ROLE OF DOMESTIC ANIMALS IN THE EPIDEMIOLOGY OF HUMAN AFRICAN TRYPANOSOMIASIS (HAT) IN KIGOMA –TANZANIA

BY

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ABSTRACT

Human African trypanosomiasis (HAT) is fatal if untreated and causes severe morbidity. In Tanzania HAT is caused by *Trypanosoma b. rhodesiense*. Trypanosomiasis in livestock is the major impediment to livestock farming and it limits the full potential of agricultural development in Tanzania. This study was undertaken in Kasulu district of Kigoma region, an area that is endemic for both human and animal trypanosomiasis. This study aimed to determine the prevalence of trypanosomiasis in domestic animals and the potential of these domestic animals as reservoirs of human infective trypanosomes. This study was undertaken in four villages namely Kagerankanda, Mvinza, Makere and Mvugwe. Eleven cattle samples (11%) of the 100 cattle sampled in the four villages were detected as positive by PCR using the ITS1 primers that identify trypanosomes to the level of species and subspecies. These were recognized as six *Trypanosoma vivax* (4%) and five *Trypanosoma congolense* forest type (3.33%). Cattle were more susceptible to trypanosome infection compared to other domestic animals like goats and dogs, because trypanosomes were not detected in these two species of domestic animals. Since *Trypanosoma brucei* species was not detected in this study, these results suggested that domestic animals that are kept in the study area do not harbour human infective trypanosomes and trypanosomiasis is prevalent in cattle.

DECLARATION

I, Jahashi Nzalawahe, do hereby declare to Senate of Sokoine University of Agriculture that the work presented here is my original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

_____ _____

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The above declaration is confirmed

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Prof: E.N. Kimbita Date

(Supervisor)

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DEDICATION

This dissertation is dedicated to my mum and wife.

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LIST OF ABBREVIATIONS

HAT	-	Human African trypanosomiasis
CNS	-	Central Nervous System
Spp	-	Species
HTC	-	Haematocrit centrifugation technique
BCM	-	Buffy coat method
mAECT	-	Mini-ion-exchange centrifugation test
PCR	-	Polymerase Chain Reaction
IFA	-	Immunoflouresence Assay
DNA	-	Deoxyribose Nucleic Acid
RNA	-	Ribonucleic Acid
kDNA	-	Kinetoplastida Deoxyribose Nucleic Acid
rDNA	-	Ribosomal Deoxyribose Nucleic Acid
rRNA	-	Ribosomal Ribonucleic Acid
SRA	-	Serum Resistant Associated gene
pg	-	Picogram
fg	-	Femtogram
°C	-	Degree celcius
RFLP	-	Restriction Fragment Length Polymorphism
ITS	-	Internal transcribed spacers
SIT	-	Sterile Insect Technique
EDTA	-	Ethylenediaminetetraacetic Acid
mm	-	Millimeter
min	-	Minutes
S	-	Seconds
ml	-	Milliliter
µl	-	Microliter
_	-	Negative
Mm	-	Millimole
µM	-	Micromole
UV	-	Ultra violet
KCl	-	Potassium chloride
HCl	-	Hydrochloric acid
$MgCl_2$	-	Magnesium chloride
bp	-	Base pair
BECANET	-	Biosciences eastern and central African network
TTRI	-	Tsetse and Trypanosomiasis Research Institute
TZS	-	Tanzanian shillings
P	-	Probability value
CI	-	Confidence interval
Df	-	Degree of freedom
DALDO	-	District Agricultural and Livestock Development Officer

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

African trypanosomiasis is a vector-borne parasitic disease, affecting human and animal in most of sub Saharan Africa. The parasites concerned are protozoa belonging to the Genus *Trypanosoma*. The main vectors responsible for transmission are tsetse fly (Genus: *Glossina*).

Animal *trypanosomiasis or nagana is an important constraint to livestock development in sub-Sahara Africa with estimated losses due to direct and indirect consequences of the disease running into $1340 million annually (Kristjanson et al., 1999).* Human African trypanosomiasis (HAT) is one of the major public health problems in sub-Saharan African countries. WHO (2005) estimates put 60 million people at risk of human African trypanosomiasis (HAT), and 500 000 people with infection. Human African Trypanosomiasis (HAT) takes two forms, depending on the parasite involved: *Trypanosoma brucei gambiense (T. b. g.)* is found in west and central Africa. This form represents more than 90% of reported cases of human African trypanosomiasis and causes a chronic infection. *Trypanosoma brucei rhodesiense* is found in eastern and southern Africa. This form represents less than 10% of reported cases and causes acute infection.

In Tanzania, human African trypanosomiasis (HAT) is caused by *T. b. rhodesiense*. The disease is endemic in Arusha, Lindi, Manyara, Mara, Kagera, Mbeya, Rukwa, Tabora, Kigoma and Ruvuma, but the disease currently tends to be concentrated in Kibondo, and Kasulu districts of Kigoma region (Kibona *et al*., 2002; Malele *et al*., 2006). About two-thirds of Tanzania is infested with tsetse fly and it is estimated that about 4 - 5 million

people living in rural areas are at risk of contracting human African trypanosomiasis. Only less than 1% of people that are at risk of infection are under regular medical surveillance (Komba *et al*., 1997). The rural populations whose livelihoods depend on agriculture, fishing, animal husbandry or hunting are the most exposed to the tsetse bites.

