COST EFFECTIVE CONTROL OF ZOONOTIC AFRICAN TRYPANOSOMIASIS IN KENYA: ANALYSING UNDERREPORTING FACTORS AND MODELING PREVALENCE IN BUSIA FOCI

CHRISTOPHER HUMPHREY WANGA, BVM, MPH&E, MBA

J80/95647/2014

A thesis submitted in fulfillment of the requirements for Doctor of Philosophy degree in Veterinary Epidemiology and Economics

of

University of Nairobi

Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, College of Agriculture and Veterinary Sciences, University of Nairobi.

2015

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Christopher Humphrey Wanga (J80/95647/2014)

Signature_____Date:_____

This thesis has been submitted for examination with our approval as the University Supervisors

1. Professor Philip Kitala, BVM, MSc., PhD.

Department of Public Health, Pharmacology and Toxicology, University of Nairobi

Signature_____Date:_____

2. Professor William Ogara, DVM, MSc., PhD.

Department of Public Health, Pharmacology and Toxicology, University of Nairobi

Signature_____Date:_____

DEDICATION

To my mother, mama Rael Atsieno Wanga

and

In memory of my father, the late Lay Canon William James Wanga

They worked tirelessly to set me off in starting my academic journey and have remained my inspiration enabling me to sustain my academic achievements to date.

ACKNOWLEDGEMENTS

First and foremost I wish to recognize my research supervisors Professor Philip Kitala and Professor William Ogara for their patience, feedback, advice and commitment in guiding me in planning ,conducting the research and preparation of this thesis. I would like to thank the Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi for registering me for this course and guiding me throughout the PhD Course. I further wish to thank the Ministry of Agriculture, Livestock and Fisheries, State Department of Livestock, State Veterinary Services for the financial and logistical support and permission to undertake this PhD Course. I wish to appreciate Pan African Tsetse and Trypanosomiasis Eradication Campaign Project (PATTEC) now Kenya Tsetse and Trypanosomiasis Eradication Council (KENTTEC) through the Acting CEO Dr Pamela Olet for supporting the field sample collection and laboratory inputs without which I would not have managed to conduct the research. I am indebted to the Busia County and Sub County Veterinary Staff led by Dr Gregory Lukhale and the Ministry of Health Staff led by Drs Godrick Onyango and Janerose Ambuchi who supported sample collection and interviews during fieldwork.

Thank you Kenya Agricultural Research Organization (KALRO) Trypanosomiasis Research Centre (TRC) Muguga Institute and Busia Alupe through Dr Grace Murilla, Joana Auma and Dr Khaluhi for availing their diagnostic facilities and laboratory back up services for my use. I thank the International Livestock Research Institute (ILRI) for availing their Resource Centre and Laboratories. I wish to recognize technical support from Albino Mutanda a statistician , Dr Sam Thumbi of Washington State University, Professor Simon Karanja of Jomo Kenyatta University of Agriculture and Technology and Dr Noreen Machila of Zambia . I recognize the inputs of Drs John Gachoi, Mark Nanyingi and Joshua Onono during data reporting and thesis compilation. Special thanks go to Dr Nelson Owuor and Professor Moses Manene of the

School of Mathematics, University of Nairobi for providing guidance on data analysis and reporting.

Thank you the Royal Veterinary College for offering a preparatory course in Modern Approaches to Surveillance in Veterinary Public Health and Advanced Modelling Techniques in Veterinary Public Health, University of Copenhagen for offering a course in Animal Health Economics, Wellcome Trust Sanger Institute and University of Cambridge for offering a in course in Mathematical Modeling for Infectious Diseases which grounded my theoretical understanding towards my PhD completion. For the animal owners from all Sub Counties of Busia County who participated in the study by availing their domestic animals on three occasions for sample collection and volunteered interview information, thank you very much.

For my family I wish to appreciate you for giving me immense support especially my wife Ruth and the young ones Nicole, Nicolas, Nikita and Nigel. May I acknowledge Dr Henry Wanga my brother for supporting my late father to meet the cost of my earlier education. To my many mentors thank you for being there for me.

To God be the glory.

TABLE OF CONTENTS

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

LIST OF PLATES

PAGE

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

ADB African Development Bank

ASDS Agriculture Sector Development Strategy

AT African Trypanosomiasis

AU African Union

AU IBAR African Union Inter African Bureau for Animal Resources

BCT Buffy Coat Technique

CBR Crude Birth Rate

CDC Centres for Disease Control

CDR Crude Death Rate

CNS Central Nervous System

CI Confidence Interval

DALY's Disability Adjusted Life Years

NTDs Neglected Tropical Diseases

DFID Department for International Development, United Kingdom

DNDi Drugs for Neglected Diseases Initiative

EAC East African Community

EDTA Ethylenediaminetetraacetic acid

EU European Union

FAO Food and Agriculture Organization

FIND The Foundation for Innovative New Diagnostics

FITCA Farming In Tsetse Controlled Areas

GIS Geographical Information Systen

GDP Gross Domestic Product

GOK Government of Kenya

KENTTEC Kenya Tsetse and Trypanosomiasis Eradication Council

HAT Human African Trypaosomiasis

HIV AIDS Acquired Immuno Deficiency Syndrome

IGAD LPI Inter Governmental Authority on Development Livestock Policy Initiative

ITS Internal Transcribed Spacer

ISCTRC International Scientific Council for Trypanosomiasis Research and Control

MDG's Millennium Development Goals (Now referred to as Sustainable Development Goals)

OIE World Animal Health Organization

OR Odds Ratio

PCR Polymerase Chain Reaction

RBC Red Blood Cells

Se Sensitivity

Sp Specificity

SRA Serum Resistant Associated Gene

SS Sleeping Sickness

TBR *Trypanasoma brucei* specific primers

WHO World Health Organization

YLD Years Lived with Disability

YLL Years of Life Lost

GLOSSARY

Basic Reproductive number (R_0) : Average number of secondary cases produced by an average infectious individual in a totally susceptible population.

Beta (β): Transmission rate of infection

Carrier: An individual who sheds an infectious agent but does not have any clinical symptoms

Compartmental model: A model in which individuals in the population are subdivided into broad subgroups (compartments) and the model tracks individuals collectively

Force of infection: The rate at which susceptible individuals become infected per unit time also known as incidence rate or hazard rate.

Incidence rate: The number of new infections in the population at risk

Prevalence: The proportion of individuals in a population that have the outcome of interest at a given time

Underreporting (UR) / Underestimation (UE) : ways in which surveillance systems fail or are unable to reflect all infections in a given population thus the number of infections estimated to have occurred in a population that have not been captured by the surveillance system for every reported case over a given time period.

Under ascertained (UA) infections occur in individuals that do not seek healthcare and hence cannot be captured by surveillance systems which are typically designed to capture cases that do seek healthcare.

ABSTRACT

The study aimed at analyzing factors contributing to underreporting of zoonotic African trypanosomiasis and quantifying the human prevalence through utilization of prevalence data of the causative parasite in the blood of domestic animals and feedback from medical practitioners under a non outbreak endemic situation in Busia County. Human African trypanosomiasis (HAT) is caused by infection with protozoan parasites *Trypanosoma brucei rhodesiense* or *gambiense* transmitted to humans by tsetse fly (*Glossina* genus) bites which have acquired their infection from human beings or from animal reservoirs harbouring the human infective trypanosome parasites. Busia Kenya has reported negligible cases of HAT inspite of the neighbouring districts in Uganda shairing porous borders with Kenya continuing to report a marked prevalence. HAT is a major constraint on human health and livestock production and health and yet its prevalence is not appropriately captured due a variety of factors leading to inadequate attention by authorities. The Polymerase Chain Reaction (PCR) test permits differentiation of human and non human infective parasites subspecies especially *T. b. rhodesiense* found in Kenya and *T. b. brucei* based on the presence or absence of the SRA gene specific for *T. b. rhodesiense.* Parasitological techniques through microscopy are routinely used in diagnosing the disease in the field despite lacking the capacity to differentiate the sub species. To gain insight into this challenge the study was split into three distinct components .

The first component of the study sought to establish whether the knowledge and practice in the identification and management of HAT by medical practitioners had an impact on diagnosis and subsequent under reporting of the disease. A cross sectional survey was conducted through the use of semi structured questionnaires administered to medical practitioners and focus group discussions. This was carried out in selected health facilities in Busia County between June 2010 and December 2011 where the curriculum of medical practitioners was also reviewed. Kakamega referral hospital was also included. Chi Square (χ^2) and logistic regression tests

expressed through odds ratios (OR) were carried out to establish relationship and association between knowledge and management practices by medical practitioners for HAT and several variables. Significance was set at $P \leq 0.05$. One hundred and twenty one (121) medical practitioners responded consisting of 72% males and 28% females. Twelve percent (12%) of the respondents reported having managed sleeping sickness before. Years of experience was statistically significant with more experienced respondents reporting to have had previous encounter with mixed zoonotic diseases including HAT as opposed to inexperienced ones (χ^2= 14.21, d.f=3, *p*=0.002).The odds of having a previous encounter with mixed zoonotic diseases including HAT was 42% greater for individuals with 5-10 years' experience or more (OR 0.58, 95% CI 0.09-0.65, *p=0.009*) and were 2.5 times more than individuals who had <1 year experience (OR 2.5, 95% CI 2.61-8.43, *p=0.018*). There exist knowledge and practice gap on HAT by medical practitioners and this could be a contributing factor to poor diagnosis and under reporting of HAT. Refresher courses on zoonotic diseases such as HAT should be conducted with more emphasis placed on zoonotic diseases in the curriculum and training of medical practitioners and veterinarians.

The second component of the study was designed as a cross sectional study with both experimental and observation characteristics to establish the prevalence of *T.b.rhodesiense* parasites in the blood of domestic animals reported using descriptive and analytic statistics. A multi stage sampling involving sub Counties as strata and sampling sites located at sub location level as clusters was done from January 2011 to March 2012. A total of 3799 blood samples from domestic animals collected in 19 sites throughout the County were screened by parasitological techniques through microscopy (later confirmed by PCR) and PCR for *T.b.rhodesiense parasites.* A total of 9 and 47 blood samples from livestock respectively were found positive for T.*b.rhodesiense* respectively for the two tests. Microscopy recorded a sensitivity of 15% and specificity 99% using PCR test as the gold standard. The comparative analysis showed that there was poor agreement between microscopy and PCR with a Cohen's

Kappa (k) value of 0.2816 in all domestic animals. The odds for being positive for any trypanosome in microscopy was 228 if one was tested positive under PCR. Human infective *Trypanosoma brucei rhodesiense* parasites were detected in 1.24 % in all sampled livestock (47/3799) under PCR and 0.24% (9/3799) under Microscopy. In cattle, PCR and microscopy detected 33 (1.05% prevalence, 95% confidence interval 0.82%-1.32%) and 6 (0.77% prevalence, 95% CI, 0.57%-1.01%) positive cases respectively. There is therefore an urgent need for development and utilization of more accurate tools such as those involving molecular techniques like PCR for the effective diagnosis of HAT. The existing tests for diagnosis especially for routine field use are not adequately sensitive due to the characteristically low number of parasites found in the blood of sleeping sickness patients.

The third component of the study developed an explanatory HAT model to estimate prevalence in humans using data from domestic animal hosts under non epidemic conditions. A mathematical model for a vector borne disease involving two vertebrate host species and one insect vector species was developed and executed through R statistical software for statistical modeling and computing. The model predicted 637 people out of total population of 780,132 corresponding to a prevalence of 0.0816% were infected with HAT parasites and 244 livestock were carriers out of the population of 328,895 livestock corresponding to a prevalence rate of 0.074% were carriers of HAT parasites by the end of the year 2014. The animal reservoir is therefore crucial in determining not only the continued occurrence of the disease in humans, but its prevalence in the human host as well. The prevalence model of HAT *T.b.rhodesiense* developed utilizes the prevalence of HAT causing parasites in livestock to estimate the prevalence in humans under non epidemic conditions which is critical in quantifying the extent of underreporting of HAT in the Busia foci and beyond. The practice by medical practioners and choice of diagnostic test directly contribute to underreporting of HAT. The occurrence of HAT infection in animal reservoirs is a critical consideration in planning for effective control of the disease and the model proposed shall provide an insight into the HAT prevalence.

CHAPTER ONE

1.0 INTRODUCTION

1. I Background

The study analysed the complex factors associated with underreporting of zoonotic African trypanosomiasis where a mathematical model was developed that's useful in estimating prevalence in humans based on the prevalence of zoonotic trypanosomiasis causing parasites in livestock, in Busia County under non epidemic conditions. African trypanosomiasis is an infectious disease of humans and animals caused by protozoan parasites of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and are transmitted by the bite of infected tsetse flies (*Glossina* sp.), (OIE, 2013; FAO, 2012).

African trypanosomiasis comprises animal and the zoonotic human types caused by *Trypanosoma brucei* species with other trypanosomiasis parasites of animals commonly being found as co infections. Zoonotic African trypanosomiasis also referred to as human African Trypanosomiasis (HAT) or sleeping sickness (SS) is caused by Trypanosoma *brucei (T.b) gambiense* and *Trypanosoma brucei (T.b) rhodesiense.* The *gambiense* HAT is localized to West and Central Africa and parts of Uganda where it is responsible for chronic sleeping sickness. The *rhodesiense* HAT is present in Eastern Africa causes an acute form of the disease. Animals are infected with *T.b.brucei* which does not infect humans although they are reservoirs for *T.b rhodesiense*, distributed throughout the entire African tsetse region especially Cenral and East Africa, (Radwanska *et al.*, 2002). The first or early stage of HAT, also known as the haemolymphatic phase, is characterised by the restriction of the trypanosomes to the blood and lymphatic system. The symptoms of this stage are fever, headaches, joint pains and itching. The second or late stage of the disease,

also known as the neurological phase, is characterized by the presence of the parasites in the cerebrospinal fluid, (WHO, 2006a). The second stage is when the typical signs of the SS disease occur which include confusion, disturbed sleep pattern, sensory disturbances, extreme lethargy, poor condition and coma. If left untreated, sleeping sickness patients die within months when infected with *T. b. rhodesiense* or within years when infected with *T. b. gambiense*. Sick humans are unable to participate in economic activities and further negative effect arising from hospitalization costs. Wild and domestic animals play a role as parasite reservoirs for human infections with *T. b. rhodesiense* trypanosomes, (Simo *et al.*, 2006). The people most exposed to the tsetse fly and therefore the disease live in rural areas and depend on agriculture, fishing, animal husbandry or hunting. HAT takes 2 forms thus chronic for *Trypanosoma brucei gambiense* or subacute for *Trypanosoma brucei rhodesiense* with the former accounting for about 95% of the reported cases. Sustained control efforts have reduced the number of new cases. Diagnosis and treatment of the disease is complex and requires specifically skilled staff, (WHO, 2015).

Trypanosomiasis is a major constraint to agriculture especially livestock production in 36 countries within the sub Saharan Africa region where also sleeping sickness occurs due to the presence of tsetse flies that transmit the disease. *Trypanasoma vivax* can also be transmitted by other biting flies while *Trypanasoma equiperdum* that affects equines is sexually transmitted. African Trypanosomiasis directly affects human health, animal health and agriculture thereby influencing the quality of rural livelihoods through ill health, reduced incomes and costs related to loss of labour and productivity, (Bett, 2008; FAO,2012).

All domestic animals can be affected by Animal Trypanosomiasis or Nagana disease and the symptoms include fever, listlessness, emaciation, hair loss and discharge from the eyes,

oedema, anaemia, and paralysis. As the illness progresses the animals weaken more and more and eventually become unfit for work as seen in Plate 1.1, hence the name of the disease "N'gana" which is a Zulu word that means "powerless or useless" rephrased to Nagana, (Winkle, 2005). Nagana disease is caused by *Trypanasoma congolense*, *Trypanasoma vivax* and *Trypanasoma brucei brucei*. In wild animals, these parasites cause relatively mild infections while in domestic animals they cause a severe, often fatal disease.

Plate 1.1 : A picture of a suckling cow confimed to be suffering from Nagana a disease caused by trypanosomes in small holder herd in Busia County.

African trypanosomiasis poses a severe negative socio economic impact throughout sub Saharan Africa with losses to production estimated at over US dollars 1.3 billion annually in terms of milk and meat in cattle, (Kristjanson *et al.*, 1999). Animal trypanosomiasis complicated by the zoonotic type is a serious constraint to productivity in Busia County where sporadic cases of human sleeping sickness cases have been reported. Between seventy and eighty percent of the potential labour of the County is engaged in subsistence mixed crop and livestock farming, (GOK, 2001; WHO, 2006b). Trypanosomiasis related losses include both the direct livestock output related such as weight loss, decrease in milk, decreased reproductive rate as well as opportunity in terms of integration of livestock into crop

production and the potential for crop improvement through loss of draught power and manure, (FITCA, 2005).

Agriculture sector is the mainstay of the Kenyan economy directly contributing 26 per cent of the Gross Domestic Product (GDP) annually and another 25% indirectly. The sector accounts for 65 per cent of Kenya's total exports and provides more than seventy percent of employment in rural areas. The agricultural sector that is dependent on human resource for to provide production factors comprises crops, livestock, fisheries, land, water, cooperatives, environment and forestry subsectors, (ASDS, 2010). Agriculture contributes directly towards the attainment of Kenya's Vision 2030 which is anchored on the political, social and economic pillars with agriculture contributing mainly through the economic pillar with beneficial effects on the others. African trypanosomiasis directly affects livestock that contribute over ten percent of Kenya's GDP and over fourty percent of agricultural GDP, (NLP, 2008). Livestock resources contributes to livelihoods in a variety of ways including through generation of cash, source of food of animal origin, generation of savings and provision of insurance, production of manure, provision of draught and hauling services, use of scarce resources available in the dry lands and social capital, (IGAD LPI,2012).

Agriculture expected to grow at 7% is the engine of the economic pillar for vision 2030 supporting the attainment of an economic growth rate of ten percent annually and sustain the same till 2030. This is when Kenya is expected to become a middle income country able to generate more resources to address the Millennium Development Goals (MDGs) now referred to as Sustainable Development Goals, (ASDS, 2010). Therefore any negative impacts on the Health of people and Agriculture such as that attributable to zoonotic and animal African trypanosomiasis must be addressed for posterity.

1.2 Statement of the problem

Routine data collection during periods of endemic transmission of HAT is lacking in many settings unlike for the epidemics or outbreak situations where there is a focused attention on the disease resulting in greater availability of data, (Monath 2006; Zuckerman *et al*., 2007). Quantification of incidence and prevalence of HAT is important in improving focus on controlling the neglected disease, because the primary reason for its neglect is that its true impact on society is not known. However, within a region or county, where transmission of a neglected disease occurs, it may assume a much greater importance as budgetary decisions are increasingly made at such decentralized levels and therefore appropriate to measure the burden of disease at this level, (Kapiriri *et al.,* 2003; Jeppson *et al.,* 2004).

The main tool used for diagnosing SS at the field level involves parasitological techniques that utilize microscopy which is characterized by a low sensitivity, (Cattand *et al.,* 2001) making estimation of the true occurrence of SS under such circumstances difficult. To date, crude estimates based on historical records of SS surveillance and case finding rates have been used. For *T.b.rhodesiense*, studies based on quantifying underreporting using data on the early to late stage ratio have shown that between 38% to 41% of *T.b. rhodesiense* cases go unreported in Uganda, with a similar picture in Tanzania, (Odiit *et al.,* 2005; Fevre *et al.,* 2005a). Kenyan statistics are missing on the level and extent of underreporting.

Unreported cases go untreated and almost invariably result in death. For neglected diseases such as Leishmaniasis, *T.b. rhodesiense* HAT and Rabies, underreporting rates have rarely been properly quantified , so progress in this regard for HAT is necessary, (Fevre *et al.,*2005b;Odiit *et al.,* 2005;Knobell *et al.,* 2005). Unfortunately, health care systems themselves may be missing many cases of HAT on presentation at non specialized units. While in some settings communities may be aware of the disease and its dangers but not

report cases, as they may be considering that drugs and treatment are not available in health units, (Kinunghi *et al.*, 2006). Detecting patients with HAT depends on disease knowledge amongst individuals and communities, which encourages patients to visit the health centre for diagnosis and appropriate management. For the health care systems, the availability of appropriate diagnostic facilities and appropriately trained staff is important, (Odiit *et al.*, 2005). In addition to the lack of estimates of levels of under detection of rhodesiense SS, it is not known what proportion of these undetected cases did seek health care and whether their medical condition was appropriately managed. Adequacy of knowledge, attitudes and practice on HAT diagnosis and management by medical practitioners needs to be established.

Important questions to be answered in this study include, if human African trypanosomiasis causing parasites *T.b.rhodesiense* can be found in the blood of domestic animals while the transmitting vector the tsetse fly is present then how come the hospitals report no cases of sleeping sickness? The Ugandan Districts of Busia and Tororo neighbouring Busia County have reported cases of Sleeping Sickness and how come the Kenyan side sharing porous borders reports no case? The explanation to these questions was provided in this study.

1.3 Overall objective

To analyse factors contributing to underreporting of the zoonotic African trypanosomiasis (HAT) and estimate prevalence of the disease in humans using data on prevalence of the causative parasite in the blood of domestic animals and feedback from medical practitioners under a non outbreak /endemic conditions in Busia County.

1.4 Specific objectives

1. To determine how the knowledge, attitude and practices of medical practitioners affect the diagnosis and management of the zoototic human African trypanosomiasis.

2. To assess the level of agreement between the outcomes of parasitological diagnostic tests under microscopy and the Polymerase Chain Reaction (PCR) test in confirmatory laboratory diagnosis of human African trypanosomiasis parasites.

3. To develop a model useful in estimating the prevalence of human African trypanosomiasis based on the parasite detection in livestock under non epidemic or endemic situation as a quantification tool for extent of underreporting of the disease.

1.5 Justification or rationale of the study

Although SS may not seem very important on the world stage to warrant equal attention with diseases such as malaria and AIDS, it remains an important disease in sub Saharan Africa as it is responsible for a considerable degree of suffering and mortality in countries and counties or districts where it is endemic. Many cases of HAT in the region are underreported and therefore there's need to validate the methods that can help quantify the level of under detection to stir action and enable effective control. Kenya shares porous borders with Tanzania and Uganda and yet she reported only 23 cases of rhodesiense HAT from 2001 to 2011 while her two neighbours reported over 4056 cases over the same period, (Simarro *et al.*, 2013).

Determination of prevalence is necessary in providing an evidence based decision making process that is effective for planning of control and interventions, (Murray and Lopez, 1996a). HAT burden can be measured in terms of impact at a range of scales at the individual level, family groups or society at large. Data on disease risk is important in decision making at all levels of society ranging from county or local, government policy, national or regional levels necessary for planning and budgetary allocation decisions . For focal diseases such as HAT, it is necessary to choose an appropriate scale at which the assessment of burden is carried out since in many sub Saharan African countries at the national level, the burden of

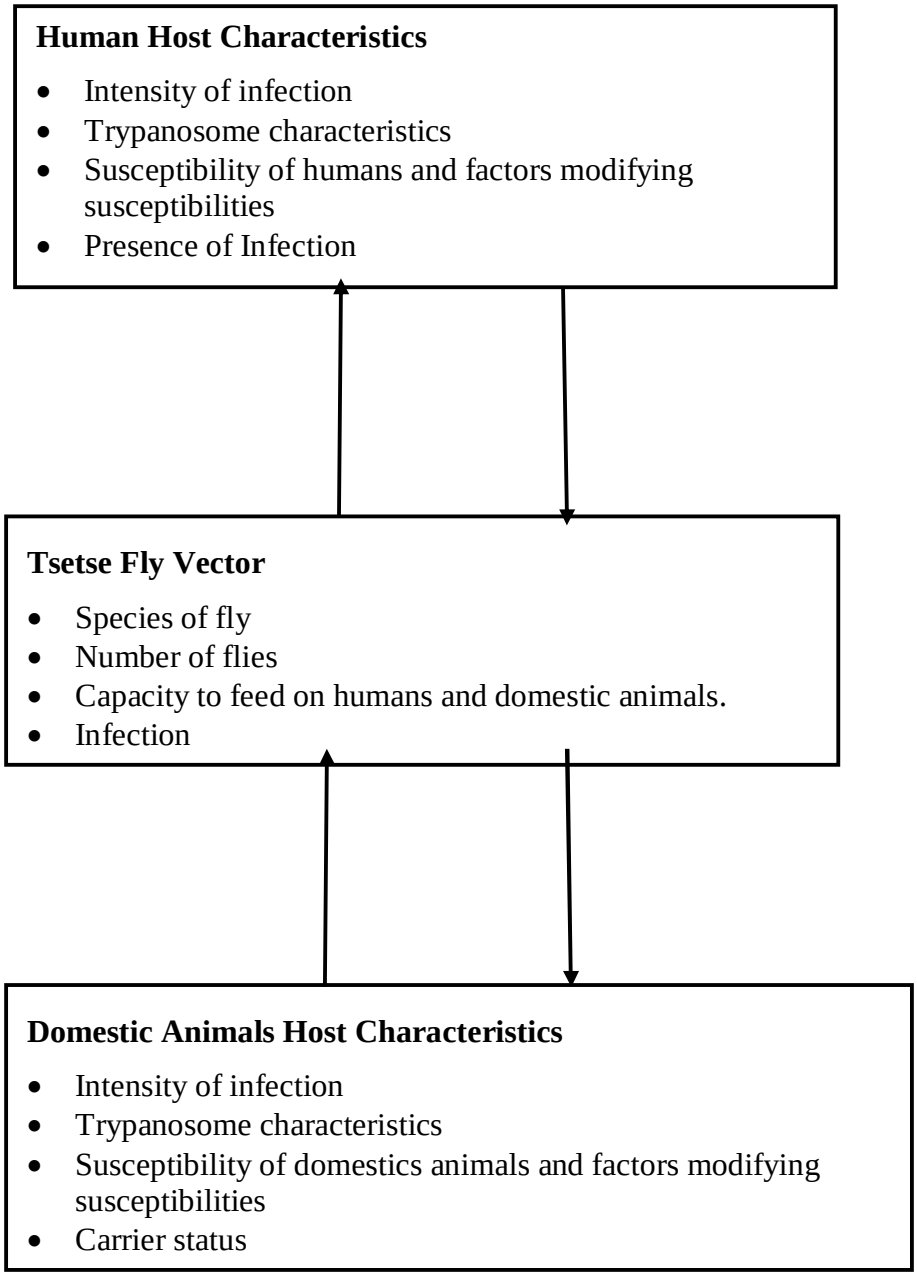
Malaria will exceed by orders of magnitude that of HAT, Leishmaniasis, Cysticercosis or many other neglected infections due to absence of critical data, (Ashford, 2000; Pawlowski, 2008). The opportunities of preventing the deaths related to SS cases are greater than for many other diseases such as AIDS and therefore it is important to ensure that the SS cases that die without having sought health care or following missed diagnosis by the healthcare system are avoided. The highest estimate of deaths because of SS where it occurs in epidemics is commonly associated with areas with poor surveillance characterized by inadequate data on prevalence of the disease, (Bossert &Beauvai, 2002; Jeppson *et al.,* 2004).

While relative disease burdens during periods of endemic and epidemic periods expressed as Disability Adjusted Life Years (DALYs) matter in themselves, it would be a mistake to calculate a DALYs value for an epidemic and assume that it would apply under endemic conditions in order to obtain a general baseline level of burden for longer term planning, (Fevre *et al.*, 2008a). When HAT burden is determined for epidemics or outbreaks this should be explicit so that generalization is avoided to non epidemic situations. The findings from this study shall help in estimating the actual prevalence of HAT in humans based on the presence of the causative parasites in animals. Estimates of the prevalence shall help determine whether by extension the disease is present, missing or under diagnosed and therefore not accurately reported.

1.6 Conceptual framework and human African trypanosomiasis causation model

Causal web model explains the basis of conceptualizing how multiple factors can combine to cause HAT consisting of multiple direct and indirect causes as shown in Figure 1.1.

Figure 1.1: Conceptual framework on the interaction between the vector and vertebrate hosts in maintaining the infectious cycle of human African trypanosomiasis



This conceptual framework above is based on a series of interconnected causal chains or web structures where the direct cause is the *Trypanasoma brucei rhodesiense* and the indirect causes are mediated through intervening variables, (Dohoo *et al.,* 2010). The dependent variable in the entire study is the number of cases of HAT detected or undetected that are influenced by several independent variables such as the host and vector populations, type of

laboratory diagnostic test and geographical location of homestead of residents. Other independent variables include access to appropriate medical care, hospital seeking behavior of infected suspects or patients, skill of the medical personnel, presence of domestic animal reservoirs or host, presence of the tsetse fly vector and the source of infection for the specific disease causing parasite. Most critical independent variables for this study were the source of infection in animal blood, the presence of the transmitting vectors, the diagnostic tests available and the expertise of the medical practitioners.

1.7 Description of the chapters

This thesis discusses the theoretical basis, procedures and methods involved in the planning and execution of the study, presents the research findings, data analysis and interpretation of the results. It is structured into six chapters. The first two chapters give the introduction, statement of the problem, justification of the study including the objectives and a review of relevant literature. Chapter three provides the methodology, research findings and discussion of the results on the knowledge, attitude and practice by Medical Practitioners on HAT. Chapter four provides the methodology, the research findings and discussion of the results on the extent of agreement between parasitological tests conducted under microscopy and Polymerase Chain Reaction (PCR) tests on identification of HAT parasites and the comparison of sensitivities between PCR and Microscopy results from livestock blood samples. Chapter five gives the findings on the developed Model for estimating prevalence useful in quantifying prevalence of HAT under non epidemic situations. Chapter six gives a summary of the findings and recommendations based on knowledge gained from the study.

CHAPTER TWO

2.0 LITERATURE REVIEW

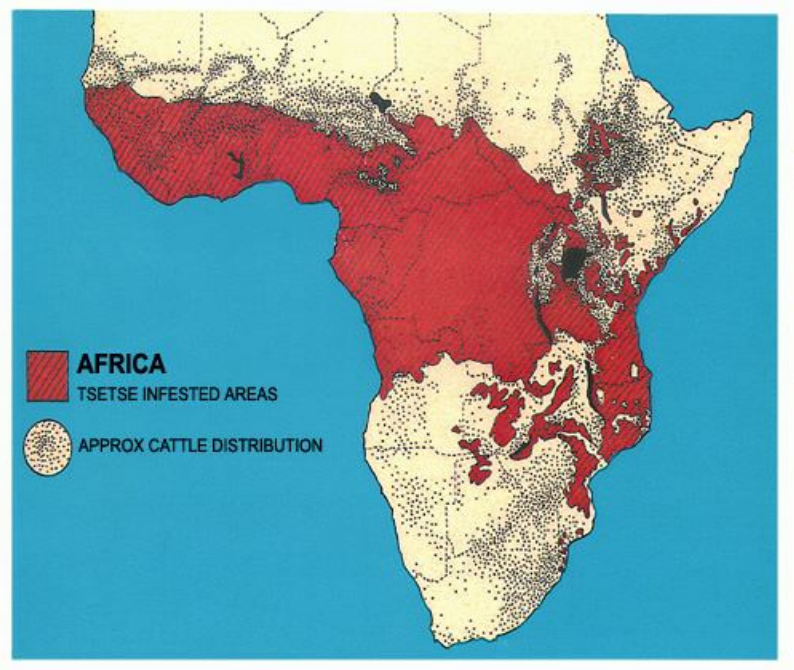
Important considerations range from the description of the vector and the disease epidemiology, burden and socioecomic importance, effective control methods, diagnostic methods, modeling and an update on interventions against African trypanosomiasis.

2.1 Tsetse and trypanosomiasis

2.1.1 Distribution

African trypanosomiasis (AT) comprising of the zoonotic HAT and the animal type is a disease complex caused by tsetse fly transmitted *Trypanosoma congolense*, *T. vivax*, *T. brucei* or mixed infections with more than one of these trypanosomes. The distribution of trypanosomiasis in Africa corresponds to the range of tsetse flies and comprises, currently an area of 8 million km^2 between 14 degrees North and 29 degrees South latitude, (Molyneux *et al.*, 1996), as shown in Figure 2.1.

Figure 2.1: Tsetse (*Glossina* spp.) distribution in Africa



Source: http://www.iaea.or.at/worldatom/Press/Focus/Tsetse/Retrieved on 02/11/11

2.1.2 Tsetse characterization

Trypanosomiasis is transmitted through the bite of an infected tsetse fly (genus Glossina). Thirty one species and subspecies of tsetse flies have been described and classified in three groups or sub genera which are generally related to different habitats. Subgenus Nemorhina, or the palpalis group, found in Western and Central Africa, live in vegetation close to a water source, such as forests, gallery forests, riverbanks and lakes, swamps and mangroves, coffee or cocoa plantations. Tsetse can even adapt to environmental changes, surviving in the periurban areas of medium and large towns and areas of intensive agriculture. This subgenus includes the main vectors of sleeping sickness including *G. palpalis palpalis* and *G. p. gambiensis* for *T. b. gambiense* and *G. fuscipes* for both *T. b. gambiense* and *T. b. rhodesiense*. *G. palpalis* is distributed in the Atlantic coast from Senegal to Angola. *G. fuscipes* is present in central Africa from Cameroon and Congo to the Rift Valley (Franco *et al.*, 2014).

