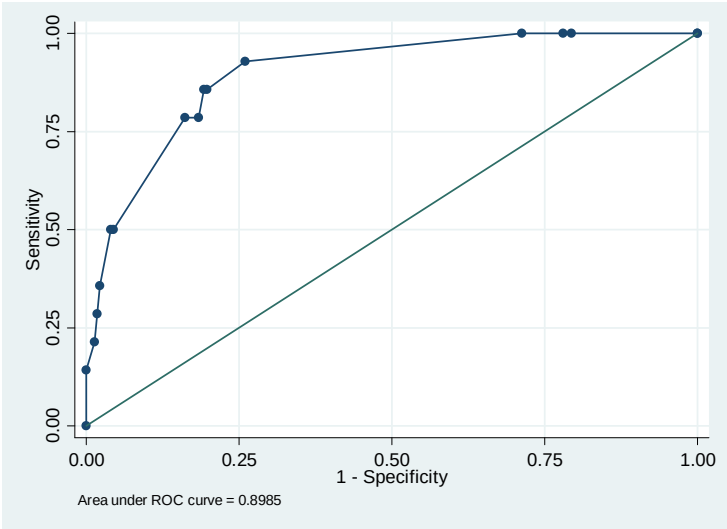


Fig 4.5: Area under the ROC curve for the multivariable model (by microscopy)

In addition to the CAT cases, microscopy detected a single case of tick-borne transmitted canine babesiosis caused by *Babesia canis* (Fig. 4.6), involving a barely 1 year old male dog from Msoro chiefdom.

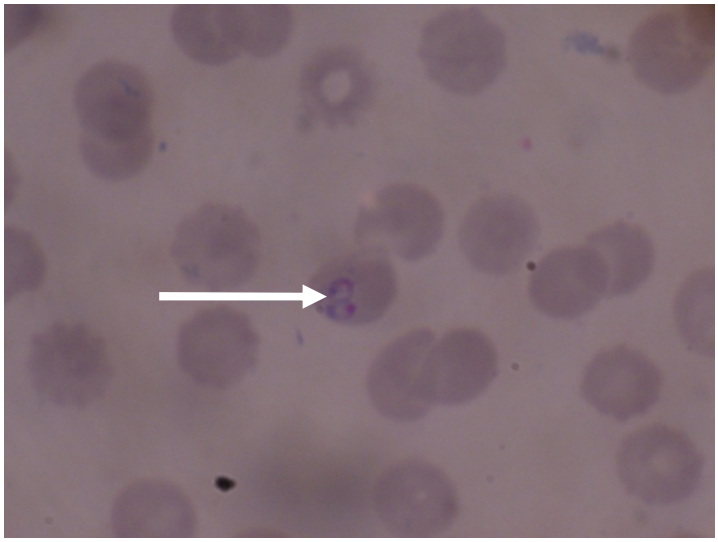


Figure 4.6: Scanty infection of *Babesia canis* (indicated by arrow) in a dog's red blood cells. Original magnification x1000

4.3 DETECTION OF AFRICAN TRYPANOSOMES IN DOG BLOOD BY LAMP

All the 237 DNA samples extracted from dog blood were also subjected to LAMP analysis using trypanosome-species-specific primers (CON2-LAMP, RIME-LAMP and SRA-LAMP). LAMP positive samples could clearly be distinguished from the negative ones through colour change i.e. bright fluorescent green (Fig4.7A-C). Accordingly, LAMP detected a total of 20 CAT cases (8.4%; 95% CI: 4.9 – 12.0%). These cases included all the 14 positive cases by microscopy plus 6 additional ones. LAMP was able to distinguish even closely related trypanosome species. According to LAMP, 13 dogs (5.5%; 95% CI: 0.7 – 10.6%) were monolytically infected, 3 with *T. congonlense*, 4 with *T. b. brucei* and 6 with *T. b. rhodesiense* (Fig. 4. 7A-C; Fig. 4.8). The other 7 dogs (2.9%; 95% CI: 0.6 – 5.9%) were co-infected, 2 with *T. congolense* and *T. b. brucei* and 5 with *T. congolense* and *T. b. rhodesiense* (Fig. 4.7B-C; Fig. 4.8). Whereas no infections of CAT were recorded from Msoro and Kakumbi chiefdoms, Munkanya, Nsefu and Malama each recorded prevalences of 3.8% (95% CI: 0.8 – 6.9%), 2.5% (95% CI: 0.5 – 4.5%) and 2.1% (95% CI: 0.3 – 4.0%), respectively.

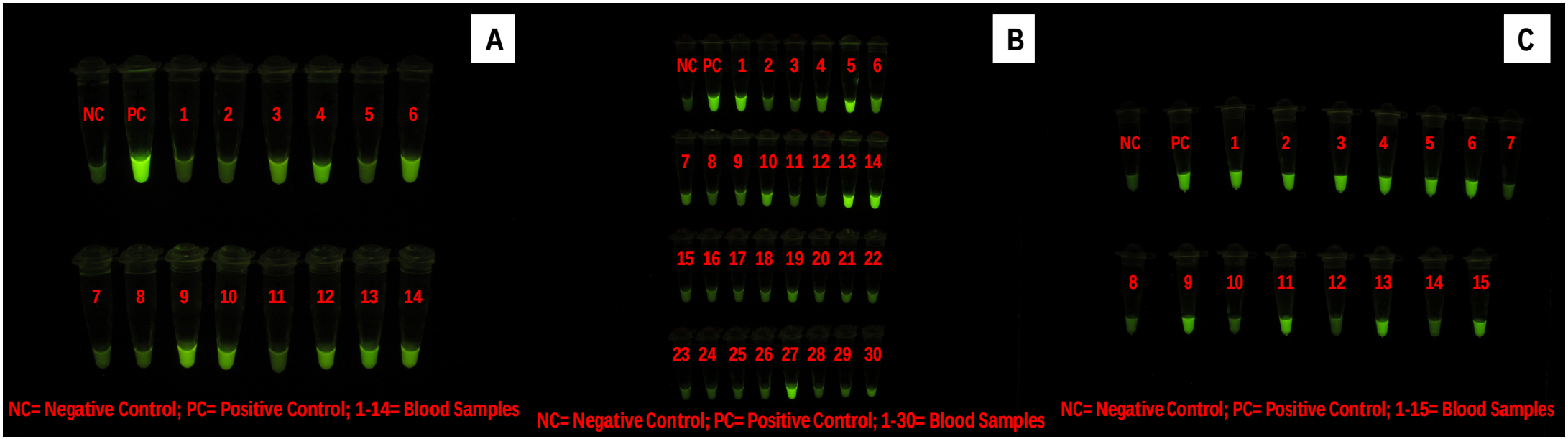


Figure 4.7: Visual appearance of representative results for CON2-LAMP (A), RIME-LAMP (B) and SRA-LAMP (C). In contrast to the light green background fluorescence in the negative samples, positive samples exhibit a bright fluorescent green colour when visualized under the transilluminator.

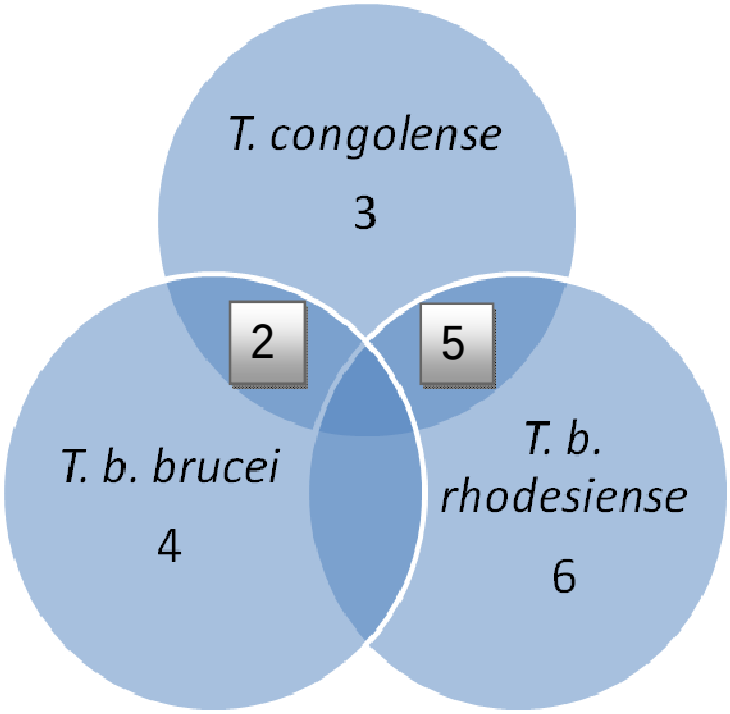


Figure 4.8: Venn diagram showing a total of 13 monolytic and 7 coinfections detected in the 20 trypanosome infected dogs.

The association between the outcome of interest (CAT by LAMP) and various independent variables (risk factors & clinical signs) was measured using Fisher's exact test and the results are presented in table 4.4 below.

Table 4.4: Measure of CAT (by LAMP) against several independent variables

LAMP					
VARIABLE	**TYPE**	**TOTAL (%) (n =237)**	**+VE (%) (n = 20)**	**95% CI**	**P-VALUE**
A. RISK FACTORS					
1. Chiefdom	Kakumbi	36 (15.2)	0 (0.0)	0.0 -0.0	**<0.001***
	Nsefu	68 (28.7)	6 (30.0)	7.9 – 52.0	
	Msoro	53 (22.4)	0 (0.0)	0.0 – 0.0	
	Malama	30 (12.6)	5 (25.0)	6.2 – 45.8	
	Munkanya	50 (21.1)	9 (45.0)	21.1 – 68.9	
2. Sex	Male	128 (54.0)	12 (60.0)	36.5 – 83.5	0.644
	Female	109 (46.0)	8 (40.0)	16.5 – 63.5	
3. Age	Young	85 (35.9)	5 (25.0)	6.2 – 45.8	0.339
	Adult	152 (64.1)	15 (75.0)	54.2 – 95.8	
4. Coat colour	Black+	20 (8.4)	3 (15.0)	4.4 – 32.1	0.232
	Brown+	207 (87.4)	15 (75.0)	54.2 – 95.8	
	White+	10 (4.2)	2 (10.0)	2.1– 24.4	
5. Used for hunting	No	12 (5.1)	3 (15.0)	4.4 – 32.1	0.069
	Yes	225 (94.9)	17 (85.0)	67.8 – 99.8	
B. CLINICAL SIGNS					
1. Body condition	Normal	206 (86.9)	14 (70.0)	47.9 – 92.0	**0.031***
	Emaciated	31 (13.1)	6 (30.0)	7.9 – 52.0	
2. Ecto-parasites	No ticks	182 (76.8)	16 (80.0)	60.8 – 99.2	1.000
	Ticks present	55 (23.2)	4 (20.0)	5.1 – 39.2	
3. Vision	Normal	232 (97.9)	17 (85.0)	67.8 – 99.8	**0.005***
	Loss of vision	5 (2.1)	3 (15.0)	4.4 – 32.1	
4. Superficial lymph nodes	Normal	213 (89.9)	16 (80.0)	60.8 – 99.2	0.129
	Swollen	24 (10.1)	4 (20.0)	5.1 – 39.2	
5. Mucous membranes colour	Normal	215 (90.7)	11 (55.0)	31.1 – 78.9	**<0.001***
	Pale	22 (9.3)	9 (45.0)	21.1 – 68.9	
6. PCV	Normal	74 (31.2)	4 (20.0)	5.1 – 39.2	0.147
	Mild	65 (27.4)	4 (20.0)	5.1 – 39.2	
	Moderate	85 (35.9)	9 (45.0)	21.1 – 68.9	
	Severe	13 (5.5)	3 (15.0)	4.4 – 32.1	

***Indicates significant p-values at $p < 0.05$.

Based on LAMP, these data suggest that the prevalence of CAT seems to be associated with 4 variables including chiefdom, body condition, vision and mucous membranes.

Using binary logistic regression, both univariable and multivariable analysis (Tables 4.5 and 4.6, respctively) were used to determine true predictors of being CAT positive by LAMP, as well as sort for confounding factors.

Table 4.5: Univariable analysis of factors associated with CAT by LAMP

VARIABLE	SUB-VARIABLE	ODDS RATIO (OR)	OR (95% CI)	P-VALUE
A. RISK FACTORS				
1. Chiefdom	Kakumbi, Msoro & Nsefu	1		
	Malama	5.0	1.4 – 17.7	**0.012***
	Munkanya	5.5	1.9 – 16.4	**0.002***
2. Sex	Female	1		
	Male	1.3	0.5 – 3.3	0.575
3. Age	Young	1		
	Adults	1.8	0.6 – 5.0	0.295
4. Coat Colour	White+	1		
	Black+	0.7	0.1 – 5.1	0.730
	Brown+	0.3	0.1 – 1.6	0.164
5. Used for hunting	Non hunter	1		
	Hunter	0.2	0.1 – 1.0	**0.049***
B. CLINICAL SIGNS				
1. Body condition	Normal	1		
	Emaciated	3.3	1.2 – 9.3	**0.025***
2. Ecto-parasites	No Ticks	1		
	Ticks	0.8	0.3 – 2.5	0.723
3. Vision	Normal	1		
	Loss of vision	19.0	3.0 – 121.4	**0.002***
4. Superficial lymph nodes	Normal	1		
	Enlarged	2.5	0.8 – 8.1	0.137
5. Mucous membrane colour	Normal	1		
	Pale	12.8	4.6 – 36.5	**<0.001***
6. PCV	Normal	1		
	Mild	1.1	0.3 – 4.8	0.850
	Moderate	2.1	0.6 – 7.0	0.242
	Severe	5.3	1.0 – 27.0	0.047*

*Indicates significant p-values at $p < 0.05$.

Note: Zero CAT prevalence in Kakumbi and Msoro causes perfect predictive failure of chiefdom as a variable. Hence these two subvariables were merged with one of the three subvariables with CAT to ensure perfect analysis.

Only significant factors were considered for entry into a multivariable logistic regression model as shown in table 4.6 below.

Table 4.6: Multivariable logistic regression model of factors associated with CAT by LAMP

VARIABLE	SUB-VARIABLE	ODDS RATIO (OR)	OR (95% CI)	P-VALUE
A. RISK FACTORS				
1. Chiefdom	Kakumbi, Msoro & Nsefu	1		
	Malama	1.8	0.4 – 8.9	0.476
	Munkanya	6.9	1.9 – 24.4	**0.003***
2. Used for hunting	Non hunter	1		
	Hunter	0.1	0.0 – 0.8	**0.029***
B. CLINICAL SIGNS				
1. Body condition	Normal	1		
	Emaciated	1.8	0.5 – 6.7	0.361
2. Vision	Normal	1		
	Loss of vision	5.6	0.5 – 65.6	0.168
3. Mucous membrane colour	Normal	1		
	Pale	10.2	2.5 – 41.8	**0.001***

* Indicates significant p-values at $p < 0.05$.

From this logistic regression analysis, CAT by LAMP was predicted by Chiefdom, hunting and colour of mucous membranes. Despite being significant at univariate analysis, body condition and vision when adjusted for confounding factors ceased to be significant. Calculations of the area under the ROC curve (0.83; Fig 4.9) and model fittiness (df = 7, AIC = 114.5; BIC = 138.7) both show an acceptable predictive ability of the model.

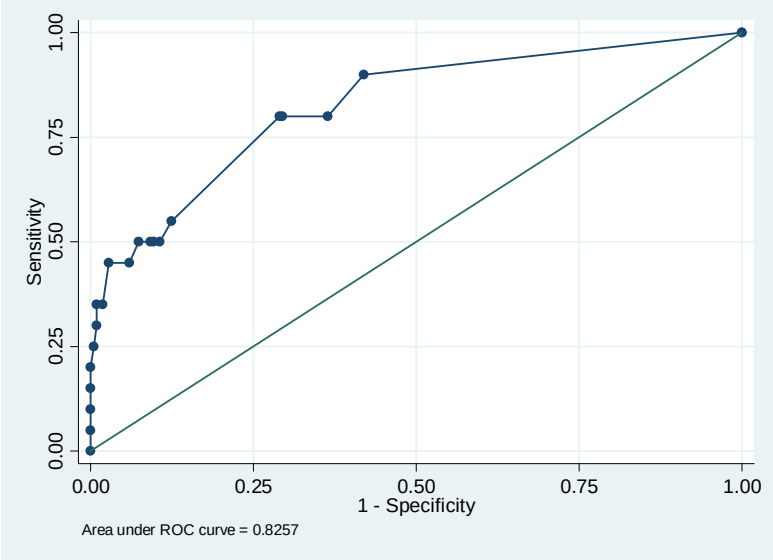


Fig 4.9: Area under the ROC curve for the multivariable model (by LAMP)

Irrespective of the detection technique used, CAT was only detected in 3 out of the 5 chiefdoms (Fig. 5.0). Whereas microscopy only detected 14 CAT cases (5.9%; 95% CI: 2.9 – 8.9%), the LAMP method increased the number of mongrel dogs with CAT to 20 (8.4%; 95% CI: 4.9 – 12.0%). LAMP further increased the cases with co-infections to 7 (2.9%; 95% CI: 0.6 – 5.9%) from the 3 (1.3%; 95% CI: 0.2 – 2.7%) that were detected by microscopy.