Kigoma region has an area of 37 037 sq. km and 56% of the land is infested with tsetse flies. All the districts in Kigoma are affected by human African trypanosomiasis, but more cases are concentrated in Kibondo and Kasulu districts. The disease has been described to occur in proportion of a large forest, which has an area of 10 368 sq. km. The disease is more concentrated on the villages along the Malagarasi river valley which has typically tsetse belt where big trees overhang open grassland. Infections are contracted mainly by those hunting, fishing, collecting honey, herding cattle, or travelling in the bush in search for basic needs of life (Kibona *et al.*, 2002). Looking into reported cases of human African trypanosomiasis (HAT) in Tanzania, many cases are from Kigoma region. From 1983 – 1992, a total of 3235 HAT cases were reported. Out of these cases, Kigoma region alone reported 2623 cases that are 81% of all cases reported in the country for a period of 10 years. From 1996 - 2005, a total of 3384 HAT cases were reported, Out of these, 2746 (81.15%) cases were from Kigoma region (Malele *et al*., 2006).

Understanding the role of domestic animals in the epidemiology of human African trypanosomiasis in this region will have implications for the design of effective control measures of the disease.

1.2 Justification

Domestic animals have been implicated to be potential reservoirs for Rhodesian sleeping sickness, and hence they have role in the epidemiology of human African trypanosomiasis.

In Uganda, Cattle, sheep, pigs and dogs have been observed serving as reservoirs for *T. b. rhodesiense* (Enyaru *et al.*, 2006; Piccozi *et al.*, 2002; Welburn *et al.*, 2001). In Kenya cattle have been shown to harbour human infective trypanosome (Onyango *et al.*, 1967). In Tanzania in areas around Serengeti national park, cattle have shown to serve as the reservoirs of *T. b. rhodesiense* (Giegy *et al.*, 1973; Magai *et al*., 2006).

Kigoma region accounts for 81% of all reported cases of human African trypanosomiasis in Tanzania, and where there are active foci of human African trypanosomiasis, the role of domestic animals in the epidemiology of human African trypanosomiasis in this region, was not known and therefore this research aimed at addressing this gap in knowledge.

1.3 Objectives

1.3.1 Overall objective

To determine the role of domestic animals in the epidemiology of human African trypanosomiasis in Kigoma region.

1.3.2 Specific objectives

(i) To isolate and characterize the trypanosome species from the domestic animals.

(ii) To determine the prevalence of animal trypanosomiasis

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aetiology

Trypanosomes are unicellular, microscopic and elongated protozoa that move by the help of a single flagellum at the base of which is found a characteristic structure known as kinetoplast, that are found in genus *Trypanosoma*. This genus consists of two divisions, Stercoraria and Salivaria. In Stercoraria division the developmental stages in the vector are completed in the posterior station, transmission is via faecal contamination and most trypanosomes in this division are non pathogenic while in Salivarian division developmental stages completed anterior station, transmission is via saliva, and trypanosomes in this division are of considerable economic importance causing disease in man and animals. The Salivarian division consists of three subgenera *Trypanozoon*, *Dutonella*, and *Nannomonas*. The subgenus *Dutonella* contains *T. vivax*, *Trypanozoon* contains *T. brucei*, two species of which *T. b. gambiense* and *T. b. rhodesiense* are responsible for sleeping sickness in man in Africa and one subspecies *T. b. brucei* for infection in domesticated animals. Other species are *T. evansi* which is found in many parts of the world in a wide variety of animals, also *T. equiperdum* and *T. suis*. In the subgenera *Nannomonas*, *T. congolense* and *T. simiae* are the important members.

The most important African trypanosomes species in Tanzania include: *T. vivax*, *T. congolense*, *T. simiae, T. b. brucei* and *T. b. rhodesiense* (Connor and Halliwel, 1987). *Trypanosoma congolense*, *T. vivax*, *T. simiae* and *T. b. brucei* are causing animal African trypanosomiasis and *T. b. rhodesiense* causing human African trypanosomiasis (Kibona *et al*., 2002). *Trypanosoma b. rhodesiense* is a zoonotic parasite, which is mainly a non-pathogenic parasite of livestock and wild animals, but which results in sleeping sickness when transmitted to humans (Piccozi *et al*., 2002).

2.2 Epidemiology

Trypanosomiasis has a sylvastic transmission cycle. The disease is maintained in ecological system which includes tsetse flies, woody vegetation and game or wild life. It is only when livestock and human are introduced into this system that tsetse fly will use the livestock and human as their food source and infect them with trypanosome. Trypanosomes except *T. equiperdum*, *T. cruzi* and *T. evansi* are transmitted cyclically by tsetse fly. Additionally, mechanical transmission can occur through tsetse or other biting flies (*Tabanus, Stomoxys*). In the case of *T. vivax*, *Tabanus* spp, and other biting flies seem to be the primary mechanical vectors outside the tsetse areas, as in Central and South America. Mechanical transmission requires only that blood containing infectious trypanosomes be transferred from one animal to another. Wild life plays an important role as natural reservoir of trypanosome infection for domestic animals and man. It is generally believed that game animals can harbor trypanosomes and hardly suffer from the disease and the mechanism of this is unknown, although differences in the electrophoretic pattern of serum protein have been described (Desowitz, 1960). Pathogenic animal trypanosomes including *T. vivax*, *T. congolense* and *T. brucei* have been isolated from wild life in East Africa (Ashcroft, 1959; Anosa and Isoun, 1983). However, various game animals such as red-buck, giraffe, bush pigs, kudu and bush buck are known reservoir hosts of pathogenic African trypanosomes.