Subgenus Glossina sensu stricto, or the morsitans group, occurs in woodland savannah and is linked to the presence of wild fauna and cattle. *G. morsitans, G. swynnertoni,* and *G. pallidipes*, all of which are found mainly in East Africa are involved in the transmission of *T. b. rhodesiense.* Subgenus Austenina, or the fusca group, lives in primary forest belts (rainforest, savannah, or coastal forests). Increasing human activity in these forests tends to make this subgenus disappear. They have not been reported to be vectors of HAT. Tsetse flies are restricted to sub-Saharan Africa, south of the Sahel desert, and north of the Namibian and Kalahari deserts. Some pockets of *G. morsitans* and *G. fuscipes* have been described in southwestern Saudi Arabia (Franco *et al.*, 2014).

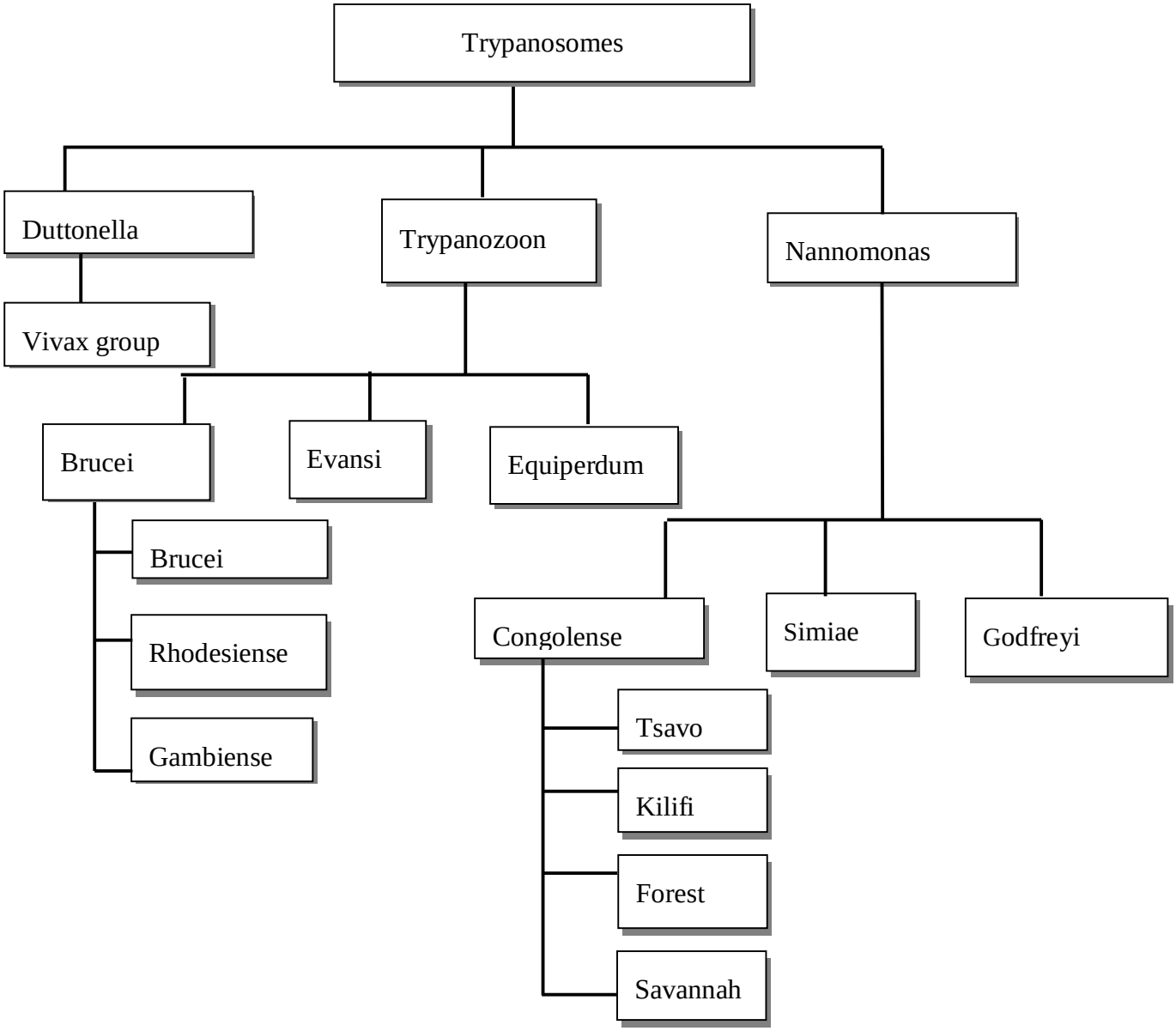
All the species of tsetse flies are potentially cyclical vectors of trypanosomes, but in nature, the infection is carried almost exclusively by *G. fuscipes, G. palpalis* and *G. morsitans*. Tsetse flies need a particular temperature range of 16°C–38°C and humidity range of 50%–80% of relative humidity to survive. Therefore, they are linked to the presence of water that increases the local humidity, allowing for the growth of vegetation that protects them from direct sunlight and wind, and attracts the animals to where tsetse feed (Franco *et al.*, 2014). The transmission of HAT involves the obligatory biological development of trypanosomes in the fly, as previously described and in only 2%–5% of cases will this development occur, with the presence of metacyclic forms in the salivary glands. Therefore, the mature infection rate among tsetse flies is quite low, with an average of less than 1% of tsetse flies infective for *T. brucei* spp. This makes the tsetse fly a relatively low competent vector for zoonotic trypanosomiasis transmission.

Nevertheless, even the low ingestion of parasites during the blood meal can result in infection in the fly and a single infected tsetse bite is sufficient for transmitting the infection to another mammalian host. However, the probability of the transmission of HAT is related to the number of trypanosomes inoculated into the mammal. Therefore, the capacity of transmission of the disease by the tsetse fly is influenced by different factors, such as the density of tsetse populations, the tsetse fly's longevity, the vector's susceptibility to infection, the tsetse fly's infestation rates, the availability of other sources of a blood meal, and the factors that determine the frequency and intensity of the host fly's contacts. *Glossina palidipes* and *Glossina fuscipes* are found in Busia, (Franco *et al.*, 2014).

2.1.3 Characterization of trypanosomes

Trypanasoma classification is as illustrated in Figure 2.2 below.

Figure 2.2: Classification of pathogenic African trypanosomes



Source: Jordan, 1986

Trypanosomes are divided into three sub genera *Nannomonas, Duttonella* and *Trypanozoon*. *Trypanosoma congolense* the most important cause of animal African trypanosomiasis belong to the subgenus Nannomonas, a group of small trypanosome parasite that is 8–25 µm long,

the undulating membrane is not obvious, free flagellum absent, posterior end rounded, kinetoplast is medium sized and terminal, often laterally positioned.Within *T. congolense*, different types or subgroups exist (savannah, forest, kilifi or Kenya coast) that have a different pathogenicity and also there is a large variation in pathogenicity within the savannah subgroup (Desquesnes *et al.,* 2012; Bengaly *et al.,* 2002). *Trypanasoma brucei rhodesiense* the cause of HAT belongs to the subgenus Trypanozoon within the family of t*rypanosomatidae,* a large group of unicellular protozoan parasitic organisms and the order of *Kinetoplastida*. *Trypanosoma brucei* is a polymorphic trypanosome species where two distinctly different forms can be distinguished, i.e. a long slender form and a short stumpy form. Often, intermediate forms, possessing characteristics of both the slender and stumpy forms, are observed. The cytoplasm often contains basophilic granules in stained specimens. *Trypanosoma brucei* (long slender form) is 17–30 µm long and about 2.8 µm wide, with an undulating membrane that is conspicuous, free flagellum present at the anterior end, posterior end pointed kinetoplast small and sub terminal. *Trypanosoma brucei* (short stumpy form) is 17–22 µm long and about 3.5 µm wide, undulating membrane is conspicuous, free flagellum absent, posterior end pointed kinetoplast small and sub terminal, (Uilenberg, 1998). *Trypanasoma vivax* transmitted by other biting flies in addition to the tsetse fly belongs to the Dutonella sub genus, *Trypanosoma vivax* is a 20–27 µm long parasite with an undulating membrane that is medium or not obvious, free flagellum present at the anterior end, posterior end rounded, kinetoplast large and terminal, (Uilenberg, 1998).

2.2 Epidemiology of African trypanosomiasis

2.2.1 Life cycle of trypanosomes

Infection of the 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. 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). Trypanosomes undergo cyclical replication while in the tsetse fly and are transmitted through tsetse fly saliva when the fly feeds on animals including humans, (Urquart and Urquart, 1987). The mammal infective forms of the parasites, the metacyclic trypanosomes, multiply at the site of inoculation into typical blood forms resulting into the formation of a raised cutaneous inflammatory swelling called the chancre, (Urquart and Urquart, 1987).

Two different trypomastigote forms can be observed in the mammalian host comprising 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 2 to 3 week life cycle in the fly during the entire life cycle. *T. brucei* cells multiply by binary fission and are considered to be exclusively extracellular, (Chappuis *et al.,* 2005).

2.2.2 Zoonotic trypanosomiasis transmission

In both forms of HAT, infection occurrence depends on the interaction of three elements within a particular environment. The first are the mammalian reservoirs of parasites (human or animal) that can be also the host suffering from the disease which are influenced by their behavioral interactions with the environment. The second element is the tsetse fly as cyclical vectors for transmission that are fully dependent on environmental factors and the third is the pathogenic parasite, the trypanosome, (Franco *et al.,* 2014). Due to reasons that are not always well known, but that are related to the interactions between these three elements, the transmission of the disease is confined to areas with quite clear spatial limits, beyond which the disease does not occur. This limited space is called a “focus”or “foci” . Trypanosomes are extracellular parasites and they can be clearly identified microscopically, but the two

subspecies that are pathogenic for human beings are morphologically indistinguishable. Since differences between these subspecies cannot be observed by microscopy, other methods have been used apart from the geographic differences, the specific molecular marker known as the serum resistance associated (SRA) gene is currently being used to differentiate between the brucei sub species, as it is always present in all *T. b. rhodesiense* isolates, (Franco *et al.*,2014).

The transmission of HAT occurs primarily in rural areas with a few exceptions and especially in areas at the furthest extremities of the formal health system creating particular problems for patients to access health care. This is important for control campaigns to have an effective outreach and importantly in the assessment of the burden of infections, hindering efforts to collect data on how many people are at risk in Kenya today, how many people are infected and what the impact of the disease is on the social environment, (Gouteux *et al.,* 1987; Abel *et al.,* 2004a). Although the disease is mostly transmitted through the bite of an infected tsetse fly, there are other ways in which people are infected with sleeping sickness which include mother to child infection with the trypanosome crossing the placenta to infect the fetus and mechanical transmission through other blood sucking insects. It is possible although it is difficult to assess the epidemiological impact of the other non traditional means of transmission and accidental infections that occur in laboratories due to pricks from contaminated needles, (Odiit *et al.,* 2004a).

2.2.3 Trypanosomiasis reservoirs

HAT is a public health problem where the vector, the parasite, its reservoir hosts and humans coexist. Wildlife and domestic animal species are known to act as reservoirs. Where wildlife is not abundant, domestic species particularly cattle are the main reservoirs with livestock demography driving outbreaks, (Fevre *et al.,* 2001). Rural populations living in regions where

transmission occurs and which depend on agriculture, fishing, animal husbandry or hunting are the most exposed to the tsetse fly and therefore to the disease. Sheep and goats are generally reported to be less susceptible to clinical trypanosomiasis however they can harbor low grade chronic trypanosome infections which can induce severe pathology when transmitted to cattle, (Taylor and Authie, 2004). Pigs are moderately susceptible to *T.congolense and T.brucei* infections. Busia being endemic for Rhodesian SS, livestock play an important role as a reservoir for human infective subspecies *T.brucei rhodesiense* frequently without displaying overt clinical signs of infection, (Wissmann *et al.,* 2011).

2.2.4 Trypanosomiasis clinical presentation

Livestock especially cattle infected by one or more of these trypanosomes manifest the disease as subacute, acute or chronic, which is characterized by intermittent fever, anaemia, rapid loss of body condition and may terminate in death if untreated, (Urquart and Urquart, 1987). *Trypanosoma brucei rhodesiense (T.b.r.)* which represents about 5% of reported cases of sleeping sickness has the first signs and symptoms observed a few months or weeks after infection with the disease developing rapidly to invade the central nervous system, (Picozzi *et al.,* 2008). The incubation period varies between trypanosomes for animal African trypanosomiasis, with *T. b. brucei* having the shortest duration from 5 to 10 days; *T. congolense* takes as long as 4 to 24 days and *T. vivax*, from 4 to 40 days, (Stephen, 1986). HAT due to *T. b. rhodesiense* infection presents as an acute (sometimes fulminant) febrile illness starting 1 to 3 weeks after the infective bite and it cannot be distinguished clinically from other tropical fevers such as malaria, enteric fever, and bacterial meningitis. Compared to *T. b. gambiense* illness, febrile episodes are more pronounced and frequent and lymphadenopathy is usually generalized. Keratitis and conjunctivitis have also been observed. There is less demarcation between first and second stage illness and Central Nervous System (CNS) involvement can be clinically limited to drowsiness and tremor. Pericarditis 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, (Chappuis *et al.,* 2005). *Trypanosoma brucei gambiense (T.b.g.)* found in West and Central Africa currently accounts for over 95% of reported cases of sleeping sickness and causes a chronic infection. A person can be infected for months or even years without major signs or symptoms of the disease. When symptoms emerge, the patient is often already in an advanced disease stage where the central nervous system is affected, (Picozzi *et al.,* 2005).

2.2.5 American trypanosomiasis

Another form of trypanosomiasis caused by *Trypanasoma cruzi* occurs mainly in 21 Latin American countries known as American Trypanosomiasis or Chagas disease whose causal organism *Trypanasoma cruzi* is a different species from those causing the African form of the disease, (Picozzi *et al.,* 2008). This disease is not a challenge in Africa more so Kenya.

2.3 Laboratory diagnosis of human African trypanosomiasis

The diagnosis of *T. b.rhodesiense* HAT follows a three step pathway consisting of screening, diagnostic confirmation and staging. The majority of control programs rely on active case detection through mass population screening. Screening tools therefore need to be sensitive, practical, quick and cheap. Diagnostic confirmation then relies on the finding of trypanosomes in the blood, lymph nodes, or cerebrospinal fluid (CSF). Unfortunately, it is estimated that many of the patients are missed by the standard parasitological techniques, (Chappuis *et al.,* 2005). Rhodesiense SS is mainly detected by passive case finding, with patients presenting at a health facility and diagnosis based on microscopic detection of trypanosomes, (WHO, 1998). The Foundation for Innovative New Diagnostics (FIND) and the WHO in 2006 launched a new initiative for the development of new diagnostic tests to

support the control of sleeping sickness. It is expected that the new tests will allow for early case detection and simplified staging and thus will improve disease management and support for the elimination of sleeping sickness as a public health problem, (Steveding, 2006).

2.3.1 Microscopy

Several parasite detection techniques are used involving microscopic examination of the wet or dry stained thick or thin blood films. Diagnostic sensitivity is increased significantly by concentrating the parasites prior to examination in combination with a phase contrast or dark ground microscope. The centrifugation parasite concentration techniques have the added advantage that the packed cell volume and hence the level of anaemia, can be determined at the individual animal and/or herd level, (OIE, 2013). The microscopic techniques currently used include:

2.3.1.1 Direct examination techniques

The simplest techniques are examination of wet, thick or thin films of fresh blood or lymph, usually obtained from lymphatic fluid, blood, lymph node and chancre aspirate for humans while when identifying the parasite from animals, ear vein, jugular vein or the tail are the source of blood. Amongst the direct examination techniques, stained thin blood films are generally regarded as more specific but less sensitive than the other two. The actual specificity and sensitivity of these techniques is directly dependent on the volume of blood actually examined, the skill and experience of the microscopist, (OIE, 2013).

a) Wet blood films

These are made by placing a droplet of blood (about 2 µl) on a clean microscope slide and covering with a coverslip (22 × 22 mm). The blood is examined microscopically at × 400 total magnifications with condenser aperture, phase contrast or interference contrast.

Approximately 50–100 fields are examined. The diagnostic sensitivity of the method is generally low but depends on the examiner's experience and the level of parasitaemia. Sensitivity can be improved significantly by lysing the Red Blood Cells (RBCs) before examination using a haemolytic agent such as Sodium Dodecyl Sulphate (SDS), (OIE, 2013).

b) Thick blood films

These are made by placing a drop of blood (5–10 µl) on a clean microscope slide and spreading it over an area of approximately 2 cm in diameter, using the corner of another slide. A dry smear should be kept dry and protected from dust, heat, flies and other insects. It is stained for 30 minutes with 4% diluted Giemsa stain in phosphate buffered saline, pH 7.2. Staining time and stain dilution may vary with stain and individual technique. The method is simple and relatively inexpensive, but results are delayed because of the staining process although commercial kits are available for quick staining. Thick smears contain more blood than thin smears and, hence, have a higher diagnostic sensitivity. Thin smears on the other hand allow easier Trypanosoma species identification, (OIE, 2013).

c) Thin blood films

A thin blood film or peripheral blood smear is a thin layer of blood smeared on a microscope slide and then stained in such a way to allow the various blood cells and parasites to be examined microscopically. Blood films are made by placing a drop of blood on one end of a slide, and using a spreader slide to disperse the blood over the slide's length. The aim is to get a region, called a monolayer, where the cells are spaced far enough apart to be counted and differentiated. The slide is left to air dry, after which the blood is fixed to the slide by immersing it briefly in methanol. The fixative is essential for good staining and presentation of cellular detail. After fixation, the slide is stained to distinguish the cells from each other.

After staining, the monolayer is viewed under a microscope using magnification up to 1000x. Individual parasite cells are examined and their morphology is characterized and recorded, (OIE, 2013).

2.3.1.2 Parasite concentration techniques

a) Microhaematocrit Centrifugation Technique (Woo method)

The Microhaematocrit Centrifugation Technique, or the Woo method (Woo, 1970), is widely used for the diagnosis of HAT. It is based on the separation of the different components of the blood sample depending on their specific gravity. The buffy coat layer examined seperates the serum from the blood cells and this is the area most parasites segment.The technique is more sensitive than the direct examination techniques, (OIE, 2013). Trypanosomes become very difficult to detect when the parasitaemia is lower than 60 trypanosomes/ml blood. To improve the separation of Red Blood Cells (RBCs), from parasites and increase the sensitivity, the specific gravity of RBCs can be increased by the addition of glycerol, (Desquesnes *et al.*, 2001).

b**) Dark Ground/Phase Contrast Buffy Coat Technique (Murray method)**

The Buffy Coat Technique or Murray method (Murray *et al.*, 1977) represents an improved technique for the detection of trypanosomes and is widely used. The buffy coat and the uppermost layer RBCs are extruded on to a clean microscope slide. Approximately 200 fields of the preparation are examined for the presence of motile trypanosomes with a dark ground or a phase contrast microscope with a ×40 objective lens. As with the microhaematocrit centrifugation technique, the buffy coat technique is more sensitive than direct examination techniques. The sensitivity of the buffy coat method can be improved by using the buffy coat double centrifugation technique. A total amount of 1500–2000 µl of blood is centrifuged,

after which the buffy coat is aspirated into a microhaematocrit capillary tube and centrifuged again. The buffy coat is examined. However, collection of the buffy coat after the initial centrifugation is a delicate step and results may vary from one technician to another. Compared with the microhaematocrit centrifugation technique, the buffy coat technique has the added advantage that preparations can be fixed and stained for more accurate identification of species and for retention as a permanent record, (OIE, 2013). Another parasite concentration method that can be used is the mini anion exchange centrifugation technique (mAECT), (Maudlin *et al.*, 2004)

2.3.2 Antibody detection

Indirect evidence for trypanosome infection can be obtained by demonstrating specific antibodies in the blood, plasma, or serum of infected hosts. Trypanosomes have a complex antigenic structure and elicit the production of a large spectrum of antibodies. *T. b. rhodesiense* specific immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies are present in high concentrations and are directed mainly against the immunodominant surface glycoprotein antigens of the parasite. Current serological tests detect antibodies after 3 to 4 weeks of infection. Seropositivity must be interpreted with caution in previously treated patients since antibodies can persist for up to 3 years after cure. Several antibody detection techniques have been developed to detect trypanosomal antibodies for the diagnosis of African trypanosomiasis especially the animal type, with variable sensitivity and specificity, (OIE, 2013).

a) Indirect Fluorescent Antibody Test

The technique for the preparation of trypanosomal antigens involves fixation of live trypanosomes using a mixture of 80% cold acetone and 0.25% formalin in normal saline,

(OIE, 2013). The test has a high sensitivity and genus specificity. Their species specificity is generally low, but may be improved by using a standardised set of the three species specific tests. It detects immune responses to current and past infections and can, therefore, only provide a presumptive diagnosis of active infection. However, persistence of antibodies after a curative treatment or a self-cure is estimated to be on the average of 3–4 months in young and adult cattle infections (Desquesnes *et al.*, 2012).

b) Antibody detection Enzyme Linked Immunosorbent Assay (ELISA)

The original antibody ELISA has been further developed for use in large scale surveys of trypanosomiasis. Recommendations have been made that allow antigen production and standardization of the test on a local basis in the sampled area. The standard antigen for trypanosomiasis antibody tests is derived from bloodstream form trypanosomes. Both antibody detection tests have high sensitivity and genus specificity. Their species specificity is generally low, but may be improved by using a standardized set of the three species specific tests. They detect immune responses to current and past infections and can, therefore, only provide a presumptive diagnosis of active infection, (Desquesnes *et al.*, 2001).

2.3.3 Use of Polymerase Chain Reaction (PCR)

2.3.3.1 Capacity of Polymerase Chain Reaction

PCR diagnostic assays overcome the low sensitivity limitations of parasitological techniques. They are powerful tools for identification and diagnosis of trypanosomes in their hosts and vectors although its costs and the need for elaborate expertise has delayed its adoption, (Thumbi *et al.*, 2010). PCR assays able to detect all pathogenic trypanosome species in a single reaction have been developed, (Cox *et al.*, 2005). These reduce the costs of screening a

sample from an endemic area by up to five times and have been suggested as suitable for large scale epidemiological studies, (Thumbi *et al.*, 2008).

2.3.3.2 Polymerase Chain Reaction tests for human African trypanosomiasis identification

Initially, the internal transcribed spacer region PCR (ITS-PCR) detects and identifies the trypanosome species affecting livestock. The second (TBR-PCR) is specific for *T. brucei*. Highly conserved ribosomal RNA (rRNA) genes are also useful for comparisons between closely related species. The internal transcribed spacer regions (ITS) in particular are relatively small, show variability among related species and are flanked by highly conserved segments to which PCR primers can be designed. Individual variations in inter species length makes the ITS region a useful marker for identification of multiple trypanosome species within a sample, (Njiru *et al.,* 2005). The ITS-PCR has enabled differentiation to the species level of all trypanosomes circulating in an animal host by means of a single PCR reaction, which is based on identification of Internal Transcribed (ITS) regions of the parasite genome, (Cox *et al.,* 2005).

Up to 1,000 different genes encoding the variant surface glycoproteins are present in the *T. brucei* genome. This phenomenon explains the fluctuating number of circulating trypanosomes in the patient's blood, which contributes to the limited sensitivity of parasite detection methods in clinical practice, (Chappuis *et al.,* 2005). Differentiation of the subspecies of *Trypanosoma brucei* has recently become possible with the observation that one of the two human infective subspecies, *T. b. rhodesiense,* carries a gene that protects the parasite from lysis in the human host, called the Serum Resistance Associated gene (SRA) (Xong *et al.,* 1998; Vanhamme *et al.,* 2003). This gene has not been found in any other species of trypanosome and has been developed as a diagnostic marker for the identification

of *T. b. rhodesiense* infections as indeed the gene has never been identified in any other *T. b. brucei* genetic background, (Welburn and Odiit, 2002). Further, currently the SRA gene has been shown to be a ubiquitous marker for *T. b. rhodesiense* parasites throughout East Africa, both within the human host and the domestic animal reservoir, (Welburn *et al.*, 2001 ; Gibson *et al.*, 2002).

2.4 Use Geographical Information System (GIS) in mapping human African trypanosomiasis

Georeferenced datasets and spatial analysis techniques are widely recognized as essential tools to support the planning and implementation of interventions against human and animal diseases, including the zoonotic African trypanosomiasis (FAO, 2012).The use of Geographical Information Systems (GIS) software now makes it cheaper and easier to produce maps which can serve as useful tools for policy discussion and allow for analysis of factors that would influence the disease pattern, (Hardy, 2003). Results obtained from molecular studies, associated with geo referenced information concerning vector, cattle distribution and relevant environment parameters, combined in a GIS have the potential of providing more informative study results. This new approach of studying complex pathogenic system is argued to lead to a better evaluation of the risk of infection, allows for effective risk communication and gives scientific outputs in ways that are understandable to non scientists, (Bell *et al.*, 2006).

2.5 Treatment of human African trypanosomiasis

Succesful management of HAT relies on treatment of infected or carrier animals to remove the source of infection. The type of treatment of HAT depends on the stage of the disease. The drugs used in the first stage of the disease are of lower toxicity and easier to administer.

The earlier the disease is identified, the better the prospect of a cure. Treatment success in the second stage depends on a drug that can cross the blood-brain barrier to reach the parasite. Such drugs are toxic and complicated to administer. Four drugs are registered for the treatment of sleeping sickness and are provided free of charge to endemic countries, (WHO, 2006c). First stage treatment of *T.b. gambiense* sleeping sickness is done with Pentamidine which is generally well tolerated by patients while Suramin is used for the treatment of the first stage of *T.b. rhodesiense* although it provokes allergic reactions and certain undesirable effects in the urinary tract.

Second stage treatment for both forms of HAT is done with Melarsoprol derived from arsenic although it has many undesirable side effects with the most dramatic being reactive encephalopathy (encephalopathic syndrome) which can be fatal in a range of 3% to 10%. An increase in resistance to Melarsoprol has been observed in several foci particularly in Central Africa. Eflornithine molecule registered in 1990 is less toxic and more recent than melarsoprol but it is only effective against *T.b.gambiense*,(WHO, 1998). A combination treatment of nifurtimox and eflornithine was introduced by the WHO in 2009 for *T.b. gambiense* disease. In the case management of SS, detection of trypanosomes in gland or lymph node aspirates or blood is always followed by lumbar puncture to determine whether there is involvement of the central nervous system (CNS) ,(WHO 2012).

2.6 Cost effective control to reduce burden of human African trypanosomiasis

2.6.1 Burden of tsetse and trypanosomiasis on livelihoods

The burden comprises wider economic effects, impacts on agriculture and effects on human health. African trypanosomiasis which comprises the animal and human disease has profoundly affected settlements and economic development in much of the African continent, especially south of the Sahara desert where it is transmitted mainly by tsetse flies. Overlaying

a map of the distribution of livestock is that of tsetse infestation highlighting the extent to which tsetse and trypanosomiasis impede livestock development, (Odiit *et al.*, 2004b). Unlike cattle, sheep and goats are kept in a very broad range of agro ecological zones where they contribute considerably to the rural economies as a source of meat, milk, manure and readily disposable income are also affected by African trypanosomiasis. Early reports suggested that trypanosomiasis was not an important disease in small ruminants however, more recent studies have clearly demonstrated that sheep and goats acquire natural infections and suffer economic loss, (Irungu *et al.*, 2002). Trypanosomes have also been shown to infect pigs, with reports of natural infections in different regions of Africa, (Masiga *et al.*, 2002).

Data on humans is scanty and therefore devising effective methods to maximize hospital attendance and reduce the number of unreported cases in the community is thus a priority, (Katunguka, 1996). The nature of human fly contact and the propensity of the disease to affect individuals in particular occupations affects households since it affects the main providers who go out to work. Sick individuals require a lot of care and those successfully diagnosed require to be accompanied during treatment and if the correct diagnosis is not made the patient needs to be cared for upto death. Payments for pretreatment drugs such as vitamins plus other costs such as transport, food provided during hospitalization and treatment. Other indirect costs include time lost to the diagnosed patients and those accompanying or caring for them (Maudlin *et al.*, 2004).

2.6.1.1 Wider economic effects

The introduction and continual spread of the disease can lead to devastating economic impacts. The economic burden of livestock trypanosomiasis has been reviewed and it has been noted that applying treatments to the animal reservoir specifically as a public health measure has been shown to be cost effective, with great added benefits when these integrated

interventions also contribute to improved animal health and productivity, (Kristjanson *et al.,* 1999 and Budd, 1999). Previously, a large scale cattle targeted intervention has been implemented in Uganda and Kenya to control the spread of *T.b. rhodesiense*, (Kabasa, 2007). There are many diseases around the world that are considered to be the "forgotten or neglected " because a majority of the population does not know that they still exist and that they are having an immense impact on the areas in which they are found. Most of these "forgotten diseases" can be found in Africa and perhaps one of the most menacing among them is human African trypanosomiasis, more commonly known as sleeping sickness. Worldwide, HAT was known to kill over 50,000 people every year with reports of epidemics wiping out entire communities, (Kabayo, 2002).

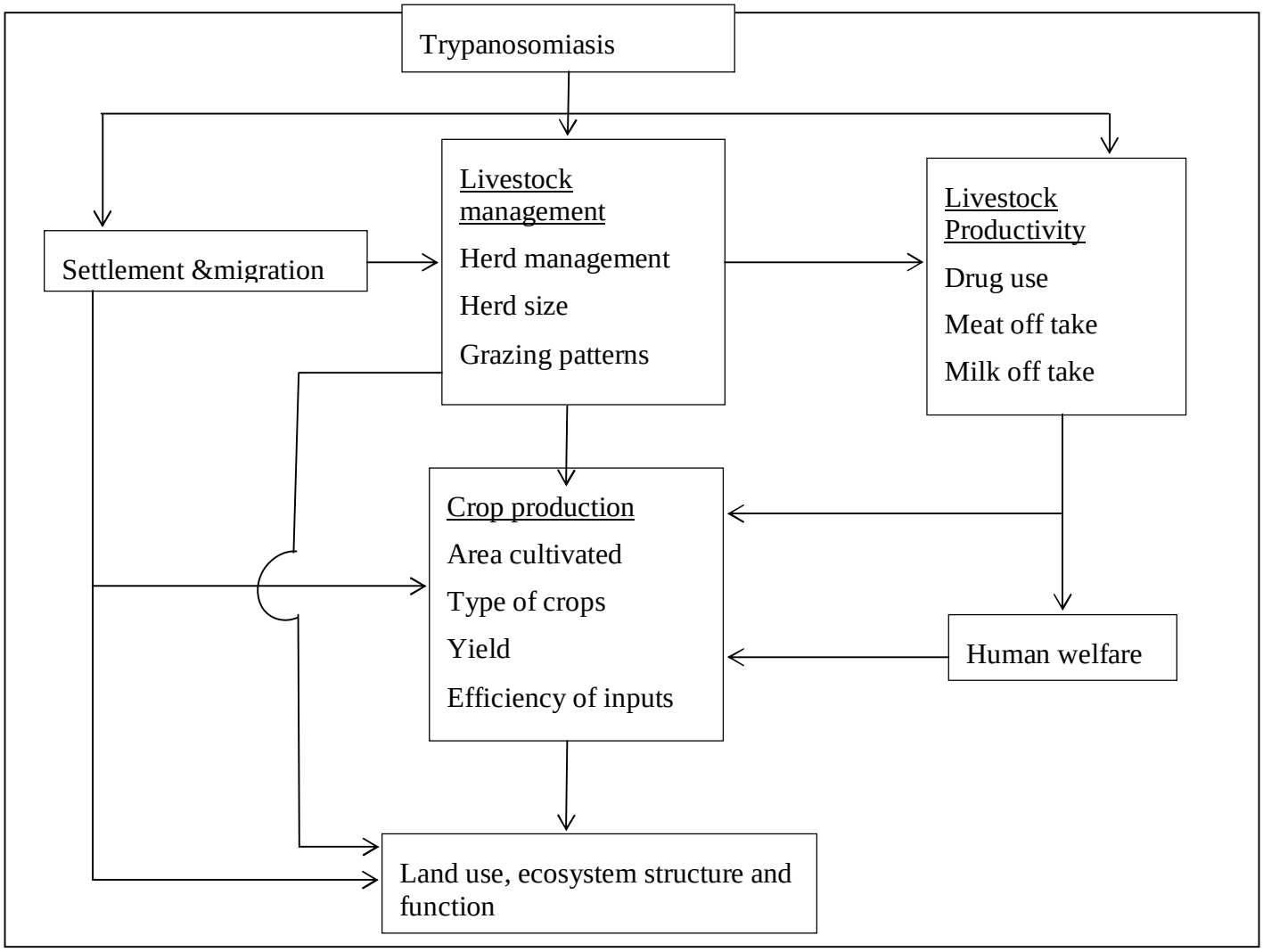
Sleeping sickness has had huge socioeconomic impacts on the areas afflicted with it and is acknowledged as "one of Africa's greatest constraints to socio-economic development", (Gubler, 1998). Kenya is one of the many countries suffering the effects of HAT where growth and development stagnates in the affected areas. The disease causes a huge loss in livestock, huge increases in capital spent on treatments and an increase in rural urban migration to escape infested areas. There are four key consequences of human actions that are impacting the spread of the disease which includes population growth, migration from rural areas, inadequate exploitation of resources such as land and lack of proper health policies, (Lutumba *et al.*, 2005). Annually, Nagana which is the cattle variety of the disease kills about 3 million cattle in Africa. Not only does this cause a drastic decrease in meat production, but it also means a decrease in the amount of milk and other animal products available for consumption by African citizens, (Pearce, 2002).