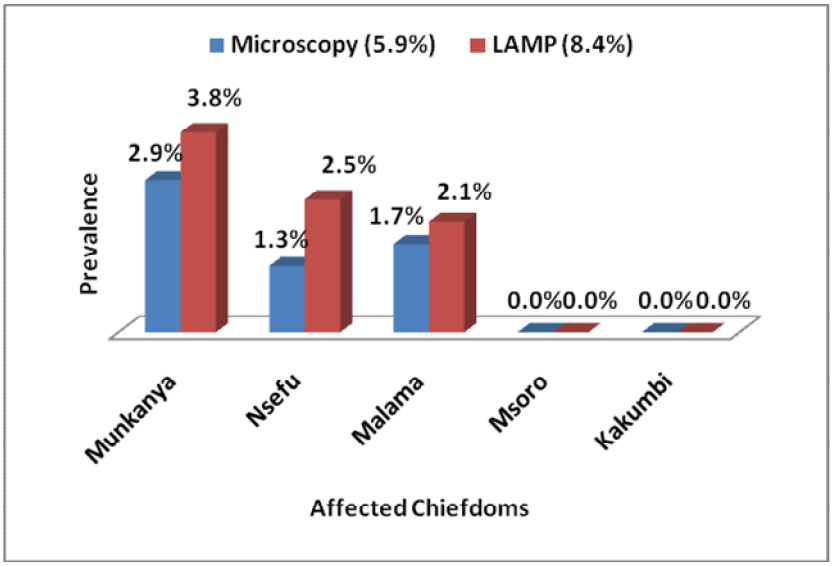


Figure 5.0: Comparative determination of CAT prevalence in dogs in chiefdoms of Mambwe district by microscopy and LAMP techniques.

More importantly, LAMP detected and distinguished *T. b. rhodesiense* and *T. b. brucei*. The human-infective *T. b. rhodesiense* was detected in dogs from Malama, 5/30 (16.7%; 95% CI: 2.5 – 30.8%); Munkanya, 3/50 (6.0%; 95% CI: 3.0 – 9.1%) and Nsefu, 3/68 (4.4%; 95% CI: 1.9 – 7.8%) chiefdoms (Fig. 5.1).

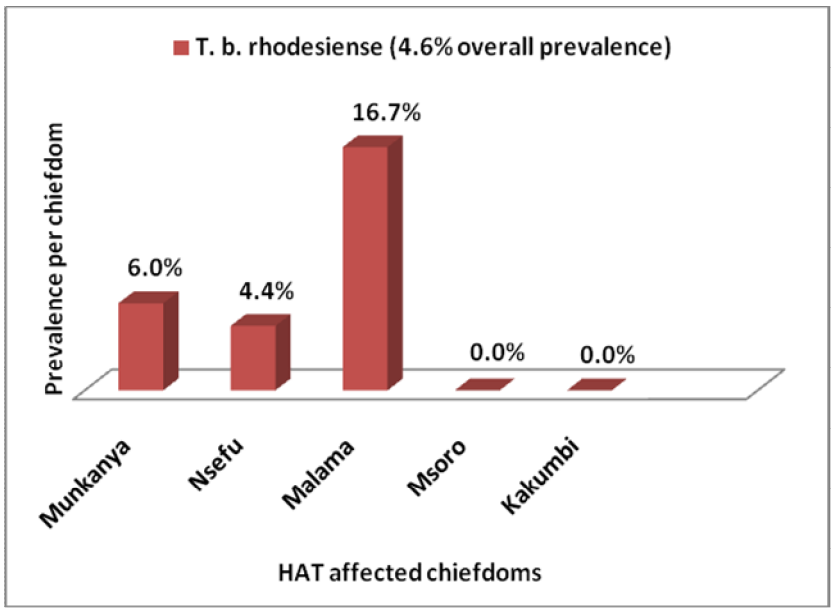


Figure 5.1: Determination of the prevalence of *T. b. rhodesiense* (HAT) in dogs in chiefdoms of Mambwe district by LAMP.

The diagnostic accuracy of LAMP (index) was determined against microscopy (reference standard) from calculations based on the 2 x 2 table 4.7 below.

Table 4.7: Diagnostic accuracy of LAMP and microscopy

	Microscopy		
	CAT present	CAT absent	Total
LAMP-positive	True Positive (TP) = 14	False Positive (FP) = 6	**TP+FP = 20**
LAMP-negative	False Negative (FN) = 0	True Negative(TN) = 217	**TN+FN = 217**
Total	**TP+FN = 14**	**TN+FP = 223**	**237**

Sensitivity = TP / (TP + FN) = 100%

Specificity = TN / (TN + FP) = 97.3%

Positive predictive value (PPV) = TP / (TP + FP) = 70%

Negative predictive value (NPV) = TN / (TN + FN) = 100%

Positive likelihood ratio (LR+) = sensitivity / (1 – specificity) = 37

Negative likelihood ratio (LR-) = (1 – sensitivity) / specificity = 0

The sensitivity of LAMP was measured based on the proportion of dogs that tested positive for CAT by microscopy and were also positive by LAMP. LAMP was found to be 100% (14/14) sensitive as an index test. The proportion of dogs that tested negative by microscopy and also negative by LAMP was used to determine the specificity of LAMP which was found to be 97.3% (217/223). The positive and negative predictive values which are proportions of true positives and true negatives, were determined to be 70% (14/20) and 100% (217/217), respectively. The positive likelihood ratio (LR) which is a ratio of proportions of dogs with CAT and was LAMP positive to the proportion of dogs without CAT and was LAMP positive, was determined to be 37. Equally, the negative LR which is the ratio of proportions of dogs with CAT and was LAMP negative to the proportion of dogs without CAT and was LAMP negative, was found to be 0. A positive LR > 10 and a negative LR < 0.1 can exert highly significant changes in probability that is capable of altering clinical management (Florokowski, 2008). LAMP had a positive LR of 37 and a negative LR of 0, suggesting that its use in CAT diagnosis can greatly improve the management of this disease especially in endemic areas.

CHAPTER 5: DISCUSSION

The potential role of domesticated dogs as reservoirs for parasitic zoonoses has been recognized as a significant public health problem worldwide (Traub *et al*., 2005). Unlike in developed nations, the lack of zoonotic awareness and veterinary attention in many developing countries has exacerbated the risks of disease transmission especially in socioeconomically disadvantaged communities living in rural settings (Nonaka *et al*., 2011). Among many parasitic canine zoonoses affecting humans and livestock in rural settings is African trypanosomiasis, which has received less public health awareness and attention (Brun *et al*., 2010; WHO, 2010; Mwanakasale *et al*., 2013). Although trypanosomiasis in farm animals such as cattle and goats is commonly reported (Simukoko *et al*., 2007; Marcoty *et al*., 2008), CAT is rarely reported in literature (Matete, 2003; Gow *et al*., 2007; Keck *et al*., 2009). The few available reports on naturally acquired CAT in East and West Africa indicate that indigenous breeds of dogs are carriers of zoonotic *T. b. rhodesiense* and *T. b. gambiense*, respectively (Matete, 2003; Njiru *et al*., 2004; Samdi *et al*., 2006). In Zambia, CAT has never been reported until recently when Namangala *et al*., (2013) documented naturally acquired *T. b. rhodesiense* in exotic breeds of dogs from the Luangwa and Zambezi valleys.

To the best of our knowledge, the current study was the first of its kind to determine the prevalence of African trypanosome species affecting indigenous dogs in Zambia. This was achieved by employing microscopy and LAMP as detection techniques. Based on the current results, LAMP increased the overall CAT prevalence to 8.4% (20/237) from the parasitological prevalence of 5.9% (14/237), suggesting an improved sensitivity by the former. In addition LAMP was more instrumental in trypanosome speciation and was able to distinguish even closely related parasite species such as *T. b. brucei* and *T. b. rhodesiense*. As such, the detection of co-infections also increased under LAMP. These findings are consistent with those of other studies and further support the idea that LAMP is more

sensitive than microscopy (Thekisoe *et al*., 2007; Matovu *et al*., 2010; Bukowe *et al*., 2012; Namangala *et al*., 2013).

LAMP amplifies DNA with high sensitivity relying on *Bst* DNA polymerase with strand displacement activity under isothermal conditions (Thekisoe *et al*., 2007). Additionally, it uses four to six specially designed primers recognising six to eight regions of the target DNA sequence, hence a high specificity. The auto-cycling reactions lead to accumulation of a large amount of the target DNA and other reaction by-products, such as magnesium pyrophosphate, that allow rapid detection using varied formats (Mori *et al*., 2001; Poon *et al*., 2006; Wastling *et al*., 2010; Njiru, 2012). When compared to PCR, LAMP has the advantage of being simpler, more rapid, cheaper and easier to perform as it only requires a heating device for incubation and may therefore be performed even in the field (Wastling *et al*., 2010; Namangala *et al*., 2012**a**). The LAMP assay shows high tolerance to biological products such that DNA extraction may not be necessary (Poon *et al*., 2006). Thus LAMP may be more practical for resource-limited communities (including Zambia) in regions where trypanosome infection is endemic (Bukowe *et al*., 2012). In addition, LAMP does not only detect pathogens of medical and veterinary importance, but also plant parasitic diseases, genetically modified products, including tumour and embryo sex identification (Njiru, 2012).

Literature shows that CAT may manifest itself as asymptomatic, chronic or acute based on the clinical signs (Boyt, 1988). This study made use of the clinical signs seen from the examined dogs to determine the status of CAT. It was interesting to note that over 68% of the sampled dogs exhibited pale mucous memebranes, which together with PCV is an indicator of anaemia (Matete, 2003; Abenga *et al*., 2005; Ezeokonkwo *et al*., 2010). Anaemia is the most important pathogenic consequence of infection with African trypanosomes (Boyt, 1988; Uilenberg, 1998; Abenga *et al*., 2005). Although most dogs were anaemic and had swollen lymph nodes, statistical analysis of results did not show any association between CAT and these exhibited clinical signs. Our findings are consistent with those of Ezeokonkwo *et al*., (2010)

who found that despite being characteristic and typical of trypanosomiasis in animals, these signs are not pathognomonic. Uilenberg (1998) further suggests that anaemia occurs in several other diseases, particularly infections with other blood parasites and certain intestinal worms. This also accords with observations in the present study, which showed a number of dogs harbouring ecto- and endo-parasites. Ticks of *Rhipicephalus* species observed mainly in the ears and interdigital spaces of several dogs transmit intra-erythrocytic protozoa, *B. canis* that cause canine babesiosis, resulting in anaemia (Nalubamba *et al*., 2010). Even though this study only detected a single case of *B. canis* infection in Msoro chiefdom, it was evident that the parasites circulate in indigenous dogs of Mambwe district. Secondly, during sampling many dogs passed stool containing endo-parasites (tape and round worms) (Ezeh *et al*., 2009; Bwalya *et al*., 2011; Nanoka *et al*., 2011). Such helminths infected dogs may also present with chronic anaemia, emaciation and poor general physical condition (Boyt, 1988; Bwalya *et al*., 2011; Traversa, 2012). In addition, several dogs looked malnourished as a result of living in resource limited communities (Traub *et al*., 2005; Nanoka *et al*., 2011). Starvation in dogs can cause anaemia and emaciation (Pointer et al, 2013). Therefore, despite most of the observed clinical signs in this study being reported as attributes of CAT (Matete, 2003; Abenga *et al*., 2005; Samdi *et al*., 2006; Akpa *et al*., 2008; Ezeh *et al*., 2009), they can be confounded by many other factors and as a result are not pathognomonic (Ezeokonkwo *et al*., 2010).

However, analysis of data using Fisher's exact test revealed that CAT was statistically associated with clinical signs (loss of vision due to corneal opacity and mucous membrane colour; by both microscopy and LAMP), and body condition (emaciation; only by LAMP). At univariable logistic regression analysis, predictors of being positive with CAT remained unchanged. However, after adjusting for confounding factors in the multivariable logistic regression model, only loss of vision and mucous membrane colour still remained significant by microscopy and LAMP, respectively. According to the calculated odds ratios, dogs with corneal opacity were between 5 – 17 times more likely to harbour trypanosomes than dogs with

normal vision. Equally the likelyhood that dogs with pale mucous membranes would harbour trypanosomes was about 4 – 10 times more than the normal dogs. Despite not being significant (p = 0.361), emaciated dogs were twice more likely to be infected than the non-emaciated dogs. Nonetheless, these three clinical signs stand as better predictors of CAT in dogs of Mambwe district. For instance, the use of loss of vision due to corneal opacity as a predictor of CAT corroborates the observations made by others that have associated it to infection caused by *T. brucei* subspecies (Boyt, 1988; Matete, 2003; Samdi *et al*., 2006; Ezeh *et al*., 2009; Ezeokonkwo *et al*., 2010). This may not be surprising as members of *T. brucei* subspecies can traverse the vascular tissue and cause damage to various extra-vascular tissues such as the liver, spleen, kidneys, lungs, eyes and the central nervous system (Horchner *et al*., 1985; Vershney *et al*., 1998). In contrast, *T. congolense* tends to be confined to the blood and lymphatic vessels (Matete, 2003; Ezeokonkwo *et al*., 2010; Namangala *et al*., 2013). Therefore, such manifestations of corneal opacity in dogs living in tsetse infested Mambwe district may indirectly indicate infection of either zoonotic *T. b. rhodesiense* or animal-infective *T. b. brucei* (Boyt, 1988; Matete, 2003). Furthermore, Matete (2003) suggests that pronounced corneal opacity in dogs can be used as preliminary sentinel warnings against the transmission of future HAT outbreaks.

When analysed for potential risk factors using Fisher's exact test, preliminary findings suggested that CAT was significantly associated with chiefdom (both by microscopy and LAMP) and age (only by microscopy). At univariable logistic regression analysis, predictors of being positive with CAT remained unchanged for microscopy and LAMP, except for hunting which at this point showed statistical significance. After adjusting for confounding factors in the multivariable logistic regression model, the three factors still remained significant. Interestingly, though significantly associated with CAT (p = 0.029), hunting dogs had a reduced likelyhood of 0.1 as compared to non hunters. Our data suggest that adult dogs, which are more likely to go into the bush and hence stand a chance of being bitten by tsetse flies, were 13 times more likely to be infected with trypanosomes than the young

dogs. As regards to chiefdoms, Munkanya was at least seven times more likely to have CAT infected dogs than the others. Therefore, the three risk factors stand as better predictors of dogs acquiring CAT in Mambwe district. As suggested by Simukoko *et al*., (2007), the distribution and impact of trypanosomiasis varied from one location to another, possibly due to the levels of tsetse-host interactions. It was interesting to note that out of the 5 chiefdoms (Kakumbi, Malama, Msoro, Munkanya and Nsefu), only 3 (Malama, Munkanya and Nsefu) recorded CAT. Although all the 5 chiefdoms were tsetse infested, there appeared to be disparities in the levels of interaction with dogs. A possible explanation for this could be that such interactions are highly influenced by the practice of illegal hunting in the Lupande GMA. Hunting is recognised as one of the predisposing factors to acquiring trypanosomiasis in tsetse infested areas (Samdi *et al*., 2006; Ezeh *et al*., 2009; WHO, 2010). Indigenous dogs are an integral part of most communities in Mambwe district and are mostly used for illegal hunting. This was evidenced by the fact that over 94% of the sampled dogs in this study were recorded as hunters. In order to control illegal hunting in the Lupande GMA, ZAWA personnel have been deployed in all chiefdoms. However, in Kakumbi and Msoro chiefdoms, there was heavy presence of ZAWA personnel that monitor acts of illegal hunting, with the former experiencing more. The situation in the other 3 chiefdoms was more bearable due to lower ZAWA presence and as a result illegal hunting could be on the higher side. This aspect seemed to influence the levels to which dogs in chiefdoms were exposed to infective tsetse fly bites and in turn the CAT prevalence.

Furthermore, the intrinsic characteristics of chiefdoms seemed to influence CAT. For instance, Malama chiefdom lies within a wildlife zone where no other domestic animals are kept except dogs; Kakumbi and Nsefu chiefdoms are within wildlife zones and livestock had been introduced; Msoro and Munkanya chiefdoms are at the edge of wildlife zones and livestock was present (Simukoko *et al*., 2007). What this entails is that, in the absence of wildlife, tsetse flies will switch to alternative blood meals within their reach (Munang'andu *et al*., 2012). Where both cattle and dogs exist, tsetse flies tend to feed more on cattle than dogs (Simukoko *et al*.,

2007). This is because larger objects are more attractive to tsetse flies than smaller ones (Hargrove, 1980). Thus, the size and strength of odours cattle produce inevitably attracts tsetse flies towards them (Boyt, 1988; Aksoy, 2003; Simukoko *et al*., 2007; Tirados *et al*., 2011). This may explain why the role of cattle as reservoirs of HAT is better understood than that of dogs (Njiru *et al*., 2004; Abenga *et al*., 2005; Fèvre *et al*., 2008; Brun *et al*., 2010). Therefore, the absence of CAT in Kakumbi and Msoro does not necessarily mean absence of AAT in cattle. Past AAT surveys in these chiefdoms have confirmed the presence of trypanosome species in cattle (Ministry of Livestock & Fisheries Development, 2010). Other factors considered as possible risks in this study were sex and coat colour. Based on tsetse behaviour, it was assumed that different coat colours would influence CAT since tsetse flies perceive shape, size, contrast with background, colour and movement (Simukoko *et al*., 2007; Tirados *et al*., 2011). Dog colour was therefore an important CAT factor in this study as it attracts and induces landing responses of flies on dogs (Schofield and Maudlin, 2001). All colours are known to divert tsetse from their usual upwind direction path, with black, red and blue being more attractive but the former having the strongest landing responses (Mramba *et al*., 2013). However, according the present study, none of these factors were statistically associated with CAT in Mambwe district.