For the case of human Africa trypanosomiasis in Tanzania, both domestic animals and wildlife are the potential reservoirs for *T. b. rhodesiense* that causes HAT in Tanzania. The studies which were done in Serengeti national park and its bordering villages by Geigy *et al*. (1971) have shown that hartebeest, hyena and lion were harboring *T. b. rhodesiense* and those of Mwambu and Mayande (1971) and Geigy *et al*. (1973) shown that cattle as well were harboring human infective *T. b. rhodesiense*. A recent study done by Magai *et*

al. (2006) has shown that warthogs and cattle harbor the human infective *T. b. rhodesiense* in Serengeti. These studies have also shown that wild animals harbor *T. congolense, T. vivax* and *T. b. brucei* which are pathogenic to domestic animals.

Tsetse flies are the main vector of trypanosomiasis in Tanzania. The most common species of tsetse flies in Tanzania are *Glossina morsitans, G. pallidipes* and *G. brevipalpis*; less wide spread are *G. swynnertoni, G. austeni, G. fuscipes* and *G. longipennis* (Silayo, 1997). These species have more defined distribution. *Glossina morsitans, G. pallidipes* and *G. swynnertoni* are members of the *morsitans* group; the distribution and abundance of this group is dependent on the host availability (Leak, 1999). *Glossina pallidipes* is regarded as a highly mobile species, inhabiting a wide climatic range, although in dry areas it preferentially inhabits areas close to rivers. *Glossina swynnertoni* is common in Tanzania game parks where it is a threat to people working in the parks and tourism by transmitting HAT to travelers (Ripamonti *et al*., 2002). This species is confined to areas of northern Tanzania and southern Kenya. Its fragmented distribution is limited to high altitudes between 900 and 1800 m above sea level, in open woodland, characterized by tree of *Acacia*, *Combretum* and *Commiphora* species, often with the presence of small thickets of vegetation (Marquez *et al*., 2006). *Glossina morsitans* is common in many parts of Tanzania; the success of this specie relies on the ability to inhabit many areas with different climatic conditions and feed of a wide range of mammals (Leak, 1999). *Glossina brevipalpis* is a member of the *fusca* group of tsetse flies, and this species requires thick vegetation and large amount of shade, and is normally found in low lying areas, between 90 and 400m above sea level (Leak, 1999).

2.3 Life Cycle

Infection of mammalian host starts with the bite of an infected tsetse fly (*Glossina* spp.), which injects the metacyclic trypomastigote form of the parasite in its saliva before taking

its blood meal (Chappuis *et al*., 2005). The trypanosomes multiply locally at the site of the bite for a few days before entering the lymphatic system and the blood stream, through which they reach other tissues and organs including the central nervous system (CNS). Two different trypomastigote forms can be observed in the mammalian host, a long, slender proliferative form and a short, stumpy non dividing form. Both forms are taken up by the tsetse fly, but only the latter is able to complete the complex two to three weeks life cycle in the fly (Chappuis *et al*., 2005).

The trypanosomes, after being ingested by a tsetse fly during a blood meal, then lose the surface coat proteins, and develop into procyclic forms. The trypanosomes undergo development and proliferations in the midgut of the fly. Individuals then migrate and enter ectoperitrophic space which separates the midgut epithelium from peritrophic membrane. These forms migrate to the proventriculus where they enter the endoperitrophic space. The mechanism by which trypanosomes cross this membrane is unknown. For *T. brucei* species migration continues to the salivary glands. Parasites in the salivary glands differentiate into epimastigotes, which attache to the lining of salivary glands.

These epimastigotes mature to infective matacyclics ready for transmission in the saliva during blood meal. While *T. congolense* after the development in the midgut migrate to the proboscis and not to the salivary glands. *Trypanosoma vivax* does not have midgut stage at all but develops in the proboscis.

2.4 Clinical Signs

Animal African trypanosomiasis, which takes the form of an acute or chronic status, is normally characterized by fever, anaemia and loss of productivity; with cattle being the most susceptible domestic animal. While *T. b. rhodesiense* HAT usually presents as an

acute febrile illness that is fatal within weeks or months if left untreated. HAT due to *T. b. rhodesiense* infection presents as an acute (sometimes fulminant) febrile illness starting from 1 to 3 weeks after the infective bite; it cannot be distinguished clinically from other tropical fevers such as malaria, enteric fever, and bacterial meningitis (Marnitez *et al*., 2007). There is less demarcation between first and second-stage illness, and CNS involvement can be observed clinically. Pancarditis with congestive heart failure, arrhythmia, and pericardial effusion can kill the patient before pronounced CNS involvement becomes apparent. Most deaths (>80%) occur within 6 months of onset of illness (WHO, 2010).

2.5 Diagnosis

Accurate diagnosis of trypanosomes is essential for understanding the epidemiology and pathogenesis (Gibson, 2007). With sound knowledge of prevalence of trypanosomes, high risk areas can be identified and limited resources can be targeted efficiently. Accurate diagnosis is also necessary for effective treatment and to monitor patterns of resistance to trypanocidal drugs (Gall *et al*., 2004).

2.5.1 Parasitological tests

Parasitological diagnosis is made by microscopic examination of wet preparations of lymph node aspirate, blood or cerebral spinal fluid (CSF). Thin and thick films can be made from blood (Murray *et al.,* 1977). Concentration methods provide a better chance to find the parasite (Janinn and Cattand, 2004). The most sensitive concentration method is the mini-ion-exchange centrifugation test (mAECT). The haematocrit centrifugation technique (HTC) and buffy coat method (BCM) are less sensitive but widely used in the field because of their low cost and rapid performance (Janinn and Cattand, 2004).