2.6.1.2 The Impacts of human African trypanosomiasis on Agriculture

It is hard for people to understand the magnitude of the problem since it is not widely publicized or in the media, but it is definitely a massive problem. An increase in the number of humans infected with sleeping sickness would result in a tremendous drop in the workforce of the area, also leading to further decline in the economy. An increase in the number of cows infected with Nagana would result in a decrease in animal labor also causing a decrease in productivity which would ultimately lead to further decline in the economy completing the loop, (Pearce, 2002). The loop is a never ending cycle that will continue to go on unless something is done to prevent further spread of human African trypanosomiasis and the impacts it has on the surrounding areas. Without serious intervention, the negative imparcts shown in this loop remain uncontrollable, (Kennedy, 2004; Kabayo, 2002). The decrease in animal products contributes to a protein shortage as well as risk of food security for African nations, (Gombe, 2003). As livestock become sick, people lose the labour that they perform for them. Oxen are used for ploughing and transportation of goods. However, when animals fall sick or die off, these jobs do not get fulfilled and once again, a person's well being worsens. Without these livestock, production decreases and poverty increases. When the economy continues to decline because of human African trypanosomiasis, the amount of money available for the creation of vector control programs also decreases resulting in fewer such programs.The impacts are illustrated in Figure 2.3 below. Since the tsetse fly infests livestock as well as humans and transmits disease that makes raising these livestock in infected areas nearly impossible, (Picard, 2000).

With a decline in the number of vector control programs, there is an increase in the number of tsetse flies. As the number of these tsetse flies increase, there is also an increase in the number of both humans and domestic animals that are infected with the disease, (Kabayo, 2002).

Figure 2.3 Impacts of human African trypanosomiasis on Agriculture



Source: Swallow, 1999.

2.6.1.3 Incidence of human African trypanosomiasis

Over 70,000 cases occurred annually (WHO, 2006b) including unreported cases with a previous estimate being 300,000 (WHO, 1998) with the disparities in these estimates illustrating the need for formal methods to quantify the substantial hidden burden of HAT. Current estimates are as stated in Table 2.1 below.

Table 2.1: The number of new cases of human African trypanosomiasis in Kenya due to *T.b. rhodesiense* reported to the WHO as at 2012

Year/Country	Kenya	Tanzania	Uganda	Total Cases
2001	10	277	426	713
2002	11	228	329	568
2003	0	113	338	451
2004	0	159	335	494
2005	0	186	473	659
2006	1	127	261	389
2007	0	126	119	245
2008	0	59	138	197
2009	1	14	129	144
2010	0	4	112	116
2011	0	1	82	83
Total	23	1294	2742	4059

Source: WHO, 2013

2.6.1.3.1 Use of Disability Adjusted Life Years (DALYs) in estimation of human African trypanosomiasis impact

Total DALYs for each cause, age, sex, and group are calculated as the sum of the non fatal burden, Years of Life with Disability (YLD) and the burden of premature mortality, Years of Life Lost (YLL). DALY = YLD + YLL. Although there is no thorough review of economic studies of HAT with DALYs and other measures, in themselves, they allow adverse health outcomes to be rated against each other. While DALYs is a practical method for appropriate use as measures of disease burden, they are an outcome in cost effectiveness analyses in terms of for example, dollars spent per DALYs averted useful in estimating the economic burden of HAT, (Fox and Hanson, 2001; Goodman *et al.*, 1999). DALYs studies are few and far between for *T.b.rhodesiense* and have tended to focus on *T.b. gambiense*. At lower

prevalence, active screening may not be cost effective in the short term, emphasizing that control efforts for this disease must take a long term perspective, (WHO, 2006c).

HAT, without distinction between *T.b. gambiense* and T.b. rhodesiense, was considered in the first Global Burden of Disease assessments, (World Bank, 1993) and estimated to result in 1.78 million DALYs lost across Africa, using a standard West 26 life table, a disability weight per episode of 0.35, (Murray and Lopez, 1996a) and an annual incidence of 32,000 reported cases, including 24,000 deaths. Life tables describe age-specific mortality in a population and determine, for example, the number of years of life lost following death at a given age (Murray and Lopez, 1996b) .Subsequent iterations of the DALY provided revised estimates such as 1.34 million DALYs lost to HAT in 2000, (WHO, 2008) and 1.54 million DALYs lost in 2002 (WHO, 2003), using an average disability-weight of 0.191 and 48,511 deaths (Mathers *et al.*, 2007).

2.6.1.3.2 Limitations in the use of Disability Adjusted Life Years

Other issues arise with regional and local studies when conducting evaluations of disease burden within a region such as relative burden of Malaria and HAT in East Africa, it is appropriate to use life tables that relate to the population under study, (Hyder and Morrow, 2006). For HAT, which exists only in sub Saharan Africa, this is less of a problem and for regional studies, using regional life tables may be more appropriate as doing so does not over estimate burden in these communities, (Paalman *et al.*, 1998). A significant limitation of incidence figures, and subsequent estimates of burden based on these, published through the World Health Organization and originating from national bodies such as ministries of health is that they relate only to what the hospital referral system picks. HAT cases are best diagnosed when they are identified in community screening exercises through active detection or passively, (WHO, 2012).

2.6.2 Effective tsetse and trypanosomiasis control methods

Control of HAT is based on the elimination of the disease in the domestic animal reservoirs as opposed to treating human patients which is costly and can be fatal due to late diagnosis of the disease and toxicity of the medicines. The variety of cost effective methods includes:

2.6.2.1 Vector control

Aerial and ground spraying were historically the major forms for tsetse control until the 1980s when the methods have recently been brought into question because of undesirable environmental effects on one hand and lack of funds to manage such large scale programmes on the other hand , (Barrett and Okali, 2006). Development of odour baited traps and targets aimed at addressing environmental concerns and consequent reduction in spraying requiring support through community based management is currently in practice, (Barrett and Okali, 2006). Although the method of tsetse control is coupled with challenges of maintenance of the traps, theft, damage from people, fire and reduction of attractant in the impregnated targets from rain water, it has been found very effective in reduction of tsetse populations. The technical constraints to use of traps and targets are that they only offer a temporary reduction of tsetse fly populations whose numbers can increase again over time if the trap and target use is not maintained, (Barrett and Okali, 2006). Use of insecticide sprayed on cattle is a control option against tsetse flies and is referred to as 'live-bait' or 'moving targets'. Insecticides which are mainly synthetic pyrethroids are available as a dip formulation or can be used as 'pour-ons'. The synthetic pyrethoids increasingly available at the farmer level currently are efficacious in tsetse control depending on community uptake as a public good .

The private good benefits in terms of tick and nuisance fly control are the more significant factors motivating farmers in their use. They may not be appropriate for tsetse control in all areas as their efficacy appears to depend on the relative spatial distribution of cattle and tsetse infestation and the proportion of tsetse blood meals taken from cattle, (McDermott and

Coleman, 2001). Widespread uptake of these technologies by smallholder farmers may be explained in that they represent "private good" interventions, with benefits accruing directly to the individuals who use them rather than to the community as a whole, (Irungu *et al.*, 2002). Concerns have been expressed that extensive use of synthetic pyrethroids on cattle may compromise control of ticks and tick borne diseases, either through accelerated development of tick populations resistant to this class of compounds or through the loss of enzootic stability to tick borne diseases, (Nga'yo *et al.*,2005).

2.6.2.2 Trypanotolerant cattle

Increasing interest has been paid in recent years to the trypanotolerance trait in a number of cattle breeds, particularly the taurine breeds of West Africa such as the N'dama. Although the trypanotolerant trait is not absolute, crossbreeding indigenous cattle with these breeds improves resistance to disease, (Franco *et al.*, 2014). Under conditions of light tsetse natural challenge, whereas 75% of Zebu cattle died, 98% of N'dama cattle survived. The utilisation of trypanotolerant livestock is often supplemented by the use of trypanocidal drugs in areas of heavy tsetse challenge, (Bett, 2008).

2.6.2.3 Use of trypanocidals in domestic animals

Trypanocidal drugs are clearly regarded as a priority by smallholder farmers. It is estimated that farmers spend $30 million per annum on trypanocidal drugs in Africa. In Kenya, there is now an expanding informal market in trypanocidal drugs, with many small scale pharmacies selling products directly to farmers, (Machila, 2005). Farmers have been shown to have varying levels of understanding of the aetiology of trypanosomiasis and other disease and farmers also lack knowledge on appropriate drug use. Despite wide availability, trypanocidal drugs are either not used or used improperly, e.g. to treat conditions for which they are ineffective, (Machila, 2005).

Misuse or overuse of drugs is uneconomic, environmentally unsound and may lead to drug resistance and toxicity. Over half of the cattle raised under trypanosomiasis risk are not given treatments of trypanocidal drugs. On the other hand, where treatments are given, this may be primarily on the basis of farmers' ability to pay, the breeds of cattle involved, and whether or not transhumance is practised, rather than on the magnitude of the disease risk. Studies in Uganda indicated that trypanocidal drug treatments are not given appropriately and the treatment rate may not reflect the prevalence of diseases and treatments that may be given unnecessarily, (Machila, 2005). The choice between use of therapeutic and prophylactic drugs may be made on the basis of cost per dose, without a clear understanding by farmers of the advantages of prophylactic drugs used in appropriate circumstances. Ethnoveterinary medicine is largely restricted to ASAL regions where nomadic pastoralists reside and to a lesser extent in some settled but marginalised rural communities, (Machila, 2005).

Products used to treat animals include Quinapyramine, Isometamidium, Homidium and Diminazene. Cross resistance between homidium, pyrithidium and isometamidium is prominent, these drugs belong to the same chemical family the phenanthridinium group. Treatment will be more effective in a well fed and rested animal, in which the immune system is not adversely affected by stress and lack of food. The efficiency of the vital detoxifying processes of animals that are ill may be reduced and a drug may be much more toxic for an animal with damaged liver or kidneys than for a healthy animal. The toxicity of drugs differs in different species of animals. For example, the margin of toxicity to quinapyramine in dogs is quite low while in pigs it is many times higher. Horses are known to be the most sensitive of the domestic animals to drugs which irritate at the site of inoculation, and one must always be careful of the subcutaneous route in horses because of this sensitivity. Diminazene aceturate has given fatal reactions in camels, horses, donkeys and dogs at doses which are considered to be normal and harmless in cattle, (FAO, 2012; Maudlin *et al.,* 2004).

2.6.2.4 Policy issues associated with animal health interventions in trypanosomiasis control

Animal health services are critical in the control of trypanosomiasis. Livestock diseases including trypanosomiasis control is integrated within the delivery systems of Veterinary Services in Kenya and across much of sub Saharan Africa. Veterinary Services were traditionally provided by the public sector either free of charge or at highly subsidised levels, (Holden, 1999). Kenya government carried out reforms in the 80's where Structural Adjustment Programmes (SAPs) led to withdrawal by government from services, whose activities are amenable to private sector provision, e.g. clinical services that included treatments and the supply of veterinary pharmaceuticals and some extension activities. This therefore predicted whether or not it would be profitable and sustainable for the private sector to invest in these activities guiding the extent of government withdrawal which directly affected disease control. Livestock remains an important reservoir for human infective *T.b. rhodesiense* disease and its treatment to free them from trypanosomes is important in HAT control, (Holden, 1999; Perry *et al.,* 2002). The major change recommended was to privatise some of the services and products or goods provided by the public sector which was based on the economic principles of excludability and rivalry or subtractability, (Wanga *et al.,* 2013).

In this view, livestock diseases are categorized into epidemics such as Foot and Mouth disease (FMD), Contagious Bovine Pleuropneumonia (CBPP) and endemic ones to include Trypanosomiasis, Helminthiases and East Coast Fever (ECF) while zoonotic ones include Tuberculosis and Brucellosis among others, (Rushton, 2009). To determine the extent of privatization of whichever veterinary or agricultural extension activities as classified in Table 2.2 below were to be privatized, the services and/or goods were viewed in form of either a public or private good.

Table 2.2: Economic Characteristics of public and private animal health services that determine who meets their cost of delivery

		Excludability	
		Low	**High**
Rivalry (or subtractability)	**Low**	**Public goods** Public funding - Epidemic or zoonotic disease control (including surveillance, movement control, quarantine services) - Control of food borne diseases - Drug quality control - Mass media information - Time insensitive production information, management information of wide applicability - Some research	**Toll goods** Private finance - Vaccine production - Diagnostic services - Veterinary clinics - Dips - Time sensitive production information, management information of wide applicability
	High	**Common pool goods** Public funding - Tsetse control on communal land using traps, targets or aerial spraying - Information embodied in locally available resources or inputs - Information on organisational development	**Private goods** Private finance - Enzootic disease prevention and control - Sales of drugs and vaccines - Some extension - Some research - Information embodied in commercially available resources or inputs - Client specific information or advice

Sources: adapted from Holden, 1999; Rushton 2009

The rationale for responsibility of disease control has been that epidemic diseases remain largely in the public domain as they are considered to be diseases important for trade. Therefore they are of greater economic importance if concerned governments wish to participate in wider international livestock markets. Zoonotic diseases control requires to remain a government priority because of public health implications, apart from the welfare of the livestock affected, (Wanga *et al.,* 2013). Endemic diseases are increasingly regarded as production or management diseases and thus, their control is considered to be a private good and should therefore be taken care of by the affected livestock owners with reason to believe that livestock owners are willing to pay for these services (Politi *et al.,* 1995; Rushton, 2009). Trypanosomiasis can benefit public investments since it is also a zoonotic disease. Pure agricultural information intended to improve existing production and management practices is usually provided to farmers through traditional extension approaches and is therefore considered to be a toll good as its supply to a target group does
not reduce its availability to other farmers, (Rushton, 2009).

2.7 Modelling as a method of quantifying human African trypanosomiasis disease burden

The first attempt to estimate under detection for *T. b. rhodesiense* SS was based on a framework of developing a decision tree model taking into account the progression of the disease and using SS case records. Public health professionals and decision makers have been expected to use this model to define which regions in the country need prioritization and funding for SS control activities, (Fevre *et al.,* 2008a).

2.7.1 Modeling sleeping sickness during outbreaks

To formally quantify the level of under detection of HAT sleeping sickness (SS) during an epidemic in Uganda, a decision tree (under detection) model was developed concurrently, to

quantify the subset of undetected cases that sought health care but were not diagnosed, a deterministic (subset) model was developed, (Odiit *et al.*, 2005). The values of the under detection model parameters were estimated from previously published records of the duration of symptoms prior to presentation and the ratio of early to late stage cases in 760 SS patients presenting at LIRI hospital, Tororo, Uganda during the 1988–1990 epidemic of SS. For the observed early to late stage ratio of 0.47, it was estimated that the proportion of under detection in the catchment area of LIRI hospital was 0.39 (95% CI 0.37–0.41) thus 39% of cases were not reported. Based on this value, it was calculated that for every one reported death of SS, 12.0 (95% CI 11.0–13.0) deaths went undetected in the LIRI hospital catchment area translating to 92% of HAT related deaths not being reported, (Odiit *et al.,* 2005). The deterministic (subset) model structured on the possible routes of a SS infection to either diagnosis with effective management or death through the health system or out of it was developed. The model showed that out of a total of 73 undetected deaths, 62 (CI 60–64) (85%) entered the healthcare system but were not diagnosed, and 11 (CI 11–12) died without seeking health care from a recognized health unit. The measure of early to late stage presentation provides a tractable measure to determine the level of rhodesiense SS under detection and to gauge the effects of interventions aimed at increasing treatment coverage, (Odiit *et al.,* 2005).

2.7.2 Generalized application of vector borne diseases' models

A general mathematical model of a vector borne disease involving two vertebrate host species and one insect vector species was easily extended to other situations involving more than two hosts and one vector species. The model, which was developed from the single host model for malaria described by Aron & May (1982), has been applied to the African trypanosomiasis. It allows for incubation and immune periods in the two host species and for variable efficiency of transmission of different trypanosome species from the vertebrates to the vectors and vice

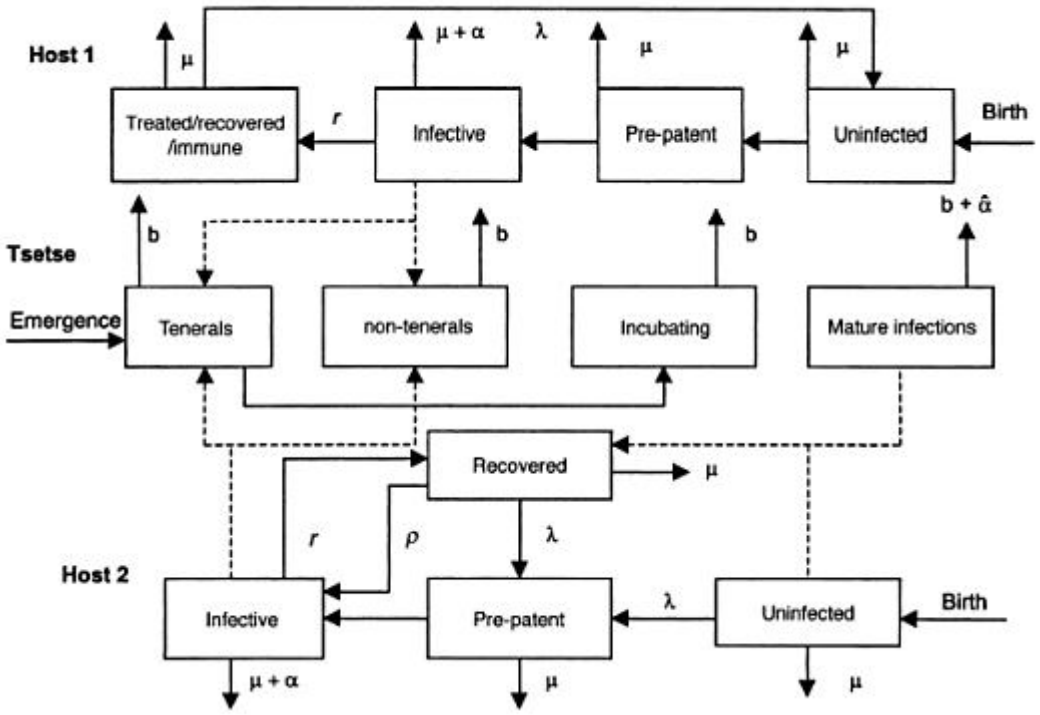
versa to be determined. Equations are derived for equilibrium disease prevalence in each of the species involved with model predictions examined by 3-dimensional phase plane analysis, which is presented as a simple extension of the 2-dimensional phase plane analysis of the malaria model, (Rodgers, 1988).

2.7.3 Trypanosomiasis transmission moodels

The involvement of several species of trypanosomes, tsetse and vertebrate hosts in trypanosomiasis has discouraged the development of mathematical models of the sort that have been available for other vector borne diseases, (Rodgers, 1988). Compartmental models using variables based on Susceptible, Exposed, Latent, Recovered individuals involving two vertebrate, one vector and multiple trypanosome species have been developed, (Rodgers, 1988; Milligan and Baker, 1988; Milligan, 1990; Artzrouni and Gouteux, 2001). Models vary by the vertebrate hosts used. Rodgers (1988) used pigs and humans; Milligan & Baker (1988) used cattle and wild hosts and evaluated the impact of using trypanocides on disease transmission, while Artzrouni and Gouteux (2001) used humans versus other pool of hosts. Milligan (1990) outlined a compartmental model for tsetse transmitted trypanosomiasis of cattle presented in Figure 2.4.

Parameter values appropriate for the African trypanosomiasis were derived from the literature where a typical West African village situation was considered with 300 humans, 50 livestock and an average population of 5000 tsetse flies. The model predicted equilibrium prevalence of *Trypanosoma vivax*, *T. congolense* and *T. brucei* of 47-0, 45-8 and 28-7% respectively in the animal hosts, 24-2, 3-4 and 0-15% in the tsetse vectors and a 7-0% infection of humans with human infective *T brucei* ,(Rodgers, 1988).

Figure: 2.4 Compartmental model of animal trypanosomiasis



Source: Milligan 1990

Description of the parameters used to build the mathematical model shown in Figure 2.5

μ- baseline mortality rate of vertebrate host per day
r- recovery rate per day
α -parasite induced mortality of vertebrate host per day
i - rate of progression to infectiousness per day
λ -force of infection per day
γ- rate of loss of immunity/resistance per day
b- baseline mortality rate of tsetse per day
ά -parasite induced mortality of tsetse
β- tsetse biting rate per day
ρ- recrudescence to infectious state

Solid arrows denote movement of individuals while broken ones represent transmission of trypanosomes between the two hosts via tsetse. Host 1 could be taken to be cattle and host 2 game. In game, recrudescence of infection is allowed at rate, ρ.

The force of infection in a vertebrate host is derived based on:

(i) Tsetse: host ratio, m,

(ii) Rate of tsetse feeding on host i, a_i

(iii) Infection prevalence in tsetse, y,

(iv) Probability that host i gets infected from an infective bite from tsetse, ω_i,

(Rodgers, 1988).

This equates to:

$$\lambda_i = a_i . m_i y_i . \omega_i$$

The force of infection in the vector, λ, depends on the rate at which tsetse feed on the available hosts and the prevalence of the disease in human or domestic animal hosts. Considering two host types, this parameter is estimated by:

$$\lambda_i = c(a_i . x_i a_i . x_2)$$

Where: c – probability that a fly gets infected from an infected blood meal,

a_1 - rate of feeding on host 1

x_1 - prevalence of the disease in host 1

a_2 - rate of feeding on host 2

x_2 - prevalence of the disease in host 2.

2.7.4 Linking animal hosts for human African trypanosomiasis parasite with prevalence in humans through modeling

There is a possibility of predicting the prevalence of HAT in humans given the prevalence of HAT parasites in animals with a certain vector population, given a transmission rate based on the frequency of bites. The effect of changing average fly density on equilibrium disease prevalence can be examined, together with the effect of seasonal changes in fly numbers on disease incidence. Seasonal changes in fly mortality rates affect both future population size

and infection rate. Peak disease incidence lags behind peak fly numbers and that the less favoured host lags behind that in the more favoured host, (Rodgers, 1988).

Near the threshold fly density for disease transmission disease incidence is even greater than at higher fly densities and may even exceed equilibrium prevalence at the same average fly density because most hosts are susceptible at the time that fly numbers begin their annual increase. Identifying the precise role of the animal reservoir may suggest that treating such livestock will achieve a greater reduction of human sleeping sickness than direct treatment of the humans alone. Statistically significant results of control campaigns may also be more easily shown by monitoring the non human reservoirs. Modeling provides a means by which a correct perspective view can be obtained of the complex epidemiology and epizootiology of the African trypanosomiasis, (Rodgers, 1988). The contribution to the basic rate of reproduction of the human infective *T. brucei* was estimated at only 0-11 from the human hosts and 2-54 from the animal hosts, indicating that in the situation modeled human sleeping sickness cannot be maintained in the human hosts alone. The animal reservoir is therefore crucial in determining not only the continued occurrence of the disease in humans, but its prevalence in the animal hosts as well, (Rodgers, 1988).

2.8 Estimation of underreporting

Reporting completeness refers to the proportion of cases attending healthcare whose health event was correctly diagnosed and appropriately reported. Under ascertainment can be split into two distinct levels as represented by the surveillance pyramid as under ascertainment (UA) of infections occurring at the community level and underreporting (UR) of infections occurring at the healthcare level. Underreported (UR) infections are those in individuals that do seek healthcare, but whose health event is not captured by the surveillance system and

therefore not notified through the notification system, (Hardnett *et al.,* 2004; MacDougall *et al.,* 2008).

UR can be estimated as the number of infected individuals attending healthcare services whose health event is not reported to the appropriate public health body for every attending a case whose health event is reported. UR can be due to under diagnosis which accounts for the cases attending healthcare but whose infection or pathogen is not diagnosed or misdiagnosed, (Hardnett *et al.,* 2004 ; MacDougall *et al.,* 2008). Under ascertainment (UA) can be estimated as the number of infections occurring in individuals that either attend or do not attend healthcare services against every case that attends and is identified. There is a symptomatic fraction of all under ascertained cases that do not attend healthcare due to mild symptoms with the knowledge that the illness is self limiting or for some other reason, and an asymptomatic fraction that do not seek healthcare as they are not aware of their infection status due to lack of symptoms, (ECDC,2010). One systematic way to decide on the best method for estimating UE or choosing Multiplication Factors (MFs) is to use the Delphi method or expert consensus. The most appropriate MFs should be disease, country, age and sex specific because underestimation rates are disproportionately distributed between diseases, countries with differing surveillance systems and reporting procedures and between demographic groups, (Cheryl *et al.,* 2014). Figure 2.6 below divided into sections a and b is a morbidity surveillance pyramid and a decision tree that can be used to estimate under ascertainment of disease.

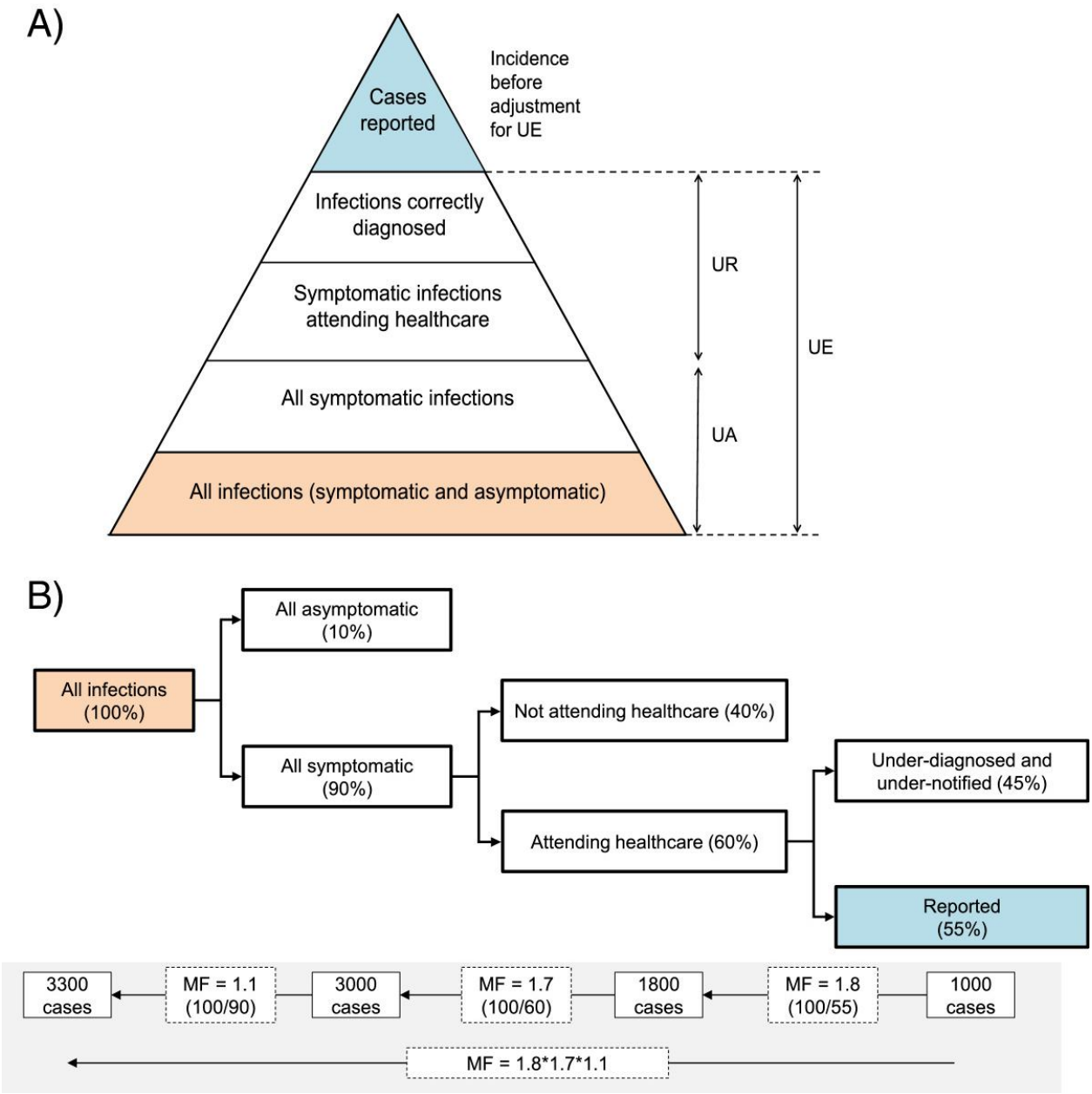
Section A) The morbidity surveillance pyramid is often used to illustrate the availability of morbidity data at each surveillance level. With each ascending level from the community, to healthcare institutions (general practitioners, hospital, laboratory), to regional and national

public health agencies data availability shrinks and only a fraction of cases from the level below is captured . In contrast to the narrow tip of the pyramid which represents data held by national public health agencies, the base is wide as it holds all infections in the community. The difference between the number at the tip and base can be considered cases lost to 'underestimation' (UE), Cheryl *et al.,* (2014).

Section B) The proportions of infections that are symptomatic, that attend healthcare, and that are reported are represented in this decision tree model. Here, only 55% of all infected individuals attending healthcare are reported through the notification system. If 1000 cases were reported then a Multiplication Factor (MF) of 1.8 (=100/55) could be derived and would correct for those underreported cases. The true number attending healthcare would be 1800 cases. Likewise, if only 60% of symptomatic cases attended healthcare, then a MF of 1.7 (=100/60) would correct for under ascertainment of symptomatic cases. The true number of cases attending healthcare would be 3000 symptomatic cases (=1.7*1800), (Cheryl *et al.,* 2014).

All infections' shaded in orange in Figure 2.6A represents the same population as the orange box in Figure 2.6B. 'Cases reported' in blue in Figure 2.6A represents the same population as the blue box in Figure 2.6B.

Figure 2.5: Deriving multiplication factors from the morbidity surveillance pyramid



Source: Cheryl *et al.*, 2014

Finally, since 90% of infections were symptomatic, a MF of 1.1 (=100/90) would correct for under-ascertainment of asymptomatic cases. The true number of infections would be 3300 (=1.1*3000). A MF to correct for total underestimation of symptomatic cases in one step would be 3.06 (=1.8*1.7) and for all infections 3.4 (=1.8*1.7*1.1)

2.9 Global and continental African trypanosomiasis control efforts

The Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) Initiative was established by a Decision AHG/156 (XXXVI) of African Heads of State and Government during the 36th Ordinary Summit of the African Union (AU), Lome, Togo, in July 2000. The African Union Commission was given the mandate to coordinate the activities leading to the implementation of the PATTEC Initiative and the AU assigned the coordination role to the AU-PATTEC Coordination Office with support from FAO and the African Development Bank (ADB). The objective of the programme was to create tsetse and trypanosomiasis free areas in all affected countries in Africa and ensuring that reclaimed areas are sustainably, equitably and economically exploited. The objective of the programme was to be achieved through the application of principles of the area wide approach, employing effective and environmentally safe tsetse suppression methods that are appropriately integrated with other medical and veterinary inputs to achieve elimination of both the vector and the diseases its transmits.

African Union Inter African Bureau for Animal Resources (AU IBAR) is the secretariat of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). The ISCTRC was established in the 60s as a vehicle of cooperation and implementation across national, regional and continental barriers. ISCTRC is a statutory council of the African Union acting as a platform for knowledge sharing and information dissemination on trypanosomiasis research and control. The ISCTRC organizes biennial scientific conference to evaluate progress sustained since 1949, (AU, 2014).

DFID's Animal Health Programme and the Livestock Production Programme have been working together in East Africa where the disease in livestock, known as Nagana is confined to southeast Uganda and western Kenya. But the risk of the disease is spreading due to

climate change effects is real. The DFID research programmes identified a more cost effective method for treating cattle to restrict the spread of the disease. This is done by restrictive application of an insecticide to the legs of cattle, rather than treating the whole animal since flies feed mostly on the legs. DFID provided £6.5 million to the Drugs for Neglected Diseases Initiative (DNDi) over 3 years. This extremely important initiative was not for profit drug development organization that focuses on some of the most neglected diseases in the World such as Sleeping Sickness, Chagas disease and Leishamaniasis. DNDi aims to improve the health and quality of life of people suffering from neglected diseases through research into better drugs. DNDi is evaluating the usefulness of different drug combinations to treat sleeping sickness and is exploring the possibility of identifying new drug candidates with possible relevance to sleeping sickness, (DFID, 2006).

CHAPTER THREE

3.0: KNOWLEGDE, ATTITUDE AND PRACTICE OF MEDICAL PRACTITIONERS IN DIAGNOSIS AND MANAGEMENT OF ZOONOTIC AFRICAN TRYPANOSOMIASIS

The chapter focusses on a cross sectional survey of medical practitioners in which the background is provided, methodology, findings, discussion and conclusion.