In agreement with previous reports (Boyt, 1988; Matete, 2003; Gow *et al*., 2007; Eloy and Lucheis, 2009; Namangala *et al*., 2012**b**), CAT in the present study was caused by *T. congolense, T. b. brucei* and *T. b. rhodesiense*. From the determined prevalence by LAMP of 8.44%; 5.49% was due to monolytic infections of the 3 trypanosome species and 2.95% due to co-infections of *T. congolense* with *T. b. brucei* and *T. congolense* with *T. b. rhodesiense.* This observation partly supports the postulation that many CAT infections in the field are caused by more than one trypanosome species (Ezeokonkwo *et al*., 2010). In agreement with previous reports (Boyt, 1988; Gow *et al*., 2007; 't Hooft, 2008), *T. congolense*-infected indigenous dogs tend to exhibit chronic infections with comparatively less severe clinical symptoms as compared to exotic breeds. Such chronically infected dogs may act as

a source of infection to other domestic animals including domestic ruminants (Abenga *et al*., 2005; Simukoko *et al*., 2007; Namangala *et al*., 2012**b**) in tsetse-infested areas such as the Luangwa and Zambezi valleys. In cattle, sheep, pigs and goats, *T. congolense* causes a debilitating disease (*nagana*) that has serious economic implications in livestock production in endemic regions (Simukoko *et al*., 2007; Marcotty *et al*., 2008). For instance, in Eastern Zambia (within the Luangwa valley), about 34% of cattle, 27.6% of sheep, 20.9% of pigs and 10.2% of goats have *nagana*, mainly caused by *T. congolense* (Simukoko *et al*., 2007).

Based on experimental findings in co-infected dogs, *T. brucei* subspecies are thought to dominate and interfere with *T. congolense* in establishing parasitaemia and consequently its pathogenic effects (Ezeokonkwo *et al*., 2010). Unlike *T. congolense* dominated CAT, the presence of either *T. b. brucei* or *T. b. rhodesiense* infections in dogs were characterized by higher parasitemia and in some cases bilateral corneal opacity and scrotal oedema. This observation conforms to that of Ezeokonkwo *et al*., (2010). Furthermore, Boyt (1988) confirms that *T. b. brucei* infection in dogs produces a severe disease often ending in death. Indeed recent cases of naturally acquired *T. b. rhodesiense* infection in Zambia involving exotic dog breeds exhibited acute fatal forms of CAT. This was characterized by massive parasitosis, nervous signs, laboured breathing and ultimately death four days after onset of clinical symptoms (Namangala *et al*., 2013). Post mortem examination of these cases revealed severe inflammation, congestion and enlargement of internal organs (Namangala *et al*., 2013). In contrast, however, indigenous dogs seem to be more trypanotolerant, exhibiting either only mild symptoms or may be asymptomatic (Abenga *et al*., 2005).

Detection of the SRA gene in trypanosomes isolated from 11 indigenous dogs in this study is intriguing and suggests their ability to act as reservoirs of HAT (Matete, 2003; Njiru *et al*., 2004; Brun *et al*., 2010; Namangala *et al*., 2012**b**). The concerns raised by Anderson *et al*., (2011) of HAT establishing in Nyamaluma (within Malama chiefdom) and surrounding areas after finding an SRA positive African buffalo were

further highlighted by detecting the SRA gene in 5 hunting dogs within that chiefdom. Hunting dogs have been implicated to act as reservoirs of HAT in Nigeria (Samdi *et al*., 2006). Based on the postulation by Matete (2003) that sporadic and very low prevalence of human infective trypanosomes in dogs closely reflects disease occurrence in humans, the prevalence of *T. b. rhodesiense* infection in hunting dogs in Malama (16.7%), Munkanya (6.0%) and Nsefu (4.4%) chiefdoms could suggest similar occurrence in human communities. Although *T. b. rhodesiense* infection in dogs is usually acute and fatal (Matete, 2003; Njiru *et al*., 2004), indigenous dog breeds as observed in this study, exhibited levels of trypanotolerance (Abenga *et al*., 2005) as compared to the exotic breeds reported by Namangala *et al*., (2013). This increases the likelihood of tsetse flies to pick the disease from dogs and transmit it to any other susceptible hosts (Namangala *et al*., 2013).

In view of the close relationship shared with humans, infected dogs are a potential source of zoonotic HAT in communities living within GMAs. It is therefore important to conduct HAT surveillance in humans wherever *T. b. rhodesiense* infection has been diagnosed to facilitate effective prevention and control measures against HAT. In agreement with this postulation, a HAT case involving a 21 year-old male from Munkanya chiefdom was recently detected at Kamoto General Hospital (personal communication). This is in addition to other sporadic HAT cases being reported in the same region. Unfortunately, several tourist activities such as safari hunting, lodging and camping mainly take place within these chiefdoms. Since 2010-2012, over six HAT cases have been reported in returning tourists after visiting this area (Cottle *et al*., 2012; Simarro *et al*., 2012; Richter *et al*., 2012). Therefore, these should be considered as high risk areas for contracting HAT by residents and visitors of Mambwe district and the SLNP (Munang'andu *et al*., 2012; Richter *et al*., 2012). It is commonly assumed that humans must enter the normal woodland habitat of the flies to become infected, but recent studies in Zimbabwe found that tsetse (*G. m. morsitans* and *G. pallidipes*) frequently attack humans inside buildings (Vale *et al*., 2013). In consequence, it seems that the contact between tsetse and humans in

houses and other buildings is an important and distinctive venue for the transmission of sleeping sickness.

The transmission of CAT in these indigenous hunting dogs may not be entirely due tsetse fly bites but possibly through oral transmission as well. Literature shows that *Trypanozoon*, especially *T. brucei* subspecies and *T. evansi* which parasitize both blood and tissue, may be transmitted mechanically through oral contamination (Uilenberg, 1998). This mechanism could apparently occur quite easily when the oral mucosa of predators is damaged (Mbaya *et al*., 2008). Often times hunting dogs and wild carnivores develop abrasions in their oral mucosa caused by bone splinters of their prey (Mbaya *et al*., 2008; Anderson *et al*., 2011). Previous studies have shown that for oral transmission to be successful, feeding of infected carcasses by non-infected predatory carnivores must be done immediately after death for the trypanosomes to remain infective (Mbaya *et al*., 2009). This is because "carrion" feeding does not favour this mode of transmission (Baker, 1968; Sasaki *et al*., 1995). Consequently, dogs and cats living in the vicinity of slaughterhouses could be infected by eating fresh meat, blood, offal or bones of trypanosomiasis infected animals (Desquesnes et al., 2013). To prove this phenomenon, two cats and a bush baby were experimentally infected with *T. b. rhodesiense* after allowing them to kill and eat infected laboratory rats (Duke *et al.,* 1934; Heisch, 1963). Furthermore, Moloo *et al*., (1973) demonstrated that the penetration of trypomastigote forms of the parasite can occur through the normal oral mucosa, in which *T. brucei* was able to multiply in cats and dogs after consuming infected goat meat. Elsewhere, oral infections involving *T. evansi* (not found in Zambia), have equally been reported in captive carnivores, hunting dogs, cats and rodents that were fed with infected meat and blood (Desquesnes et al., 2013). Since, trypanosomes cannot withstand and survive the pH conditions in the stomachs of carnivores and rodents, parasite penetration must therefore inevitably occur through the oral mucosa (Desquesnes et al., 2013).

CHAPTER 6: CONCLUSIONS AND RECOMMENDATION

To the best of our knowledge, this is the first study to report on CAT in indigenous dogs in Mambwe district and Zambia as a whole. Compared to microscopy, LAMP exhibited higher sensitivity and specificity to detect CAT. Thus unlike microscopy, LAMP was able to distinguish *T. b. brucei* from *T. b. rhodesiense* infection. Detection of the SRA gene in dogs from Munkanya, Nsefu and Malama chiefdoms suggests that the 3 chiefdoms were risk factors for contracting HAT and that indigenous dogs could act as reservoirs of zoonotic *T. b. rhodesiense*. The act of hunting by dogs in these chiefdoms could have increased their chance of getting infected either cyclically or orally. In the presence of tsetse flies, *T. b. rhodesiense* can easily be transmitted from infected hunting dogs to humans. Pronounced corneal opacity in dogs is associated with *T. brucei* subspecies infection. These findings suggest that indigenous dogs may be involved in the re-emergence of zoonotic HAT in Mambwe district, which is a historic HAT focus. This study has therefore provided baseline data on CAT in indigenous dogs and their possible roles in transmitting AAT and HAT in tsetse-infested Mambwe district.

As a measure of preventing and controlling possible AAT and HAT outbreaks in Mambwe district, the following recommendations are made based on the findings of this study:

1. Because of the apprehensive behaviour exhibited by most dog owners towards researchers examining their hunting dogs during sampling, there is need for community sensitization in tsetse-infested regions such as GMAs on the public health significance of some of the pathogens, including *T. b. rhodesiense* that dogs may harbour. More importantly, keeping of dogs in wildlife-livestock interface areas should be discouraged, not only to prevent poaching but also for public health reasons.

2. There is urgent need to conduct HAT surveillance in such tsetse-infested region as Mambwe district, using user-friendly, sensitive and specific tests such as LAMP.

3. These data should guide policy makers in the Ministry of Health and the Department of Veterinary and Livestock Development to formulate policies on surveillance, treatment, prevention and control of both human and animal trypanosomiasis in GMAs.

4. In tsetse-infested areas, domestic animals should be regularly screened for trypanosome infections, with positive ones being treated promptly.

5. Dog owners should ensure that their dogs are fed on thoroughly cooked meat and bones.

Future research should focus on the following:

1. The parasite detection sensitivity may be further improved by centrifugation techniques that use the buffy coat as source of DNA.

2. CAT surveillance at various time points during the year to determine the influence of seasonality on CAT prevalence.

3. To evaluate the role of the oral route of transmission of CAT in dogs.

REFERENCES

Abenga, J.N., David, K., Ezebuiro, C.O.G. and Lawani, F.A.G. 2005. Observations on the tolerance of young dogs (puppies) to infection with *Trypanosoma congolense*. *Afr J Clin Exper Microbiol*; 6: 28-33.

Adeiza, A.A., Maikai, V.A. and Lawal, A.I. 2008. Comparative haematological changes in experimentally infected Savannah brown goats with *Trypanosoma brucei* and *Trypanosoma vivax*. *Afr J Biot*; 7: 2295-2298.

Aitcheson, N., Talbot, S., Shapiro, J., Hughes, K., Adkin, C., Butt, T., Sheader, K. and Rudenko, G. 2005. VSG switching in *Trypanosoma brucei*: antigenic variation analysed using RNAi in the absence of immune selection. *Mol Microbiol*; 57: 1608-1622.

Akpa, P.O., Ezeokonkwo, R.C., Eze, C.A. and Anene, B.M. 2008. Comparative efficacy assessment of pentamidine isethionate and diminazene aceturate in the chemotherapy of Trypanosoma brucei brucei infection in dogs. *Vet Parasitol*; 151: 139-149.

Aksoy, S. 2003. Control of tsetse flies and trypanosomes using molecular genetics. *Vet Parasitol*; 115: 125-145.

Aksoy, S., Gibson, W.C. and Lehane, M.J. 2003. Interactions between tsetse and trypanosomes with implications for the control of trypanosomiasis. *Adv Parasitol*; 53: 1-83.

Anderson, N.E., Mubanga, J., Fevre, E.M., Picozzi, K., Eisler, M.C., Thomas, R. and Welburn, S.C. 2011. Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia. *PLoS Negl Trop Dis*; 6: e1211.

Anonymous, 1968. Sleeping sickness in Rhodesia. *Br Med J*; 4: 467.

Auty, H., Anderson, N.E., Picozzi, K., Lembo, T., Mubanga, J., Hoare, R., Fyumagwa, R.D., Mable, B., Hamill, L., Cleaveland, S. and Welburn, S.C. 2012. Trypanosome diversity in wildlife species from the serengeti and Luangwa Valley ecosystems. *PLoS Negl Trop Dis*; 6: e1828.

Awuoche, E.O. 2012. Tsetse fly saliva: Could it be useful in fly infection when feeding in chronically aparasitemic mammalian hosts. *Open Vet. J*; 2: 95-105.

Baker, J.B. 1968. Trypanosomes of wild mammals in the neighbourhood of the Serengeti National Park. Proceedings of the Zoological Society of London, 26-29 September, London, United Kingdom. pp. 34-38.

Barret, M.P. 2003. The Trypanosomoses. *Lancet*; 362: 1469-1480.

Barrett, M.P., Boykin, D.W., Brun, R. and Tidwell, R.R. 2007. Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. *Br J Pharmacol*; 152: 1155-1171.

Bisser, S., Ouwe-Missi-Oukem-Boyer, O.N., Toure, F.S., Taoufiq, Z., Bouteille, B., Buguet, A. and Mazier, D. 2006. Harbouring in the brain: A focus on immune evasion mechanisms and their deleterious effects in malaria and human African trypanosomiasis. *Int J Parasitol*; 36: 529-540.

Boyt, W. 1988. A field guide for the diagnosis, treatment and prevention of African animal trypanosomiasis (FAO).

Brun, R., Blum, J., Chappuis, F. and Burri, C. 2010. Human African Trypanosomiasis. *Lancet*; 375: 148-159.

Bukowa, K.M., Sugimoto, C., Simukoko, H., Sinyangwe, L., Chitambo, H., Mataa, L., Moonga, L., Fandumu, P., Silawve, V., Inoue, N. and Namangala, B. 2012. Detection of re-emerging bovine trypanosomiasis in southern Zambia by loop-mediated isothermal amplification (LAMP). *BAHPA*; 60: 265-272.

Butler, D. 2003. Tropical diseases: raiding the medicine cabinet. *Nature*; 424: 10-11.

Bwalya, E.C., Nalubamba, K.S., Hankanga, C. and Namangala, B. 2011. Prevalence of canine gastrointestinal helminths in urban Lusaka and rural Katete Districts of Zambia. *Prev Vet Med*; 100: 252-255.

Cano, J., Descalzo, M.A., Ndong-Mabale, N., Ndongo-Asumu, P., Boboukasi, L., Buatiche, J.N., Nzambo-Ondo, S., Ondo-Esono, M.A. and Roche, J. 2007. Spatial and Temporal variability of the *Glossina palpalis palpalis* population in the Mbini focus (Equatorial Guinea). *Int. J. Health Geogr*; 6: 36-41.

Cecchi, G., Paone, M., Franco, J.R., Fèvre, E.M., Diarra, A., Ruiz, J.A., Mattioli, R.C. and Simarro, P.P. 2009. Towards the Atlas of human African trypanosomiasis. *Int J Health Geogr*; 8: 15.

Chappuis, F., Loutan, L., Simarro, P., Lejon, V. and Büscher, P. 2005. Options for field diagnosis of human african trypanosomiasis. *Clin Microbiol Rev*; 18: 133-146.

Chitanga, S., Marcotty, T., Namangala, B., Van den Bossche, P., Van Den Abbeele, J. and Delespaux, V. 2011. High Prevalence of Drug Resistance in Animal Trypanosomes without a History of Drug Exposure. *PLoS Negl Trop Dis*; 5: e1452.

Cleaveland, S., Laurenson, M.K. and Taylor, L.H. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Philos Trans R Soc Lond B Biol Sci*; 356: 991-999.

Cottle, L.E., Peters, J.R., Hall, A., Bailey, J.W., Noyes, H.A., Rimington, J.E., Beeching, N.J., Squire, S.B. and Beadsworth, M.B. 2012. Multiorgan dysfunction caused by travel-associated African trypanosomiasis. *Emerg Infect Dis*; 18: 287-289.

Couto, C.G. and Wellman, M. 1998. Anaemia: how can the CBC help me? *Proc North Am Vet Conf*; 12: 333-334.

Cox, A.P., Tosas, O., Tilley, A., Picozzi, K., Coleman, P., Hide, G. and Welburn, S.C. 2010. Constraints to estimating the prevalence of trypanosome infections in East African zebu cattle. *Parasit Vectors*; 3: 82.

Dantas-Torres, F. 2008. Canine vector-borne diseases in Brazil. *Parasit Vectors*; 1: 25.

Deborggraeve, S., Koffi, M., Jamonneau, V., Bonsu, F.A., Queyson, R., Simarro, P.P., Herdewijn, P. and Büscher, P. 2008. Molecular analysis of archived blood slides reveals an atypical human Trypanosoma infection. *Diagn Microbiol Infect Dis*; 61: 428-433.

Despommier, D.J., Gwadz, R.W., Hotez, P.J. and Knirsch, C.A. 2005. Parasitic Diseases; African trypanosomiasis. 5th edition. Apple trees production, LLC New York.