2.5.1.1 Stage Determination: Cerebrospinal Fluid Examination

In the absence of sufficiently specific clinical signs and blood tests indicating the evolution from first to second stage HAT, staging of patients still relies on examination of CSF obtained by lumbar puncture (Chappuis *et al.,* 2005).

2.5.2 Serological tests

The serological tests, which are commonly used, include Immunoflouresence assay (IFA) and Enzyme – linked immunosorbent assay (ELISA). Specie-specific monoclonal antibodies have been used to develop ELISA for South America trypanosomes (Voller *et al*., 1975), African trypanosomes (Nantulya *et al*., 1987) and *T. evansi* (Verloo *et al*., 2000). However, antibodies to most trypanosome species have not been developed and, by their nature, antibodies require extensive testing of potential cross reactivity against a range of unrelated species. Nevertheless, serological techniques still have a potential because they are cheaper than PCR and do not require electricity (Ouma *et al*., 2000). The requirement of sophisticated equipment for IFA and ELISA methods limits their use to reference laboratories for remote testing of samples collected on the field survey (Janinn and Cattand, 2004).

2.5.3 Animal inoculation

Rats and mice are very susceptible to most strains of *T. brucei.* The high cost and the long delay before obtaining the result are definite obstacles to routine field use of these techniques**.**

2.5.4 Molecular techniques

The greater accuracy of molecular techniques has addressed the problems of using microscopic and serological techniques and has provided highly sensitive identification

methods. Widespread application of these identification methods to large numbers of field collected samples now demands high throughput approaches. Meanwhile, it has also become increasingly clear that unknown species are present in the trypanosome populations recovered from the field, necessitating the development of generic rather than species-specific methods for identification.

2.5.4.1 DNA probes

Species-specific DNA probes were the first molecular methods with sufficient sensitivity for direct identification of the small numbers of organisms found in the naturally infected vertebrate and invertebrate hosts, without the requirement of culturing large numbers of cells. The method involved preparing dot blots of unpurified samples of tissues collected directly from infected vertebrate and invertebrate hosts followed by hybridization, usually with radioactively- labeled DNA fragments. DNA probes are able to detect around 1000 trypanosomes. Each DNA probe is a species-specific DNA fragment and thus, to ensure specificity of hybridization, each trypanosome species of interest requires an individual DNA sequence to be identified and tested for specificity. Typically, non-coding repetitive DNA elements make good DNA probes as they are less conserved than coding regions among related species and their repetitive nature offers the advantage of high sensitivity. For African trypanosomes, satellite DNA repeats that form the bulk of the minichromosomes fulfill these criteria and were easy to isolate from most species; other repetitive DNA fragments were used for *T. vivax* (Gibson, 2002). Trypanosomes share a unique mitochondrial DNA-containing organelle, the kinetoplast. This has provided another fruitful source of species-specific DNA probes. Kinetoplast DNA (kDNA) minicircles have a high copy number with an estimated 5000 to 10 000 minicircles per trypanosome and the packaging of these minicircles into organelle makes for easy of isolation. Minicircles were initially believed to have no coding function, but are now

known to encode the guide RNAs used for editing mitochondrial DNA transcripts (Simpson *et al*., 2000). The level of sequence variation in kDNA minicircles has proved useful for solving problems of identification of some kinetoplastids including *T. cruzi* and *Leishmania* species, but has not been widely applied to tsetse-transmitted trypanosomes. Despite the high sensitivity and specificity of DNA probe methodology, it was quickly superseded by PCR-based methods after the introduction of this revolutionary technique in 1987 (Mullis and Faloona, 1987).

2.5.4.2 Species-specific PCR

The application of PCR-based techniques greatly aided by molecular species identification, as its high sensitivity allowed identification from low numbers of organisms. The first PCR-based studies used pairs of primers designed to amplify a region-specific to each species. Identification is made by the presence of a PCR product of specific size on an agarose gel. For many trypanosomes species, the species-specific DNA elements previously used as DNA probes were the ones targeted for PCR. The rapid increase in available DNA sequences data, generated from studies in cell biology, taxonomy and genome projects have provided additional targets for these tests. For example, specific primers have been developed from other repetitive elements, such as telomeric repeats of *T. cruzi* (Chiurillo *et al*., 2003) and rDNA sequences. The discovery of a single gene, the serum resistance associated (SRA) gene that enables *T. b. rhodesiense* to survive in humans (Xong *et al*., 1998), led to the development of a specific PCR test for it (Welburn *et al*., 2001). This is especially important as it enables this species to be differentiated from morphologically identical *T. b. brucei* which share the same host range, but is unable to infect humans. Picozzi *et al.* (2007) developed a multiplex PCR to discriminate between *T. b. brucei* and zoonotic *T. b. rhodesiense*. This PCR contains two sets of primers; one targeting the SRA gene, the diagnostic gene for *T. b. rhodesiense*, and the other set targets

a single copy gene which indicates if the DNA is of sufficient quality to amplify single copy gene. These tests are highly specific and can also be extremely sensitive, with detection down to 0.1pg DNA, equivalent to one trypanosome (Masiga *et al*., 1992). These methods were readily applicable to material collected directly from the hosts in the field. Species-specific tests, because of their high specificity and sensitivity have been used extensively in the diagnosis, such as Salivarian trypanosomes in the tsetse guts or mouth parts. These tests have been used to identify trypanosomes from field sample in many studies including those of Woolhouse *et al*., 1994; Masiga *et al*., 1992; Malele *et al*., 200, and Lehane *et al*., 2000.