3.1 Introduction

Many factors have been reported as contributing to under diagnosis and under reporting of zoonotic diseases particularly in the sub Sahara African region. These include poor disease surveillance coverage, poor diagnostic capacity, the geographical distribution of those most affected, skills and attitudes of medical practitioners and lack of clear strategies to address the plight of zoonotic diseases. The current study investigates how the knowledge and practice of medical practitioners on human African trypanosomiasis is a potential contributing factor to under diagnosis of the disease and consequent underreporting. There's inadequate information on the prevalence of animal and human trypanosomiasis in Busia in official records at the County and National levels.

This has necessitated the utilization of information from previous surveys and published scientific findings. Diagnosis and treatment of patients in Kenya is the responsibility of a range of health personnel including specialists and consultants, medical officers, clinical officers (medical assistants), nurses and other paramedics. Translation of knowledge into the practice of proper management of patients is among the critical areas in health care delivery. This is only possible if health service providers have the right knowledge and attitude of addressing the health problems they are dealing with. Considering that formal training is an

integral part influencing thecapacity of medical practitioners in diagnosing diseases, assessing the knowledge of practitioners could be an important step in addressing challenges associated with underreporting.

3.2 Methodology

3.2.1 The Study area

The study area is shown in Figure 4.1 conducted in 21 health facilities of Busia County comprising of 3 per sub County and the Kakamega General Referral Hospital. Inclusion of Kakamega was justified based on it being previously the only Provincial General Hospital that received most referral patients. The health facilities were selected from the entire Busia County covering a total land area of 1694 square kilometers and a water mass of 215 square kilometers. In 2009 during the national census the Busia population was 743,946 comprising of 356,122 males and 387,824 females. The total estimated population as at 2012 was 809,988 consisting of 387,693 males (42%) and 422,295 females (58%), (CIDP, 2014; KNBS, 2013). The rural population that is more exposed to HAT was 677,235 against the urban population of 77,426.

About 70.7% of Busia residents lived 5km and beyond a health facility,19% lived between 1.1 to 4.9km while 10.3% lived less than 1km from a health facility. The doctor to population ratio was 1:41,200 while the nurse to population ratio was 1:13 and the HIV prevalence was 7.4%. The morbidity rate was 46.5%. The life expectancy for the Busia County inhabitants was 51 against the national figure of 61 years for female while for males it was 54 years against the national figure of 58years. The Crude Birth rate for the County was 46/1000 against the national rate of 38.4/1000 while the Crude Death rate was 12.6/1000 against the national rate of 10.4/1000. The absolute poverty level for Busia residents was 64.2 %

contributing 2.83% of the national poverty. Agriculture employed over 580,278 people and wage employment caters for 163,668, (CIDP, 2014).

3.2.2 Study design

A descriptive cross sectional survey was conducted between June 2010 and December 2011 among medical practitioners in the facilities identified through the use of semi structured questionnaire as shown in appendix I and focused group discussions following the format shown in appendix II. It focused on the sub county hospitals and health centres, referral health facility of Kakamega who often received patients referred from Busia, major private hospitals and some dispensaries within Busia County in Kenya which is endemic for HAT. On ethics, research authorization was obtained from the University of Nairobi, National Council of Science and Technology, Department of Veterinary Services, Ministry of Health and authorities of the individual health facilities. Informed consent was sought from the medical practitioners identified before being interviewed.

3.2.3 Study population

The research population consisted of medical practitioners working in all existing medical facilities within the County based on administrative units of the seven sub Counties. These comprised of 82 public health facilities, 14 private health facilities and one former Provincial Referral Hospital (CIDP, 2014). The medical practitioners consisted of Consultants or Specialists, Medical Officers, Clinical Officers, Nurses (those involved in disease diagnosis) and Laboratory Technologists latter only from the Level 4 and 5 Hospitals, (CIDP, 2014). Specialists are trained beyond a bachelors or first degree, medical officers have training to a degree level while assistant medical officers and clinical officers are trained to diploma level lasting at least 3 years. For the purpose of this study, all these categories of staff have been referred to as medical practitioners.

3.2.3.1 Target population

The target population comprised of all medical practitioners in the three purposively selected health centres or hospitals from each of the seven sub Counties as shown in Table 3.1 .All the medical practitioners who volunteered to take part in the study were interviewed. Medical practitioners from Kakamega referral Hospital were also voluntarily interviewed.

Table 3.1 Health facilities sampled

Sub County	Health Facility	Classification
Matayos	Busia County Hospital	Level five referral
	Tanaka	Private nursing home
	Matayos	Health centre
Teso North ,	Kocholia	Level four referral
	Moding'	Health centre
	Angu'rai	Health centre
Teso South	Alupe Hospital	Level four referral
	Amukura	Health centre
	Likolis	Health centre
Nambale	Nambale	Level 4 referral
	Malanga	Health centre
	Khayo	Health centre
Butula	Khunyangu	Level four referral
	Butula Mission	Private hospital
	Bumala B	Hospital
Funyula	Nangina Mission	Private hospital
	Sio Port	Level four referral
	Nambuku	Health centre
Budalangi	Port Victoria	Level four referral
	Mukhobola	Health centre
	Sirimba	Dispensary

3.2.3.2 Accessible population

The accessible population consisted of all the medical practitioners who were on duty during the diverse dates when the interviews were conducted and questionnaires administered in the selected health facilities and were willing to be part of the study.

3.2.3 Sampling

3.2.3.1 Sampling frame

The sampling units consisted of all medical practitioners in the health facilities comprising of Kakamega Referral Hospital and 21 health facilities from Busia as shown in Table 3.1 out of the possible 83 shown in Table 3.2.

Table 3.2: Sampling frame for the health facilities

Health Facility	Number
Provincial referral	1
Level five referral	1
Level four referral	7
NGO/Mission hospitals	2
Health centres	12
Dispensaries	46
Private clinics/nursing homes	14
Total	83

Further all eligible medical practitioners based on the inclusion criteria for the research population based on Kothari (2004), as provided in Table 3.3 were interviewed.

Table 3.3: Sampling Frame for the Medical Practitioners

Medical Practitioners	Busia County	Kakamega Referral Hospital
Specialists	6	5
Medical Officers	11	10
Clinical Officers	53	Not interviewed
Laboratory Technicians	5	Not interviewed
Nursing Officers diagnosing patient conditions	66	Not interviewed
Total	141	15

3.2.3.2 Sampling procedure

A multi stage sampling process was conducted involving the seven sub Counties where each was identified as a stratum from which clusters of three health facilities were picked. The health facilities were purposively identified based on whether it was level 5 and 4 hospital or the biggest health center within the sub County. The health facility served as a cluster from which all the medical practitioners in the identified health facility were recruited in a census sampling format. Key informant discussions and interviews were held with the Provincial Medical Officer of Health, Medical Superintendents and the Directors of private health facilities.

3.2.3.3 Sample size

Sample size was calculated based on the formula

$n=Z^2\ p\ q\ N\ /\ e^2\ (N-1)\ +Z^2\ p\ q$, (Kothari, 2004)

Where:

n is the desired sample size

N: size of population which is the number of households

p: population reliability (or frequency estimated for a sample of size n), where p is 0.5 is used

and $p + q= 1$

e: margin of error considered as acceptable at 5% for this study.

$Z\ \alpha\ /2$: normal reduced variable at 0.05 level of significance z is 1.96

Therefore

$n=1.96^2$ x 0.5x 0.5 x 156 $/(0.05)^2(156-1) + 1.96^2$ x0.5x0.5 = 149.8224/1.3479 = 111.15≈112

In this study a sample of one hundred and twenty one (121) out of (141) medical practitioners were interviewed having increased the sample size to improve representativeness from the acceptable minimum number of one hundred and twelve.

3.2.4 Data collection

Field pre testing of the questionnaire was conducted in June 2010 at Siaya Level Five Hospital in Siaya County which was not included in the study.Data collection was done by interviews and through a semi structured questionnaire that was developed to assess knowledge of the causes, clinical features and diagnosis of HAT. Primary information in the questionnaires comprised of the sections on the socio demographic profile to provide the background of the respondent to aid in analysis. Confidentiality of any personal information obtained during the entire study was maintained. The focus of the questionnaire was on medical practitioners' knowledge considered important for identification and diagnosis of HAT as one of the neglected zoonoses.

On clinical features questions were asked about classical and pathognomonic features of human African trypanosomiasis in humans and finally data was collected on knowledge of diagnostic protocols for HAT with differentials providing additional information. The questions in most of the sections were structured or close ended, in order to save time and cost while a few open ended questions were used to probe for extra detail. I visited the identified medical practitioners individually and hand delivered the self administered questionnaires on appointment and collected them after a mutually agreed period on the same day. Focus Group Discussions comprising five participants convened by the hospital administrators were conducted as a follow up to the questionnaire administration to validate the findings between the researcher and the various heads of departments. All the medical practitioners present on the first day of the visit and who agreed to participate with the study

were recruited. Time to fill in the questionnaire was allocated at thirty minutes based on the average time recorded during pre testing. This was done to minimize sharing of knowledge and referring to text books that could provide misleading findings from the study.

3.2.5 Data analysis

Qualitative and quantitative data obtained from the field was cleaned, coded, stored and analysed. Various numbers were assigned to the several responses while a specific number was assigned to all missing data. Open questions were scanned in advance and codes for likely responses assigned. Coding was done to facilitate quantitative analysis techniques using the computer program. The Statistical Package for the Social Sciences (SPSS) was used for data analysis. Double entry followed by comparison of the two files to detect any inconsistencies was done and a spreadsheet was used. Frequency data were analyzed using likelihood ratio and the chi square test in Minitab version 14. Percentages, means, standard deviations, tables, pie charts, bar charts, graphs of frequencies and proportions were used to report and present findings. Ranking of opinions was done.

All the responses were assessed in relation to the information provided by zoonoses text books, (Martin and Hugh 1995) and were assigned as: "True" if the response was the same or very similar to the documented, "False" if it was not. Medical practitioners were classified into levels of training as specialists, medical officers, clinical officers, laboratory technicians and nursing officers involved in disease diagnosis. For descriptive statistics, the general characteristics of the study sample were expressed using frequencies, graphs and tables that were used quantify the scores and to present results.

On Inferential Analysis odd's ratios and chi Square tests were used. Those variables that showed statistically significant differences between them at 95% confidence were subjected

to logistic regression to determine the exact differences within each variable with the outcome variable being the extent of knowledge or practice on the subject under investigation. The diagnostic odds ratio ranges from zero to infinity, although for useful tests it is greater than one and higher diagnostic odds ratios are indicative of better performance. Diagnostic odds ratios less than one indicate that the test can be improved by simply inverting the outcome of the test if it is in the wrong direction. While a diagnostic odds ratio of exactly one means that the test is equally likely to predict a positive outcome whatever the true condition the test gives no information.

3.3 Results

Medical practitioners paid limited attention and focus on HAT when carrying out routine diagnostic procedures on patients visiting health facilities while encountering patients with similar symptoms.

3.3.1 Demographics

One hundred and twenty one (121) medical practitioners responded to the questionnaire comprising of 72% males and 28% females. The median age was 28 years with Inter Quartile Range of 27-35 with 62% of them below 30 years of age, 19% between 30-39 years, 14% aged 40-49 years while 5% were 50 or more years.

Table 3.4 Numbers of medical practitioners sampled

Medical Practitioner	Numbers Sampled
Specialists	10 (8%)
Medical Officers	17 (14%)
Clinical Officers	39 (32%)
Nurses	50 (42%)
Laboratory Technicians	4 (4%)
Total	121

The average number of respondents' years of experience was 6.2 years with standard deviation (s.d) being 4.4 years while the average number of years the respondents had spent at their current station was 2.7 years with sd 1.8 years. At the time of the study, 16% of the respondents had less than 1 year experience, 45% had between 1-5 years' experience, 13% had between 5-10 years' experience and 26% had 10 and above years' experience. Prior to the interview, 28% of the respondents had spent less than 1 year at their current station, 60% had between 1-5 years, 3% had between 5-10 years and 9% had stayed at their current duty station for 10 or more years.

3.3.2 Ranking of zoonoses

Zoonoses were ranked by the respondents on their perceived relative importance as demonstrated in Table 3.5 below.

Table 3.5: Shows how the respondents rated the importance in occurrence of 5 Zoonotic Diseases in their current areas of work:

Zoonotic Disease	Very Important	Important	Average	Unimportant	Very unimportant
Amoebiasis	-	3%	3%	-	6%
Anthrax	16%	12%	8%	11%	-
Ascariasis	-	-	4%	9%	-
Bird Flu	-	3%	-	-	-
Brucellosis	36%	8%	17%	-	5%
Ebola	5%	-	-	-	-
Elephantiasis	-	3%	3%	-	-
Hydatidosis/Echinococcus Granulosis	5%	10%	10%	35%	18%
Rabies	23%	26%	17%	-	-
Schistosomiasis	5%	3%	12%	-	-
Trypanosomiasis	5%	12%	6%	11%	56%
Typhoid	3%	12%	15%	29%	5%
Toxoplasmosis	2%	-	4%	-	6%
Yellow Fever	-	-	-	3%	-
Not Sure	-	8%	1%	2%	4%

Brucellosis at 36%, Rabies at 23% and Anthrax 16% were perceived as the highest rated zoonotic diseases as very important by respondents while 5% of the respondents rated Ebola, Trypanosomiasis, and Hydatidosis as very important while only 2% vouched for Toxoplasmosis. Rabies was perceived as the highest rated zoonotic disease as important in occurrence by 26% of the respondents, while Anthrax, Trypanosomiasis and Typhoid were rated as important by 12% each, while Hydatidosis (*Echinococcus Granulosus)* was rated as important by 10% of the respondents. For those infections that were rated as average by occurrence, 17% of the respondents cited Rabies, Brucellosis and Typhoid, while 12% cited Schistosomiasis and 10% cited Hydatidosis, while Anthrax and Trypanosomiasis were rated as average by 8% and 6% of the respondents respectively. For infections that were perceived

and rated as unimportant by occurrence at the respondent workstations, Hydatidosis was cited by 35%, Typhoid by 29%, Trypanosomiasis and Anthrax by 11% and Ascariasis 9%. For infections that were perceived as very unimportant by the respondents, Trypanosomiasis was cited by the largest proportion at 56%, Hydatidosis by 18%, typhoid and Brucellosis by 5% each and toxoplasmosis by 6%.

3.3.3 Opinion of medical practitioners on prioritization of common diseases

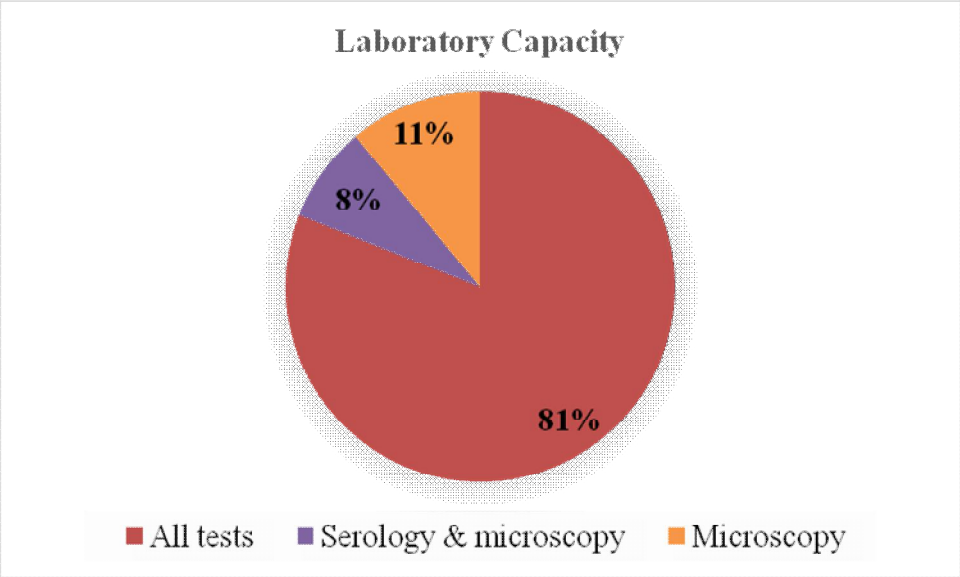
The participants were able to identify the following diseases as important namely Malaria, HIV/AIDS, Water and Food borne infections at their current work stations with the following specific responses. Majority respondents at 96% reported that Malaria was rated as very important while the remainder (4%) felt that Malaria was ranked as important. Most (89%) of the study participants reported that HIV/AIDS was very important, 9% said HIV/AIDS was ranked as important while 2% ranked it as being of average importance. For water and food borne diseases, 27% of respondents reported that they ranked it as very important, 55% as important while 18% rated it as of average importance. Despite trypanosomiasis being endemic in Busia from published data, it was not mentioned by any respondent as being a common disease, (Wissmann, 2011).

3.3.4 Utilization of laboratory services

When asked about how often they used laboratory tests to confirm clinical diagnosis, 62% of the respondents reported they used it always, 36% used it on some occasions while 2% rarely used the laboratory. The respondents were asked to rank in their opinion what would improve diagnosis amongst proper laboratory facilities, increased number of medical personnel and continuous professional development. Upto 64% respondents ranked all the combined factors as equally important, 32% cited proper laboratory services, 3% cited continuous professional

development while 1% cited increasing medical personnel. In Figure 3.1 below, 81% of the respondents reported that the laboratories at their current work stations had the capacity to carry out both serology and microscopy tests, 8% stated their labs could carry out all tests while 11% stated that their labs could only carry out microscopy.

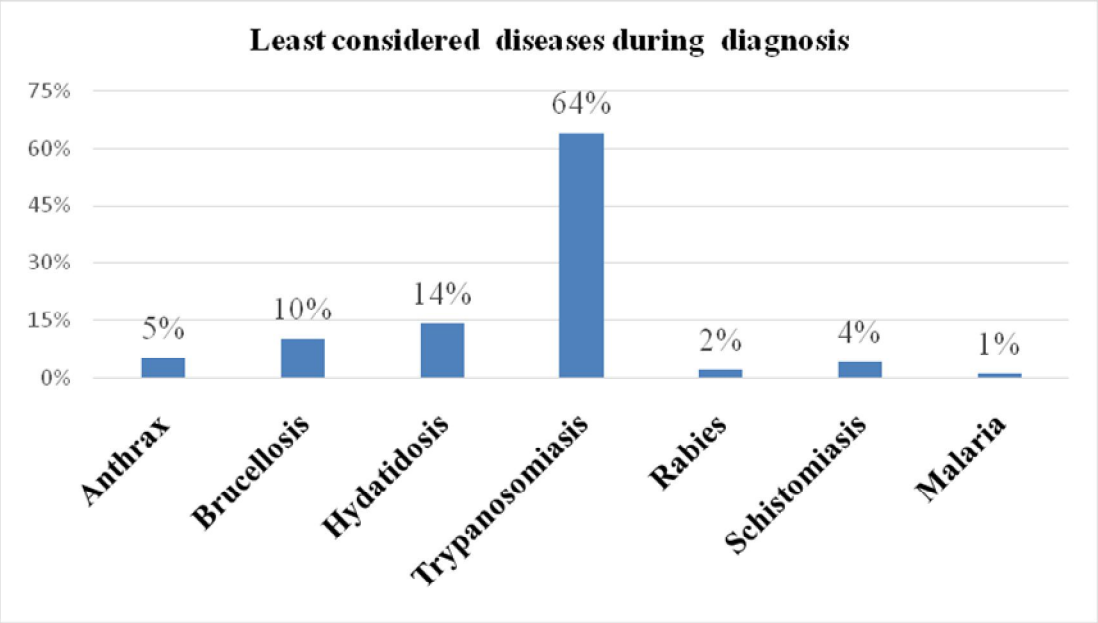
Figure 3.1: Capacity of laboratory services



3.3.5 Capacity by medical practitioners to carry out HAT diagnosis

Figure 3.2 below shows the reported level of neglect by medical practitioners during diagnosis when they encounter diseases presenting with similar symptoms to HAT.

Figure 3.2: Diseases considered as differential diagnosis in routine diagnostic work of cases presenting with common signs such as fever



The respondents were asked about the stage at which trypanosomiasis was diagnosed, 50% reported that it was diagnosed at the laboratory, 33% reported that it was hardly ever diagnosed while 7% and 10% said it was diagnosed at the 1st and 2nd consultation respectively. The respondents were also asked to list the clinical diagnostic features for human sleeping sickness where 57% listed excessive sleep or somnolence, 28% cited fever, headache and confusion was cited by 6% of the respondents each while 9% cited drowsiness. The findings reveal that trypanosomiasis at 64% is the least thought about disease when medical practitioners are routinely diagnosing diseases with related common symptoms such as fever which may contribute to its neglect during identification by clinicians while hydatidosis, was reported the second most neglected disease according to 14% of the respondents while 10% and 5% of the respondents recognized Brucellosis and Anthrax as neglected respectively.

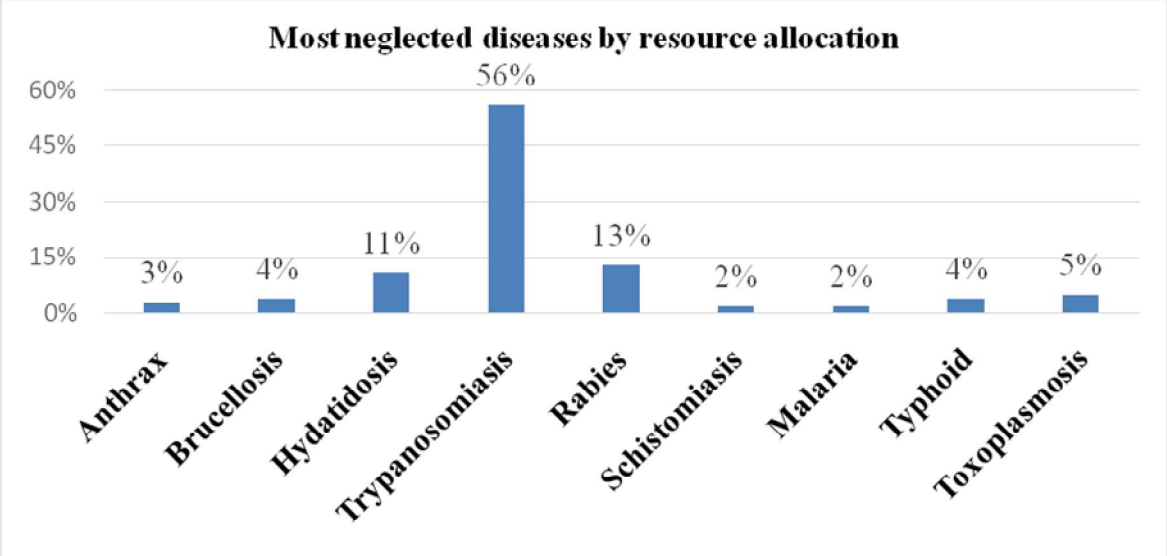
Majority of the respondents at over 91% correctly identified blood as the sample taken for diagnosis of HAT and there were no significant differences between the respondents' age, sex, specialty and years of experience and the knowledge on the type of sample for HAT diagnosis. However, statistically significant differences at 95% confidence level in opinion were observed between laboratory technicians and specialists on one hand and medical , clinical officers and nurses on the other hand regarding the ideal point of HAT diagnosis (χ^2= 14.12, d.f=1, p=0.008 where χ^2 represents chi square, d.f, degrees of freedom and p probability). Laboratory staff and specialists were 3 times likely to be the respondents who correctly identified the laboratory as the point for HAT diagnosis compared to medical and clinical officers and nurses [odds ratio (OR) 3.1, 95% confidence interval (CI) 2.76-8.11, *p=0.038*] . Consequently, while no significant differences were noted by age and sex regarding what would improve diagnosis, of respondents who said proper laboratory services, laboratory personnel and consultants were 1.7 more likely to consider this option as compared to medical and clinical officers and nurses (OR 1.7, 95% CI 0.99-5.23, p<0.001).

Conversely, for those who felt increasing medical personnel would improve diagnosis, medical , clinical officers and nurses are 11% more likely to opt for this as compared to laboratory staff and consultants (OR 0.11, 95% CI 0.04-0.51, p <0.001). Among respondents who cited HAT as the most neglected diseases by medical practitioners during diagnosis, clinical officers were significantly higher (48%) compared to other specialties (*p=<0.001).* Only 12% respondents reported having managed sleeping sickness before while 87% had not previously managed sleeping sickness and 1% was not sure. 55% of those who had managed sleeping sickness before had diagnosed it at the laboratory, 38% had diagnosed it using clinical features while 7% had diagnosed it using a combination of both clinical features and a laboratory confirmatory test.

3.3.6 Perception of respondents on the most neglected disease through resource allocation

Results in Figure 3.3 below shows that 56% of the respondents reported that trypanosomiasis was the most neglected disease by the government in terms of resource allocation followed by Rabies at 13%. Hydatidosis was ranked as the third most neglected diseases in terms of resource allocation by 11% of the respondents. This inadequate resource allocation contributes further to under detection a position that was corroborated by the line budgeting of the Ministry of Health for Busia during the study period from the annual budget estimates, (GOK, 2010). Further, those who cited HAT as the most neglected disease by the government in terms of resource allocation were more likely to be medical officers and consultants (*p=0.012)*.

Figure 3.3: Zoonotic diseases control most affected through inadequate resource allocation by government



3.3.7 Most commonly diagnosed diseases

Malaria was ranked as the most commonly diagnosed disease by 96% of the respondents while 4% ranked HIV/AIDS as the most commonly diagnosed disease. HIV/AIDS was ranked as the second most diagnosed disease by 52%, typhoid was ranked second by 45% while TB was ranked second by 3% of the respondents. 23% and 19% of the respondents ranked HIV/AIDS and tuberculosis as the third most diagnosed diseases at their workstations. The second least and least diagnosed disease was sleeping sickness as cited by 26% and 23% of the respondents respectively. Most of the respondents at 77% were not sure of the least commonly diagnosed disease at their work station. The respondents were further asked to rank the diseases listed in Table 3.6 below from the most diagnosed to the least diagnosed the results of which were found to corroborate with the medical records accesed in the course of hospital visits during the study.

Table 3.6: Ranking of routinely diagnosed diseases

Disease	Most diagnosed	Second	Third	Fourth	Fifth	Least diagnosed
Malaria	96%	-	-	-	-	-
Cholera	-	-	5%	13%	-	-
Typhoid	-	45%	14%	-	-	-
HIV/AIDS	4%	52%	23%	-	-	-
TB	-	3%	19%	21%	-	-
Sleeping sickness	-	-	-	-	26%	23%
Not Sure	-	-	39%	66%	74%	77%

As a follow up to ranking of the most and least diagnosed diseases, the respondents were asked to tell the stage at which the diseases they had identified were diagnosed. Table 3.7 below shows a summary.

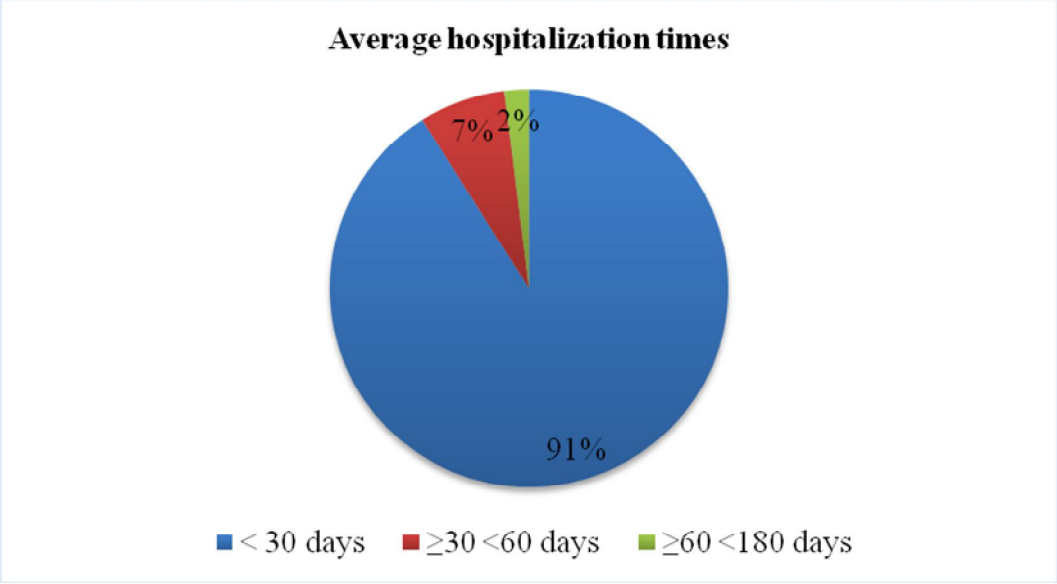
Table 3.7: Stage of diagnosis of the disease

Disease	First Visit	Second Visit	Laboratory	Postmortem
Malaria	72%	-	28%	-
Cholera	61%	5%	34%	-
Typhoid	42%	7%	51%	-
HIV/AIDS	29%	5%	66%	-
Tuberculosis	21%	23%	56%	-
Sleeping sickness	5%	15%	71%	9%

Malaria was reported by 72% and 28% of the study participants to be diagnosed at the first visit and laboratory respectively, 61% reported that Cholera was diagnosed at the 1st visit while 5% and 34% reported that Cholera diagnosis took place at the 2nd visit and laboratory respectively. Only 5% of the respondents reported that sleeping sickness was detected at the 1st visit, 15% and 71% reported diagnosis at the 2nd visit and during laboratory diagnosis while 9% reported that sleeping sickness diagnosis occurred during post mortem.

Based on the opinion of the respondents, the period patients spent admitted is as shown in Figure 3.4 below.

Figure 3.4: Average hospitalization periods



At least 91% of the respondents reported that the longest average hospitalization periods at their current station were less than 30 days as depicted in Figure 3 above. Only 7% of the patients were hospitalized for between 1-2 months while 2% were hospitalized for between 2 to 6 months. HIV/AIDS accounted for 81% of the prolonged hospitalization times, Tuberculosis (Tb) accounted for 9%, complicated meningitis 3% and protein malnutrition accounted for 7%. For those admitted with HIV/AIDS, Tb accounted for 92% of the complicating infections, 4% organ failure, 3% herpes and 1% meningitis with no report of SS as a secondary infection. Mixed zoonotic infections were reported to have been encountered by 32% of the respondents with 72% of the respondents who had encountered mixed zoonotic infections reporting that their biggest challenge was diagnosis, 24% cited getting appropriate drugs while 4% indicated that getting the source of infection was their biggest challenge.

3.3.8 Knowledge of mixed zoonoses including human African trypanosomiasis

A significant statistical difference was noted between years of experience and having encountered mixed zoonotic diseases (χ^2= 14.21, d.f=3, p=0.002). Logistic regression analysis showed that individuals with 10 or more years of experience had a 1.58 likelihood of having encountered mixed zoonotic diseases over individuals who had less between 5-10 years' experience (OR 1.58, 95% CI 1.09-1.65, *p=0.009*) . Those with over 10 years experience were 2.5 times more likely to have encountered mixed zoonotic diseases compared to those respondents who had less than 1 year experience (OR 2.5, 95% CI 1.61-8.43, *p=0.018*). Similarly, significant differences were noted between age and previous encounter with mixed zoonotic diseases (χ^2= 9.22, d.f=2, p=0.04). Respondents who were ≥ 40 years were 2 times likely to have diagnosed mixed zoonotic diseases compared to respondents below the age of 30 years (OR 2.2, 95% CI 1.15-6.41, *p=0.009*). However, previous encounter with zoonotic diseases was not associated with sex and specialty of the respondents.

3.4 Discussion

A research gap was identified by the WHO on the need for quantifying the dual burden of infections of African trypanosomiasis to livestock and humans, (WHO, 2006c). This was important in order to provide added benefits to humanity when control is implemented by generating data in this under researched area that requires novel metrics and systematic data gathering across the range of required data. The study objective on establishing the level of knowledge attitude and practice of medical practitioners in identifying and managing HAT addressed part of this need. This study documented the contribution of medical practitioners to underreporting in their routine practice. With the average number of respondents' years of experience being over 6 years, the findings obtained could easily be generalized over a wider population of medical practitioners.

In an era of evidence based medicine, decision makers need high quality data to support decisions about whether or not to use a diagnostic approach in a specific clinical situation and, if so, which approach. Many quantitative indicators of test performance have been introduced, comprising sensitivity and specificity, predictive values, chance corrected measures of agreement, likelihood ratios, area under the receiver operating characteristic curve and many more. All are quantitative indicators of the test's ability to discriminate patients with the target condition (usually the disease of interest) from those without it, resulting from a comparison of the test's results with those from the reference standard in a series of representative patients. In most applications, the reference standard is the best available method to decide on the presence or absence of the target condition. In this study the odds ratio and likelihood ratios were used as a single indicators of compairing the performance of some of the measures for medical practitioners on their extent of knowledge, attitudes and practice. As such it also can be applied to express the strength of the association

between the independent and dependent variables assessed from medical practitioners. The likelihood ratios can come in handy for comparing the diagnostic capabilities.