Desquesnes, M., Holzmuller, P., Lai, D.H., Dargantes, A., Lun, Z.W. and Jittaplapong, S. 2013. *Trypanosoma evansi* and Surra: A Review and Perspectives on Origin, History, Distribution, Taxonomy, Morphology, Hosts, and Pathogenic Effects. *Biomed Res Int*; 2013: 194176.

d'Ieteren, G.D., Authié, E., Wissocq, N. and Murray, M. 1998. Trypanotolerance, an option for sustainable livestock production in areas at risk from trypanosomosis. *Rev Sci Tech*; 17: 154-175.

Docampo, R. and Moreno, S.N. 2003. Current chemotherapy of human African trypanosomiasis. *Parasitol Res*; 90: 10-13.

Duke, H.L., Mettam, R.W.M and Wallace, J.M. 1934. Observations on the direct passage from vertebrate to vertebrate of recently isolated strains of *Trypanosama brucei* and *Trypanosoma rhodesiense*. *Trans Reg Soc Trop Med Hyg*; 28: 77-84.

Duleu, S., Vincendeau, P., Courtois, P., Semballa, S., Lagroye, I., Daulouède, S., Boucher, J.L., Wilson, K.T., Veyret, B. and Gobert, A.P. 2004. Mouse strain susceptibility to trypanosome infection: an arginase-dependent effect. *J Immunol*; 172: 6298-6303.

Duscher, G., Peschke, R., Wille-Piazzai, W. and Joachim, A. 2009. Parasites on paper--The use of FTA Elute ((R)) for the detection of Dirofilaria repens microfilariae in canine blood. *Vet Parasitol*; 161: 349-351.

Eloy, L.J. and Lucheis, S.B. 2009. Canine trypanosomiasis: etiology of infection and implications for public health. *J Venom Anim Toxins incl Trop Dis*; 15: 591.

Evison, C. and Kathuria, K.D.S. 1982. An introduction to the status and control of tsetse (Glossina spp) in Zambia, (Occasional paper # 1), Department of veterinary and tsetse control services, Lusaka Zambia.

Ezeh, I.O., Agbo, L.I., Emehelu, C.O., Nweze, E.N., Ezeokonkwo, R.C. and Onah, D.N. 2009. Berenil – Resistant *Trypanosoma brucei brucei* infection in a dog in Nsukka area of Enugu state, Nigeria. *NVJ*; 29: 34-42.

Ezeokonkwo, R.C., Ezeh, I.O., Onunkwo, J.I., Obi, P.O., Onyenwe, I.W. and Agu, W.E. 2010. Comparative haematological study of single and mixed infections of mongrel dogs with Trypanosoma congolense and Trypanosoma brucei brucei. *Vet Parasitol*; 173: 48-54.

Fèvre, E.M., Wissmann, B.V., Welburn, S.C. and Lutumba, P, 2008. The burden of human African trypanosomiasis. *PLoS Negl Trop Dis*; 2: e333.

Florokowski, C.M. 2008. Sensitivity, Specificity, Reeceiver-Operating Characteristic (ROC) curvesand Likelihood Ratios: Communicatiing the performance of Diagnostic tests. *Clin Biochem Rev*; 29 Suppl (i).

Geiger, A., Cuny, G. and Frutos, R. 2005. Two Tsetse fly species, *Glossina palpalis gambiensis* and *Glossina morsitans morsitans*, carry genetically distinct populations of the secondary symbiont *Sodalis glossinidius*. *Appl Environ Microbiol*; 71: 8941-8943.

Gibson, W. 2007. Resolution of the species problem in African trypanosomes. *Int J Parasitol*; 37: 829-838.

Goddard, J. 2003. Mosquito vector competence and West Nile Virus transmission. Cliggott publishing, division of CMP healthcare media.

Gow, A.G., Simpson, J.W. and Picozzi, K. 2007. First report of canine African trypanosomosis in the UK. *J Small Anim Pract*; 48: 658-661.

Grébaut, P., Bena, J.M., Manzambi, E.Z., Mansinsa, P., Khande, V., Ollivier, G., Cuny, G. and Simo, G. 2009. Characterization of sleeping sickness transmission sites in rural and periurban areas of Kinshasa (République Démocratique du Congo). *Vector Borne Zoonotic Dis*; 9: 631-636.

Hargrove, J.W. 1980. The effect of model size and ox odour on the alighting response of Glossina morsitans Westwood and Glossina pallidipes Austen (Diptera: Glossinidae). *Bull Entomol Res*; 70: 229-234.

Health Protection Agency. 2010. Cases of sleeping sickness diagnosed in UK travellers 2000-2010. http://www.hpa.org.uk/hpr/archives/2010/hpr4210.pdf.

Heisch, R.B. 1963. Presence of trypanosomes in bush babies after eating infected rats. *Nature*; 169: 118-119.

Horchner, F., Zillmann, U., Metzner, M., Schonefeld, A. and Hehlitz, D. 1985. West African dogs as a model for research on trypanotolerance. *Trop Med Parasit*; 36: 257-258.

Joshi, P.P., Shegokar, V.R., Powar, R.M., Herder, S., Katti, R., Salkar, H.R., Dani, V.S., Bhargava, A., Jannin, J. and Truc, P. 2005. Human trypanosomiasis caused by *Trypanosoma evansi* in India: the first case report. *Am J Trop Med Hyg*; 73: 491-495.

Kagira, J.M., Maina, N., Njenga, J., Karanja, S.M., Karori, S.M. and Ngotho, J.M. 2011. Prevalence and types of coinfections in sleeping sickness patients in Kenya (2000/2009). *J Trop Med*; 2011: 248914.

Keck, N., Herder, S., Kaba, D., Solano, P., Gomez, J., Cuny, G. and Davoust, B. 2009. Epidemiological study of canine trypanosomosis in an urban area of Ivory Coast. *Parasite*; 16: 305-308.

Kennedy, P.G. 2008. The continuing problem of human African trypanosomiasis (sleeping sickness). *Ann Neurol*; 64: 116-126.

Kennedy, P.G. 2013. Clinical features, diagnosis, and treatment of human African trypanosomiasis (sleeping sickness). *Lancet Neurol*; 12: 186-194.

Konnai, S., Mekata, H., Odbileg, R., Simuunza, M., Chembensof, M., Witola, W.H., Tembo, M.E., Chitambo, H., Inoue, N., Onuma, M. and Ohashi, K. 2008. Detection of

Trypanosoma brucei in field-captured tsetse flies and identification of host species fed on by the infected flies. *Vector Borne Zoonotic Dis*; 8: 565-573.

Krafsur, E.S. 2009. Tsetse flies: Genetics, evolution, and role as vectors. *Infect Genet Evol*; 9: 124-141.

Kuepfer, I., Hhary, E.P., Allan, M., Edielu, A., Burri, C. and Blum, J.A. 2011. Clinical presentation of *T. b. rhodesiense* sleeping sickness in second stage patients from Tanzania and Uganda. *PLoS Negl Trop Dis*; 5: e968.

Leak, S.G.A. 1999. Tsetse biology and ecology; their role in the epidemiology and control of trypanosomiasis. CABI publishing in association with International livestock research, Nairobi Kenya.

Lindner, A.K. and Priotto, G. 2010. The unknown risk of vertical transmission in sleeping sickness--a literature review. *PLoS Negl Trop Dis*; 4: e783.

Lutumba, P., Makieya, E., Shaw, A., Meheus, F. and Boelaert, M. 2007. Human African trypanosomiasis in a rural community, Democratic Republic of Congo. *Emerg Infect Dis*; 13: 248-254.

Mambwe district council profile. 2008. Local government association. www.lga-zambia.org.zm.

Marcotty, T., Simukoko, H., Berkvens, D., Vercruysse, J., Praet, N. and Van den Bossche, P. 2008. Evaluating the use of packed cell volume as an indicator of trypanosomal infections in cattle in eastern Zambia. *Prev Vet Med*; 87: 288-300.

Matete, G.O. 2003. Occurrence, clinical manifestation and the epidemiological implications of naturally occurring canine trypanosomosis in western Kenya. *Onderstepoort J Vet Res*; 70: 317-323.

Matovu, E., Kuepfer, I., Boobo, A., Kibona, S. and Burri, C. 2010. Comparative detection of trypanosomal DNA by loop-mediated isothermal amplification and PCR from flinders technology associates cards spotted with patient blood. *J Clin Microbiol*; 48: 2087-2090.

Mbaya, A.W., Aliyu, M.M., Nwosu, C.O. and Ibrahim, U.I. 2008. Captive wild animals as potential reservoirs of haemo and ectoparasitic infections of man and domestic animals in the arid-region of Northeastern Nigeria. *Vet arhiv*; 78: 429-440.

Mbaya, A.W., Aliyu, M.M. and Ibrahim, U.I. 2009. The clinico-pathology and mechanisms of trypanosomosis in captive and free-living wild animals: A review. *Vet Res Commun*; 33: 793–809.

MD Travel Health. 2010. Sleeping sickness in Zambia. http://www.mdtravelhealth.com/recent_health_alerts.php.

Ministry of Agriculture and Co-operatives. 2002. Annual report – Kakumbi tsetse and trypanosomiasis research station, DVLD – Mambwe District.

Ministry of Agriculture and Livestock Development. 2012. Personal communication. Mambwe District.

Ministry of Livestock and Fisheries Development. 2010. Annual report – Kakumbi tsetse and trypanosomiasis research station, DVLD – Mambwe District.

Moore, D.A., Edwards, M., Escombe, R., Agranoff, D., Bailey, J.W., Squire, S.B. and Chiodini, P.L. 2002. African trypanosomiasis in travelers returning to the United Kingdom. *Emerg Infect Dis*; 8: 74-76.

Morrison, W.I., Murray, M., Sayer, P.D. and Preston, J.M. 1981. The Pathogenesis of Experimentally Induced *Trypanosoma brucei* Infection in the Dog: I. Tissue and Organ Damage. *Am J Pathol*; 102: 168-181.

Munang'andu, H.M., Siamudaala, V., Munyeme, M. and Nalubamba, K.S. 2012. A review of ecological factors associated with the epidemiology of wildlife trypanosomiasis in the Luangwa and Zambezi valley ecosystems of Zambia. *Interdiscip Perspect Infect Dis*; 2012: 372523.

Mwanakasale, V. and Songolo, P. 2011. Disappearance of some human African trypanosomiasis transmission foci in Zambia in the absence of a tsetse fly and trypanosomiasis control program over a period of forty years. *Trans R Soc Trop Med Hyg*; 105: 167-172.

Mramba, F., Oloo, F., Byamungu, M., Kröber, T., McMullin, A., Mihok, S. and Guerin, P.M. 2013. Standardizing Visual Control Devices for Tsetse Flies: East African Species *Glossina swynnertoni*. *PLoS Negl Trop Dis*; 7: e2063.

Nalubamba, K.S., Hankanga, C., Mudenda, N.B. and Masuku, M. 2010. The epidemiology of Canine *Babesia* infections in Zambia. *J Prevet Med*; 99: 240-244.

Namangala, B. 2009. Surveillance of the re-emerging sleeping sickness in Luangwa valley, Zambia – Progress report. University of Zambia, School of Veterinary Medicine.

Namangala, B. 2011. How the African trypanosomes evade host immune killing. *Parasite Immunol*; 33: 430-437.

Namangala, B. 2012. Contribution of innate immune responses towards resistance to African trypanosome infections. *Scand J Immunol*; 75: 5-15.

Namangala, B., Hachaambwa, L., Kajino, K., Mweene, A.S., Hayashida, K., Simuunza, M., Simukoko, H., Choongo, K., Chansa, P., Lakhi, S., Moonga, L., Chota, A., Ndebe, J., Nsakashalo-Senkwe, M., Chizema, E., Kasonka, L. and Sugimoto, C. 2012a. The use of Loop-mediated Isothermal Amplification (LAMP) to detect the re-emerging Human African Trypanosomiasis (HAT) in the Luangwa and Zambezi valleys. *Parasit Vectors*; 5: 282.

Namangala, B., Oparaocha, E., Inoue, N., Mweene, A.S., Moonga, L., **Lisulo, M**. and Sugimoto, C. 2012b. Detection of the human-infective *Trypanosoma brucei rhodesiense* in dog blood from Chiawa, Zambia. *JST*; 16: 9-14.

Namangala, B., Oparaocha, E., Kajino, K., Hayashida, K., Moonga, L., Inoue, N., Suzuki, Y. and Sugimoto, C. 2013. Preliminary Investigation of Trypanosomosis in Exotic Dog Breeds from Zambia's Luangwa and Zambezi Valleys Using LAMP. *Am J Trop Med Hyg*; 5: 13-0078.

Namangala, B., Sugimoto, C. and Inoue, N. 2007. Effects of exogenous transforming growth factor beta on *Trypanosoma congolense* infection in mice. *Infect Immun*; 75: 1878-1885.

Ngoma, M.S., Nalubamba, M., Kowa, S., Minyoi, D., Mubanga, J., Mwansa, J., Musuka, J., Chintu, C., Bhat, G., Kapakala, T., Mumba, P. and Nyalungwe, K. 2004. Congenital trypanosomiasis. *J Trop Ped*; 50: 6.

Njiru, Z.K. 2012. Loop-mediated isothermal amplification technology: towards point of care diagnostics. *PLoS Negl Trop Dis*; 6: e1572.

Njiru, Z.K., Mikosza, A.S., Armstrong, T., Enyaru, J.C., Ndung'u, J.M. and Thompson, A.R. 2008. Loop-mediated isothermal amplification (LAMP) method for rapid detection of *Trypanosoma brucei rhodesiense*. *PLoS Negl Trop Dis*; 2: e147.

Njiru, Z.K., Mikosza, A.S., Matovu, E., Enyaru, J.C., Ouma, J.O., Kibona, S.N., Thompson, R.C. and Ndung'u, J.M. 2008. African trypanosomiasis: sensitive and rapid detection of the sub-genus *Trypanozoon* by loop-mediated isothermal amplification (LAMP) of parasite DNA. *Int J Parasitol*; 38: 589-599.

Njiru, Z.K., Ndung'u, K., Matete, G.O., Ndungu, J.M. and Gibson, W.C. 2004. Detection of *Trypanosoma brucei rhodesiense* in animals from sleeping sickness foci in East Africa using the serum resistance associated (SRA) gene. *Acta Trop*; 90: 249-254.

Njiru, Z.K., Traub, R., Ouma, J.O., Enyaru, J.C. and Matovu, E. 2011. Detection of Group 1 *Trypanosoma brucei gambiense* by loop-mediated isothermal amplification. *J Clin Microbiol*; 49: 1530-1536.

Nonaka, N., Nakamura, S., Inoue, T., Oku, Y., Katakura, K., Matsumoto, J., Mathis, A., Chembesofu, M. and Phiri, I.G. 2011. Coprological survey of alimentary tract parasites in dogs from Zambia and evaluation of a coproantigen assay for canine echinococcosis. *Ann Trop Med Parasitol*; 105: 521-530.

Pays, E., Laurent, M., Delinte, K., Van Meirvenne, N. and Steinert, M. 1983. Differential size variations between transcriptionally active and inactive telomeres of *Trypanosoma brucei*. *Nucleic Acids Res*; 11: 8137-8147.

Peacock, L., Ferris, V., Bailey, M. and Gibson, W. 2012. The Influence of Sex and Fly Species on the Development of Trypanosomes in Tsetse Flies. *PLoS Negl Trop Dis*; 6: e1515.

Pointer, E., Reisman, R., Windham, R. and Murray, L. 2013. Starvation and the clinicopathologic abnormalities associated with starved dogs: a review of 152 cases. *J Am Anim Hosp Assoc*; 49: 101-107.

Poon, L.L., Wong, B.W., Ma, E.H., Chan, K.H., Chow, L.M., Abeye- wickreme, W., Tangpukdee, N., Yuen. K.Y., Guan, Y., Looareesuwan, S. and Peiris, J.S. 2006. Sensitive and inexpensive molecular test for Falciparum malaria: detecting Plasmodium falciparumDNA directly from heat-treated blood by Loop-mediated isothermal amplification. *Clin Chem*; 52: 303-306.

Richter, J., Göbels, S., Göbel, T., Westenfeld, R., Müller-Stöver, I. and Häussinger, D. 2012. A returning traveller with fever, facial swelling, and skin lesions. *BMJ*; 344: e2092.

Samdi, S.M., Abenga, J.N. and Kalgo, A.M. 2006. Trypanosomiasis in hunting dogs in Kaduna, north central Nigeria: implications on the disease in humans. *J Biomed Invest*; 4: 15-18.

Samdi, S.M., Abenga, J.N., Wayo, B., Mshelia, W.P., Musa, D., Haruna, M.K., Musa, B.U., Bala, I. and Adeyemi, K. 2011. The complementary roles of biting flies and reservoirs of infection: In the resurgent of African animal trypanosomosis in Keffi local government area of Nassarawa State, Nigeria. *Asian J Anim Vet Adv*; 6: 316.

Sasaki, H., Kange, E.K. and Kanburia, H.F.A. 1995. Blood meal source of *Glossina pallidipes* and *Glossina longipennis* (Diptera: *Glossinidae*) in Nguruman, Southwest Kenya*. J Med Ent*; 32: 390-393.

Schofield, C.J. and Maudlin, I. 2001. Trypanosomiasis control. *Int J Parasitol*; 31: 615-620.

Sharma, R., Gluenz, E., Peacock, L., Gibson, W., Gull, K. and Carrington, M. 2009. The heart of darkness: growth and form of Trypanosoma brucei in the tsetse fly. *Trends Parasitol*; 25: 517-524.