Studies using species-specific tests have transformed our understanding of trypanosomiasis epidemiology and underlined the value of applying PCR-based identification widely as possible to field collected samples. However, the numbers of species-specific PCR reactions required for each DNA sample can make this method time consuming and expensive.

2.5.4.3 Loop mediated isothermal amplification

A promising development is the application of loop – mediated isothermal amplification (LAMP), a novel method for gene amplification (Notomi *et al*., 2000). The technique relies upon auto-cycling strand displacement by Bst DNA polymerase under isothermal conditions (60˚C – 65 ˚C), and is extremely sensitive, able to detect down to 1fg of DNA, equivalent to 0.01 trypanosomes (Thekisoe *et al*., 2007). Large amounts of DNA are produced by six primers amplifying eight parts of the target DNA within 30-60 minutes. Although amplified DNA can be observed by conventional gel electrophoresis, visualization can also be made by inclusion of fluorescent dye such as SYBR Green 1. The advantages make it an ideal technique for parasite diagnosis and identification in remote

terrain. This technique has adapted for identification of medically important members of *Trypanozoon* (Njiru *et al*., 2007). Thekisoe *et al*. (2007) reported LAMP test for other medically important trypanosomes such as *T. congolense*, *T. evansi* and *T. b. gambiense.*

2.5.4.4 Generic PCR based methods

To overcome the limitations of the species-specific approach, a range of methods have been developed that use generic primers in conserved areas of the genome to amplify sections of DNA from any, or specific groups of, trypanosome species. The trypanosomes species are then identified either by the length of one or more PCR products, either directly or after restricts digestion of PCR-amplified fragments. Identification can also be achieved by sequencing the amplified fragment. Generic approaches have not only been used to study trypanosomes of medical and veterinary importance, but have also been used to study the wider diversity of trypanosomes (Gibson, 2007). The generic PCR based methods includes PCR restriction fragment Length polymorphism (RFLP), Sequence analysis and Ribosomal DNA spacer, ITS. This latter method was adopted in this study. The ITS can be easily amplified using the PCR primers complementary to the conserved regions of the 18S, 28S or 5.8S rRNA genes and is therefore a popular choice as a target for species identification. An identification system using ITS was proposed to replace the multiple species-specific tests for identification of African trypanosomes species by Desquesnes *et al*. (2001). There is a sufficient interspecies length variation in the ITS1 region to enable identification of trypanosome species by the size of the PCR – amplified product using agarose gel electrophoresis. It is quicker and cheaper than the specie specific technique, because the number of PCRs required per sample is greatly reduced, and therefore large number of samples can be processed. It is also able to detect mixed infections in a single PCR, which can be identified by the presence of multiple bands. New species could potentially be identified if their ITS region differs in size from the known

trypanosomes. Redesign of the original primers (Desquesnes *et al*., 2001; Njiru *et al*., 2005), improved the ability to identify *T. vivax*, a common cattle pathogen. Although use of generic PCR test should reduce analysis to a single test per sample, the sizes of ITS1 PCR bands for some species cannot be distinguished (Desquesnes *et al*., 2001) and thus it is often necessary to confirm findings by using species-specific primers, adding an extra step.

2.6 Treatment

There are currently only three trypanocides available for treatment of animal trypanosomiasis, these includes, isometamidium and homodium which have both prophylactic and therapeutic effects and diminazene which has only therapeutic properties. There are two drugs that are currently used for treatment of HAT, these includes suramin which is used in early-stage *T. b. rhodesiense* infections while Melarsoprol is used in late stages (Janinn and Cattand, 2004).

2.7 Control

The control of Africa trypanosomiasis is often seen as synonymous with the control of tsetse vector (Steverding, 2008). To be effective, control procedures must be carried out over areas large enough to delay the re-appearance of tsetse for many years (Marquez *et al*., 2006). In the mid-1950s to 1980s large – scale control programmes, including aerial spraying to eliminate tsetse, were active, and disease eradication seemed a possibility at that time (Torr *et al*., 2005). However, during the 1990s aerial spraying was curtailed in favour of bait technologies that could be applied by the local communities. Unfortunately, this meant that the cover of the control strategies was not as widely spread; tsetse populations and, therefore disease risk grew. Currently, combinations of strategies are used to control the tsetse populations. These range from the use of insecticides applied by aerial

or directly onto cattle; deployment of target traps (Ndengwa and Minok, 1999) and sterile insect technique (SIT) (Goossens *et al*., 2006). This latter technique involves the sterilization and mass release of male flies which make the females produce infertile pupae. This technique has been already used in Tanzania on the island of Zanzibar; the project was successful with the complete eradication of *G. austeni* from the island (Verysen, 2001). This technique has been suggested for the eradication of tsetse flies over the whole of Africa; however there is a fierce opposition. Some argue that this technique is too expensive, and fear reinvasion of tsetse flies from other areas without eradication. In addition SIT requires the use of insecticide-based techniques to suppress the tsetse population by around 90% initially in order for SIT to be successful. Torr *et al*. (2005) argue that the insecticide alone would be able to eliminate a population, and is a far cheaper method of control.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

This study was carried out in Kasulu district of Kigoma region. The district is located in the between latitudes (4°, 30'S,) and longitudes (30°, 00') and it is served by Malagarasi river valley which is a typical tsetse belt with forest, game reserve and open grass land.