Lack of unanimity by the respondents on the stage at which HAT is diagnosed depicted inadequate knowledge of the disease features. Diagnosis and hence reporting of diseases depend largely on the level of understanding of the disease. Therefore lack of unanimity on clinical features of the disease contributes to poor disease identification. Estimating the true occurrence of SS under such circumstances is, therefore, difficult. To date, crude estimates based on historical records of SS surveillance and case finding rates have been used. The finding that the respondents paid limited attention and focus on HAT when carrying out routine diagnostic procedures on patients visiting health facilities led to low prioritization of sleeping sickness by medical practitioners. The revelation that HAT is the least considered disease when medical practitioners are routinely diagnosing diseases with related common symptoms such as fever may contribute to its neglect during identification by clinicians. For instance, if a practitioner is not well informed of how the diseases manifests or does not know how to investigate for its presence, there is a higher chance of misdiagnosis as one would not know which disease to investigate and how. A physician attending to an ill veterinarian or a zookeeper will immediately suspect a wide array of diseases other than zoonoses while a pediatrician attending to a sick child who recently received a puppy will not suspect an animal transmitted disease.

The results of the study indicate that it is possible some zoonoses are missed by those entrusted with the duty of identifying them since focus is on endemic and seemingly more important diseases such as Malaria, HIV AIDS and foodborne infections that were reported as more important during ranking. The low ranking of HAT in opinion as an important zoonotic disease by medical practitioners contributes to its underreporting. Most medical practitioners

were of the opinion that the most important disease in their stations was Malaria probably due to its high prevalence and morbidity therefore receiving greater attention.

Despite HAT being endemic in Busia from published data, (Wissmann, 2011) it was not mentioned by any respondent as being a common disease. Many reasons could explain these findings. The practice by medical practitioners concentrate on some endemic and prioritized diseases or diseases that have been common in their areas and ignoring zoonoses that are of great public health importance points to a possibility that teaching curricula in medical training institutions does not put due emphasis on zoonoses. This information is corroborated by the few and sometimes indistinct hours allocated to zoonoses in the respective curriculum. The location of practitioners in the distant underprivileged rural areas could also explain the indifference in the level of knowledge of HAT. Refresher programmes, seminars and workshops are more convenient and cheaper means to capacity build staff who are in employment whether in urban or rural areas, (Kunda *et al.*, 2008).

Considering that majority medical practitioners used laboratory tests to confirm clinical diagnosis and reported it as the most reliable method of improving diagnosis of HAT, it is critical that studies be conducted to establish the basis of unreliability in utilizing this service. The unanimity that a blood sample is the most appropriate diagnostic sample of choice for HAT depicted good knowledge however the reasons for the absence of laboratory requests routinely needs to be determined. Since most of the respondents reported that the laboratories at their current work stations had the capacity to carry out serology and microscopy tests essential for HAT, the inability to utilize laboratory diagnostic services requires an explanation. Inadequate laboratory inputs and poor attitude towards submitting samples to the laboratory are important attributable factors to underreporting since HAT is mainly diagnosed

during laboratory testing as reported in this study. In Kenya, like in other sub Sahara African countries, there is a poor diagnostic capacity for many diseases including HAT in the laboratories however this is more pronounced in the rural areas where the majority population resides. Despite HAT having no pathognomonic pathological lesion to aid in post mortem, 9% respondents cited post mortem as the stage at which HAT is identified implying inadequate knowledge in this aspect.

Laboratory technicians and specialists better identified the stage of accurate HAT diagnosis since the former handle the laboratory diagnostic requests while the latter have obtained more specialized training according them better diagnostic capacity as compared to medical and clinical officers and nurses. More clinical officers cited HAT as the most neglected diseases by medical practitioners during diagnosis than any other specialties since they are comparatively the ones most commonly involved in diagnosis at the level four hospitals and health centers. The perception of majority of the respondents reporting that HAT was the most neglected disease by the government in terms of resource allocation was telling on its level of prioritization. This inadequate resource allocation contributes further to under detection a position that was corroborated by the line budgeting of the Ministry of Health for Busia during the study period from the annual budget estimates, (GOK, 2010). Majority medical officers more than any other cadres cited HAT as the most neglected disease by the government in terms of resource allocation considering that they have better access to administrative information since they commonly serve as the hospital administrators.

During a similar study in the Lake Victoria belt, it was reported that there was a lack of reliable statistics on under detection of the HAT disease in sub Saharan Africa because of, amongst other reasons, infrastructural and costs constraints resulting into disease estimates

being guesses based on a clinical diagnosis and not parasitological or other diagnostic tests, (Snow *et al.,* 1999). WHO reported that the major burden of SS is documented through morbidity and mortality statistics in hospitals a measure which is obtainable only if a patient presents to hospital, the disease is accurately diagnosed and the cause of death is documented which is hardly the case, (WHO, 2006c) . With fewer people seeking treatment and most deaths occurring outside the hospital set up there is need to device other means of accurately estimating the prevalence and subsequent burden of HAT.

Knowledge of reservoirs for zoonoses and the way they are transmitted to humans has enabled not only their diagnosis and reporting but also their control, (Fevre *et al.,* 2008b). For instance, knowledge of animal reservoir and transmission modes has enabled the identification and control of zoonoses outbreaks in the world such as Rift Valley fever in Kenya and Somalia, Nipah virus in Malaysia and Singapore , (WHO,1998). It is therefore important to optimize the diagnosis of HAT and other diseases especially neglected zoonoses that have significant socio economic impact on human life. Field data such as the one generated in this study can enable accurate estimation of the disease prevalence that would lead to a greater proportion of HAT cases being detected in good time and therefore preventing almost all of the deaths. According to WHO, between 2001 and 2011 Kenya reported only 23 incidences of HAT translating into two cases each each year while its neighbours who share open, porous borders with unrestricted movement of people and livestock reported 1294 cases in Tanzania and 2762 cases in Uganda over the same period, (Simarro *et al.,* 2013).

The more years' of experience a medical practitioner had, the more likely he had a chance of encountering mixed zoonotic diseases since it is assumed he has gained essential experience

in differentiating diseases including HAT. Experience is therefore a critical factor when deploying personnel in the various health outfits. The study has revealed that knowledge and a practice of medical practitioners are important contributing factors to under reporting of diseases such as zoonoses concurring with the findings of a similar study among rural and urban medical practitioners in Tanzania, (Kunda *et al.*, 2008). With more than half of the respondents in this study reporting HAT as the most neglected disease during diagnosis and less than 20 percent having ever diagnosed or encountered patients with the disease before this study and yet it is an endemic disease in Busia is a worrying trend. Both domestic and wild animals have been shown to be important reservoirs of zoonoses, (Kilonzo and Komba, 1993) evidenced in the study findings in chapter four which underline the importance of cattle as a reservoir for HAT. In Africa, Bovine Tuberculosis, Brucellosis, Anthrax, Rabies, African Trypanosomiasis, Plague and Echinococcosis have been documented as being among the most common zoonoses, hence need to for localized studies that help in generating data that can support allocation of resources to control these diseases (Barret and Okali, 2006).

Resource allocation is very important in management of any disease and yet over half of the respondents reported that Trypanosomiasis was the most neglected disease by the government in terms resource allocation before the Food and Agriculture Organization (FAO) and the African Union (AU) supported PATTEC project was rolled out in the region, (AU,2014). This inadequate resource allocation has contributed greatly to the level of under detection. In Uganda a scenario that may be applicable in Busia, it was reported that despite the reported increase in the number of individuals infected with *Trypanosoma brucei rhodesiense* species in the 1990s, WHO estimated that the figures represented only 10–15% of the actual number of infected individuals, (Cattand *et al.*, 2001).

HAT morbidity data relied on nationally and globally is based on hospital records which are a poor reflection of the true status considering the study findings where medical practitioners confirm that the disease is not prioritized during routine diagnostic work. During the focused group discussions it was reported that some patients on the other hand have been seeking alternative services such as those offered by traditional healers and hence delay to present to health facilities or fail to present at all making data on their disease status not available for epidemiological records which was similar to the findings of a similar study in Eastern Uganda that neighbours the study area, (Odiit *et al.,* 2004a).

Further, the study reported that poor referral systems, limited surveillance coverage, difficulty and delay in diagnosis by the health facilities have been contributing to the underreporting of zoonoses. The study findings that individuals as well as societies have been slow to act on zoonotic African trypanosomiasis due to inadequate technical capacity is in agreement with previous study findings in Western Kenya that attributed the same to insufficient systematic continuing education and opportunities to acquire new knowledge on zoonoses for those working in health institutions, (Hardy, 2003; Asano *et al.,* 2003). A previous study in Eastern Uganda during a HAT outbreak reported that approximately 85% of the patients who died undiagnosed entered the health system at some stage and that one third of those that did enter the health system died undiagnosed, (Fevre *et al.,* 2008a). This report concurs with this study where it was reported that only 15% of the respondents had previously encountered HAT patients despite having a working experience of at least 6years. The small number of respondents reported having managed sleeping sickness before is an indictment that majority may easily miss the disease due to inadequate exposure and experience.

All these underscore the fact that medical professionals do not give due consideration to animals as carriers of diseases that can be transmitted to humans resulting in poor quality of epidemiological data on zoonoses and their control measures on animal and human populations particularly in sub Saharan Africa, (Perry *et al.,* 2002). Diseases that adversely impact the health of humans, animals and the environment can only be solved through structured communication, cooperation and collaboration across disciplines and institutions.

3.5 Conclusion

Knowledge , attitude and practice of medical practitioners is an important ingredient in diagnosis and effective management of HAT and other zoonotic diseases which are important factors that can contribute under diagnosis and under reporting in Kenya. The study reported an acceptable level of knowledge by medical practitioners in identifying and managing HAT however their attitudes on the relative importance of the disease negatively affected their capacity to effectively manage the disease. With low prioritization of HAT, medical practitioners contributed substantively to underreporting of the disease in their routine practice. Estimating the true occurrence of HAT under such circumstances has therefore been difficult. To date, crude estimates based on historical records of HAT surveillance and case finding rates have been used. HAT morbidity data relied on nationally and globally is based on hospital records which are a poor reflection of the true status considering the study findings where medical practitioners confirm that the disease is not prioritized during routine diagnostic work.

The results of the study indicate that HAT among other zoonoses is missed during diagnosis by medical practitioners since focus is on endemic and seemingly more important diseases such as Malaria, HIV AIDS and foodborne infections. The practice by medical practitioners to concentrate on some endemic and prioritized diseases or diseases that have been common

in their areas and ignoring zoonoses that are of great public health importance points to a possibility that teaching curricula in medical training institutions does not put adequate emphasis on zoonoses. There exist gaps in diagnostic capacity and knowledge of zoonoses such as HAT among medical practitioners and the general communities affected in HAT foci.

Inadequate laboratory inputs and poor attitude by medical practitioners towards submitting samples to the laboratory are important attributable factors to underreporting since HAT is mainly diagnosed during laboratory testing. Poor referral systems, limited surveillance coverage, difficulty and delay in diagnosis by the health facilities have been contributing to the underreporting of HAT. Majority of the medical practitioners reported having no experience in managing the disease. Medical professionals do not give due consideration to animals as carriers of diseases that can be transmitted to humans and also fail to consult their veterinary counterparts in zoonotic disease management.

HAT was reported neglected by the government in terms of resource allocation due to low level of prioritization leading to lack of reliable data on the prevalence of the disease. With fewer people seeking treatment and most deaths occurring outside the hospital set up there is need to device other means of accurately estimating the prevalence and subsequent burden of HAT. It is therefore important to optimize the diagnosis of HAT and other diseases especially neglected zoonoses that have significant socio economic impact on human life. Field data such as the one generated in this study can enable accurate estimation of the disease prevalence that would lead to a greater proportion of HAT cases being detected in good time and therefore preventing almost all of the deaths.

One Health Approach promotes the idea that healthy productive animals lead to healthy people through provision of safe food, economic gain and companionship. Globally,

management of zoonoses is converging towards the one health approach to management of diseases, a principle that Kenya can adopt through collaborative frameworks between veterinary and medical practitioners and other stakeholders that is necessary for the effective control of zoonoses such as HAT.

CHAPTER FOUR

4.0: AGREEMENT BETWEEN THE DIAGNOSTIC TESTS OF MICROSCOPY BASED PARASITOLOGICAL TECHNIQUES AND POLYMERASE CHAIN REACTION IN ESTIMATING THE PREVALENCE OF ZOONOTIC AFRICAN TRYPANOSOMIASIS PARASITES

Results on the prevalence of HAT parasites from microscopy based parasitological tests and the PCR were compared to help estimate the true prevalence of HAT causing parasites in the blood of domestic animals in Busia County.

4.1 Introduction

HAT reports from WHO indicate that there are none or close to zero cases of sleeping sickness in Kenya from 2010 to 2014 save for two reported cases from Masai Mara in 2012. This is a statistic that raises concerns based on information from neighboring countries such as Uganda that reports many cases as seen in Table 2.1. Busia County, which is the study area in Kenya, shares a porous border with endemic Eastern Uganda that reports majority of rhodesiense HAT or sleeping sickness cases. The study seeks to explain whether the disease is actually not there or the disease is under diagnosed and therefore not accurately reported. This is in view of the high level of incidence and prevalence in the neighboring countries especially across the border in Uganda.

The contribution to underreporting under investigation is that attributable to the type of diagnostic tool used. The study will compare the level of agreement of the prevalence results from the routinely used parasitological tests supported by microscopy and PCR done to help quantify the prevalence of HAT causing parasites in the blood of domestic animals. This information is important considering that domestic animals are a source of infection to

humans. The prevalence of the HAT parasites in domestic animals can be used to estimate the prevalence in human through modeling.

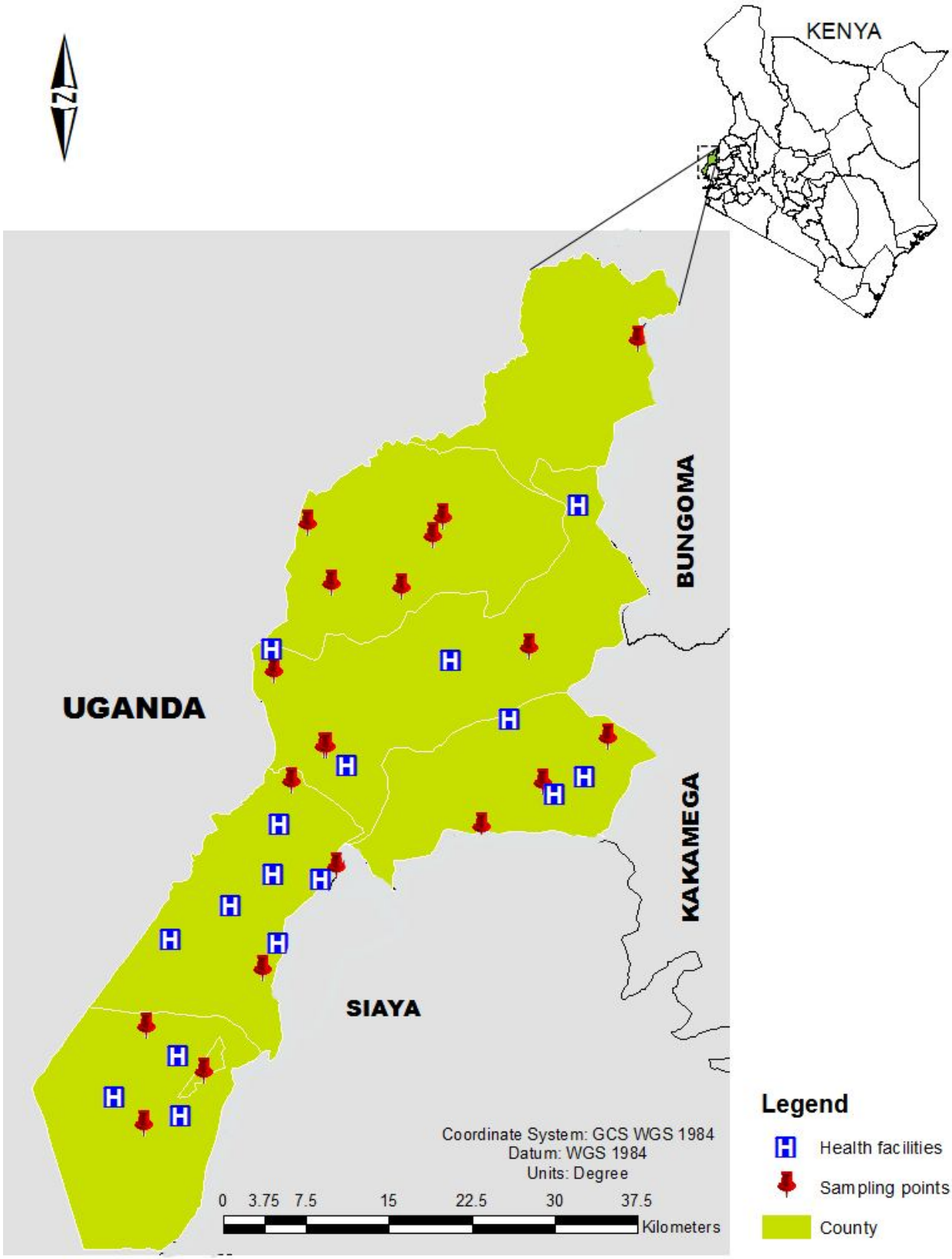
4.2 Methodology

4.2.1 Study area

The Study was conducted in Busia County containing the georeferenced sampling sites shown in Figure 4.1 below. Busia County is located between Latitude 0.4333° N and Longitude 34.1500° E bordering Kakamega County to the east, Bungoma County to the north, Lake Victoria and Siaya County to the south and Busia and Tororo Districts, Uganda (Uganda) to the west as shown in Figure 4.1 below .The Busia altitude is undulating and rises from about 1,130m above sea level at the shores of Lake Victoria to a maximum of about 1,500m in the Samia and North Teso Hills. The Central part of the County, especially Butula and Nambale Sub Counties, are occupied by a peneplains marked by low flat divides of approximately uniform height, often capped by lateritic and a shallowly incised swampy drainage system, (County Fact Sheet, 2015; CIDP, 2014).

The study was conducted in nineteen sites within the seven Sub Counties of Busia County namely Odiado, Majanja and Rumbiye in Funyula Sub County; Rugunga, Sibinga and Bududusi in Budalangi Sub County; Matayos and Mayenje in Matayos Sub County; Nambale and Namahindi in Nambale Sub County; Bukuyudi, Bukhalalire and Emauko in Butula Sub County; Chakol, Apatit, Obechun in Teso South Sub County and Obekai, Kolanya and Katelenyang’ in Teso North Sub County.

Figure 4.1: Map of Busia County showing the sampling sites and the health facilities



Source: Google Maps March 2015-Map concept

4.2.2 Socio economic characteristics

Busia County is in Western Kenya formerly Western Province. It had a total population of 743,946 People during the 2009 Census and 154,225 Households and now estimated at over 800,000 People covering an area of 1,695 Square Kilometers. The Population density is 439 per Square Kilometer and unfortunately 66% of the population lives below the poverty line. Busia County is endowed with natural resources namely arable land, livestock, water, pasture, forests, tourist attractions such as Kakapel National Monument, Lake Victoria Viewpoint with the main economic activities being trade, agriculture, tourism, fishing and commercial business ,(County Fact Sheet,2015). Trade with neighboring Uganda is a major economic activity within Busia town, the County headquarters and largest town being a cross border centre. Away from town, the County economy is heavily reliant on fishing and agriculture, with cassava, millet, sweet potatoes, beans and maize being the principal crops. Livestock rearing is mainly on small scale subsistence with an increasing presence of dairy animals. Though most residents of Busia County are ethnically Luhya, there is also a substantial population of Iteso in addition to other minority communities, (CIDP, 2014).

4.2.3 Study design

This was a cross sectional study with both descriptive and analytic characteristics carried out to establish the prevalence of *T.b.rhodesiense* in the blood of livestock. Reconnaissance surveys were conducted in the study areas in advance and a sampling frame made up of all cattle, sheep, goats and pigs constituting a herd in the study areas constructed. This was after considering information on the distribution of households, herd sizes, grazing patterns, tsetse density and trypanosomiasis prevalence from existing reports. During the reconnaissance study key stakeholders were sensitized on the pending survey and the various roles each was expected to play.

4.2.4 Sampling

A sampling frame was designed, sampling procedure set and sample size determined for the study.

4.2.4.1 Sampling frame

The sampling units comprised the entire livestock population as shown in Table 4.1 where a list of herds belonging to individual owners were identified consisting of cattle, pigs and shoats (sheep and goats) accessible at the 19 sampling sites which were targeted for collection of blood samples with the selected livestock being identified using a simple random sampling approach. The sampling considered each sub County as strata from which at least two sub locations were picked randomly. In each of the sub location picked, the local Veterinary Office identified collection sites where the biggest diversity of livestock routinely aggregate for herd health programs or procedures as a cluster for sampling .

Table 4.1: Livestock population in Busia

Livestock species	Population as at 2009
Cattle	163,795
Sheep and Goats	117,514
Donkeys	460
Pigs	48,788
Total	330,057

Source: KNBS, 2010

4.2.4.2 Sample size

The sample size was determined statistically bearing in mind the resources at hand to ensure that the required precision of the estimate , the variance in the outcome of interest, the desired level of confidence at 95% and that the estimate obtained from the sampling should be close

to the true population value. The sample size" n" was determined based on the formula below derived from Cochran (1977) and presented by James *et al.,* (2001).

Livestock Population=330,057

Susceptible population (N) ≈ 3301 based on baseline prevalence of HAT which was assumed to be 1 % based on a previous study conducted in 2009 in Busia and reported by Von Wissmann *et al.,* 2011.

$n= Z^2_{\alpha} pq/L^2$

Where

n = sample size

Z_{α} = the value of Z_{α} required for confidence = 1-α, the level of confidence (1- α) for the statistical test was set at 95% and $Z_{0.05}$=1.96

L = acceptable margin of error at 5%.

p = a priori estimate of the proportion at 0.5

q = 1-p

pq= estimate of variance

Priori minimum threshold was set at a 50 % reduction in herd level disease prevalence

Therefore

$n= 1.96^2$ x 0.5x0.5/ 0.05^2 = 384.16≈384 samples

Since n>5% of N

Correction formula was used to obtain the final minimum sample size n_1

$n_1= n / (1+n/N)$

n_1=384/ (1+384/3301) = 343.98≈344 samples

At least 400 or more samples were collected from each Sub County stratum totalling 3799 samples in the entire County.

4.2.4.3 Sampling procedure

Multistage sampling was done. Sampling was stratified by Sub Counties out of which between two and three sub locations were randomly selected within each sub county after being allocated a random number. Based on the livestock distribution I identified 3 sub locations each from Teso North, Teso South, Funyula, Budalangi and Butula Sub Counties and 2 sub locations each from Matayos and Nambale Sub Counties for sampling. The sampling sites in each selected sub location were where livestock from various farms congregate for procedures such as spraying or watering or vaccination.

The sites were randomly selected based on a frame supplied by the respective local veterinary office where at least 200 livestock were sampled representing at least 70% of the existing livestock population in the sampled sites. The distribution per species was prorated based on their respective populations.Within each site (cluster) the required samples were identified through simple random method using computer generated random numbers where identified animals were bled and information on the health status and ownership of the animal documented in the form shown in appendix III. Sick animals were treated. The study sample consisted of the livestock selected for participation in the study from which a blood sample could be obtained. Permission was sought from the owner of the selected animal before any samples were taken. The homes for visitation with zero grazed animals were also identified at random.

4.2.5 Data collection

Multi stage sampling was done after considering the sampling frame, sampling procedures and the determination of the sample size. This was done in four phases comprising of reconnaissance visit and three subsequent data collection field visits where the livestock blood samples collected were processed appropriately.

4.2.5.1 Phases of the study

In order to obtain a representative picture of the prevalence of HAT parasites in domestic animals, period prevalence of just over one year (15months) was considered appropriate conducted in three phases as opposed to point prevalence. Initial field data collection was conducted between January and March 2011 after the end of the short rains when the fly populations are their peak, the second field data collection conducted between September and November 2011 after the end of the long rains for similar reasons as in phase one and the third and final data collection conducted between January and March 2012. Tse tse fly population peak after rainy seasons. The four phases of the study beginning with the reconnaissance were:

(a) A 3 month reconnaissance period covering the baseline data phase that ran between August and December 2010 as seen in Table 4.2a was carried out. During this phase mapping of the study area, identification and engagement of stakeholders were done and each category was sensitized on the role they were expected to play when the research began.

Table 4.2 a :Scheduling of the reconnaissance visits and initial field visit phases

County	Time in Months																
	Aug 2010				Dec 2010	Jan 2011		Mar 2011						Sep 2011		N o v 2011	
	4	3	2	1	0	1	2	3	4	5	6	7	8	9	10	11	12
Busia	Baseline data phase					phase one								phase two			

(b) An initial sampling phase was carried out between January and March 2011 with the second phase being undertaken between September and November 2011 as shown in Table 4.2a. These phases involved active blood sample collection from the livestock and interviews with the animal owners to provide information about their animals.

(c) The third and last phase lasting three months was done between January and March 2012 as shown in Table 4.2b where the final livestock blood samples were taken and interviews conducted.

Table 4.2b: Scheduling of the final field visit phase

County	Time in Months											
	Jan 2012		Mar 2012									
	1	2	3	4	5	6	7	8	9	10	11	12
Busia	phase three			Data Analysis and Further Laboratory Diagnostic work								

4.2.5.2 Collection of blood samples

Animals were restrained in crushes, clinical examination done and packed cell volume (PCV) determined for all the blood samples collected on the basis of which a decision was made about which animals were to be treated with trypanocidal or not. Blood samples were mainly collected from livestock in the crushes in the common sites with minimal quantities being collected from the homesteads for pigs and zero grazed livestock since most are not grazed alongside other livestock outdoors. Further information sought was obtained by visiting the individual farms to interview the owners based on the information needed since livestock are taken out for grazing by children or employees. Information was obtained on all the livestock sampled in the study on sex, weight, age, color and name of owner of each animal. Blood samples obtained and labeled in readiness for the determination of the presence of the parasite or carrier status. Livestock were randomly sampled thrice during the three field visits during which period they were restrained in a crush and blood collected from the ear veins using a pair of heparinized capillary tubes for the parasitological tests conducted supported by microscopy by use of the Buffy Coat Technique (BCT). Whole blood samples of between 5mls-10mls each for PCR were collected from ear veins or the jugular vein into vacutainers

from all cattle, pigs, sheep and goats. In a number of livestock (mainly goats and sheep) ear vein puncture failed to draw sufficient blood due to small or collapsing ear veins. The geographic co-ordinates of each sampling site linked to the various livestock keeping homesteads, linked to a unique identification number, were recorded using a handheld global positioning system 12 (GPS 12) Personal Navigator (Garmin Ltd, Kansas, USA) with results presented later on in this chapter for each sampling site .

4.2.6 Data processing and analysis

All blood samples were subjected to parasitological tests and microscopy and PCR tests with the results displayed in Section 4.3. The geographic coordinates of each sampling site provided the information that assisted in generating the Busia County map in Figure 4.1 using the Arc GIS program. Blood for the different tests was processed as appropriate.

4.2.6.1 Microscopy

Parasitological blood samples for microscopy were examined in the laboratory and the types of trypanosomes identified after direct observation of the buffy coat. The microhaematocrit centrifugation and buffy coat techniques were used since they were particularly useful in that the packed cell volume (PCV) could be assessed at the same time. The presence of trypanosomes in the blood was determined after haematocrit centrifugation followed by subsequent examination of the buffy coat under a compound microscope. The phase contrast microscopy with the BCT as stated by Murray *et al.,* (1977) was not used. The blood concentration technique of microhematocrit centrifugation (mHCT) uses capillary tubes containing anticoagulant which are filled three quarters full with little prick blood with the dry end sealed with plasticine. Through high speed centrifugation in a hematocrit centrifuge for 6 to 8 min, trypanosomes were concentrated at the level of the white blood cells, between the plasma and the erythrocytes. Thin blood smears were used to identify the presence of all

other blood parasites although the results were not included in this study they were handed over to the County Veterinary Office to support their disease control initiatives and and also published . *T.brucei* species are morphologically indistinguishable and once the the parasitological tests are finalized all the species of trypanosome identified under microscopy was recorded. The samples that were positive for *T.brucei* under microscopy were further subjected to PCR to differentiate *T.brucei brucei* and *T.brucei rhodesiense* to enable determine the actual prevalence of *T.b.rhodesiense* prevalence under microscopy. The results were compared with those obtained directly from PCR findings on all the samples to calculate sensitivities. Ability to pick *T.b.rhodesiense* the causative organism for sleeping sickness was compared between the results of parasitological tests (through microscopy) confirmed by PCR and all samples analysed by PCR.

4.2.6.2 Polymerase Chain Reaction

On PCR assays, blood from livestock measuring 100ul blood was aliquoted from the vacutainers and processed. DNA was extracted using GeneJET ™ Genomic DNA purification Kit using the manufacturer's instructions. A volume of 5 µl of amplified product was loaded on 2% agarose gel stained with ethidium bromide and electrophoresis carried out at 100 volts for 30minutes. On PCR cycling for the Internal Transcriber Spacer 1 (ITS1) after extraction, the test was carried out in 10µl reaction mixture consisting of Dream Taq DNA polymerase in Dream Taq Green buffer, 0.2mM dNTPs, 2mM $MgCl_2$, primers at 1 µM and 2ul of DNA template. PCR conditions were those described by Njiru *et al.,* (2005) for ITS1. Gel electrophoresis method was used to separate mixtures of DNA, RNA, or proteins according to molecular size. PCR cycles for ITS1, CF and BR primers started with an initial step 94C for 5 minutes, followed by 35 cycles of 94C for 40s, 58C for 40s, 72C for 90s and final extension at 72C for 5 minutes. ITS1CF,5'CCGGAAGTTCACCGATATTG and BR,

5’TTGCTGCGTTCTTCAACGAA primer is important in identification of all types of Trypanosomes . The ITS1 primers are able to identify most trypanosomes.

The blood samples were further subjected to TBR-PCR primers used in identification of *T.brucei* species using TBR1 5’GAATATTAAACAATGCGCAG3&TBR25’-CCATTTATTAGCTTTGTTGC3’.

The conditions for TBR, 1 & 2 primers were an initial step 95C for 3 minutes, followed by 30 cycles of 92C for 30s, 60C for 45s, 72C for 45s and final extension at 72C for 4minutes.

ITS1 PCR generated PCR products of approximately 500bp useful in identification of the various trypanosome species. TBR PCR was conducted to confirm presence of the Brucei Trypanozoon. The blood samples that were confimed to contain the Trypanasoma brucei trypanozon were further subjected to Serum Resistant Antigen (SRA) PCR to confirm the *Trypanosoma brucei rhodesiense* presence.

SRA PCR cycling conditions SRA A& E primers SRA A 5’GACAACAAGTACCTTGGCGC-3’ , SRA E 5’-TACTGTTGTTGT ACC GCCGC-3’were an initial step 95C for 3 minutes, followed by 35 cycles of 95C for 30s, 60C for 45s, 72C for 60s and final extension at 72C for 2minutes.

4.2.6.3 Supportive procedures

The degree of anaemia was estimated by measuring packed cell volume % (PCV) and recorded in aform shown in appendix III. To determine the PCV after centrifugation, the microhaematocrit capillary tube (containing ear vein or jugular vein blood) was placed in a haematocrit reader. The length of the packed RBC column was expressed as a percentage of the total volume of blood. Measuring the PCV is useful for determining the degree of anaemia an indication of trypanosomiasis and other blood parasites infection. All the livestock that were found positive and those that had a PCV of < 22 were treated with

diminazene aceturate at 7 mg kg-1 body weight. Other conditions that were observed or presented by the cattle owners were also managed appropriately. Low PCV was important in identifying animals that required medication.

4.2.6.4 Statistical analysis

Data results from the positive and negative samples were recorded in a Microsoft Excel spread sheet (Microsoft Corporation, Redmond, USA). Test results were appended to this spreadsheet and samples classified as trypanosome positive if they were positive for any of the detectable trypanosome species by parasitological tests through microscopy (and confirmed by PCR) or those tested solely by PCR ITS1 for all trypanosomes, TBR PCR for *T.brucei* and PCR SRA for *T.brucei rhodesiense*. Sensitivity and specificity were calculated. To conduct a comparison between the two tests, the Cohens Kappa (k) statistic was identified as the appropriate tool to assess the level of agreement where a cross tabulation of the summary data is done for purposes of comparison of the results of the two tests. Studies that measure the agreement between two or more observations use a statistic that takes into account the fact that observers will sometimes agree or disagree simply by chance. The kappa statistic (or kappa coefficient) is the most commonly used statistic for this purpose. A kappa of 1 indicates perfect agreement, whereas a kappa of 0 indicates no agreement. Precision, as it pertains to agreement between observations (interobserver agreement), is often reported as a kappa statistic. Kappa is intended to give one a quantitative measure of the magnitude of agreement between observers. It applies especially to screening and diagnostic tests. Comparing the results from microscopy to the result on PCR assesses the validity of the former to diagnose HAT. Assessing whether the tests agree on the presence or absence HAT parasites regardless of validity assesses precision (reliability).

The Kappa Statistic was interpreted as follows:

k≤0.4 corresponds to poor agreement

k>0.4≤0.75 corresponds to good agreement

k>0.75 corresponds to excellent agreement

(Dohoo, 2010).

The value of odds ratio ranges from 0 to infinity, with higher values indicating better discriminatory test performance. A value of 1 means that the tests are not significantly different. Values lower than 1 point to improper test interpretation (more negative tests among the diseased). The inverse of the odds ratio can be interpreted as the ratio of negativity odds within the diseased relative to the odds of negativity within the non diseased.