Simarro, P.P., Franco, J.R., Cecchi, G., Paone, M., Diarra, A., Ruiz Postigo, J.A. and Jannin, J.G. 2012. Human African Trypanosomiasis in Non-Endemic Countries (2000–2010). *J Travel Med*; 19: 44–53.

Simukoko, H., Marcotty, T., Phiri, I., Geysen, D., Vercruysse, J. and Van den Bossche, P. 2007. The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomiasis on the plateau of eastern Zambia. *Vet Parasitol*; 147: 231-238.

Soulsby, E. 1982. Helminth, Arthropod and Protozoa of Domestic Animals. London, UK. Bailliere, Tindall and Cassell.

Steverding, D. 2008. The history of African trypanosomiasis. *Parasit Vectors*; 1:3.

't Hooft, E.M. 2008. Canine African trypanosoma. *J Small Anim Pract*; 49:487.

The Center for Food Security and Public Health. 2009. African animal trypanosomiasis.www.cfsph.iastate.edu/Factsheets/pdfs/trypanosomiasis_african.pdf.

Thekisoe, O.M., Kuboki, N., Nambota, A., Fujisaki, K., Sugimoto, C., Igarashi, I., Yasuda, J. and Inoue, N. 2007. Species-specific loop-mediated isothermal amplification (LAMP) for diagnosis of trypanosomosis. *Acta Tropica*; 102: 182-189.

Tirados, I., Esterhuizen, J., Rayaisse, J.B., Diarrassouba, A., Kaba, D., Mpiana, S., Vale, G.A., Solano, P., Lehane, M.J. and Torr, S.J. 2011. How Do Tsetse Recognise Their Hosts? The Role of Shape in the Responses of Tsetse (*Glossina fuscipes* and *G. palpalis)* to Artificial Hosts. *PLoS Negl Trop Dis*; 5: e1226.

Torr, S.J., Hargrove, J.W. and Vale, G.A. 2006. Towards a rational policy in dealing with tsetse. *Trends Parasitol*; 21: 537-541.

Traub, R.J., Robertson, I.D., Irwin, P.J., Menckle, N. and Thompson Andrew, R.C.A. 2005. Canine gastrointestinal parasitic zoonoses in India. *Trends Parasitol*; 21: 42-48.

Traversa, D. 2012. Pet roundworms and hookworms: A continuing need for global worming. *Parasit Vectors*; 5: 91.

Truc, P., Jamonneau, V., N'Guessan, P., N'Dri, L., Diallo, P.B. and Cuny, G. 1998. *Trypanosoma brucei* ssp. and *T congolense*: mixed human infection in Côte d'Ivoire. *Trans R Soc Trop Med Hyg*; 92: 537-538.

Uilenberg, G. 1998. A field guide for the diagnosis, treatment and prevention of African animal trypanosomiasis (FAO).

Vale, G.A., Hargrove, J.W., Chamisa, A., Hall, D.R., Mangwiro, C. and Torr, S.J. 2013. Factors affecting the propensity of tsetse flies to enter houses and attack humans inside: increased risk of sleeping sickness in warmer climates. *PLoS Negl Trop Dis*; 7: e2193.

Van Den Abbeele, J., Caljon, G., De Ridder, K., De Baetselier, P. and Coosemans, M. 2010. *Trypanosoma brucei* modifies the tsetse salivary composition, altering the fly feeding behavior that favors parasite transmission. *PLoS Pathog*; 6: e1000926.

Vershney, J.P., Varshney, V.P. and Dwivedi, S.K. 1998. Clinico-endochronological findings in clinical trypanosomiasis in dog. *J Vet Parasitol*; 12: 143-144.

von Wissmann, B., Machila, N., Picozzi, K., Fèvre, E.M., deC Bronsvoort, B.M., Handel, I.G. and Welburn, S.C. 2011. Factors associated with acquisition of human

infective and animal infective trypanosome infections in domestic livestock in Western Kenya. *PLoS Negl Trop Dis*; 5: e941.

Waiswa, C., Olaho-Mukani, W. and Katunguka-Rwakishaya, E. 2003. Domestic animals as reservoirs for sleeping sickness in three endemic foci in south-eastern Uganda. *Ann Trop Med Parasitol*; 97: 149-155.

Wastling, S.L., Picozzi, K., Kakembo, A.S.L. and Welburn, S.C. 2010. LAMP for Human African Trypanosomiasis: A Comparative Study of Detection Formats. *PLoS Negl Trop Dis*; 4: e865.

Wastling, S.L. and Welburn, S.C. 2011. Diagnosis of human sleeping sickness: sense and sensitivity. *Trends Parasitol*; 27: 394-402.

Wells, D.L. 2007. Domestic dogs and human health: An overview. *Br J Health Psychol*; 12: 145-156.

World Health Organisation. 2013. Trypanosomiasis. www.who.int/topics/trypanosomiasis_african/

World Health Organisation. 2010. African trypanosomiasis (sleeping sickness) Fact sheet N° 259.

LIST OF PUBLICATIONS

Journal Publications

1. Lisulo, M., Sugimoto, C., Kajino, K., Hayashida, K., Mudenda M., Moonga, L., Ndebe, J., Nzala, S. and Namangala, B. Under review. Determination of the prevalence of African trypanosome species in indigenous dogs of Mambwe district, eastern Zambia by Loop-mediated isothermal amplification. *Parasit Vectors.*

2. **Lisulo, M**., Masuku, M., Nzala, S. and Namangala, B. In press, Parasitological determination of haemoprotozoa in indigenous dogs of Mambwe district, Eastern Zambia. *Zambia Journal of Science and Technology*.

3. Namangala, B., Oparaocha, E., Mubemba, B., Hankanga, C., Moonga, L., Ndebe, J., **Lisulo, M**., Kajino, K., Hayashida, K. and Sugimoto, C. In press. Detection of Human-infective Trypanosomes in Acutely-infected Jack Russel from Mfuwe, within South Luangwa National Park, Zambia, by Loop-mediated Isothermal Amplification. *Tanzania Veterinary Journal*.

4. Namangala, B., Oparaocha, E., Inoue, N., Mweene, A.S., Moonga, L., **Lisulo, M.** and Sugimoto, C. 2012. Detection of the human-infective *Trypanosoma brucei rhodesiense* in dog blood from Chiawa, Zambia. *Zambia Journal of Science and Technology*; 16: 9-14.

Papers delivered as Oral Presentation at Conferences/Seminars

1. Namangala, B., Kajino, K., Hachaambwa L, Hayashida K, **Lisulo M**, Oparaocha E, Mweene AS, Choongo K, Simuunza M, Simukoko H, Moonga L, Chota A, Ndebe J, Chizema E, Kasonka L, Gwenhure L, Makaya P, Suzuki Y, Sugimoto C. Rapid detection of human-infective trypanosomes in clinical specimens from Luangwa and Zambezi river valleys using LAMP**.**

Presented at: The 4th Scientific Meeting of JST-JICA/SATREPS Zambia Tuberculosis & Trypanosomiasis Project, University Teaching Hospital – 16th October 2013.

2. **Lisulo, M**., Nzala, S. and Namangala, B. Determination of the prevalence of human infective trypanosomes in domesticated dogs in Mambwe district, Eastern province of Zambia.
   Presented at: The 7th National Health Research Conference, New Government Complex, Lusaka – 14-16 October 2013.

3. Namangala B, Hachaambwa L, Kajino K, Mweene AS, Simuunza M, Simukoko H, Choongo K, Moonga L, Gwenhure L, Makaya P, Chota A, Ndebe J, **Lisulo M**, Chansa P, Lakhi S, Nsakashalo-Senkwe M, Oparaocha E, Chizema E, Kasonka L, Suzuki Y, Sugimoto C. Sleeping sickness is re-emerging in the Luangwa and Zambezi river valleys: Detection by loop-mediated isothermal amplication (LAMP).
   Presented at: The 7th National Health Research Conference, New Government Complex, Lusaka – 14-16 October 2013.

4. Namangala B, Kajino K, **Lisulo M**, Oparaocha E, Mweene AS, Choongo K, Hayashida K, Simuunza M, Simukoko H, Moonga L, Chota A, Ndebe J, Chizema E, Kasonka L, Suzuki Y, Sugimoto C. Detection of the Re-emerging Sleeping Sickness in the Luangwa and Zambezi Valleys by Loop-mediated Isothermal Amplication.
   Presented at: The 8th European conference on Tropical Medicine and International Travel Health, 2013. Tivoli, Copenhagen, Denmark – 10-13 September 2013.

5. **Lisulo, M**., Nzala, S. and Namangala, B. Determination of the prevalence of human infective trypanosomes in domesticated dogs in Mambwe district, Eastern province of Zambia.
   Presented at: The Directorate of Research and Graduate Studies – Postgraduate Seminar Week.

School of Medicine: 20th – 21st March, 2013 (Awarded prizes as Best Oral and Poster presenter).

6. Namangala, B., Oparaocha, E., **Lisulo, M**., Mweene, A.S., Simuunza, M., Simukoko, H., Choongo, K., Hankanga, C., Zulu, V.C., Kajino, K., Suzuki, Y., and Sugimoto, C. Detection of human-infective trypanosomes in clinical samples from domestic animals and tsetse flies from Zambia's tsetse-infested regions using LAMP**.**

   Presented at: The 2013 AGM for the Veterinary Association of Zambia. Grand Place, Lusaka - 20 June 2013.

7. Namangala, B., Oparaocha, E., Mubemba, B., Hankanga, C., Moonga, L., Ndebe, J., **Lisulo, M**., Kajino, K., Hayashida, K. and Sugimoto, C. Detection of Human-infective Trypanosomes in Acutely-infected Jack Russel from Zambia's South Luangwa National Park by Loop-mediated Isothermal Amplification.

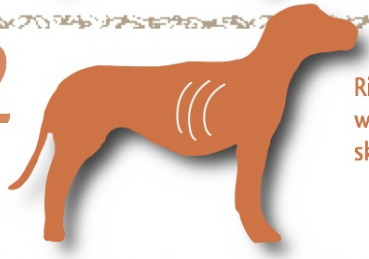
   Presented at: The 30th TVA conference on Contribution of the Veterinary profession to the improvement of human health, AICC- Arusha, Tanzania 11 – 13 December 2012.

Body Condition Scoring

1

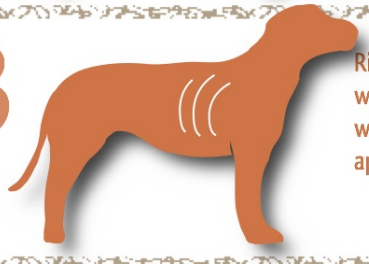
Ribs, spine and bony protrusions are easily seen at a distance. These pets have lost muscle mass and there is no observable body fat. Emaciated, bony, and starved in appearance.

2



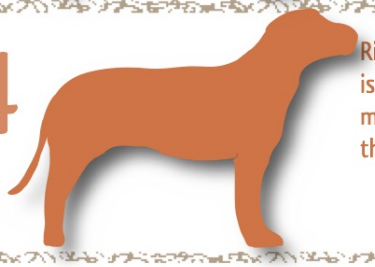
Ribs, spine and other bones are easily felt. These pets have an obvious waist when viewed from above and an abdominal tuck. Thin, lean or skinny in appearance.

3



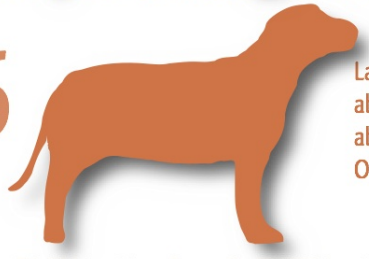
Ribs and spine are easily felt but not necessarily seen. There is a waist when viewed from above and the abdomen is raised and not sagging when viewed from the side. Normal, ideal and often muscular in appearance.

4



Ribs and spine are hard to feel or count underneath fat deposits. Waist is distended or often pear-shaped when viewed from above. The abdomen sags when seen from the side. There are typically fat deposits on the hips, base of tail and chest. Overweight, heavy, husky or stout.

5



Large fat deposits over the chest, back, tail base and hindquarters. The abdomen sabs prominently and there is no waist when viewed from above. The chest and abdomen often appear distended or swollen. Obese.

Association for Pet Obesity Prevention

www.PetObesityPrevention.com ~ 9256 Beach Drive, Calabash, NC 28467

APPENDIX B

SAMPLING RECORD SHEET

GENERAL INFORMATION

DATE	15.10.2012	VILLAGE	ALIKANGELO	GPS Latitudes (S)	13.38148
DOG OWNER	P.....	CHIEFDOM	MUNKANYA	GPS Longitudes (E)	31.95633

PARTICULARS OF DOG:

ID:	142	SEX	MALE	USED FOR HUNTING	YES
NAME	P.....	AGE	4 YEARS	ANAL TEMP (°C)	39.6
BREED	MONGREL	COAT COLOR	BROWN	PCV (%)	15

PHYSICAL EXAMINATION:

CLINICAL PICTURE	MUCOUS MEMBRANE COLOUR	LYMPH NODES	OTHER
Loss of vision due to cloudiness of the both eyes (Bilateral corneal opacity). Sign started about two weeks ago. Highly emaciated Ocular discharge observed in both eyes.	Both ocular and oral membranes look very pale.	Prescupular lymph nodes are enlarged	Ticks observed in both ears and interdigital spaces

LABORATORY RESULTS & TREATMENT/VACCINATION:

MICROSCOPY	TRYPANOSOME SPECIES	MOLECULAR	TRYPANOSOME SPECIES	TREATMENT/VACCINATION
POSITIVE	*T. b.* subspecies	POSITIVE	*T. b. rhodesiense*	3.5 mg/kg of Berenil given in intramuscularly. 1 ml of rabisin given subcutaneously

APPENDIX C

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
E-mail: unzarec@unza.zm
Assurance No. FWA00000338
IRB00001131 of IORG0000774

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

24th July, 2012.

Your Ref: 020-07-12.

Mr. Malimba Lisulo,
C/o Head of Department,
School of Medicine,
Biomedical Sciences,
PO Box 50110,
Lusaka.

Dear Mr. Lisulo,

RE: SUBMITTED RESEARCH PROPOSAL FOR ETHICAL WAIVER:
"DETERMINATION OF THE PREVALENCE OF HUMAN-INFECTIVE TRYPANOSOMES IN DOMESTIC DOGS IN MAMBWE DISTRICT, EASTERN PROVINCE OF ZAMBIA"

The above mentioned research proposal was submitted to the Biomedical Research Ethics Committee for ethical waiver on 17th July, 2012. Since the study will be conducted as part of the routine clinical care for the dogs, the waiver is granted.

CONDITIONS:

- The waiver is based strictly on your submitted proposal. Should there be need for you to modify or make changes to the proposal you will need to seek clearance from the University of Zambia Biomedical Research Ethics Committee.
- If you need any clarifications please consult this office.
- **Ensure that a final copy of the results is submitted to this Committee.**

This waiver was granted in accordance with the University of Zambia Biomedical Research Ethics Committee procedures on granting waiver of ethical review.

Yours sincerely,

Dr. J.C Munthali
CHAIRPERSON

Date of approval: 24 July, 2012 **Date of expiry**: 23 July, 2013

APPENDIX D

The University of Zambia
DIRECTORATE OF RESEARCH AND GRADUATE STUDIES

Telephone: 260-1-290258 Ext 2208
Fax: +260-1-290258
E-mail drgs@unza.zm

P O Box 32379
Lusaka, Zambia

8th April, 2013

Malimba Lisulo (531004806)
C/O School of Medicine
Biomedical Sciences
P.O. Box 32379
LUSAKA

Dear Mr. Lisulo,

LETTER OF COMMENDATION

On behalf of the Directorate of Research and Graduate Studies, am pleased to congratulate you for being awarded a prize as Best Oral presenter (School of Agricultural Sciences, Medicine and Veterinary Medicine Category) at the Postgraduate Seminar Week held from 20th to 21st March, 2013 at the University.

You should keep up the good work.

Prof. A. I. Nyambe
DIRECTOR

cc Dean, School of School of Medicine
Assistant Dean (Postgraduate), School of Medicine
Head, Department of Biomedical Sciences
Assistant Registrar (Graduate Studies)

APPENDIX E

The University of Zambia

DIRECTORATE OF RESEARCH AND GRADUATE STUDIES

Telephone: 260-1-290258 Ext 2208
Fax: +260-1-290258
E-mail drgs@unza.zm

P O Box 32379
Lusaka, Zambia

8th April, 2013

Malimba Lisulo (531004806)
C/O School of Medicine
Biomedical Sciences
P.O. Box 32379
LUSAKA

Dear Mr. Lisulo,

LETTER OF COMMENDATION

On behalf of the Directorate of Research and Graduate Studies, am pleased to congratulate you for being awarded a prize as Best Poster presenter (School of Agricultural Sciences, Medicine and Veterinary Medicine Category) at the Postgraduate Seminar Week held from 20th to 21st March, 2013 at the University.

You should keep up the good work.