3.2 Study Design

A Cross-sectional study design was employed in which prevalence of the disease at a single point in time was determined.

3.3 Sample Size Calculation

The sample size was calculated using the formula developed by Thrusfield (1995) as follows; $n=Z^2PQ/L^2$, where; n=required sample size, Z is the Z value for a given confidence level, P is the known or estimated prevalence, Q= (1-P), and L=allowable error of estimation. For the purpose of this study a confidence level assumed at 95% with an allowable error of estimation of 5%. The average prevalence of trypanosomiasis in Kigoma region in cattle, goats and sheep was estimated at 10%. Therefore; P=0.1, Q=0.9 and hence $n=1.96^2$x 0.1x0.9/0.05^2=138. The calculated sample size was 138 animals but the sample size was arbitrarily approximated to 150 animals.

3.4 Sample Selection

A purposive sampling was adopted for the selection of study villages. A total of four villages namely Kagerankanda, Mvinza, Makere and Mvugwe, with reported cases of human African trypanosomiasis and animal trypanosomiasis in Kasulu district was involved in the study. At village level the sampling of the domestic animals was based on

convenient sampling technique. The number of animals sampled in each village shown in the Table 1 below. The number of goats and dogs were much smaller than that of cattle, due to their less availability in the study area.

Table 1: Summary of the number of animals sampled in each village

Animal species	Kagerankanda	Mvugwe	Makere	Mvinza	**Sub total**
Cattle	25	25	25	25	**100**
Goats	10	10	10	10	**40**
Dogs	3	2	2	3	**10**
Sub total	**38**	**37**	**37**	**38**	**150**

3.5 Data Collection

Data collection involved blood collection for molecular diagnosis of trypanosomes.

3.5.1 Blood sample collection

From each selected animal, blood was collected from the jugular vein into vacutainer tubes containing EDTA and stored at 4ºC in a cool box and carried back to the laboratory. At the laboratory, blood sample from the vacutainer tubes was applied onto Whatman FTA cards and left to dry and then stored in sealed plastic bags at room temperature. In order to have higher chances of detecting parasites; blood sample collection was done early in the morning (Mark *et al.*, 2004).

3.5.2 PCR analysis

3.5.2.1. DNA extraction

DNA was extracted as described by Boid *et al.* (1999). A 6 mm diameter disc was cut from the blood spot on the Whatman FTA card and placed into the 0.5 ml microcentrifuge tube. 200µl of sterile water was added and the tube incubated at 37°C for 30min in a thermocyler. The tubes were then centrifuged briefly at high speed (14 000 r.p.m.) the

resulting supernatant was collected, and discarded. A second 100µl aliquot of sterile water was added to the Whatman FTA card disc in the original tube and incubated at 100°C for 30min in the thermocycler. After brief high speed centrifugation (14 000 r.p.m.) the supernatant was removed and stored at -20°C ready for PCR analysis.

3.5.2.2 Polymerase Chain Reaction (PCR)

PCR was performed using a 25µl reaction volume containing 1gm of dry taq (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 units of pure taq DNA polymerase, 200 µM of each of the four deoxynucleoside triphosphates and reaction buffer), 1µl of each primer (1µM), 2µl DNA sample and double distilled water to a final volume of 25µl. *Trypanosoma b. brucei* reference DNA as a positive control and negative control was included in each set of PCR reactions so as to rule out false positive results.

Table 2: ITS1 primer sequence and PCR conditions

Primer name	Primer sequence	PCR conditions
ITS 1 CF	CCGGAAGTTCACCGA TATTG	Initial DNA denaturation stage at 94˚C for 5min, followed by 35 cycles of final DNA denaturation stage at 94˚C for 40s, Annealing stage at 55˚C for 40s, initial extension stage at 72˚C for 1.30min and final extension stage at 72˚C for 5min
ITS 1 BR	TTGCTGCTGTCTTCA ACGAA	

For trypanosome detection, DNA extracted from the FTA cards were analyzed by PCR using ITS1 primer. Primer sequence, amplification conditions and expected PCR product size for ITS1 primer shown in (Table 2 and 3). PCR was performed as described by Njiru *et al*. (2005). All PCR products obtained were separated by electrophoresis in a 2% agarose gel, stained with ethidium bromide and photographed under UV illumination.

Table 3: Expected product size for ITS1 PCR

Trypanosoma species	Product size (bp)
Trypanosoma brucei	480
Trypanosoma congolense Savannah type	700
Trypanosoma congolense Kilifi type	620
Trypanosoma congolense Forest type	710
Trypanosoma vivax	250

3.6. Data Analysis

The obtained data were entered in Microsoft Office Excel 2007 and then imported to Epi info version 3, onto which statistical analysis was performed. The prevalence of trypanosome species and infection between animals were determined using Chi-square test at 95% confidence interval and the level of significance difference was set at 0.05.

3.7 Location and Duration

This study was carried out in Kasulu district of Kigoma region (see 3.1). It was carried for nine months starting from October 2009 to June 2010.

CHAPTER FOUR

4.0 RESULTS

4.1 Detection of Trypanosomes

All 150 animal samples were subjected to ITS1 PCR analysis for detection of trypanosomes. Eleven (11%) cattle samples out of the 100 were found infected by PCR. However, all goats and dogs samples were not infected. The infected cattle samples were from the two villages, Kagerankanda and Mvinza with six and five cases in each village respectively as shown in Table 4. The typical results of ITS1 PCR analysis are shown in Fig. 1 and 2.