4.3 Results

All the results are displayed in Appendix IV. The summary results contain the information on the levels of agreement of PCR and microscopy and the sensitivity of the latter in estimating the prevalence for all trypanosomes and *T.b.rhodesiense* in particular. Under microscopy, a total of 51 livestock were found infected with various species of trypanosomes while Polymerase chain reaction (PCR) revealed that a total of 320 livestock were infected with various species of trypanosomes as shown in Table 4.4a. Under PCR a total of 47 livestock were found carriers of human African trypanosomiasis *T.b.rhodesiense* parasites as opposed to 9 under microscopy as shown in Table 4.4b below. The results are in two main sections consisting of an initial part which is the summary of findings and related analysis the second part comprising the data report on the results from each sampling site.

4.3.1 Overall prevalence results for all trypanosomes and *T.b.rhodesiense*

Data collected in three different time periods and from 7 different sub counties in Busia County Kenya was collated, a summary of which is displayed below in Table 4.4a for prevalence of all trypanosomes and Table 4.4b for the prevalence of *T.b.rhodesiense*.

Table 4.3a: Laboratory results from PCR and microscopy for all trypanosomes

Number of livestock sampled	PCR ITSI positive for all forms of trypanosomes	PCR ITS1 negative for all forms of trypanosomes	Microscopy positive for all forms of trypanosomes	Microscopy Negative for all forms of trypanosomes
Cattle -2288	211	2077	40	2248
Shoats -917	75	842	7	910
Pigs -594	34	560	4	590
Total -3799	320	3479	51	3748

Table 4.3b: Laboratory results from PCR and microscopy for *T.b.rhodesiense*

Number of livestock sampled	PCR SRA positive for *T.brucei rhodesiense*	PCR SRA negative for *T.brucei rhodesiense*	Microscopy positive for *T.brucei rhodesiense* (confimed by PCR)	Microscopy Negative for *T.brucei rhodesiense* (confimed by PCR)
Cattle -2288	33	2255	6	2282
Shoats -917	06	911	1	916
Pigs -594	08	586	2	592
Total -3799	47	3752	9	3790

4.3.2 Sensitivity of microscopy test

The sensitivity of the test will the proportion of animal blood samples with disease the causing parasites that will have a positive result. In defining sensitivity, the interest is only in the proportion of samples with disease causing parasites that test positive. This means that the sensitivity of a test only tells us how good the test is for identifying samples with disease causing parasites when only looking at those with disease. The specificity of a test is the proportion of the blood samples without the disease that will have a negative result. In defining specificity, interest is in the proportion of blood samples without the disease causing

parasites that test negative. Specificity can only be calculated from those blood samples that do not have the disease causing parasites.

A test with a high sensitivity is useful for 'ruling out' a disease if a patient tests negative. The 100% sensitivity means that the test will detect virtually every case with the disease but its relatively low specificity means it will be falsely positive for a number of cases that actually don't have the disease. A test with a high specificity is useful for 'ruling in' a disease if a patient tests positive. A highly specific test is, therefore, most helpful to the clinician when the test result is positive. The Sensitivity calculation was as shown in Table 4.4a below. Sensitivity (Se) as the proportion of livestock carriers of HAT parasites that test positive was estimated using the formula Se = a/a+c =a/m_1. Specificity (Sp) as the proportion of non carrier livestock that test negative was estimated by Sp= d/c+d = d/m_0 , (Dohoo *et al.,* 2010) .

Table 4.4a Data layout for test evaluation

	Test positive	Test negative	Total
Disease positive	a (true positive)	a (false positive)	m_1
Disease negative	c (false positive)	d (true negative)	m_0
Total	n_1	n_0	n

Source: Dohoo *et al.,* (2010)

Sensitivity and Specificity of Microscopy test was estimated using the PCR results as a gold standard as shown in Tables 4.4b and 4.4c.

Table 4.4b Sensitivity and Specificity of microscopy for cattle blood samples

	Test positive (T+)	Test negative (T+)	Total
Disease positive (D+)	5	28	33
Disease negative (D-ve)	1	2254	2255
Total	6	2282	2288

Sensitivity= 5/33=15% or 0.15

Specificity= 2254/2255=99% or 0.99

Table 4.4c Sensitivity and Specificity of microscopy for blood samples of all livestock

	Test positive (T+)	Test negative (T-ve)	Total
Disease positive (D+)	7	40	47
Disease negative (D-ve)	2	3750	3752
Total	9	3790	3799

Sensitivity = 7/47=14.9% or 0.149

Specificity= 3750/3752=99% or 0.99

4.3.3 Comparison of level of agreement between microscopy and PCR

The Kappa Statistic inter observer variation can be measured in any situation in which two or more independent tests or observations are evaluating the same thing. The calculation is based on the difference between how much agreement is actually present ("observed" agreement) compared to how much agreement would be expected to be present by chance alone ("expected" agreement). The observed agreement is simply the percentage of results for which the two tests agree as shown in Figure 4.6a. A confidence interval for kappa, which may be even more informative, can also be calculated. Fortunately, computer programs are able to calculate kappa as well as the P value or confidence interval of kappa. Remember, though, the P value in this case test whether the estimated kappa is not due to chance. It does not test the strength of agreement. Also, P values and confidence intervals are sensitive to sample size, and with a large enough sample size, any kappa above 0 will become statistically significant.

To establish the level of agreement between the two tests, the Cohens Kappa statistic was done by cross tabulation of the those samples that are positive and negative for microscopy and those negative and positive in PCR to enable comparison of the results of the two tests as seen in table below 4.5a and 4.5b for cattle . Numbers given are the observed values in each cell, while the numbers in brackets are the expected values for the same cell.

4.3.3.1 Cattle Samples

Table 4.5a Cross tabulation for prevalence data from cattle for all trypanosomes

Cattle: Crosstabs for status for all trypanosomes	Microscopy (+ve)	Microscopy (-ve)	Total
PCR SRA (+ve)	38 (3.68)	173 (207.3)	211
PCR SRA (-ve)	2 (36.3)	2075 (2040.69)	2077
Total	40	2248	2288

Numbers given are the observed values in each cell, while the numbers in brackets are the expected values for the same cell.

Table 4.5b Cross tabulation for *T.b. rhodesiense* pravalence data from cattle

Cattle: Crosstabs for status for T.brucei rhodesiense	Microscopy (+ve)	Microscopy (-ve)	Total
PCR SRA (+ve)	5 (0.08)	28 (32.91)	33
PCR SRA (-ve)	1 (5.91)	2254 (2249.1)	2255
Total	6	2282	2288

Let p_0=sum of elements in the lead diagonal of observed values in Table 4.6a divided by total number of livestock sampled and p_E=sum of elements in the lead diagonal of expected

values for the reported observed values in Table 4.6a, divided by total number of livestock sampled.

Then, the Cohen's Kappa statistic (Dahoo, 2010) for tests on the livestock data is given by,

$$k = \frac{p_0 - p_E}{1 - p_E} = \frac{0.924 - 0.894}{1 - 0.894} = 0.2816$$

Going by the criteria set in the Cohen's Kappa statistics, we remark that since $k < 0.4$, there is poor agreement between the outcomes of PCR and Microscopy tests.

The odds ratio can also be used to determine whether a particular exposure is a risk factor for a particular outcome, and to compare the magnitude of various risk factors for that outcome.

OR=1 Exposure does not affect odds of outcome

OR>1 Exposure associated with higher odds of outcome

OR<1 Exposure associated with lower odds of outcome

The 95% confidence interval (CI) is used to estimate the precision of the OR. A large CI indicates a low level of precision of the OR, whereas a small CI indicates a higher precision of the OR. It is important to note however, that unlike the p value, the 95% CI does not report a measure's statistical significance. Nevertheless, it would be inappropriate to interpret an OR with 95% CI that spans the null value as indicating evidence for lack of association between the exposure and outcome.

To measure the extent of disagreement between Microscopy and PCR for all types of trypanosomiasis, we use the odds ratio for two groups of samples thus those that turned positive for PCR and those that turned negative for PCR. Interest is to compute the odds of being positive under Microscopy test for each PCR (positive or negative) category as shown below for Table 4.6a.

$$Odds\ (PCR+ve\ group) = \frac{Microscopy\ positive}{Microscopy\ negative}$$

$$Odds\ (PCR+ve\ group) = \frac{38}{173}$$

$$Odds\ (PCR-ve\ group) = \frac{2}{2075}$$

$$Odds\ Ratio = \frac{\left(\frac{38}{173}\right)}{\left(\frac{2}{2075}\right)} = 227.89$$

This indicates that the odds of being positive for any trypanosome under microscopy is 228% large if one was tested positive under PCR. We are almost very certain that the test would turn out to be positive under microscopy if it was positive under PCR. To measure the extent of disagreement in estimating the extent of estimating *T.b.rhodesiense* from the two tests, we use the Odds Ratio for the two different sets of tests ie those that turned positive for PCR and those that turned negative for PCR. Interest is to compute the odds of being positive under Microscopy test for each PCR (positive or negative) category.

$$Odds\ (PCR+ve\ group) = \frac{Microscopy\ positive}{Microscopy\ negative}$$

$$Odds\ (PCR+ve\ group) = \frac{5}{27}$$

$$Odds\ (PCR-ve\ group) = \frac{1}{2255}$$

$$Odds\ Ratio = \frac{\left(\frac{5}{27}\right)}{\left(\frac{1}{2255}\right)} = 1569.21$$

This indicates that the odds of being positive for *T.b.rhodesiense* under Microscopy is 1569% large if one was tested positive under PCR. We are almost certain that the test would turn out to be positive under microscopy if it turned positive under PCR.

4.3.3.2 Agreement level between PCR and microscopy for all livestock species

Other than the cattle population, we summarize the Kappa statistic result for all the livestock types as provided in Table 4.6 for all trypanosomes when the results on Microscopy are compared with PCR.

Table 4.6: Measure of agreement between PCR and microscopy by livestock types

Livestock	Kappa Statistic	Decision on Tests	Odds Ratio
Cattle	0.2816	Poor agreement	227
Shoats	0.1566	Poor agreement	245
Pigs	0.1902	Poor agreement	352
Total	0.2429	Poor agreement	242

Decision on the kappa statistic

k≤0.4 Poor agreement

k>0.4≤0.75 Good agreement

k>0.75 Excellent agreement

There was no agreement between microscopy outcome and PCR for *T.b.rhodesiense* based on the kappa statistic among different livestock types ranging from cattle, shoats and pigs as shown in Figure 4.7.

Table 4.7: Measure of agreement between PCR and microscopy by livestock types

Livestock	Kappa Statistic	Confidence Interval for k		Decision on Tests	Odd Ratio
		Lower	Upper		
Cattle	0.289	0.259	0.318	Poor Agreement	1569.20
Shoats	0.270	0.224	0.315	Poor Agreement	3427.33
Pigs	0.376	0.312	0.440	Poor Agreement	1824.93
Total	0.273	0.251	0.296	Poor Agreement	1809.14

There was no agreement between Microscopy outcome and PCR for all types of

trypanosomes among different livestock types ranging from cattle, sheep and goats, and pigs.

4.3.3.3 Choice of sampling site and influence on PCR and microscopy tests outcome

Sampling was carried out in 19 sites and as shown in Table 4.8, there was no agreement between Microscopy and PCR outcomes for the different regions or sites sampled. The choice of the site did not influence the results obtained from PCR and Microscopy for *T.b.rhodesiense*. The sampling site or region had no significant influence on detection level of HAT, there was no agreement between Microscopy outcome and PCR for different livestock types and for the different regions.

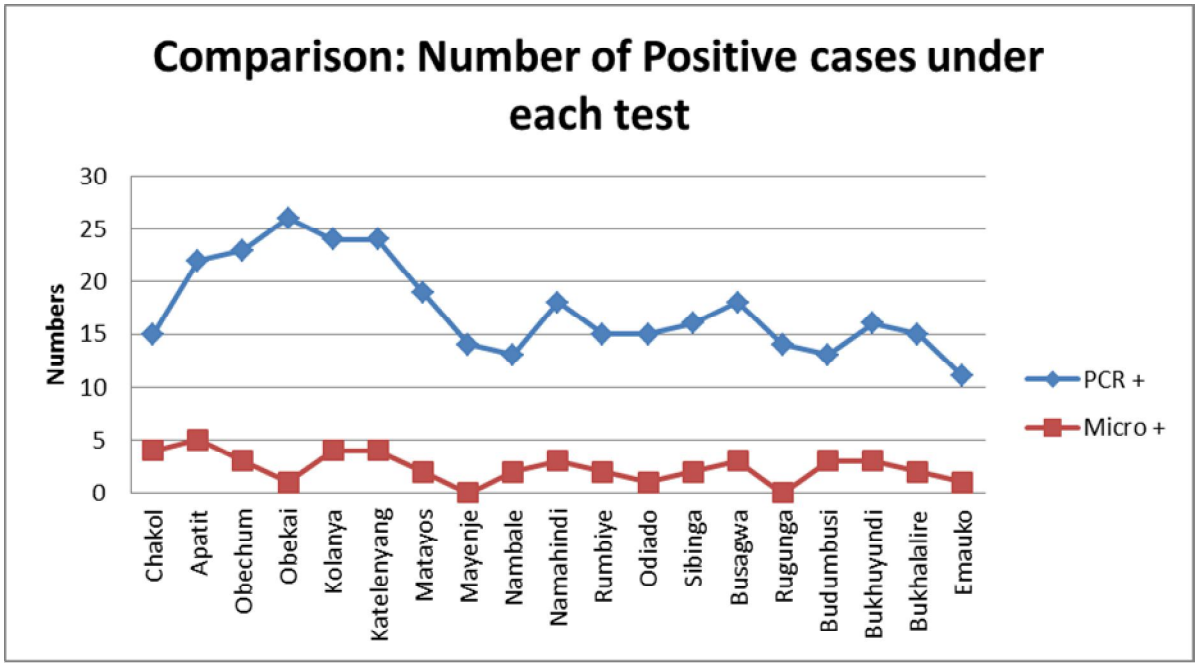
Table 4.8: Measure of agreement between PCR and microscopy by regions

				Confidence Interval for k	
Region	Kappa Statistic (k)	Decision on Tests	Odd Ratio	Lower	Upper
Teso North	0.294764957	Poor Agreement	228.0781893	0.23871733	0.350812588
Teso South	0.185202394	Poor Agreement	160.6944232	0.13866005	0.231744738
Matayos	0.099238579	Poor Agreement	202.1575563	0.05341073	0.145066423
Nambale	0.247937928	Poor Agreement	271.247619	0.18228434	0.313591521
Funyula	0.174021213	Poor Agreement	258.7454545	0.12807579	0.219966633
Budalangi	0.208726965	Poor Agreement	251.7379135	0.15709872	0.260355209
Butula	0.218729097	Poor Agreement	283.0110193	0.1673328	0.270125398

4.3.3.4 Prevalence of HAT parasites in cattle

Microscopy fails to capture many true positives as shown in figure 4.2. A comparison of the sensitivity in the diagnosis of trypanosomes especially *T.b.rhodesiense* species between PCR and Microscopy in cattle detected 33 [1.05% prevalence, 95% confidence interval (CI) =0.82%-1.32%] and 6 [0.77% prevalence, 95% CI=0.57%-1.01%] respectively. Microscopy and PCR agreed positively on 5 samples with PCR detecting an additional 28 samples as being positive.

Figure 4.2: Figure shows the number of cases that turn out positive under the two tests across the regions for all trypanosomes.



4.3.3.5 Prevalence of all trypanasome species in livestock

Results on the diagnostic tests for trypanosomes in domestic animals blood samples were as shown in Plate 4.1 for parasitological tests under microscopy and Plate 4.2 for PCR. In Plate 4.1, the T.*brucei* shown is the long slender forms with a free flagellum, which is almost one half of the length of the organism. The posterior end is pointed and the nucleus is central and the kinetoplast is placed up to in front of the posterior extremity.

Plate 4.1: *Trypanosoma brucei* species in a thin blood smear stained with Giemsa under routine parasitological tests in Busia County.

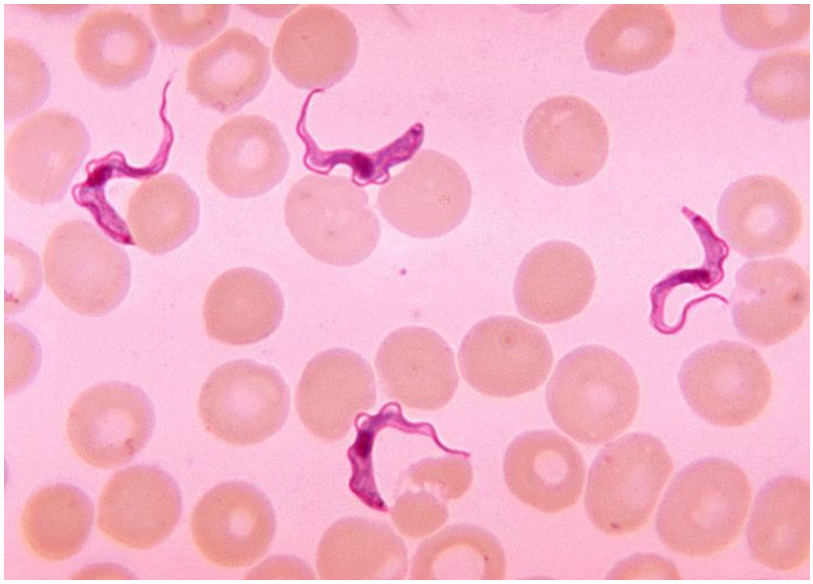
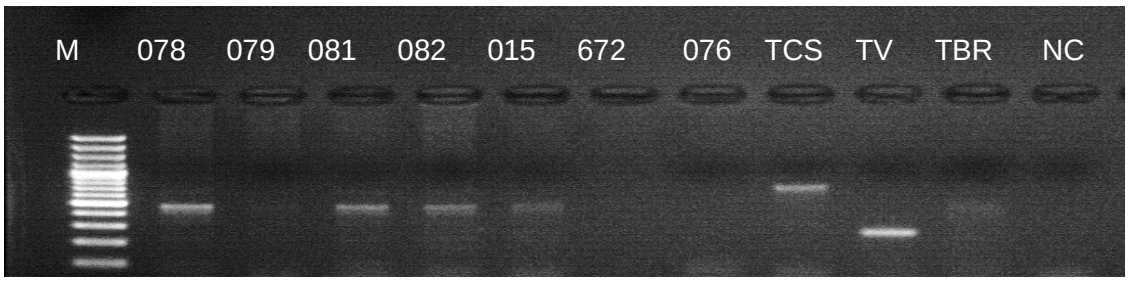


Plate 4.2 PCR outcome for the various trypanosomes analysed



Gel electrophoresis of samples and reference DNA samples amplified with ITS1CF and BR. Lane 1, 100bp marker, lane 2-8 cattle samples, Lane 9 T congolense savannah(TCS) positive control, Lane 10 T. vIvax (TV), Lane 11 T brucei sp (TBR), lane 12, NC negative control

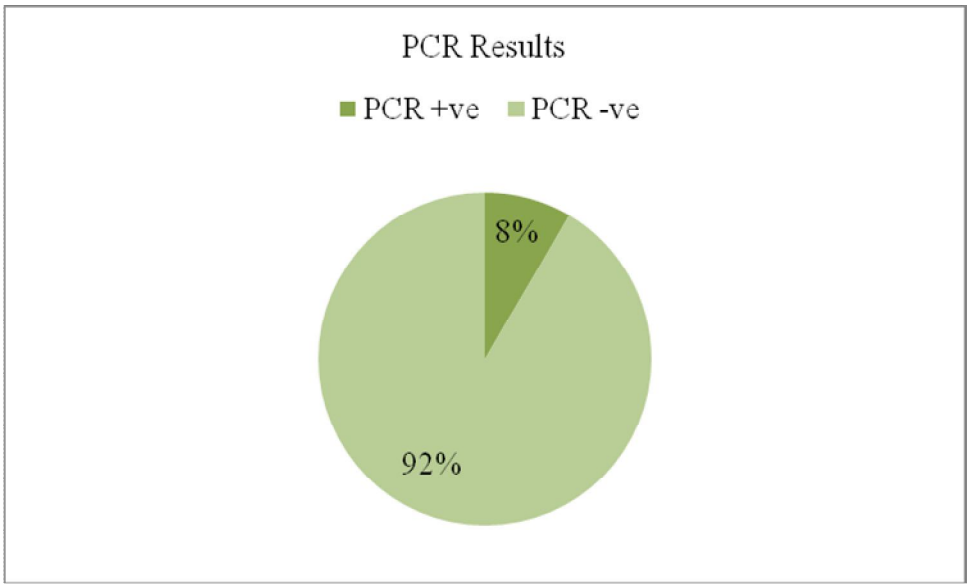
The prevalence of various types of trypanosomes documented from the blood samples under the parasitological and PCR ITS1 tests are displayed in Table 4.9.

Table 4.9: Overall numbers of trypanosome species that were reported.

Parasite	Result under ITS1 PCR	Result under Microscopy
T.vivax	105	17
T.Congolense	109	12
T.brucei	102	19
T.Simiae	4	3
Total	320	51

Out of these overall prevalence cattle contributed 9.2%, shoats 8.2% and pigs 5.7%. Percentage of the positive and negative test outcomes from actual field survey data under PCR is as shown in Figure 4.3 and Table 4.10a documenting that the prevalence of all types of trypanosomiases from field survey was 8.449% under PCR out of which HAT causing parasites comprised 14.6%. *T.b.rhodesiense* was 46% and 47% of the total *T.brucei* species prevalence under PCR and microscopy respectively.

Figure 4.3: Percentage of the positive and negative test outcomes from actual field survey data under PCR.



Prevalence of the various trypanosome species Table 4.10a illustrates the percentage differences in the results.

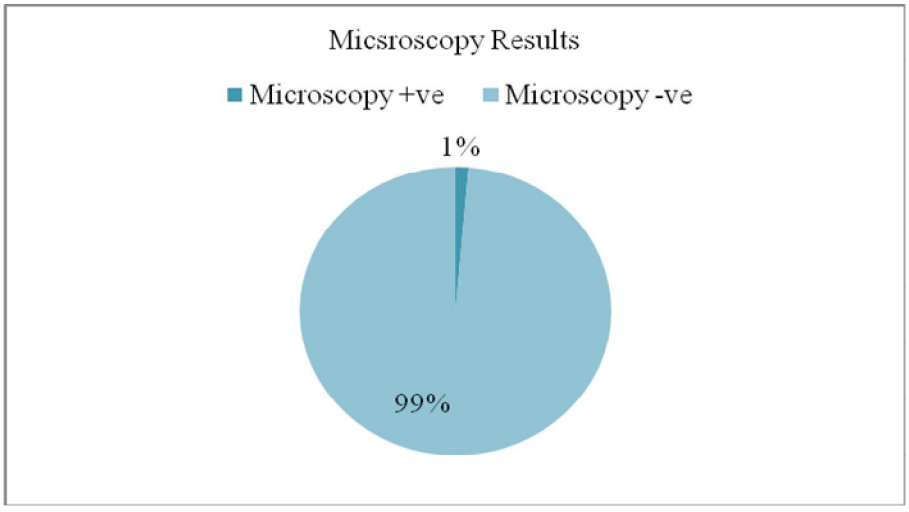
Table 4.10a: Prevalence for all types of trypanosomes in all domestic animal species

	PCR +ve	Microscopy +ve	Microscopy -ve	PCR -ve	Total
Total	320	51	3748	3479	3799
Percentage	8.42327	1.34246	98.65754	91.57673	100

4.3.3.6 Proportion of HAT positive samples identified by microscopy

As shown in Figure 4.4 the percentage of the positive and negative test outcomes from the actual field survey data from microscopy test reported AT prevalence at 1% with 15.6% being contributed by *T.b.rhodesiense*. Since *T.brucei* species are morphologically indistinguishable after parasitological examination under microscopy, the positive samples were differentiated by PCR SRA.

Figure 4.4: Percentage of the positive and negative test outcomes from actual field survey data microscopy test.



The difference in positive outcome of about 7% is understood in this context as a measure of prevalence contributing to underreporting attributable to the use of Microscopy.

4.3.3.7 Proportion of positive HAT samples identified by PCR against microscopy

PCR reveals 1.24% and microscopy 0.24% prevalence of HAT parasites in the samples collected shown in Table 4.10b.

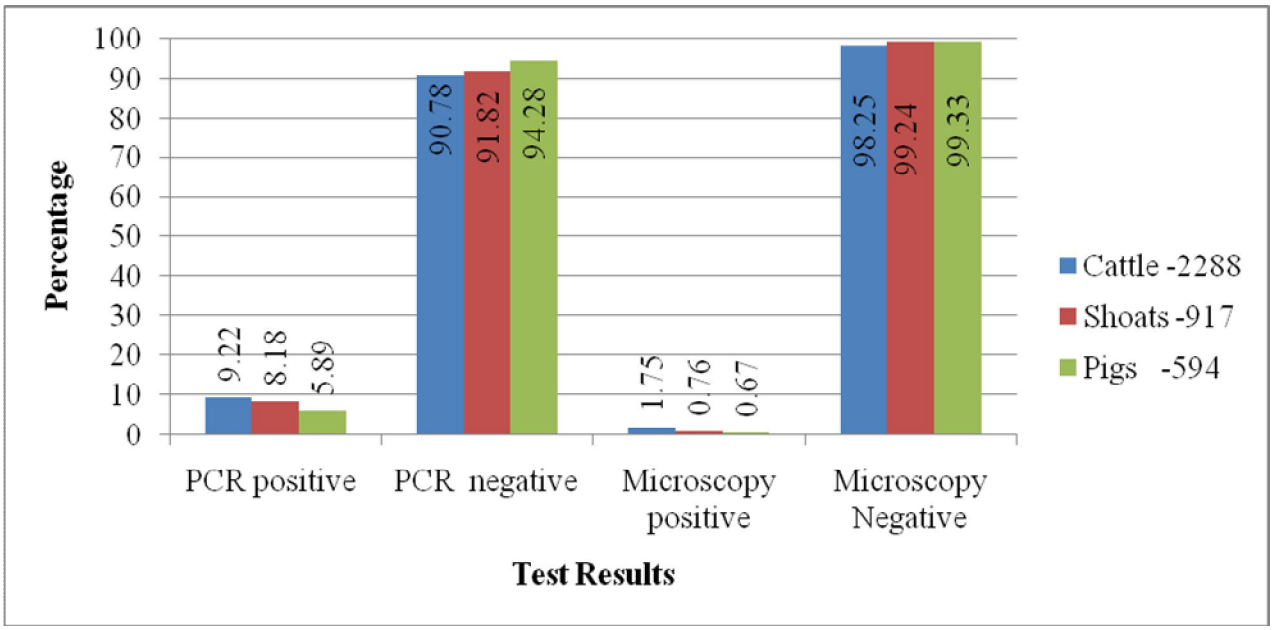
Table 4.10b: *Trypanasoma brucei rhodesiense* incidence as a percentage in all the livestock sampled

	PCR +ve	Microscopy +ve	Microscopy -ve	PCR -ve	Total
HAT Positive	47	9	3790	3752	3799
Percentage	1.24	0.24	99.76	98.76	100

4.3.4 Individual HAT parasite prevalence results from the various sampling sites based on host species group

The microscopy and PCR tests for the prevalence of HAT parasites from all types of livestock blood samples are as shown in Figure 4.5 below.

Figure 4.5: Entire Busia County



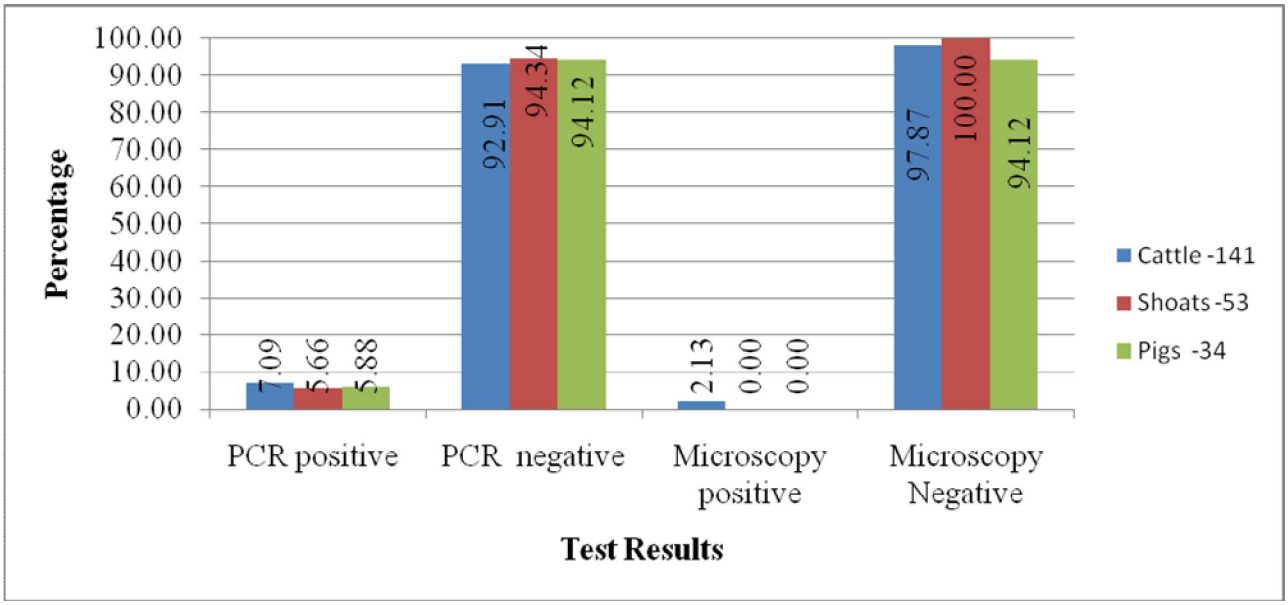
The total cattle samples were 2288 out of which a prevalence of 9.22 % and 1.75% was reported for trypanasomes under PCR and microscopy respectively out of which about 15% comprises of *T.b.rhodesiense.* Out of the 917 shoats sampled, a prevalence of 8.2% and 0.76

% respectively were positive for trypanasomes under PCR and microscopy with about 15% comprising HAT parasites. Over 5.8% and 0.67% prevalence was recorded out of the total 594 sampled pigs for contributing to trypanosomiasis prevalence.

4.3.4.1 HAT parasites prevalence Chakol

The communal grazing site in Chakol where sampling was done is located on Latitude 0.515^0 North and 34.152^0 East, at an altitude of 1176 metres above sea level. At least 5% prevalence was noted under PCR for all species of livestock sampled as shown in Figure 4.6 below in Chakol site within Teso South Sub County.

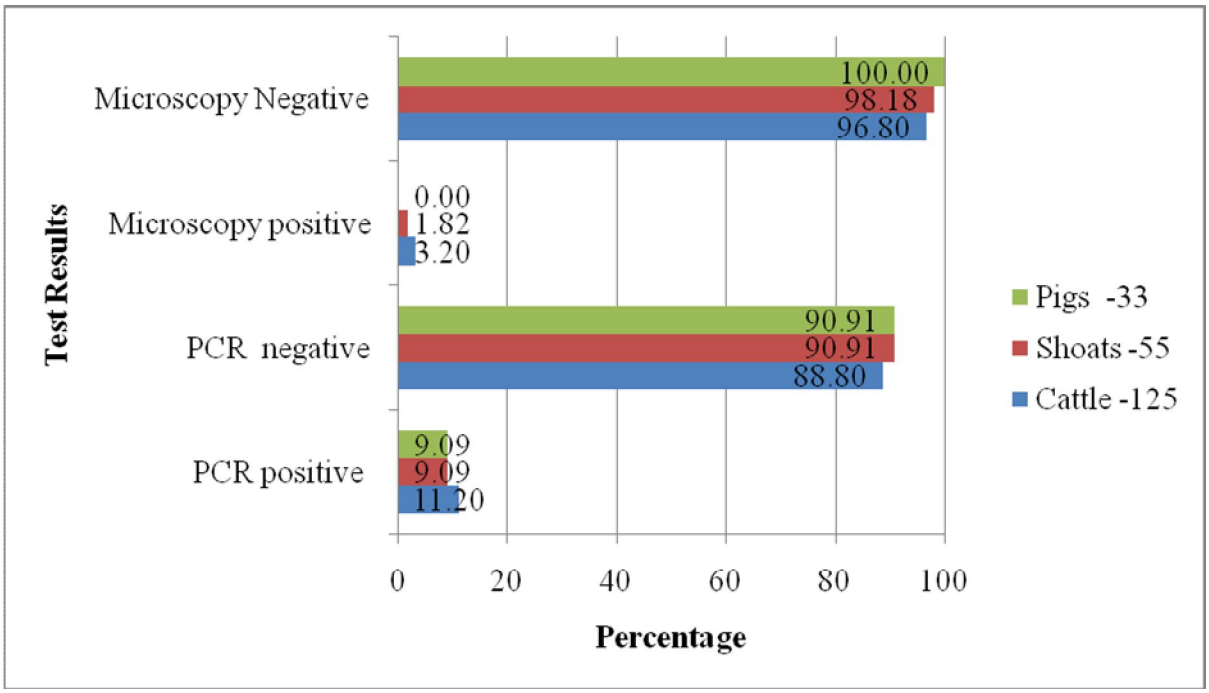
Figure 4.6: Chakol results



4.3.4.2 HAT parasites prevalence in Appatit

Figure 4.7 shows the findings. Sampling at Appatit was conducted at a functional crush pen located on Latitude 0.553^0 North and 34.234^0 East at an altitude of 1348 metres above sea level. Results depict a high prevalence with each species registering at least 9% under PCR and cattle recording 3.2% under Microscopy in Appatit site of Teso South Sub County.