Prof. A. I. Nyambe
DIRECTOR

cc Dean, School of School of Medicine
Assistant Dean (Postgraduate), School of Medicine
Head, Department of Biomedical Sciences
Assistant Registrar (Graduate Studies)

APPENDIX F

Parasitological determination of haemoprotozoa in indigenous dogs of Mambwe district, Eastern Zambia

Malimba Lisulo[1], Maxwell Masuku[2], Selestine Nzala[3], Boniface Namangala[4]*
[1]Department of Biomedical Sciences, School of Medicine, University of Zambia, P.O. Box 50110, Lusaka, Zambia; [2]Department of Clinical studies, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia; [3]Department of Community Medicine, School of Medicine, University of Zambia, P.O. Box 50110, Lusaka, Zambia; [4]Department of Paraclinical studies, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia

*Corresponding author: Tele: +260 211 293727
E-mail: boniface_1020@yahoo.com; b.namangala@unza.zm (B. Namangala)

Abstract

Vector-borne haemoprotozoa are among the most important parasites affecting domestic animals in tropical and subtropical regions. In this study, Giemsa-stained blood smears from a total of 237 local dogs (mongrels) comprising 128 males and 109 females from five chiefdoms of Mambwe district, eastern Zambia, were examined parasitologically for the presence of haemoprotozoan parasites. We report that 6.3 % (95% CI: 2.9 – 10.7%) of the dogs had haemoprotozoan infections, the majority of which were subclinically infected with trypanosomes (5.9 %; 95% CI: 2.9 – 8.9%). Munkanya (14.0%; 95% CI: 8.5 – 18.4%) and Malama (13.3%; 95% CI: 8.8 – 19.8%) chiefdoms recorded higher prevalence of mainly *Trypanosoma congolense*, although no haemoprotozoa were recorded in Kakumbi. Only a single case of babesiosis (1.9%; 95% CI: 0.04 – 4.7%) was recorded in Msoro chiefdom. Such haemoparasite-infected mongrels may act as a source of infection to livestock and even humans. We envisage that this study will stimulate research to investigate the influence of seasonal variation on the prevalence of wild and domestic animal haemoprotozoa in wildlife-livestock interface areas, using more sensitive and specific molecular techniques.

Key words: Babesiosis; Dog; Game management area; Trypanosomosis; Zambia.

Introduction

Dogs are the most successful canids adapted to human habitation worldwide. In most tropical and subtropical regions, dogs are kept for various reasons including security, hunting or just as companion animals. However, dogs harbor a number of infectious agents including protozoan parasites (e.g. *Babesia*, *Leishmania, Trypanosoma* species), bacteria (e.g. *Anaplasma*, *Ehrlichia* species) and helminthes (e.g. *Dirofilaria, Dipylidium* species) (Matete, 2003; Dantas-Torres, 2008; Bwalaya *et al*, 2011; Nalubamba *et al*, 2011; Namangala *et al*, 2012a, 2013).

Babesia species that commonly affect dogs include *Babesia canis* and *B. gibson* which cause clinically indistinguishable diseases although the parasites may be distinguished microscopically in that the former is larger (4-5 µm) than the latter (1-2.5 µm). According to Abdullahi *et al*, (1990), canine babesiosis (CB) in tropical and subtropical regions is transmitted by *Rhipicephalus* ticks while *Haemaphysalis*

ticks transmit the disease only in southern Africa. The disease may range from asymptomatic, chronic to hyperacute fatal forms (Abdullahi *et al*, 1990).

African trypanosomes that affect dogs include *Trypanosoma brucei* subspecies, *Trypanosoma congolense* and *Trypanosoma evansi* (Matete, 2003; Gow *et al*, 2007; Namangala *et al*, 2012a, 2013). The main vectors of *T. brucei* subspecies and *T. congolense* are tsetse flies (*Glossina* species), whose distribution is restricted to sub-Saharan Africa. However, *T. evansi* is mechanically transmitted by any haemotophagous arthropod and cause canine trypanosomosis (CT) in Asia, Middle East, Latin America and the Northern and North-Eastern parts of Africa. Canine trypanosomosis may also range from asymptomatic, chronic to acute fatal forms (Matete, 2003; Gow *et al*, 2007; Kech *et al*, 2009; Namangala *et al*, 2013).

Although haemoprotozoa may be diagnosed by the more sensitive molecular techniques such as polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) or by serology, microscopy remains the most reliable, available and affordable test in the resource-limited endemic areas. Canine haemoprotozoa are rarely reported in Zambia. In particular, there is currently no published data on the prevalence of dog haemoprotozoa in Mambwe, an important tourist district adjacent to South Luangwa National Park (SLNP) in Zambia's Eastern province. This study was thus designed to address the paucity of information on the prevalence of haemoprotozoan parasites in locally bred dogs (mongrels) within Mambwe district.

Materials and methods

During the month of October 2012, a total of 237 mongrels comprising 128 males and 109 females were sampled in five chiefdoms (Kakumbi, Malama, Msoro Munkanya and Nsefu) of the tsetse-infested Mambwe district, Zambia. Mambwe, with a surface area of 4840 km², lies between latitudes 10° and 15° South and longitudes 30° and 33° East, adjacent to SLNP. Convenient samples were obtained from pets whose owners agreed to the sampling and after obtaining informed consent to participate in the survey. After clinical examination and body condition scoring as described by Munang'andu *et al* (2011), blood was collected from the individual dog's cephalic vein into heparinized tubes. During the sampling exercise, data for animal identification and clinical history of individual dogs were captured on a form. The packed cell volume (PCV) values were determined and Giemsa-stained thin blood smears from each dog were examined as described by Nalubamba *et al* (2011). As observed by Bwalya *et al* (2011), most of the examined dogs in this study were also seen passing tape and round worms in their stool during sampling. All the sampled dogs were vaccinated against rabies for free.

Data (± standard error [SE]) captured on record sheets were entered, stored and statistically analyzed using STATA version 11.0. Mean rectal temperature and PCV in infected and non-infected subjects were compared using the one-way analysis of variance. All statistical tests were considered significant at $p<0.05$.

Results

About 95% (95% CI: 92.1 – 97.7%) of the examined dogs were hunters that were generally thin, with poor body condition scores. The overall prevalence of haemoprotozoa in the sampled dogs was 6.3% (95% CI: 2.9 – 10.7%). All the sampled dogs that tested positive for haemoprotozoa were treated with diminazene acerturate (Berenil®) (Sigma-Aldrich, Germany) for free.

Dogs that were positive for haemoprotozoa had slightly lower PCVs (29±9.4%) when compared to test negative mongrels (32±8.7%). There was no significant difference (p = 0.06) in the mean rectal temperatures between dogs positive for haemoprotozoa (39.21±0.8 ^{o}C) and those that were negative (39.18±0.5 ^{o}C). Furthermore, lymphadenopathy was only observed in 10% (95% CI: 6.3 – 14.0%) of all the sampled dogs.

Twenty three percent (95% CI: 17.8 – 28.6%) of the dogs had *Rhipicephalus* tick species (identified according to Soulsby, 1982) in their ears as well as in their interdigital spaces. However, tick-borne infection, babesiosis, was only detected in one male dog which was barely one year old, from Msoro chiefdom, with a low PCV (20%). Morphological characterization of the *Babesia* parasites (Soulsby, 1982) in the infected dog revealed that it was the larger-size *B. canis* (Fig. 1A).

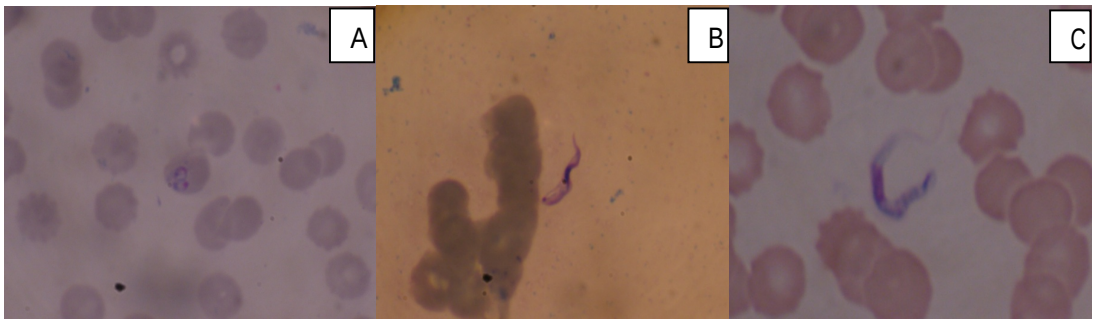


Figure 1. Microscopic examination of Giemsa-stained blood smears from indigenous dogs, arrows showing haemoprotozoa: A. *Babesia canis*. B. *Trypanosoma congolense*. C. *Trypanosoma brucei*. Original magnification x 100.

About 5.9% (95% CI: 2.9 – 8.9%) of the Mambwe dogs were parasitologically positive for CT (Table 1). The Trypanosomes were morphologically identified according to Cornor and Halliwell (1987). It was found that ~4.2% (95% CI: 1.00 – 6.40%) of the mongrels were infected with *T. congolense* (Fig. 1B), 1.3% (95% CI: 0.17 – 3.4%) with *T. brucei* (Fig. 1C) while 0.4% (95% CI: -1.7 – 2.00%) were co-infected with *T. congolense* and *T. brucei* parasites (Table 1).

Table 1: Prevalence of haemoprotozoa in indigenous dogs of Mambwe district, eastern Zambia

Chiefdom	N^{o} of dogs	Mean PCV (%) of infected dogs	Infected with				Overall prevalence of infection
			B. canis	*T. congolense*	*T. brucei*	*T. congolense + T. brucei*	
Kakumbi	36	N/A	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0%
Malama	30	30±14.1	0 (0.0%)	3 (10.0%)	1 (3.3%)	0 (0.0%)	13.3%
Msoro	53	20±0.0	1 (1.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.9%
Munkanya	50	27±8.4	0 (0.0%)	5 (10.0%)	1 (2.0%)	1 (2.0)	14.0%
Nsefu	68	32±5.7	0 (0.0%)	2 (2.9%)	1 (1.5%)	0 (0.0%)	4.4%
Total	**237**	**29±9.4**	**1 (0.4%)**	**10 (4.2%)**	**3 (1.3%)**	**1 (0.4)**	**6.3%**

N/A: not applicable

Munkanya had the highest dog haemoprotozoa prevalence (14.0%; 95% CI: 8.5 – 18.4%), followed by Malama (13.3%; 95% CI: 8.8 – 19.8%), Nsefu (4.4%; 95% CI: 2.05 – 7.8%) and Msoro (1.9%; 95% CI: 0.04 – 4.7%) while no dog was found with haemoprotozoa in Kakumbi chiefdom (Table 1). A statistical significance difference (P=0.003) in the prevalence of haemoprotozoa infection in dogs among chiefdoms was observed.

Discussion

The majority of local inhabitants of Mambwe are rural poor people who are subsistence farmers, fishermen and/or hunters. As such, the local inhabitants of Mambwe still keep dogs for hunting. This is despite the fact that keeping dogs in game management area (GMA) is prohibited by law (Namangala *et al*, 2013). The current survey has provided baseline data on the prevalence of haemoprotozoa among the indigenous dogs of Mambwe district. To our knowledge, this is the first report of haemoprotozoa among the local Mambwe dogs. According to our data, only 6.3 % of the sampled dogs had haemoprotozoan infections, the majority of which were infected with trypanosomes, which may not be surprising in view of the abundant tsetse vector and other biting flies in the study area. CT is rarely reported in literature, particularly in such indigenous dog breeds (Gow *et al*, 2007; Horchner *et al*, 1985). Considering the fact that parasitaemia is generally low in naturally infected animals and that microscopy may fail to detect haemoprotozoa during low parasitaemia, it is possible that the prevalence of CT could be higher if more sensitive molecular techniques were used (Chappuis *et al*, 2005).

Most of the infected dogs did not manifest clinical disease apart from poor body condition and anaemia, manifested by low PCV and pale visible mucous mebranes. For instance, all the parasitologically positive dogs recorded normal body temperatures. These data support the notion that indigenous dog breeds in tsetse-infested regions such as Mambwe, may be tolerant to trypanosome infections (Horchner *et al*, 1985). Ideally, those dogs should have shown more severe clinical symptoms considering the fact that (i) they generally had a poor body condition score, (ii) most of them never received any veterinary services, hence the observed tick burden and worm infestation (both round and tape worms were observed in the stool of some dogs during sampling) which could have contributed to anaemia (Bwalya *et al*, 2011). Nonetheless, the majority of those dogs were either asymptomatic or only exhibited mild signs, with no case of acute CT. As such, they could act as a source of infection for other domestic animals (cattle, goats, pigs) and even for humans (Matete, 2003; Simukoko *et al*, 2007; Dantas-Torres, 2008; Bukowa *et al*, 2012; Namangala *et al*, 2013, 2012a, 2012b).

The variation in the prevalence of CT among the five chiefdoms was intriguing, with higher prevalence being reported in Munkanya (14%), followed by Malama (13.3%) and Nsefu (4.4%) while no CT case was reported in Kakumbi and Msoro. The difference in CT distribution may be a result of (i) the proximity to SLNP and availability and abundance of tsetse flies and other biting insects, (ii) the occurrence of larger domestic livestock which may be more preferable for tsetse feeding than small dogs (Simukoko *et al*, 2007), (iii) the frequency of hunting by dogs and (iv) the availability of veterinary services for mongrels. Thus the fact that no domestic animals other than dogs are kept in Malama may partially explain the higher prevalence whereas the presence of livestock in wildlife zones in Nsefu,

Kakumbi and Msoro (Simukoko *et al*, 2007) as well as the availability of veterinary services for dogs in Kakumbi and Msoro may explain the absence or lower prevalence of CT.

Compared to CT, CB is more frequently reported in literature and has a wider geographically distribution (Inokuma *et al*, 2004; Dantas-Torres and Figueredo, 2006; Nalubamba *et al*, 2011). In the present study, only 1 out of the 237 dogs was diagnosed with CB. According to a recent report (Nalubamba *et al*, 2011), this may not be surprising since the peak cases of CB in Zambia seem to occur during the rainy (November-March) and cold dry (June/July) seasons, coinciding with high tick infestation rates. The present study was carried out early in the month of October during which period the CB cases may have been lower. According to previous reports (Maia *et al*, 2007), the high ambient temperatures during the month of October in Mambwe, coupled with the low relative humidity, could induce vector tick morphogenetic diapause which inhibits reproduction and questing behaviour, resulting in reduced chances of infecting hosts. Interestingly, the CB-infected dog from Msoro chiefdom reported herein was male, barely 1 year old. The dog seemed clinically sick, with a pyrexia of 40.3^{o}C and was severely anaemic. This is in agreement with previous reports that male dogs younger than 1 year of age, were more likely to be positive for CB than other categories (Bashir *et al*, 2009; Nalubamba *et al*, 2011). The paired piroplasms in Giemsa-stained erythrocytes found in the present study are characteristic of the larger-sized *B. canis* which is more frequently reported on the African continent (Abdullahi *et al*, 1990) whereas the small-sized *B. gibson* has only been reported in East Africa (Kjemtrup *et al*, 2000). Wildlife seems to be the principal reservoirs of *Babesia* species although stressed animals succumb to the infection and die ((Kjemtrup *et al*, 2000; Penzhorn, 2006).

Conclusion

In this preliminary study, we report the occurrence of canine haemoprotozoa (*Trypanosoma* and *Babesia* species) in indigenous dogs of Mambwe district in eastern Zambia, with an overall prevalence of 6.3%. The majority of these infections were caused by *Trypanosoma* species. Future studies should investigate the influence of seasonal variation on vector burden and activity and their impact on the prevalence of domestic animal haemoprotozoa, using more sensitive and specific molecular techniques. In addition, the role of wildlife in the persistence of domestic livestock haemoparasites in wildlife-livestock interface areas should be investigated.

Acknowledgements

This study (field work) received financial support from Southern Africa Consortium of Research Excellence (SACORE). The authors would also like to thank the technical staff in the Department of Paraclinical Studies, Eastern Province Veterinary Officer, Mambwe District Veterinary Officer and the support field staff from the Department of Veterinary and Livestock Development, Eastern Province Principal Tsetse Biologist, Zambia Wildlife Authority officers and the dog owners who agreed to participate in the study and kindly allowed their pets to be sampled.

References

Abdullahi SU, Mohammed AA, Trimnell AR, Sannusi A, Alafiatayo R (1990). Clinical and haematological findings in 70 naturally occurring cases of canine babesiosis. *J Small Anim Pract*. **31:** 145–147.

Bashir IN, Chaudhry ZI, Ahmed S, Saeed MA (2009). Epidemiological and vector identification studies on canine babesiosis. *Pakistan Vet J*. **29:** 51–54.

Bukowa KM, Sugimoto C, Simukoko H, Sinyangwe L, Chitambo H, Mataa L, Moonga L, Fandumu P, Silawve V, Inoue N, Namangala B (2012). Detection of re-emerging bovine trypanosomiasis in southern Zambia by loop-mediated isothermal amplification (LAMP). *BAHPA*. **60:** 265-272.

Bwalya EC, Nalubamba KS, Hankanga C, Namangala B (2011). Prevalence of canine gastrointestinal helminths in urban Lusaka and rural Katete Districts of Zambia. *Prev Vet Med.* **100:** 252-255.

Chappuis F, Loutan L, Simarro P, Lejon V, Büscher P (2005). Options for field diagnosis of human African trypanosomiasis. *Clin Microbiol Rev*. **18:** 133-146.

Connor RJ, Halliwell RW (1987). Bovine trypanosomiasis in southern Tanzania: parasitological and serological survey of prevalence. *Trop Anim Health Prod.* **19:** 165-172.