Table 4: Summary of the number of animals infected in each village

Animal species	Kagerankanda	Mvugwe	Makere	Mviza
Cattle	6	0	0	5
Goats	0	0	0	0
Dogs	0	0	0	0

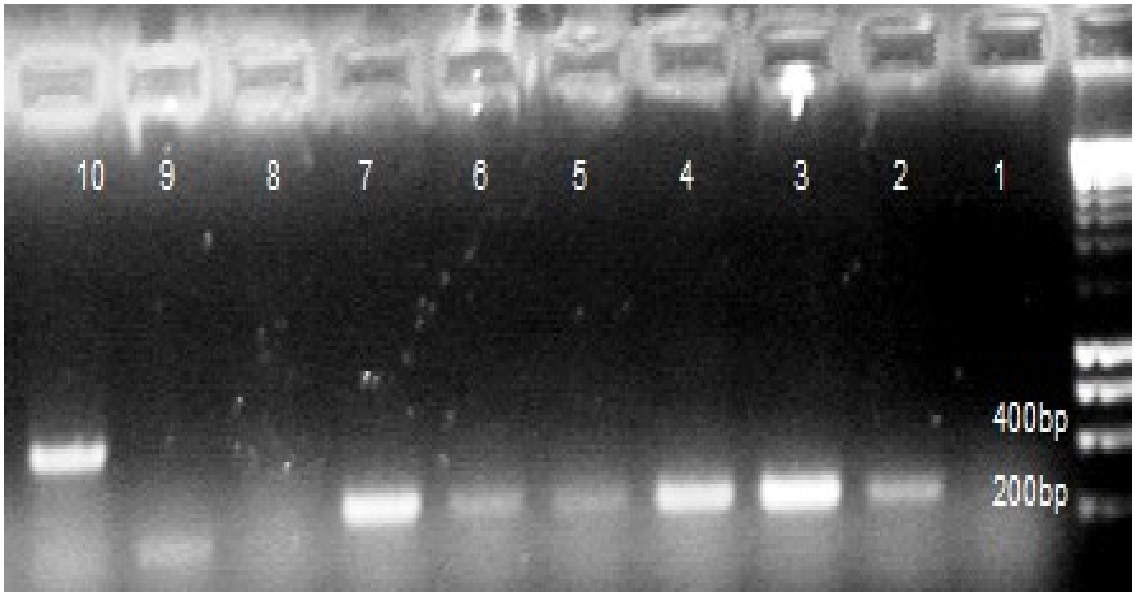

Figure 1: Analysis of PCR products obtained using ITS1 primers of 10 blood samples (on Whatman FTA cards) taken from cattle in Kasulu district of Kigoma region in Tanzania. Samples in lanes 2, 3, 4, 5, 6 and 7 were positive for *T. vivax*. Lanes 8 and 9 were negative. Lane 1 was negative control and lane 10 was the *T. b. brucei* which used as positive control. Bioline hyperladder I was used as the DNA marker.

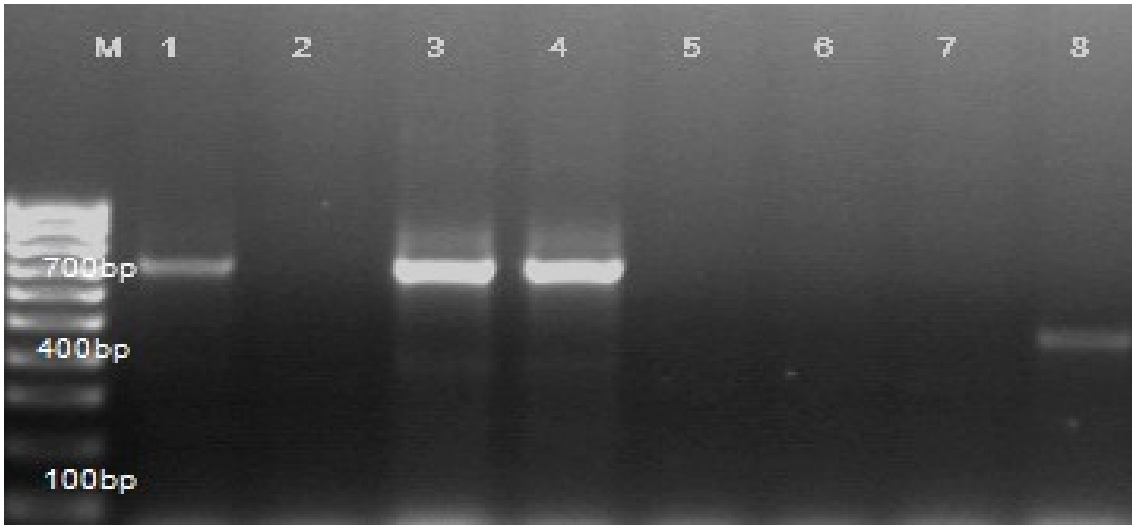

Figure 2: Analysis of PCR products obtained using ITS1 primers of eight blood samples (on Whatman FTA cards) taken from cattle in Kasulu district of Kigoma region in Tanzania. Samples in lanes 1, 3 and 4 were all positive for *T. congolense* forest type. Lanes 2, 5 and 6 were negative. Lane M represented a Bionline DNA ladder IV while lane 7 was a negative control and lane 8 was the *T. b. brucei* used as the positive control.