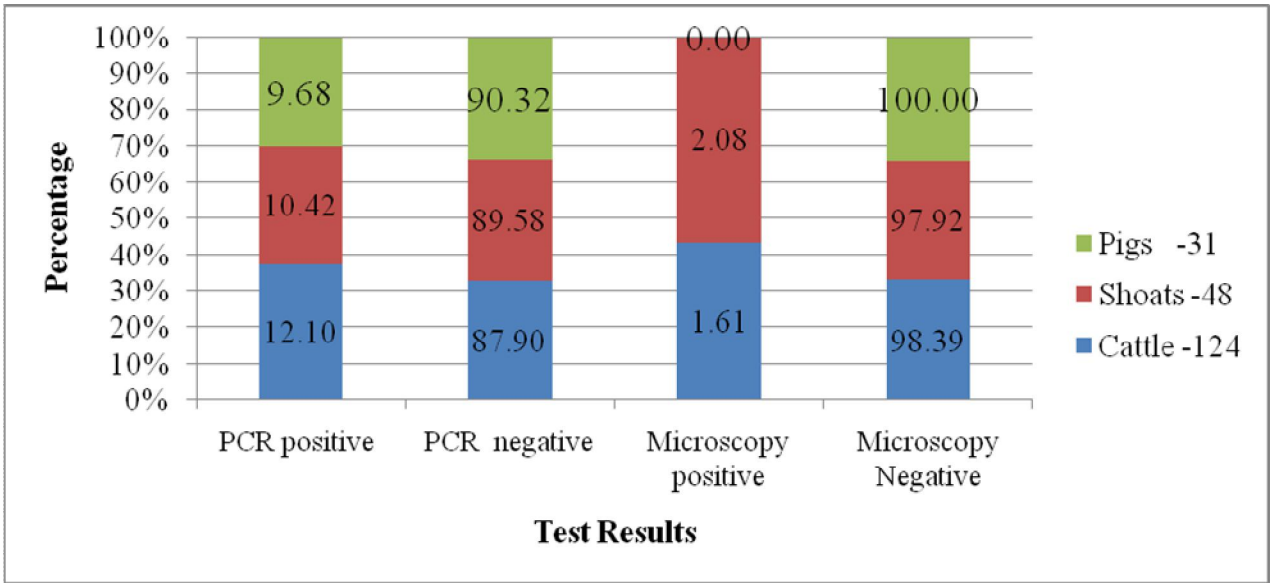
Figure 4.7: Appatit results



4.3.4.3 HAT parasites prevalence Obechun

The findings were as shown in Figure 4.8.

Figure 4.8: Obechun results



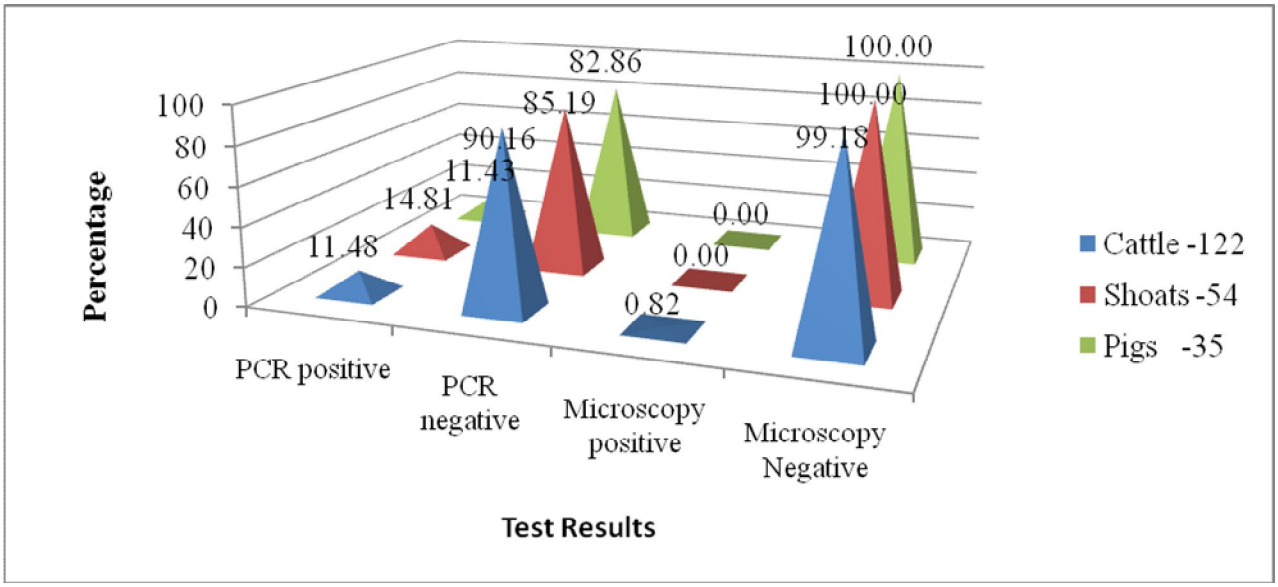
Sampling at Obechun was done on a communal grazing ground where routine herd health programs are conducted located on Latitude 0.563^0 North and 34.132^0 East at an altitude of 1155 metres above sea level. Figure 4.8 shows a high prevalence with each species

registering at over 9% under PCR and shoats recording 2.08 % under Microscopy in Obechun of Teso South Sub County.

4.3.4.4 HAT parasites prevalence Obekai

Samples were collected in crushes at a communal grazing ground and located on Latitude 0.568^0 North and 34.242^0 East at an altitude of 1351 metres above sea level. The results as reported in Figure 4.9 shows the second highest prevalence with each species registering over 11 % under PCR while Microscopy recorded a very low prevalence in Obekai of Teso North Sub County.

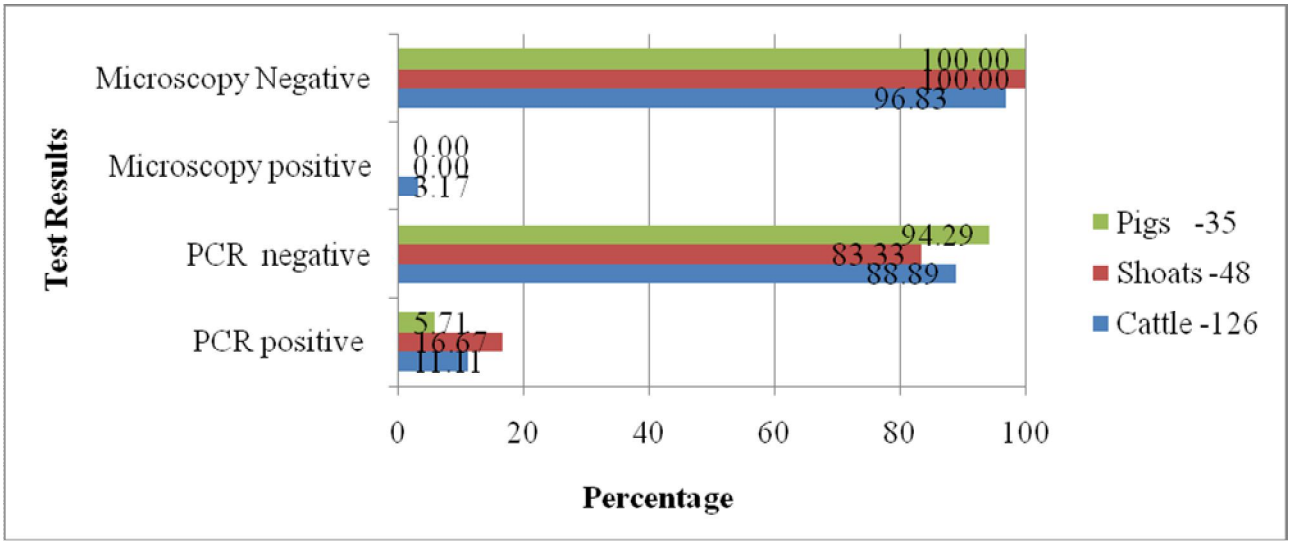
Figure 4.9: Obekai results



4.3.4.5 HAT parasites prevalence Kolanya

See figure 4.10 below. The livestock blood samples in Kolanya were collected at a disused cattle dip that is routinely used for communal herd health programs located on Latitude 0.712^0 North and 34.401^0 East at an altitude of 1411 metres above sea level.

Figure 4.10: Kolanya results

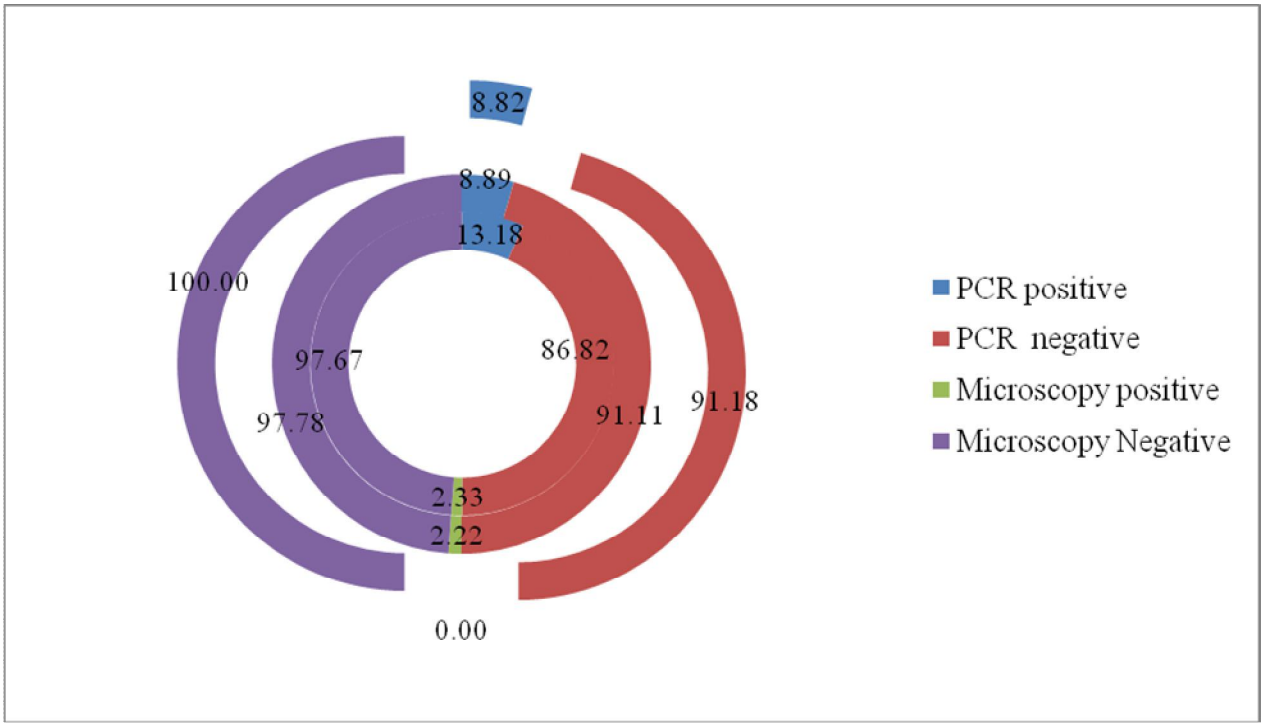


The results reported in Figure 4.10 shows the highest prevalence in shoats registering at over 16 % under PCR with Microscopy recording over 3% prevalence in cattle.

4.3.4.6 HAT parasites prevalence Katelenyang

Results are shown in figure 4.11 below.

Figure 4.11: Katelenyang results

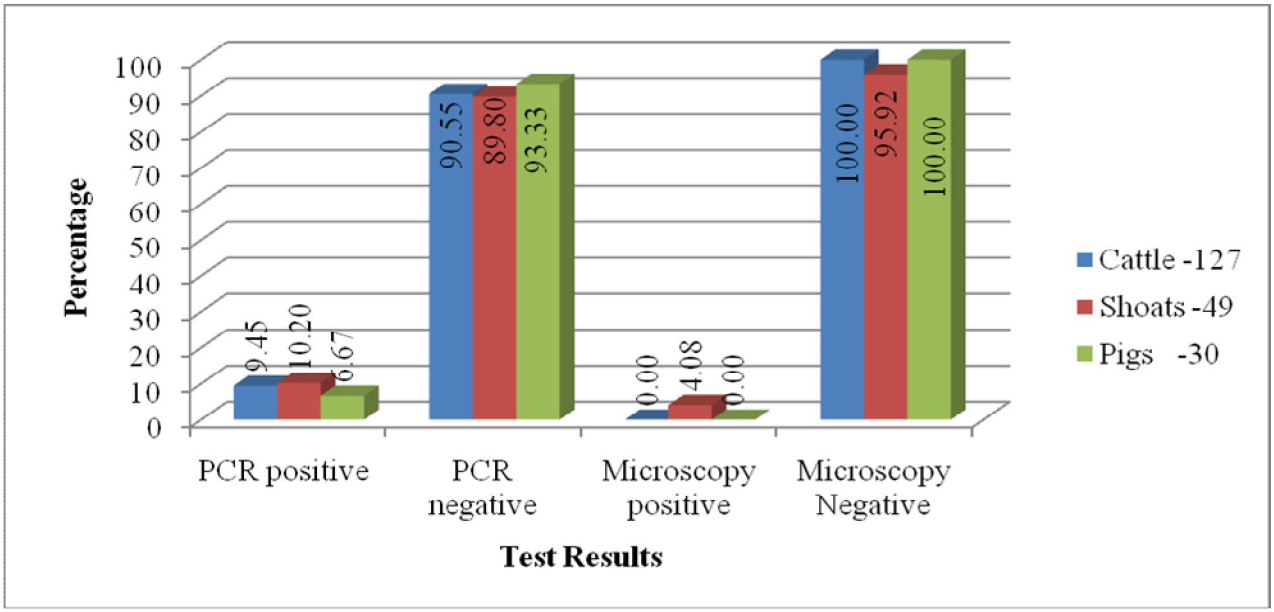


The livestock blood samples in Katelenyang were collected in mobile crushes at a communal grazing ground along the river located on Latitude 0.382^0 North and 34.146^0 East at an altitude of 1162 metres above sea level. The results as shown in Figure 4.11 reveal a consistently high prevalence from PCR in all species with at least 8% with Microscopy recording only upto 2.3% in Katelenyang of Teso North Sub County.

4.3.4.7 HAT parasites prevalence Matayos

The results in Figure 4.12 shows high prevalence in shoats registering at over 10 % under PCR with Microscopy recording over 4% prevalence in shoats in Matayos of Matayos Sub County.

Figure 4.12: Matayos results

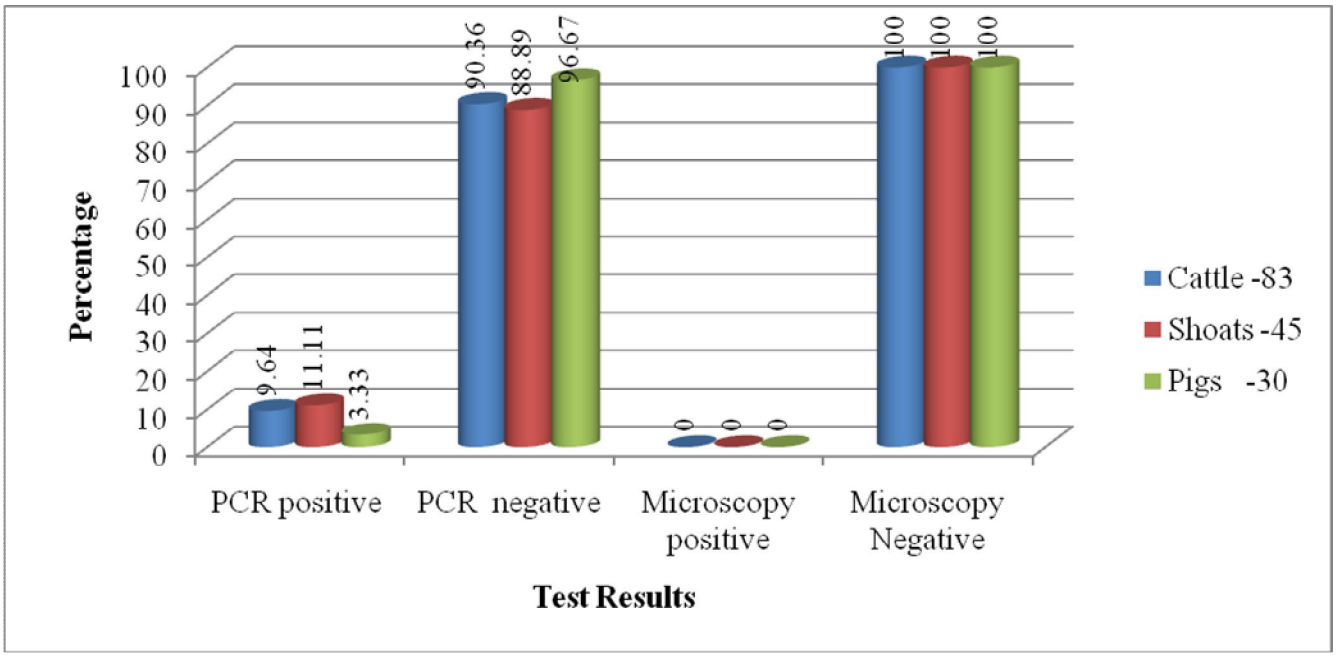


The livestock blood samples in Matayos were collected at a crush in communal grazing ground located on Latitude 0.382^0 North and 34.148^0 East at an altitude of 1262 metres above sea level.

4.3.4.8 HAT parasites prevalence Mayenje

The livestock blood samples in Mayenje were collected at a communal watering and grazing ground located on Latitude 0.443^0 North and 34.105^0 East at an altitude of 1218 metres above sea level. The results as shown in Figure 4.13 show a high prevalence in shoats registering at over 11 % under PCR while Microscopy picked no parasites in Mayenje of Matayos Sub County.

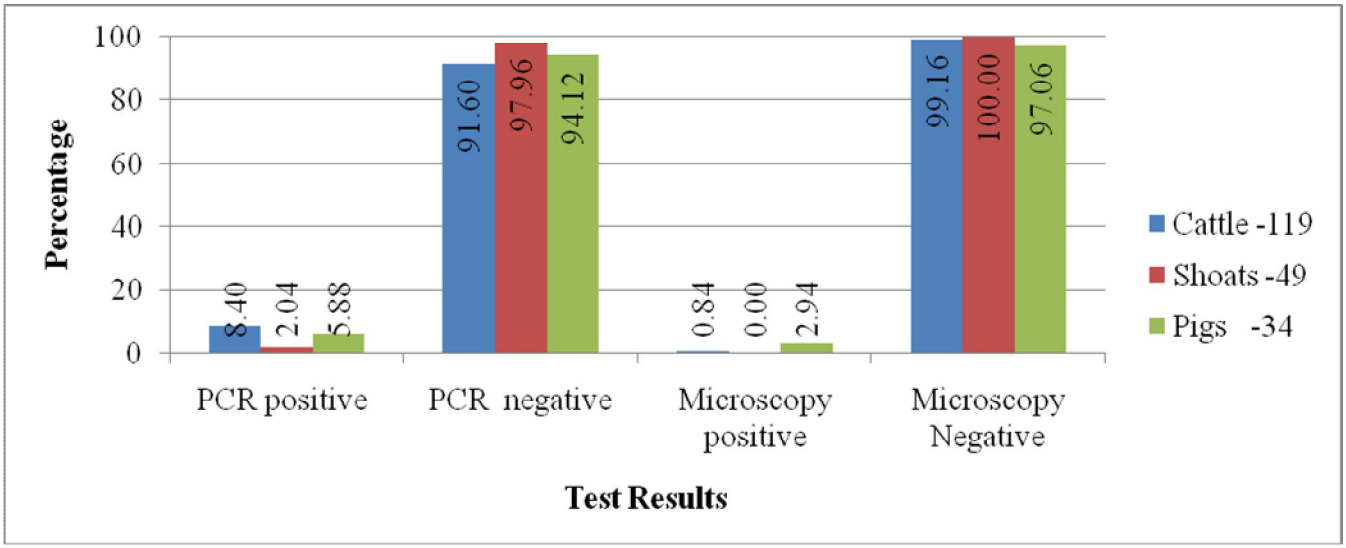
Figure 4.13: Mayenje results



4.3.4.9 HAT parasites prevalence Nambale

As shown in Figure 4.14 below, samples in Nambale were collected in a crush at a communal ground used for grazing and herd health programs located on Latitude 0.511^0 North and 34.209^0 East at an altitude of 1180 metres above sea level. The results in Figure 4.14 show a moderate prevalence in all species at above 5%. However for shoats under PCR with microscopy there was 2.04 % and zero prevalence respectively while in pigs it was 5.88 % and 2.94 % respectively in Nambale Centre, site of Nambale Sub County.

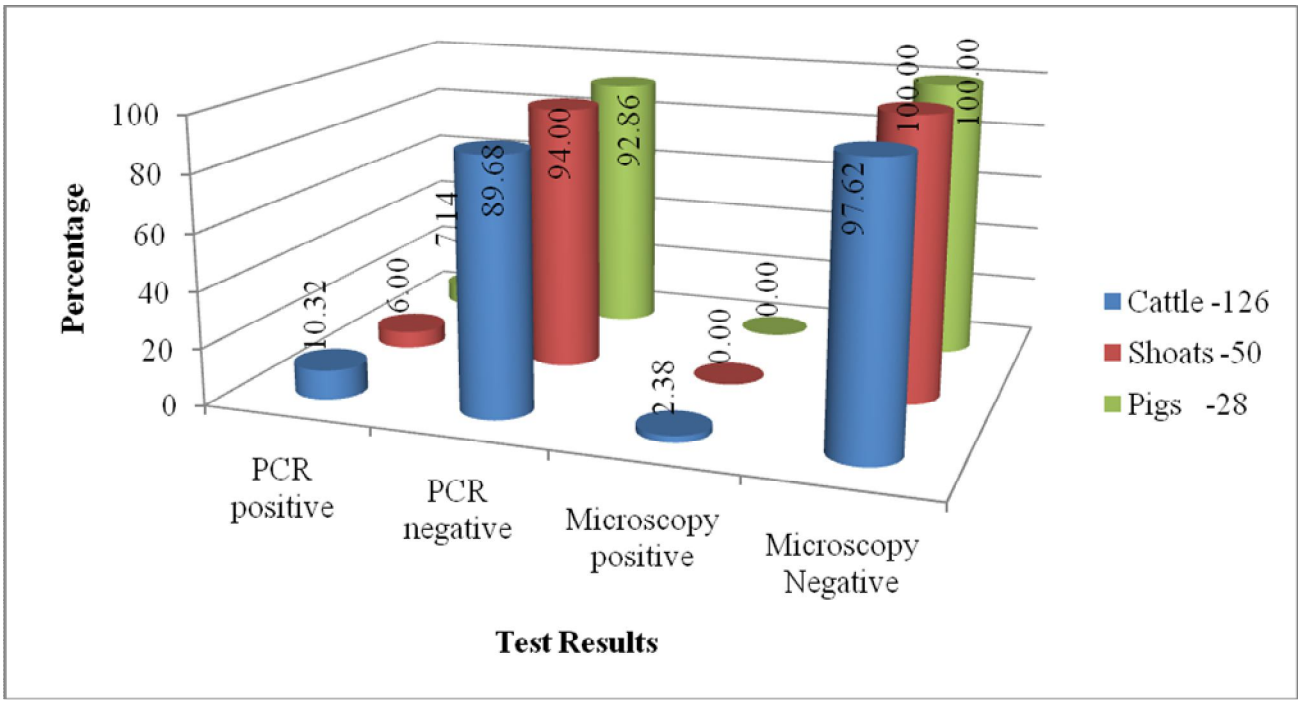
Figure 4.14: Nambale results



4.3.4.10 HAT parasites prevalence Namahindi

Figure 4.15 shows the results from Namahindi.

Figure 4.15: Namahindi results



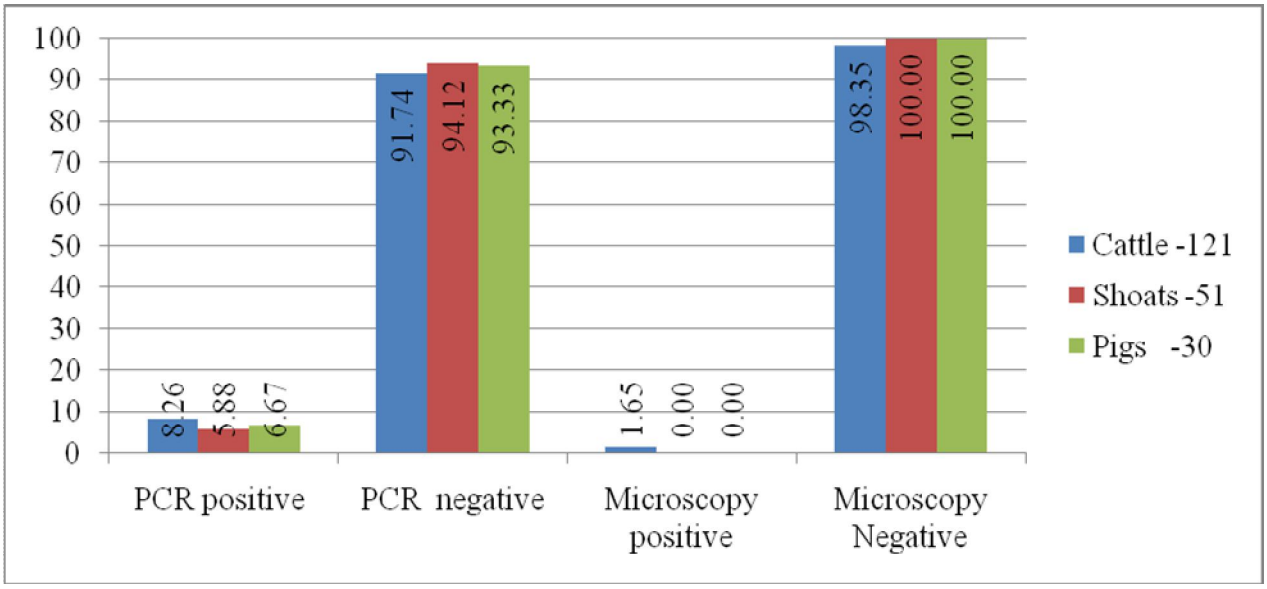
Samples in Namahindi were collected at a communal ground used for grazing and herd health programs located on Latitude 0.463^0 North and 34.312^0 East at an altitude of 1216 metres above sea level. Figure 4.15 shows there was a moderate to a high prevalence of over 6 % in

all species under PCR with Microscopy giving only 2.38 % prevalence in cattle in Namahindi of Nambale Sub County.

4.3.4.11 HAT parasites prevalence Rumbiye

Samples in Rumbiye were collected at a communal ground used for grazing and herd health programs located on Latitude 0.202^0 North and 34.096^0 East at an altitude of 1231 metres above sea level as presented in results in Figure 4.16 below.

Figure 4.16: Rumbiye results

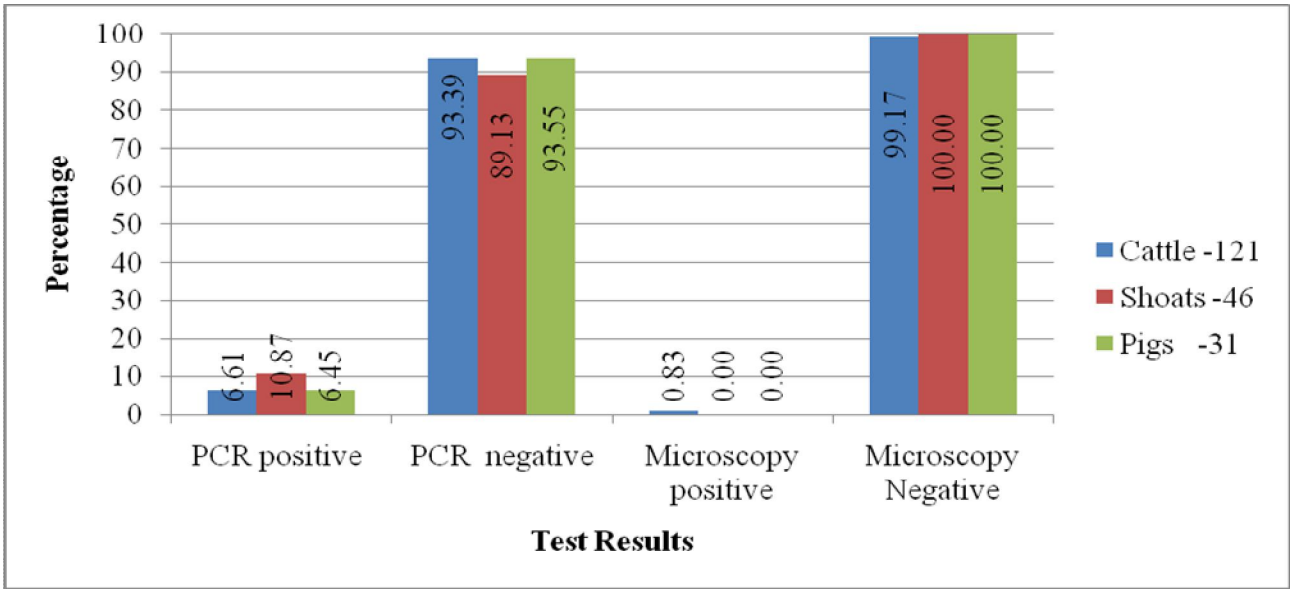


The results show a moderate prevalence of between 5.88 to 8.26 % in all species under PCR with Microscopy giving only 1.68 % in cattle in Rumbiye of Funyula Sub County.

4.3.4.12 HAT parasites prevalence Odiado

Domestic animal samples in Odiado were collected at a communal watering and herd health programs grounds located on Latitude 0.285^0 North and 34.156^0 East at an altitude of 1296 metres above sea level with results shown in Figure 4.17. The findings show a moderate to high prevalence of between 6.45 to 10.87 % in all species under PCR with microscopy giving only 0.83 % in cattle in Odiado of Funyula sub County.

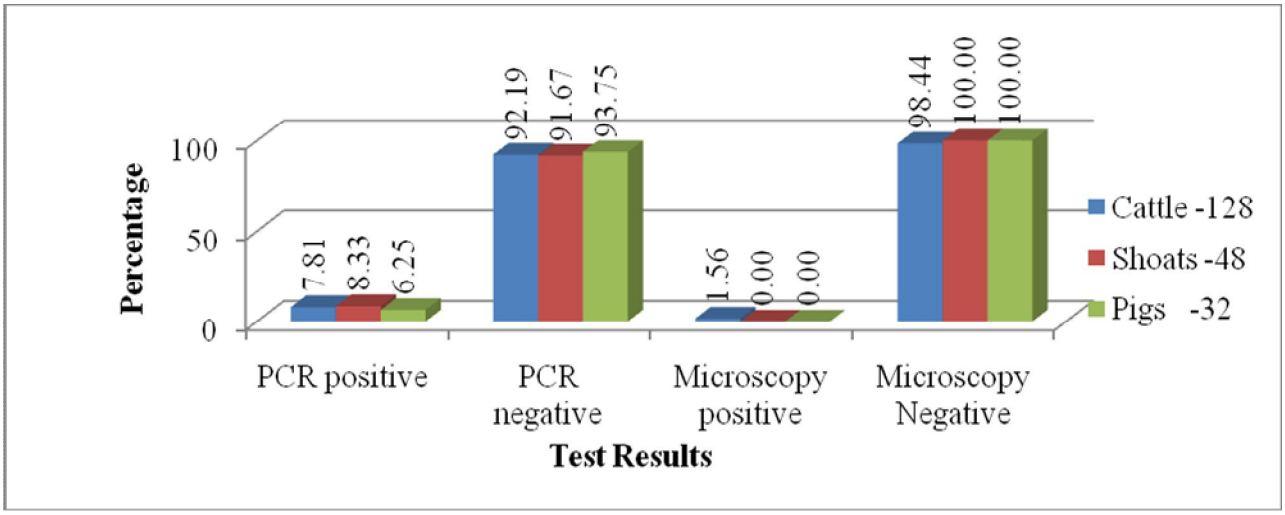
Figure 4.17: Odiado results



4.3.4.13 HAT parasites prevalence Sibinga

Findings are reported in Figure 4.18 below.