Dantas-Torres F (2008). Canine vector-borne diseases in Brazil. *Parasit Vectors*. **1:** 25.

Dantas-Torres F, Figueredo LA (2006). Canine babesiosis: a Brazilian perspective. *Vet Parasitol*. **141:** 197–203.

Gow AG, Simpson JW, Picozzi K (2007). The first report of canine African trypanosomosis in the UK. *J Small Anim Pract.* **48:** 658-661.

Horchner F, Zillmann U, Metzner M, Schonefeld A, Hehlitz D (1985). West African dogs as a model for research on trypanotolerance. *Trop Med Parasitol*. **36:** 257-258.

Inokuma H, Yoshizaki Y, Matsumoto K, Okuda M, Onishi T, Nakagome K, Kosugi R, Hirakawa M (2004). Molecular survey of Babesia infection in dogs in Okinawa, Japan. *Vet Parasitol*. **121:** 341–346.

Keck N, Herder S, Kaba D, Solano P, Gomez J, Cuny G, Davoust B (2009). Epidemiological study of canine trypanosomosis in an urban area of Ivory Cost. *Parasite*. **16:** 305-308.

Kjemtrup AM, Kocan AA, Whitworth L, Meinkoth J, Birkenheuer AJ, Cummings J, Boudreaux MK, Stockham SL, Irizarry-Rovira A, Conrad PA (2000). There are at least three genetically distinct small piroplasms from dogs. *Int J Parasitol*. **30:** 1501–1505.

Maia MG, Costa RT, Haddad JPA, Passos LMF, Ribeiro McFB (2007). Epidemiological aspects of canine babesiosis in the semiarid area of the state of Minas Gerais, Brazil. *Prev Vet Med*. **79:** 155–162.

Matete GO (2003). Occurrence, clinical manifestation and the epidemiological implications of naturally occurring canine trypanosomosis in western Kenya. *Onderstepoort J Vet Res*. **70:** 317-323.

Munang'andu HM, Munyeme M, Nambota AM, Nalubamba KS, Siamudaala VM (2011). Detection of Babesia spp. in free-ranging Pukus, *Kobus vardonii*, on a game ranch in Zambia. *Korean J Parasitol*. **49:** 437-440.

Nalubamba KS, Hankanga C, Mudenda NB, Masuku M (2011). The epidemiology of canine Babesia infections in Zambia. *Prev Vet Med*. **99:** 240-244.

Namangala B, Hachaambwa L, Kajino K, Mweene AS, Hayashida K, Simuunza M, Simukoko H, Choongo K, Chansa P, Lakhi S, Moonga L, Chota A, Ndebe J, Nsakashalo-Senkwe M, Chizema E, Kasonka L, Sugimoto C (2012a). The use of Loop-mediated Isothermal Amplification (LAMP) to detect the re-emerging Human African Trypanosomiasis (HAT) in the Luangwa and Zambezi valleys. *Parasit Vectors*. **5:** 282.

Namangala B, Oparaocha E, Inoue N, Mweene AS, Moonga L, Lisulo M, Sugimoto C (2012b). Detection of the human-infective *Trypanosoma brucei rhodesiense* in dog blood from Chiawa, Zambia. *UNZAJST*. **16:** 9-14.

Namangala B, Oparaocha E, Kajino K, Hayashida K, Moonga L, Inoue N, Suzuki Y, Sugimoto C (2013). Preliminary Investigation of Trypanosomosis in Exotic Dog Breeds from Zambia's Luangwa and Zambezi Valleys Using LAMP. *Am J Trop Med Hyg*. **89:** 116-118.

Penzhorn BL (2006). Babesiosis of wild carnivores and ungulates. *Vet Parasitol*. **138:** 11-21.

Simukoko H, Marcotty T, Phiri I, Geysen J, Vercruysse J, Van de Bossche P (2007). The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomosis on the plateau of eastern Zambia. *Vet Parasitol*. **147:** 231-238.

Soulsby E (1982). Helminth, Arthropod and Protozoa of Domestic Animals. London, UK. Bailliere, Tindall and Cassell.

APPENDIX G

Determination of the prevalence of African trypanosome species in indigenous dogs of Mambwe district, eastern Zambia, by loop-mediated isothermal amplification

Malimba Lisulo[1§], Sugimoto Chihiro[2*], Kajino Kichi[2*], Hayashida Kyouko[2*], Macarthy Mudenda[1*], Ladslav Moonga[3*], Joseph Ndebe[4*], Selestine Nzala[5*] and Boniface Namangala[3*]

[1]Department of Biomedical Sciences, School of Medicine, University of Zambia, P.O. Box 50110, Lusaka, Zambia; [2]Research Centre for Zoonisis Control, Hokkaido University, Kita-Ku, Sapporo 001-0020, Japan; [3]Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia; [4]Department of Disease Control, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka, Zambia; [5]Department of Community Medicine, School of Medicine, University of Zambia, P.O. Box 50110, Lusaka, Zambia.

*These authors contributed equally to this work
[§]Corresponding author

Email addresses:
ML: malimbalisulo@yahoo.co.uk
SC: sugimoto@czc.hokudai.ac.jp
KK: kiichi@czc.hokudai.ac.jp
HK: kyouko-h@czc.hokudai.ac.jp
MM: mudendamacerthy@yahoo.com
LM: ladslavm@yahoo.com
JN: j.ndebe@yahoo.com
SN: shnzala@unza.zm
BN: b.namangala@unza.zm; boniface_1020@yahoo.com

Abstract

Background

Dogs have been implicated to serve as links for parasite exchange between livestock and humans, and remain an important source of many emerging and re-emerging diseases such as African trypanosomiasis. Yet, canine African trypanosomiasis (CAT), particularly in indigenous dogs (Mongrel breed) remains under reported in literature. This research evaluated the performance of loop-mediated isothermal amplification (LAMP) in detecting trypanosomes in blood from indigenous dogs of tsetse-infested Mambwe district in eastern Zambia.

Methods

A cross sectional survey of CAT was conducted within 5 chiefdoms (Msoro, Kakumbi, Munkanya, Nsefu and Malama) of Mambwe district. Blood samples from indigenous hunting dogs were conveniently collected and screened using microscopy (the gold standard) and LAMP (index test). Data were analysed using Stata version 11.0 and statistical significance was accepted at 95% confidence level.

Findings

A total of 237 dogs were screened for CAT. Only 14 tested positive by both microscopy (5.9%; 95% CI: 2.9 – 8.9%) and LAMP. Six other dogs tested positive by LAMP but were negative by microscopy, bringing the total CAT positives to 20 (8.4%; 95% CI: 4.9 – 12.0%). Irrespective of the detection method used, CAT was only recorded from 3 chiefdoms (Munkanya, Nsefu and Malama) out of the 5. According to LAMP, these infections were caused by *Trypanosoma congolense*, *Trypanosoma brucei brucei* and zoonotic *Trypanosoma brucei rhodesiense* with the human serum resistance-associated (SRA) gene, causing human African trypanosomiasis (HAT). Although these CAT cases generally did not manifest clinical illness, an association was observed between infection with *Trypanosoma brucei* subspecies and occurrence of corneal opacity.

Conclusions

Indigenous dogs in eastern Zambia are potential sources of re-emerging HAT within the Luangwa valley were sporadic cases in humans have been reported. Detection of the SRA gene in these dogs indicates the risk of humans contracting HAT in Mambwe district. LAMP was more sensitive in detecting trypanosomes than microscopy and was able to distinguish even closely related *Trypanosoma brucei brucei* from *Trypanosoma brucei rhodesiense*. Hence the need for continuous trypanosome surveillances in animals, humans and tsetse flies using sensitive and specific tests such as LAMP.

Keywords

CAT, Indigenous dogs, LAMP, Trypanosomes, Mambwe district, Zambia.

Background

In almost all societies, dogs are widely utilised and offer several benefits to man with the main one being security [1]. However, from a public health perspective, dogs have been sources of zoonotic parasites including haemoparasites [2, 3]. As such, dogs have served as a link for parasite exchange among livestock, wildlife and humans, and remain an important source of emerging and re-emerging infectious diseases [4, 5].

In tsetse-infested sub-Saharan African countries, pathogenic protozoan trypanosome species are transmitted to a wide range of susceptible mammalian hosts, including dogs, through infective tsetse fly (*Glossina*) bites when taking blood meals [6, 7]. Specifically, dogs are affected by *Trypanosoma congolense*, *Trypanosoma evansi* and *Trypanosoma brucei* subspecies [8-10], causing canine African trypanosomiasis (CAT). In exotic breeds of dogs, *T. brucei* subspecies tend to cause acute CAT [5] while infections caused by *T. congolense* appear to be more chronic [5, 9, 11]. According to Abenga et al. [6], indigenous dog breeds in tsetse-infested regions of sub-Saharan Africa seem to be trypano-tolerant. Although such dogs get infected with trypanosomes, they either exhibit subclinical signs or may not exhibit any overt clinical signs of the disease at all. Consequently, such dogs may also act as sources of infection to other domesticated animals and, more importantly, those with the human-infective *T. brucei rhodesiense* and *T. brucei gambiense* may serve as a source of infection for humans [5, 8, 11-13].

In our recent communication on CAT in exotic dog breeds [5], we reported for the first time in Zambia the occurrence of CAT caused by *T. congolense* and *T. brucei*

subspecies. Through the use of LAMP, we had observed that some dogs were infected with the human-infective *T. b. rhodesiense*, suggesting their potential to act as sources of HAT. Two of the dogs had contracted CAT from the tsetse-infested Mambwe district in eastern Zambia, with one of them testing positive for *T. b. rhodesiense* infection.

To further address the paucity of data on CAT, we extended our investigations in this research to a relatively larger sample of indigenous dogs, within the vicinity of South Luangwa National Park (SLNP) in Mambwe district. We further evaluated the performance of the trypanosome species-specific LAMP, using parasite DNA obtained from the indigenous dog blood samples, against microscopy, which is the gold standard.

Methods

Study site and design

During the month of October 2012, a cross sectional survey of CAT involving a total of 237 indigenous dogs was conducted in 47 villages within 5 chiefdoms (Msoro, Kakumbi, Nsefu, Munkanya, and Malama) of Mambwe district. The dogs comprised of 128 males and 109 females, most of which were hunting dogs aged between 3 months and 16 years. Mambwe district is situated in the eastern province of Zambia along the Luangwa valley which supports a high density of tsetse flies and is a historic HAT focus. It lies within Lupande game management area (GMA) between latitudes 10° and 15° South and longitudes 30° and 33° East [14] adjacent to SLNP and is a popular tourist destination.

Sample collection and microscopy

Blood samples were conveniently collected from indigenous dogs whose owners consented to participate in the survey. A majority of other dog owners totally refused to participate due to mistrust or suspicion that our research team would kill their pets, as most of them were used for wildlife poaching. Yet for others, the refusal was merely based on myths that routine rabies vaccinations result in their dogs becoming ineffective hunters or leads to blindness.

Each participating dog was clinically examined and its body condition scored as described by [15]. About 2 ml of blood was drawn from the cephalic vein of each dog into heparinised capillary tubes [5] and packed cell volume (PCV) values were determined. Thin giemsa-stained smears of each sample were made and examined by microscopy as described by Matete and Nalubamba et al. [8, 16]. Datum specific for each dog was captured on record sheets. All the participating dogs in this research were freely vaccinated against rabies.

DNA extraction and loop-mediated isothermal amplification

DNA was extracted from all the 237 blood samples and used for the LAMP assay as described by Namangala et al. [5], using specific primers targeting the 18S rRNA gene of *T. congolense* (CON2-LAMP) [17], the repetitive insertion mobile element (RIME) gene of the *Trypanozoon* subgenus group (RIME-LAMP) [18] and the human serum resistance-associated (SRA) gene uniquely expressed by *T. b. rhodesiense* (SRA-LAMP) [19], respectively. All RIME-LAMP positive

samples were screened for *T. b. rhodesiense* using SRA-LAMP. Samples that were RIME-LAMP positive and SRA-LAMP negative were considered to be *T. b. brucei*.

Data analysis

The captured data were entered, stored and statistically analysed using STATA version 11.0. The Fisher's exact test was used to determine whether an association existed between the outcome variable (microscopy and LAMP results) and categorical variables under consideration (risk factors and clinical signs). Binary logistic regression was used to determine the true predictors of being positive for CAT by microscopy and LAMP. P values <0.05 were considered statistically significant; however, variables with P values ≤0.10 were still included in the multivariable regression model to take care of confounders.

Ethical clearance

Approval to conduct this study was granted by the University of Zambia Biomedical Research Ethics Committee under Ref: 020-07-12. Informed consent was sought from dog owners to participate in the survey and collect blood from their dogs.

Results

Clinical appearance of the examined indigenous dogs

Physical examinations revealed that a total of 31 (13.1%; 95% CI: 8.8 – 17.4%) out of the 237 dogs were emaciated with pale mucous membranes. Furthermore, 55 dogs (23.2%; 95% CI: 17.8 – 28.6%) were infested with ecto-parasites, mainly ticks of *Rhipicephalus* species, predominantly found in the ears and inter digital spaces. As was observed in a separate study by Bwalya et al. [20], we also observed in this research several dogs whose actual number was not recorded with infections of adult tape and round worms passed in their stool during sampling. About 24 dogs (10.1%; 95% CI: 6.3 – 14.0%) had enlarged superficial lymph nodes while 5 (2.1%; 95% CI: 0.3 – 4.0%) exhibited bilateral corneal opacity as shown in (Figure 1; Clinical examination of an indigenous dog showing evidence of bilaterial corneal opacity) and 1 adult male dog had scrotal oedema.

Detection of African trypanosomes by microscopy versus loop-mediated isothermal amplification

According to microscopy [11], 14 out of 237 mongrels (5.9%; 95% CI: 2.9 – 8.9%) were found to be infected with trypanosomes. All those 14 cases were also found to be positive for trypanosome infection by LAMP. In addition, LAMP detected 6 other CAT cases, bringing the total number of cases to 20 (8.4%; 95% CI: 4.9 – 12.0%). Although no CAT cases were recorded from Msoro and Kakumbi chiefdoms, Munkanya, Nsefu and Malama each recorded CAT prevalences of 3.8% (95% CI: 0.8 – 6.9%), 2.5% (95% CI: 0.5 – 4.5%) and 2.1% (95% CI: 0.3 – 4.0%) by LAMP, respectively. According to LAMP, 13 indigenous dogs (5.5%; 95% CI: 0.7 – 10.6%) were monolytically infected, 3 with *T. congonlense*, 4 with *T. b. brucei* and 6 with *T. b. rhodesiense*. The other 7 dogs (2.9%; 95% CI: 0.6 – 5.9%) were co-infected, 2 with *T. congolense* and *T. b. brucei* and 5 with *T. congolense* and *T. b. rhodesiense* (Table 1).

A tendency towards lower PCV of CAT positive (29±9.4) *versus* negative indigenous dogs (32±8.7) was observed. However, there was no significant difference in the mean rectal temperatures (p = 0.06) of indigenous dogs with CAT (39.21±0.8) compared to those without (39.18±0.5).

The diagnostic accuracy of LAMP (index) was determined against microscopy (reference standard) from calculations based on the 2 x 2 (Table 2). LAMP was found to be very sensitive and specific, and had a positive likelihood ratio (LR) of 37 and a negative LR of 0, suggesting that its use in CAT diagnosis can greatly improve the management of this disease especially in endemic areas.

Discussion

The present research evaluated the performance of LAMP against microscopy which is the gold standard in detecting trypanosomes in the blood collected from indigenous dogs of Mambwe district. Whereas microscopy detected 14 CAT cases (5.9%), LAMP detected 20 CAT cases (8.4%) out of the 237 mongrels that were available for sampling in the present study, including all the 14 parasitologically positive cases, suggesting that LAMP is a reliable test. Furthermore, LAMP was not only able to distinguish different trypanosome species such as *T. congolense* and *T. brucei* subspecies, but was also able to distinguish between closely related *T. b. brucei* and *T. b. rhodesiense*.

According to LAMP, about 35% of the CAT cases were co-infections, supporting the notion that many CAT infections in the field are caused by more than one trypanosome species [13]. Based on experimental findings in co-infected dogs, *T. brucei* subspecies are thought to dominate and interfere with *T. congolense* in establishing parasitaemia and subsequently their pathogenic effects [13]. In further agreement with previous reports [8, 13], the presence of either *T. b. brucei* or *T. b. rhodesiense* infections in dogs was characterized by relatively higher parasitaemia and in some cases bilateral corneal opacity and scrotal oedema, unlike monolytic infections with *T. congolense*. As previously reported [8, 11, 13, 21, 22], the loss of vision due to corneal opacity is associated with *T. brucei* subspecies induced CAT. This observation supports our finding that a statistical association exists between CAT and corneal opacity. Dogs with corneal opacity were 18 times more likely to be CAT infected than the dogs with normal vision (Odds ratio 18; 95% CI: 1.2 – 267.3; p=0.037). This may not be surprising as members of *T. brucei* subspecies, unlike *T. congolense*, can traverse the vascular tissue and cause damage to various extra-vascular tissues, including the eyes [5, 8].