4.2 Prevalence of trypanosomiasis in different animal species

The prevalence of Bovine trypanosomiasis in Kasulu district was 11% (95% CI, n=100), while the prevalences of trypanosomiasis in goats (95% CI, n=40) and dogs (95% CI, n=10) were 0%. The trypanosome species detected in the study were *T. congolense* forest type and *T. vivax.* Cattle where the only animals infected, this could be due to the fact that, cattle are severely affected by *T. congolense* and *T. vivax* compared to goats which are moderately affected. Dogs are known to have refractory to *T. vivax* infections and moderately affected by *T. congolense*. Specific infections rates based on trypanosome species were 4 % (95% CI, n=11) and 3.33 %(95% CI, n=11) for *T. vivax* and *T. congolense* forest type respectively. A highly significant infection rate in cattle was found in Kageranka and Mvinza villages (P=0.340 at 95% CI, df=1 and χ^2 =0.91). This was mainly due to the fact that the two villages are bordering Malagarasi forest reserve which harbors a large population of tsetse flies.

CHAPTER FIVE

5.0 DISCUSSION

This study has established that domestic animals in Kasulu do not harbor the human infective trypanosomes and trypanosomiasis is prevalent in cattle in Kasulu while the small ruminants and dogs were not infected. This presumably was due to the fact that small ruminants and dogs were less exposed to the tsetse challenge compared to cattle, because of the different grazing patterns of the small ruminants, being tethered in areas closer to the homesteads and dogs spending more time in homes. The trypanosome species detected in the study were *T. congolense* forest type (45.45%) and *T. vivax* (54.54%) being the most prevalent species in cattle of Kasulu. The infected cattle were found in two villages out of four study villages and these were Kagerankanda and Mvinza, the latter two villages have high tsetse challenge since they are bordering Malagarasi forest reserve that harbor large tsetse populations. However, these results are in line with other studies by Nonga and Kambarage (2009), and Msolla *et al*. (2001), who reported that *T. vivax* was the most prevalent species in cattle followed by *T. congolense*. This presumably was an effect of mechanical transmission acting with much ease on *T. vivax* than on *T. congolense*, and as well as non-tsetse flies transmission of *T. vivax.*

The prevalence of trypanosomiasis in small ruminants was zero percent and this could be the reason why the small ruminants survives better than cattle under medium tsetse challenge, however studies in area of high tsetse fly challenge have shown that small ruminants succumb to trypanosomiasis and that heavy economic loss is occasioned (Ng'ayo *et al*., 2005). The different grazing patterns of small ruminants, being grazed closer to the villages, and hence being less exposed to tsetse challenge and the less preference of tsetse flies feeding on small ruminants, due to the anti-feeding behaviour of the goats and sheep (including leg kicking and stamping, tail and ear flicks, skin rippling),

explains as to why small ruminants were no infected. However, the prevalence of trypanosomiasis established in this study was lower compared to the study done by Connor and Halliwell (1987), who reported a prevalence of 16% in southern Tanzania. The lower prevalence might be attributed to regular use of trypanosome chemoprophylaxis as most farmers (75%) were using isometamedium chloride since they were aware of the disease and its control. Moreover sustained deployment of odour baited targets treated with deltamethrin insecticide was done by the Kasulu district council since 1995 to date, has contributed to the reduction of tsetse flies by 95% (Rufurenge, F. personal communication, 2010). For instance, in the study conducted by Msolla (2001), a reduction of 93% of tsetse flies in Melela was reported following the use of alpha cypermethrin 10% preparation. For the past 10 years, many agro-pastoralists from Shinyanga and Mwanza regions have migrated to Kasulu district looking for fertile land to grow crops as well as good pasture for their livestock. This has contributed to increased clearing of bushes in preparation for agriculture and settlements in Kasulu district. This phenomenon may have also contributed significantly to tsetse reduction and hence low prevalence of trypanosomiasis. Moreover, the weekly use of pyrethroid acaricides against ticks probably has contributed to the reduction of tsetse fly density.

This study was conducted during the rainy season and livestock farmers' experience in Kasulu, was that they get very few cases of trypanosomiasis during the rainy season and more cases during the dry season. The reason being during the rainy season there are plenty of pastures and livestock farmers avoid grazing their animals in areas where there is high tsetse challenge, and as a result they experience few cases of trypanosomiasis. However, during the dry season there are limited pastures and farmers have no choice, they have graze their animals even in areas of high tsetse challenge hence more cases of trypanosomiasis during the dry season.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

It is clearly shown in this study that domestic animals do not play a role in the epidemiology of human African trypanosomiasis in Kasulu district, while animal trypanosomiasis was found to be prevalent in cattle. Only cattle of Kagerankanda and Mvinza study villages were found to be positive for trypanosomiasis, the two villages borders Malagarasi forest reserve which harbour large tsetse populations. Hence cattle at these villages were exposed to higher tsetse challenge, compared to those in study villages which do not border Malagarasi forest reserve.

Despite the continuous deployment of odour-baited traps in communal grazing areas, the use of chemoprophylaxis, bush clearing and other methods of tsetse control, animal African trypanosomiasis is still prevalent in cattle populations in Kasulu district. With the existence of other disease control methods, the most desirable and effective means of controlling the disease is through controlling the vector to avoid their contact with cattle and human.

It is therefore recommended that, controlling of tsetse flies should aim at a significant reduction of tsetse flies density both in communal grazing areas and forest reserves in order to avoid the re-invasion of tsetse flies from forest reserves to communal grazing areas. For the better understanding of epidemiology of human African trypanosomiasis in Kasulu district further studies on wild animals and tsetse blood meal analysis should be done. These studies will generate information which will save as baseline data for designing effective human African trypanosomiasis control strategies in Kasulu district.

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