The present findings are consistent with those of other studies and further support the idea that LAMP tends to be more sensitive than microscopy [5, 17, 23, 24]. Furthermore, LAMP has the advantage over other sensitive and specific molecular techniques such as polymerase chain reaction (PCR) in that it is simpler, more rapid and cheaper to perform as it only requires a heating device for incubation and may therefore be performed even in the field [25, 26]. Since our laboratory extracts DNA by simply boiling the Elute FTA disks at 95^{o}C for 30 minutes (Whatman FTA® Elute Cards, Whatman, UK), we are in the process of preparing LAMP reagents that would conveniently enable us conduct the assay in the field. Furthermore, the LAMP assay shows high tolerance to biological products such that DNA extraction may not be necessary [27]. Thus LAMP may be more practical for routine diagnosis of CAT and other neglected tropical protozoan diseases in resource-limited communities of sub-Saharan Africa where such infections are endemic [24].

Our findings show that location (chiefdom; $p \leq 0.012$), age ($p = 0.025$) and illegal hunting ($p = 0.029$) were significant risk factors that predisposed indigenous dogs to CAT. Out of the 5 chiefdoms surveyed, CAT was only detected in Munkanya (18%), Malama (17%) and Nsefu (9%) while no CAT cases were reported in Kakumbi and Msoro. The human-infective *T. b. rhodesiense* was detected in each of the 3 chiefdoms where CAT was reported. The difference in CAT distribution may be as a result of (i) the proximity to SLNP, availability and abundance of tsetse flies and other haematophagous arthropods, (ii) the occurrence of larger domestic livestock which may be more preferable for tsetse feeding than the smaller mongrels [14], (iii) the frequency of hunting by dogs and (iv) the heavy presence of Zambia wildlife authority (ZAWA) personnel that monitor acts of illegal hunting in Kakumbi and Msoro chiefdoms. Thus the fact that no domestic animals other than dogs are kept in Malama may partially explain the higher CAT prevalence whereas the presence of livestock in wildlife zones [14] and the availability of veterinary services for dogs mainly in Kakumbi and Msoro may explain its absence.

In conformity with a recent report of CAT in exotic dogs [5], this research reports two main trypanosome species causing CAT in indigenous dogs, which are *T. congolense* and *T. brucei* subspecies. However, whereas *T. brucei* subspecies reportedly cause acute CAT in exotic dog breeds [5, 8], no acute CAT was observed among indigenous Mambwe dogs. In fact, most of the CAT cases in this research did not manifest clinical disease other than corneal opacity in some cases. These data suggest that indigenous dogs in tsetse-infested regions such as Mambwe district may be relatively tolerant to trypanosome infections [5, 6, 8]. Ideally, those dogs should have shown more serious clinical signs considering the fact that most of them were malnourished, generally had poor body condition scores and never received veterinary services. Nonetheless, the majority of those dogs were either asymptomatic or only exhibited mild signs, with no case of acute CAT. As such, they could serve as a reservoir of infection for other domestic animals and humans [5, 21].

The detection of the SRA gene in trypanosomes isolated from 11 indigenous dogs in this research is a source of public health concern in view of the close relationship between dogs and humans. Matete [8] postulated that "sporadic and very low prevalence of human-infective trypanosomes in dogs closely reflects disease occurrence in humans". It is worth mentioning that in Nyamaluma (within Malama chiefdom) where Anderson et al [28] had earlier reported an SRA positive case involving an African buffalo, we detected the same gene in 5 indigenous hunting dogs. These findings indicate the risk of contracting HAT by the local communities, ZAWA officials and tourists. For instance, unpublished confirmed sporadic cases of HAT have previously been reported in Mambwe district [29], with the most recent case involving a 21 year-old male from Munkanya chiefdom (unpublished report). In 2008 alone, between the months of March and July, about 12 HAT cases involving ZAWA officials were reported in the Luangwa valley [30]. Furthermore, at least 6 HAT cases have been reported among tourists visiting SLNP since 2010 [31-33]. Collectively, these data show that HAT is re-emerging in the old foci within the Luangwa river valley [25, 31-33].

Conclusions

Despite the many benefits indigenous dogs may offer to the local communities of Mambwe district, our research findings show that they are potential links for tryapanosome exchange between livestock and humans. For instance, the detection

of the SRA gene in trypanosomes from these dogs indicates the risk of humans contracting re-emerging HAT in Mambwe district. LAMP is a more reliable test in detecting trypanosome species than microscopy, which is the gold standard. It has a higher sensitivity and specificity than microscopy which failed to distinguish members of the closely related *T. brucei* subspecies. According to LAMP, the prevalence of CAT in Mambwe district was 8.4% with *T. congolense*, *T. b. brucei* and the zoonotic *T. b. rhodesiense* causing infections in dogs. Although these CAT cases generally did not manifest clinical illnesses, an association between infection with *T. brucei* subspecies and occurrence of corneal opacity was observed.

In order to facilitate effective prevention and control measures of trypanosomiasis, there is need to increase both active and passive surveillance of HAT, using sensitive and specific tests such as LAMP, in domestic and wild animals, humans and tsetse vectors. It is particularly important to sensitize the local community of the potential dangers of keeping dogs that are just left to scavenge without receiving any veterinary services in such a prohibited place like the GMA. Such dogs may harbour several other zoonoses in addition to *T. b. rhodesiense*, with potential serious implications to human health.

Abbreviations

CAT: Canine African trypanosomosis; HAT: Human African trypanosomiasis; SLNP: South Luangwa national park; SRA: Serum resistance-associated gene; GMA: Game management area; ZAWA: Zambia wildlife authority; LAMP: Loop-mediated isothermal amplification; RIME: Repetitive insertion mobile element; PCR: Polymerase chain reaction

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

ML helped to conceive the study, participated in its design, obtained funding, collected samples, performed microscopy, purified the DNA from dog blood on FTA elute cards, performed the LAMP Assays, analysed data and drafted the manuscript. MM, LM, and JN were involved in the purification of DNA from dog blood on FTA elute cards and performed the LAMP Assays. SN co-supervised the study and helped in editing the manuscript. BN supervised and helped to conceive the study, participated in its design, collected samples, performed the LAMP Assays and edited the manuscript. SC, KK and HK helped to conceive the study, participated in its design and assisted in obtaining funding. All the authors read and approved the final manuscript.

Acknowledgements

This research was part of the main project entitled “Establishment of rapid diagnostic tools for Tuberculosis and Trypanosomiasis and screening of candidate compounds for Trypanosomiasisin Zambia”, supported by the Japanese International Cooperation Agency (JICA). It also received financial support from the Centre for Zoonosis Research, Hokkaido University, Japan, through the fund under the project entitled “Surveillance studies of emerging and re-emerging zoonoses”. The field work of this research was funded by the Southern Africa Consortium for Research Excellence (SACORE) and the Medical Education Partnership Initiative (MEPI) in

Zambia. We are thankful to all the study participants and the staff at Kakumbi Tsetse and Trypanosomiasis Research Station in Mambwe district who were involved in this research for their assistance and cooperation.

References

1. Wells DL: **Domestic dogs and human health: An overview.** *Br J Health Psychol* 2007, **12**: 145-156.
2. Dantas-Torres F: **Canine vector-borne diseases in Brazil.** *Parasit Vectors* 2008, **1:** 25.
3. Nonaka N, Nakamura S, Inoue T, Oku Y, Katakura K, Matsumoto J, Mathis A, Chembesofu M, Phiri IG: **Coprological survey of alimentary tract parasites in dogs from Zambia and evaluation of a coproantigen assay for canine echinococcosis**. *Ann Trop Med Parasitol* 2011, **105**: 521-530.
4. Cleaveland S, Laurenson MK, Taylor LH: **Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence.** *Philos Trans R Soc Lond B Bio lSci* 2001, **356**: 991-999.
5. Namangala B, Oparaocha E, Kajino K, Hayashida K, Moonga L, Inoue N, Suzuki Y, Sugimoto C: **Preliminary Investigation of Trypanosomosis in Exotic Dog Breeds from Zambia's Luangwa and Zambezi Valleys Using LAMP.** *Am J Trop Med Hyg* 2013, **89:** 116-118.
6. Abenga JN, David K, Ezebuiro COG, Lawani FAG: **Observations on the tolerance of young dogs (puppies) to infection with *Trypanosoma congolense*.** *Afr J Clin Exper Microbiol* 2005, **6**: 28-33.
7. Brun R, Blum J, Chappuis F, Burri C: **Human African Trypanosomiasis.** *Lancet* 2010, **375**: 148-159.
8. Matete GO: **Occurrence, clinical manifestation and the epidemiological implications of naturally occurring canine trypanosomosis in western Kenya.** *Onderstepoort J Vet Res* 2003, **70**: 317-323.
9. Gow AG, Simpson JW, Picozzi K: **First report of canine African trypanosomosis in the UK.** *J Small Anim Pract* 2007, **48**: 658-661.
10. Eloy LJ, Lucheis SB: **Canine trypanosomiasis: etiology of infection and implications for public health.** *J Venom Anim Toxins incl Trop Dis* 2009, **15**: 591.
11. Boyt W: **A field guide for the diagnosis, treatment and prevention of African animal trypanosomiasis (FAO).** 1988
12. Uilenberg G: **A field guide for the diagnosis, treatment and prevention of African animal trypanosomiasis (FAO).** 1998
13. Ezeokonkwo RC, Ezeh IO, Onunkwo JI, Obi PO, Onyenwe IW, Agu WE: **Comparative haematological study of single and mixed infections of mongrel dogs with *Trypanosoma congolense* and *Trypanosoma brucei brucei*.** *Vet Parasitol* 2010, **173**: 48-54.
14. Simukoko H, Marcotty T, Phiri I, Geysen D, Vercruysse J, Van den Bossche P: **The comparative role of cattle, goats and pigs in the epidemiology of livestock trypanosomiasis on the plateau of eastern Zambia.** *Vet Parasitol* 2007, **147**: 231-238.

15. Munang'andu HM, Munyeme M, Nambota AM, Nalubamba KS, Siamudaala VM (2011). Detection of Babesia spp. in free-ranging Pukus, *Kobus vardonii*, on a game ranch in Zambia. *Korean J Parasitol.* **49:** 437-440.
16. Nalubamba KS, Hankanga C, Mudenda NB, Masuku M: **The epidemiology of Canine *Babesia* infections in Zambia.** *J Prevet Med* 2010, **99**: 240-244.
17. Thekisoe OM, Kuboki N, Nambota A, Fujisaki K, Sugimoto C, Igarashi I, Yasuda J, Inoue N: **Species-specific loop-mediated isothermal amplification (LAMP) for diagnosis of trypanosomosis.** *Acta Tropica* 2007, **102**:182-189.
18. Njiru ZK, Mikosza AS, Matovu E, Enyaru JC, Ouma JO, Kibona SN, Thompson RC,Ndung'u JM: **African trypanosomiasis: sensitive and rapid detection of the sub-genus Trypanozoon by loop-mediated isothermal amplification (LAMP) of parasite DNA.** *Int J Parasitol* 2008, **38**: 589-599.
19. Njiru ZK, Mikosza AS, Armstrong T, Enyaru JC, Ndung'u JM, Thompson AR: **Loop-mediated isothermal amplification (LAMP) method for rapid detection of *Trypanosoma brucei rhodesiense*.** *PLoS Negl Trop Dis* 2008, **2**: e147.
20. Bwalya EC, Nalubamba KS, Hankanga C, Namangala B: **Prevalence of canine gastrointestinal helminths in urban Lusaka and rural Katete Districts of Zambia.** *Prev Vet Med* 2011, **100**: 252-255.
21. Samdi SM, Abenga JN, Kalgo AM: **Trypanosomiasis in hunting dogs in Kaduna, north central Nigeria: implications on the disease in humans.** *J Biomed Invest* 2006, **4**: 15-18.
22. Ezeh IO, Agbo LI, Emehelu CO, Nweze EN, Ezeokonkwo RC, Onah DN: **Berenil – Resistant *Trypanosoma brucei brucei* infection in a dog in Nsukka area of Enugu state, Nigeria.** *NVJ* 2009, **29**: 34-42.
23. Matovu E, Kuepfer I, Boobo A, Kibona S,Burri C: **Comparative detection of trypanosomal DNA by loop-mediated isothermal amplification and PCR from flinders technology associates cards spotted with patient blood.** *J Clin Microbiol* 2010, **48**: 2087-2090.
24. Bukowa KM, Sugimoto C, Simukoko H, Sinyangwe L, Chitambo H, Mataa L, Moonga L, Fandumu P, Silawve V, Inoue N, Namangala B: **Detection of re-emerging bovine trypanosomiasis in southern Zambia by loop-mediated isothermal amplification (LAMP).** *BAHPA* 2012, **60**: 265-272.
25. Namangala B, Hachaambwa L, Kajino K, Mweene AS, Hayashida K, Simuunza M, Simukoko H, Choongo K, Chansa P, Lakhi S, Moonga L, Chota A, Ndebe J, Nsakashalo-Senkwe M, Chizema E, Kasonka L, Sugimoto C: **The use of Loop-mediated Isothermal Amplification (LAMP) to detect the re-emerging Human African Trypanosomiasis (HAT) in the Luangwa and Zambezi valleys.** *Parasit Vectors* 2012, **5**: 282.
26. Wastling SL, Picozzi K, Kakembo ASL, Welburn SC: **LAMP for Human African Trypanosomiasis: A Comparative Study of Detection Formats.** *PLoSNegl Trop Dis* 2010, **4**: e865.
27. Poon LL, Wong BW, Ma EH, Chan KH, Chow LM, Abeye- wickreme W, Tangpukdee N, Yuen KY, Guan Y, Looareesuwan S, Peiris JS: **Sensitive and inexpensive molecular test for Falciparum malaria: detecting *Plasmodium falciparum* DNA directly from heat-treated blood by Loop-mediated isothermal amplification.** *Clin Chem* 2006, **52**: 303-306.

28. Anderson NE, Mubanga J, Fevre EM, Picozzi K, Eisler MC, Thomas R, Welburn SC: **Characterisation of the wildlife reservoir community for human and animal trypanosomiasis in the Luangwa Valley, Zambia**. *PLoS Negl Trop Dis* 2011, **6**: e1211.
29. Ministry of Livestock and Fisheries Development: **Annual report – Kakumbi tsetse and trypanosomiasis research station, DVLD – Mambwe District.** 2010.
30. Zambia wildlife authority (ZAWA) report, 2008. **Trypanosomiasis (Sleeping Sickness) infection among Bambanda-Zaro Sanctuary (BZS) staff.**
31. Cottle LE, Peters JR, Hall A, Bailey JW, Noyes HA, Rimington JE, Beeching NJ, Squire SB, Beadsworth MB: **Multiorgan dysfunction caused by travel-associated African trypanosomiasis.** *Emerg Infect Dis* 2012, **18**: 287-289.
32. Richter J, Göbels S, Göbel T, Westenfeld R, Müller-Stöver I, Häussinger D: **A returning traveller with fever, facial swelling, and skin lesions.** *BMJ* 2012, **344**: e2092.
33. Simarro PP, Franco JR, Cecchi G, Paone M, Diarra A, Ruiz Postigo JA, Jannin JG: **Human African Trypanosomiasis in Non-Endemic Countries (2000–2010).** *J Travel Med* 2012, **19**: 44–53.

FIGURE LEGENDS:

Figure 1; Clinical examination of an indigenous dog showing evidence of bilaterial corneal opacity

Table 1: Prevalence of Trypanosome species in indigenous dogs of Mambwe district, eastern Zambia, by LAMP

Chiefdom	N° of dogs	Mean PCV (%) of infected dogs	Infected with					Overall prevalence of infection
			T. c	*T. b. b*	*T. b. r*	*T. c* + *T. b. b*	*T. c* + *T. b. r*	
Kakumbi	36	N/A	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0%
Malama	30	30±14.1	0 (0.0%)	0 (0.0%)	3 (10.0%)	0 (0.0%)	2 (6.7%)	16.7%
Msoro	53	20±0.0	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0%
Munkanya	50	27.7±8.4	2 (4.0%)	2 (4.0%)	1 (2.0%)	2 (4.0%)	2 (4.0%)	18.0%
Nsefu	68	32±5.7	1 (1.5%)	2 (2.9%)	2 (2.9%)	0 (0.0%)	1 (1.5%)	8.8%
Total	**237**	**29±9.4**	**3 (1.3%)**	**4 (1.7%)**	**6 (2.5%)**	**2 (0.8%)**	**5 (2.1%)**	**8.4%**

N/A: Not applicable; *T. c: Trypanosoma congolense; T. b. b: Trypanosoma brucei brucei; T. b. r: Trypanosoma brucei rhodesiense*

Table 2: Diagnostic accuracy of LAMP and microscopy

	Microscopy		
	CAT present	CAT absent	**Total**
LAMP-positive	True Positive (TP) = 14	False Positive (FP) = 6	**TP+FP = 20**
LAMP-negative	False Negative (FN) = 0	True Negative(TN) = 217	**TN+FN = 217**
Total	**TP+FN = 14**	**TN+FP = 223**	**237**

Sensitivity = TP / (TP + FN) = 100%
Specificity = TN / (TN + FP) = 97.3%
Positive predictive value (PPV) = TP / (TP + FP) = 70%
Negative predictive value (NPV) = TN / (TN + FN) = 100%
Positive likelihood ratio (LR+) = sensitivity / (1 – specificity) = 37
Negative likelihood ratio (LR-) = (1 – sensitivity) / specificity = 0