Figure 4.18 Sibinga results

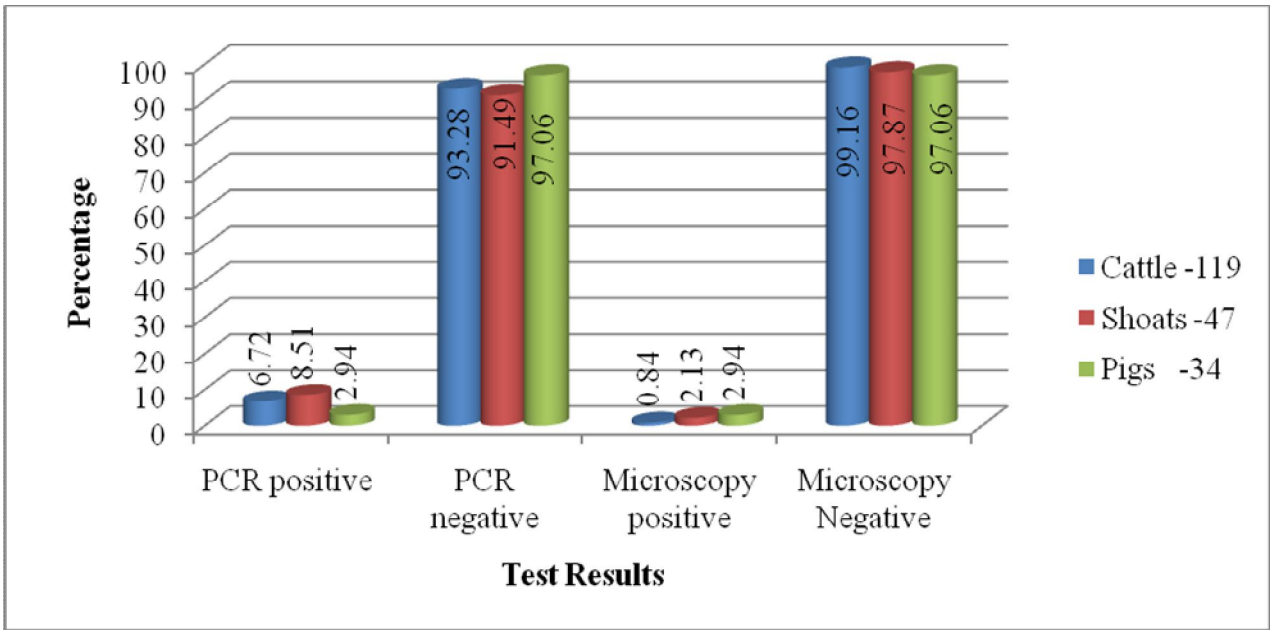


Samples in Sibinga were collected at a working crush pen located on Latitude 0.354^0 North and 34.119^0 East at an altitude of 1159 metres above sea level. The results reveal a moderate prevalence of between 6.25 to 7.81 % in all species under PCR with Microscopy giving only 1.56 % prevalence in cattle in Sibinga of Funyula Sub County.

4.3.4.14 HAT parasites prevalence Budumbusi

Samples in Nambale were collected at a communal ground used for grazing and herd health programs located on Latitude 0.511^0 North and 34.209^0 East at an altitude of 1180 metres above sea level. Figure 4.19 shows a moderate prevalence of between 2.94 to 8.51 % in all species under PCR with Microscopy giving a high of 2.94 in pigs in Budumbusi.

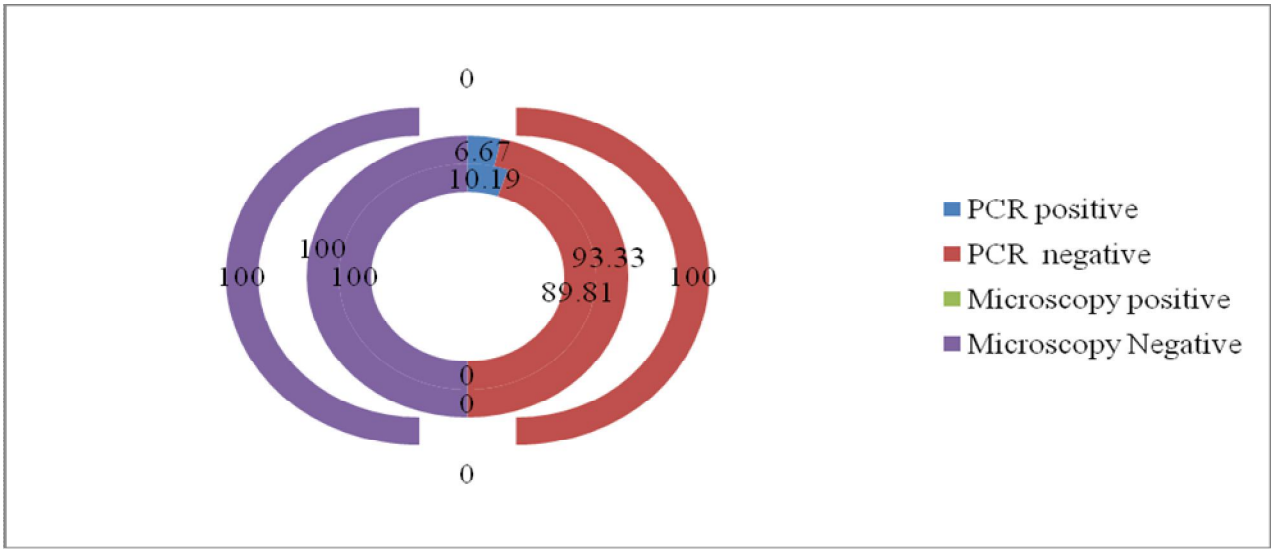
Figure 4.19: Budumbusi results



4.3.4.15 HAT parasites prevalence Rugunga

Domestic animal samples in Rugunga were collected at a communal ground used for grazing and herd health programs located on Latitude 0.076^0 North and 33.999^0 East at an altitude of 1142 metres above sea level. Figure 4.20 shows a moderate to high prevalence of up to 10.19 % in cattle under PCR with Microscopy recording no parasites in Rugunga of Budalangi Sub County.

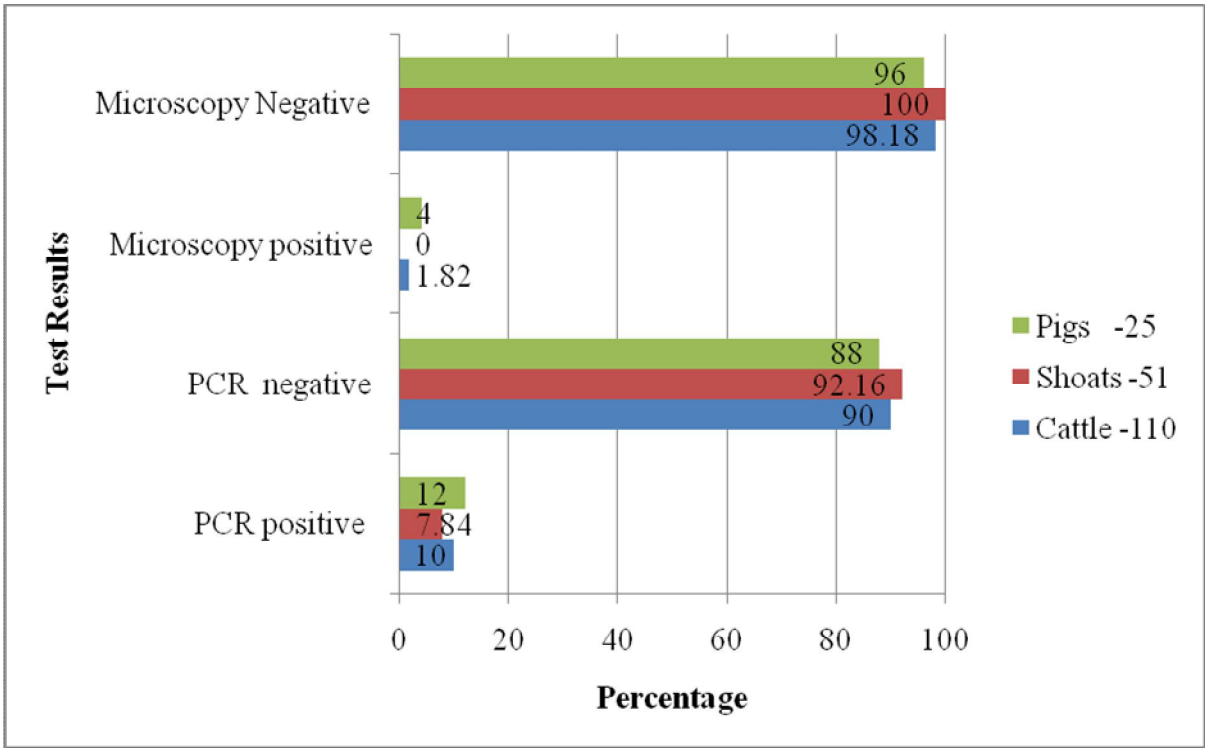
Figure 4.20: Rugunga results



4.3.4.16 HAT parasites prevalence Busangwa

See Figure 4.21 below.

Figure 4.21: Busangwa results



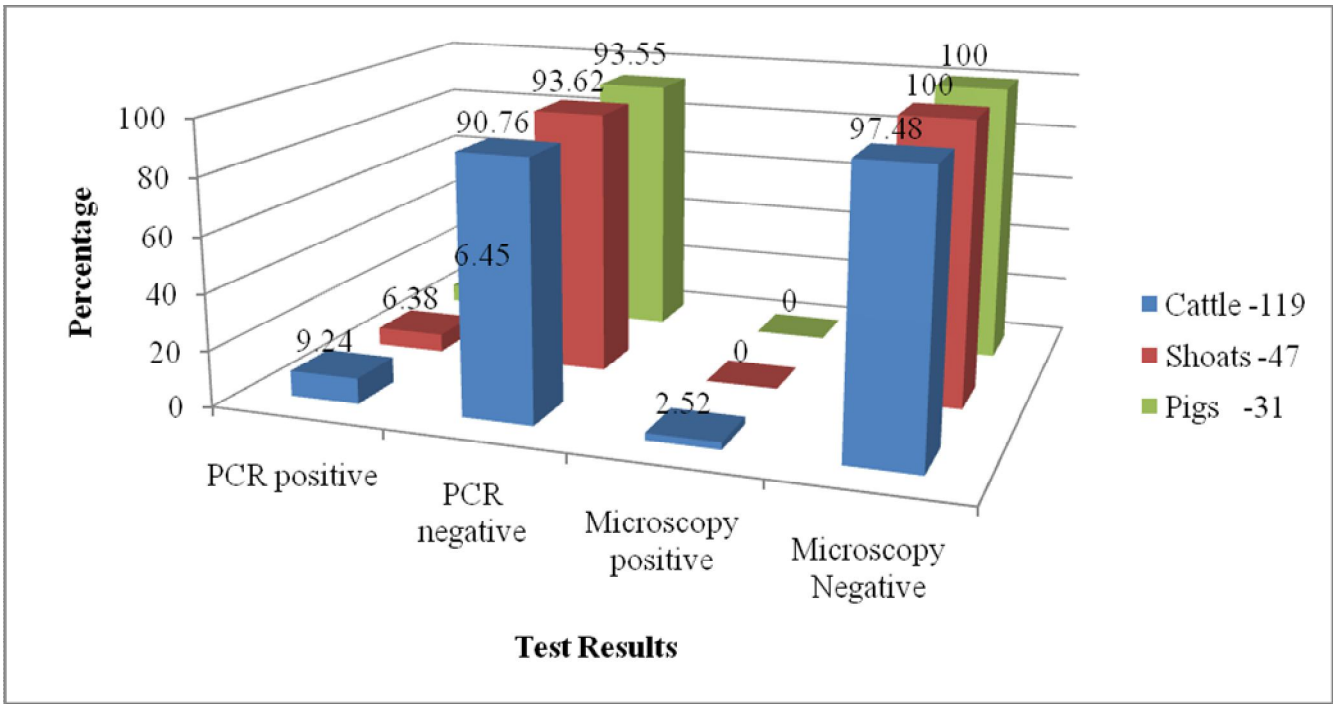
Domestic animal blood samples in Busangwa were collected at a communal ground used for grazing and herd health programs located on Latitude 0.118^0 North and 34.048^0 East at an

altitude of 1146 metres above sea level. The results reveals a moderate to high prevalence of up to 10.00 % in cattle under PCR with Microscopy recording upto 4% prevalence of parasites in pigs sampled in Busangwa of Budalangi sub County.

4.3.4.17 HAT parasites prevalence Bukuyudi

Results are displayed in Figure 4.22.

Figure 4.22: Bukuyudi results

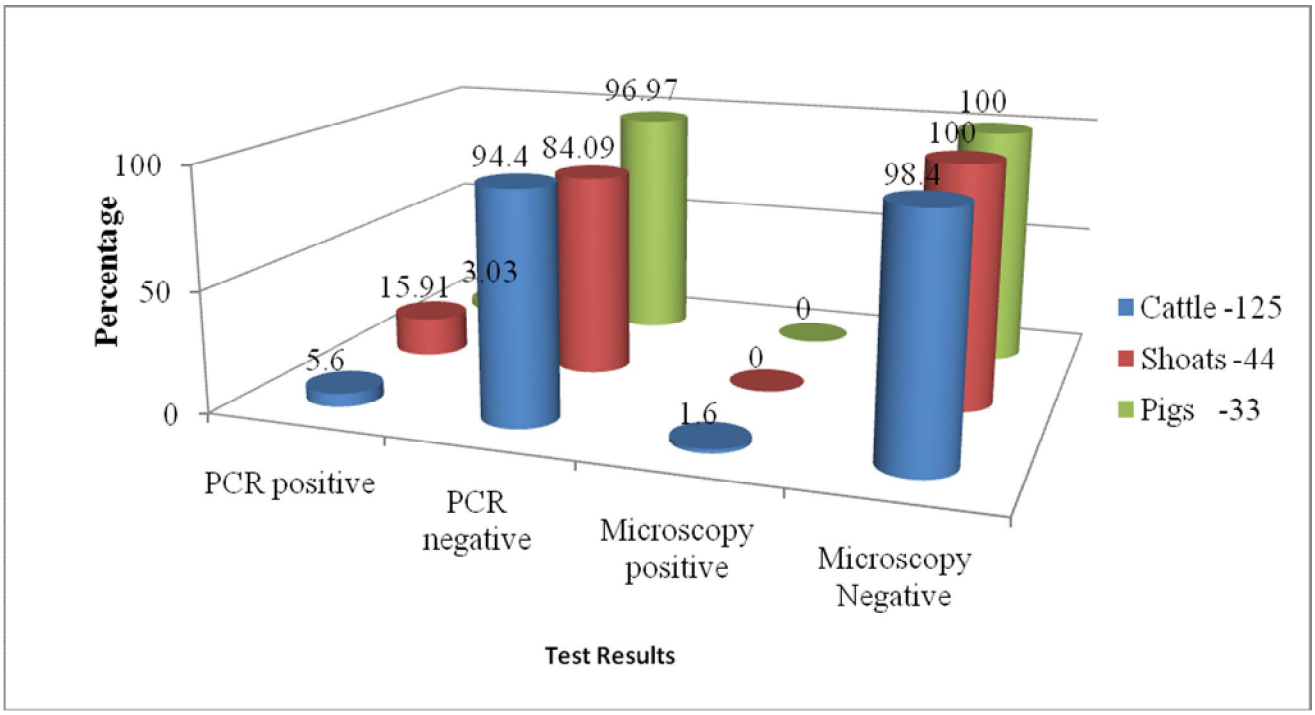


Livestock blood samples in Bukuyudi were collected at a communal ground used for grazing and herd health programs located on Latitude 0.353^0 North and 34.323^0 East at an altitude of 1254 metres above sea level. Figure 4.22 shows a moderate to high prevalence of between 6.45 to 9.24 % in all species under PCR with Microscopy giving 2.52% in cattle in Bukuyudi site of Butula Sub County.

4.3.4.18 HAT parasites prevalence Bukhalalire

Livestock blood samples in Bukhalalire were collected at a communal ground used for grazing and herd health programs located on Latitude 0.318^0 North and 34.274^0 East at an altitude of 1315 metres above sea level. Figure 4.23 shows a moderate to very high prevalence of between 3.03 to 15.91 % in all species under PCR, Microscopy giving 1.6% in cattle in Bukhalalire of Butula sub County.

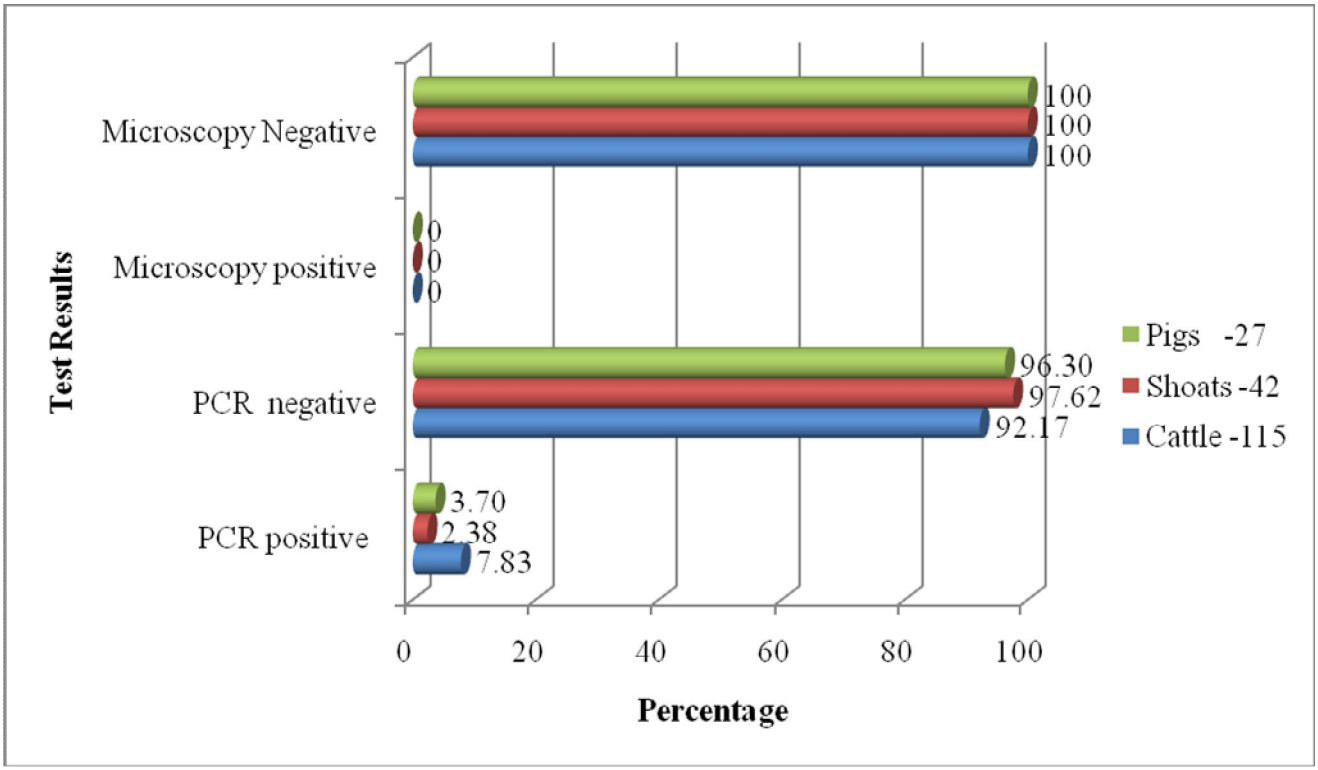
Figure 4.23: Bukhalalire results



4.3.4.19 HAT parasites prevalence Emauko

Livestock blood samples in Bukuyudi were collected at a communal ground used for grazing and herd health programs located on Latitude 0.390^0 North and 34.376^0 East at an altitude of 1269 metres above sea level. Figure 4.24 shows a low to moderate prevalence of between 2.38 to 7.83 % in all species under PCR, Microscopy recording no parasites in Emauko of Butula sub County.

Figure 4.24: Emauko results



4.4 Discussion

The study analyzed the extent of agreement between microscopy and PCR as diagnostic tests for HAT and further determined the sensitivity and specificity of microscopy. Diagnosing *T.b rhodesiense* HAT has traditionally been based on the use of clinical examination, parasitological techniques and microscopy where an attempt is made to detect parasitaemia in peripheral blood especially in early infections. As reported in this study during the focus group discussions in chapter three, adequate knowledge of animal reservoirs and transmission routes enables medical practitioners to focus on key areas related to the disease and hence reach the definitive diagnosis easier and earlier enough for prompt management of the disease, similar to an earlier finding when measuring disease in populations in East Africa, (Hyder and Morrow, 2006). The extensive cross sectional data set collected for the present study, achieved coverage of over 70% of the livestock population in the sampling sites ensuring representativeness. This permitted a detailed analysis of the prevalence of

trypanosome parasites at the local level using PCR a sensitive molecular tool, which increased the number of trypanosome infections detected at least six fold when compared directly to the microscopy results from the same set of samples This was higher than the two fold increase earlier reported in a similar study on the use of PCR assay for the detection of *trypanosoma spp* in naturally infected dairy cattle, (Clausen *et al.*, 1998).

Cattle were identified as the livestock species with the highest prevalence of all trypanosome parasites or infection at 9.2%, whereas the prevalence in shoats and pigs was at 8.1% and 5.7 % respectively. The prevalence in pigs differed significantly in all the sampling sites contributing to a potential active source of infection for humans as shown in the results from individual site prevalences of HAT parasites in the porcine hosts. In a previous study of the densely populated agropastoral district of Busia, livestock were found to be the main trypanosome reservoir of epidemiological significance since wild animals are in negligible in population, (Wissmann, 2011). The study reported 8.4% and 1.3% prevalence for all trypanosomes was found in livestock under PCR and microscopy respectively creating the need for development and use of sensitive tests that can provide the true disease picture.

Under PCR SRA a total of 47 livestock were carriers of human African Trypanosomiasis, *T.b.rhodesiense* parasites comprising of 33 cattle, 6 shoats and 8 pigs as seen in table 4.4b above. In a previous study in the same area in Busia, Wissman reported that Human infective *T. b. rhodesiense* were detected by PCR in a total of 19/1260 cattle (1.5%) and 9 out of the 312 pig samples (2.9%) a finding corroborated in this study. PCR can detect subclinical infections with very low parasitaemia, (Wissman *et al.,* 2011). However even low grade infections must still be regarded as transmissible as only a single trypanosome is required to infect a tsetse fly, (Wellburn and Maudlin,1989) and it has been demonstrated that even during chronic, low parasitaemic phases of *T. brucei* infections in cattle, sufficient parasites

are present to infect tsetse. Cattle are the most important reservoir of *T. b. rhodesiense* in this region, (Hide *et al.,* 1996), with up to 18% of cattle infected in an epidemic focus in Uganda, (Welburn *et al.,* 2001). Therefore the results obtained are extremely important for planning control programs.

The study findings creates a need for active surveillance of the disease in humans to validate the reports from National and WHO reports , as shown in Table 2.1 indicating that there is no HAT in Busia while the routine passive surveillance is based on pasitological tests through microscopy. PCR yields a better test outcome over microscopy indicative of the contribution of the choice of the diagnostic test towards under reporting of HAT and AT cases in Busia County due to the fact that microscopy is largely used in the laboratories of the health facilities. Previous observations of a high prevalence of PCR detected trypanosomiasis in shoats at between 20–25% in Busia District by Ng'ayo *et.al,* in 2005 were not replicated in this study. The present results support previous findings in a similar study in Western Kenya and Eastern Uganda, in which cattle were identified as the most important reservoir of trypanosomiasis, with low levels of infection being detected in small ruminants and highly variable infection prevalence depending on sampling sites seen in pigs, (Wissmann *et al.,* 2011).

Differences in trypanosome prevalence between livestock species have previously been attributed to reduced susceptibility of small ruminants resulting in a low or transient parasitaemia, or lower exposure of small ruminants to tsetse bites. The latter was supported by the identification of cattle and pigs as the major source of blood meals of both *Glossina fuscipes fuscipes* and *Glossina pallidipes* in this region, (Waiswa *et al.,* 2003). Protection of cattle from pathogenic trypanosome infections is at the centre of productivity motivated control strategies in this region, (Wamwiri *et al.,* 2007). In recent years PCR has become

widely applied for the detection of trypanosomes overcoming many of the constraints of parasitological and serological techniques, being highly sensitive and specific for trypanosome detection. Individual species specific multicopy trypanosome DNA sequences can be targeted to easily identify the parasites, (Welburn *et al.,* 2006). PCR assays able to detect all pathogenic trypanosome species in a single reaction have also been developed, (Njiru *et al.,* 2005). These reduce the costs of screening a sample from an endemic area by up to five times and have been suggested as suitable for large scale epidemiological studies, (Thumbi *et al.,* 2008). The study utilized the PCR ITS1 test to identify all the pathogenic trypanosome species. In recognition of these research findings PCR SRA results was utilized in this study as the gold standard for testing sensitivity of parasitological results through microscopy.

The use of PCR for detecting trypanosome DNA is the most reliable and accurate technique available for the specific identification of natural animal infections for most trypanosome species and sub species, (Njiokou *et al.,* 2004). To further discriminate *T. b. brucei* from *T. b. rhodesiense* PCR reactions targeting the single copy serum resistance associated (SRA) gene has been developed and applied, (Xong *et al.,* 1998). These human infective parasites coexist in all species other than man, with the morphologically identical parasite species *T. b. brucei* which only infects non human mammalian hosts. All subspecies of *T. brucei* are able to coexist with a range of other morphologically distinct trypanosomes which are pathogenic only to livestock such as *T. vivax, T. congolense* and *T. simiae* (in pigs) which affect livestock, (Welburn *et al.,* 2006). This explains the need to characterize the whole range of trypanosomes found in the blood of domestic animals to obtain the actual prevalence and clear disease burden.

The use of ITS-PCR in diagnosis of African trypanosomes allows the identification of several trypanosome species in the same reaction. This is both a saving in time and cost, as less PCR reactions need to be carried out to gain an understanding of the prevalence of trypanosomes in an area. Although FTA cards have simplified collection and transport of blood samples but since often DNA is not spread homogenously over the matrix, (Picozzi *et al*., 2002 and Cox *et al.,* 2010), I chose to use vacutainer bottles to carry blood samples. Although this can be overcome by taking several punches from each FTA card, it can be time consuming and expensive.

Use of parasitological techniques confirmed by microscopy, produces a relatively smaller proportion of livestock blood samples found to be infected or as carriers of various species of trypanosomes estimated at 16% of those from PCR results. The very low sensitivity and very high specificity values of the parasitological tests compared to PCR creates a need for a clear policy shift when identifying the suitable methodology for routine diagnosis of HAT. Routine diagnosis under laboratory field conditions for trypanosomiasis using classical parasitological approaches shows poor sensitivity. This is in part, due to the normally very low peripheral parasitemia of naturally infected animals (Wastling and Welburn,2011 and Masake 2002). The limitations of microscopy for both human and animal trypanosomiasis screening led to the development of a range of serological tests such as the complement fixation test (CFT), indirect fluorescent antibody test (IFAT), card agglutination test (CATT) and enzyme linked immunosorbant assay (ELISA). However, these methods are unable to differentiate between existing infections and previous exposure to infection, and may lack specificity (Luckins 1977 and Welburn *et al*., 2001).

PCR has been become widely applied for the detection of trypanosomes overcoming many of the constraints of parasitological and serological techniques, being highly sensitive and

specific for trypanosome detection. Individual species specific multicopy trypanosome DNA sequences can be targeted to identify parasites. Highly conserved ribosomal RNA (rRNA) genes are also useful for comparisons between closely related species. The internal transcribed spacer regions (ITS) in particular are relatively small, show variability among related species and are flanked by highly conserved segments to which PCR primers can be designed (Luckins 1977 and Welburn *et al*., 2001).

The kappa statistic (or kappa coefficient) is the most commonly used statistic to measure agreement of diagnostic tests. Though kappa may be affected by the prevalence of the finding under observation, methods to overcome this limitation have been adopted. The effect of prevalence on Kappa is less than that on predictive values of the disease under consideration. The choice of this analytic method was important considering that the level of agreement between microscopy and PCR, where the prevalence of HAT causing parasites is supposedly very low, although the residents normally feel the disease is present. Understanding of kappa and recognizing this important limitation will allow one to better analyze this results in reporting interobserver agreement. Kappa makes no distinction among various types and sources of disagreement. Because it is affected by prevalence, it may not be appropriate to compare kappa between different studies or populations. Nonetheless, kappa provides much more information than a simple calculation of the raw proportion of agreement or related statistical methods. There was no agreement between microscopy and PCR results for all types of trypanosomes among different livestock types ranging from cattle, sheep and goats, and pigs implying that many positives results from PCR are missed by microscopy.

The sampling site or region had no significant influence on detection level of HAT with there being no agreement between microscopy outcome and PCR for different livestock types and

for the different regions. This confirms the hypothesis that PCR yields a better test outcome over parasitological results through microscopy thereby contributing to under reporting of HAT and AT cases in Busia County, a result that is not influenced by the choice of area or region sampled. Other than the cattle population, we summarize the Kappa statistic result for all the livestock types as provided in Table 4.7 for all trypanosomes when the results on microscopy are compared with PCR. There was no agreement between microscopy outcome and PCR for all types of trypanosomes among different livestock types ranging from cattle, sheep and goats, and pigs. The Kappa statistic for comparison of the incidence for the two tests on *T.b.rhodesiense* in the other livestock types is provided in Table 4.8 above when the results on microscopy are compared with PCR. There was no agreement between microscopy outcome and PCR for *T.b.rhodesiense* among different livestock types ranging from cattle, shoats and pigs.

The very high specificity and very low sensitivity of microscopy reported in this study creates the need for a clear interpretation. Different cut points yield different sensitivities and specificities where the cut point determines how many subjects will be considered as having the disease. The cut point that identifies more true negatives will also identify more false negatives and the cut point that identifies more true positives will also identify more false positives. If the diagnostic (confirmatory) test is expensive or invasive there is need to minimize false positives or use a cut point with high specificity. If the penalty for missing a case is high and the disease is fatal and treatment exists, or disease easily spreads then maximize true positives that is, use a cut point with high sensitivity. Diagnostic testing can be used to discriminate cases with a target disease from the select population without it such as sensitivity and specificity.

Since using paired indicators can be a disadvantage in comparing the performance of competing tests, especially if one test does not outperform the other on both indicators, I in addition used the odds ratio as a single indicator of diagnostic performance. To measure the extent of disagreement between microscopy and PCR for all types of trypanosomiasis, the study utilized the odds ratio for two groups of samples thus those that turned positive for PCR and those that turned negative for PCR. The diagnostic odds ratio is closely linked to existing indicators, it facilitates formal meta analysis of studies on diagnostic test performance, and it is derived from logistic models, which allow for the inclusion of additional variables to correct for heterogeneity. The odds ratio is an important option that is not prevalence dependent, and may be easier to understand, as it is a familiar epidemiologic measure. The diagnostic odds ratio (DOR) of a test is the ratio of the odds of positivity in disease relative to the odds of positivity in the non diseased or can also be considered as the ratio of the odds of disease in test positives relative to the odds of disease in test negatives. These characteristics lend the DOR particularly useful for comparing tests whenever the balance between false negative and false positive rates is not of immediate importance. Sensitivity and specificity are expressions of the conditional hit rates of the test.

The odds of being positive for any trypanosome under microscopy is 228% large if one was tested positive under PCR. We are almost very certain that the test would turn out to be positive under microscopy if it was positive under PCR. To measure the extent of disagreement in estimating the extent of estimating *T.b.rhodesiense* from the two tests, we use the odds ratio for the two different sets of tests thus those that turned positive for PCR and those that turned negative for PCR. Interest is to compute the odds of being positive under microscopy test for each PCR (positive or negative) category. This indicates that the odds of being positive for *T.b.rhodesiense* under microscopy is 1569% large if one was tested positive under PCR. We are almost certain that the test would turn out to be positive under

microscopy if it turned positive under PCR. There was no agreement between microscopy and PCR outcomes for the different regions or sites sampled. The choice of the site did not influence the results obtained from PCR and microscopy limiting the differences observed to be due to the sensitivities of the tests and the variety host species for *T.b.rhodesiense*.

Anaemia status identified through display pallor of mucous membranes and further characterized by packed cell volume (PCV) has been shown to be moderately sensitive for the detection of trypanosome infections, with a sensitivity of 51% being recorded using a cutoff point of PCV below 34% as indicator of a blood parasite infection especially Trypanosomiasis, (Simarro *et al.*,2008). In this study low PCV indicative of anaemia was useful in helping determine which livestock received treatment with trypanocidals or any other medications which in turn motivated animal owners to keep participating in the study due to the potential benefit if the livestock were found positive for trypanosomiasis or any other disease especially the tick borne diseases during the survey. Although anaemia can be caused by factors other than tsetse transmitted trypanosomiasis, it remains one of the most important indicators of trypanosomiasis in cattle. As trypanosomiasis is a herd problem, the PCV profile of a herd is influenced by the number of trypanosome infected livestock and can be used to indicate differences in disease challenge. The average PCV is also influenced by the age and level of genetic susceptibility of cattle. While in the present study pallor of the mucous membranes was elected as an indicator of anaemia to reflect the criteria on which veterinary clinicians or animal health workers would base their treatment decisions, the previous finding meant additional clinical findings were necessary.

The advantages reported through the use PCR help in detection of a significant proportion of subclinically infected cattle, which contributes to the reservoir of trypanosome infection in this endemic area is important in surveillance and control programs. With very low average

profit margins on livestock production, there is limited scope for sophisticated diagnostic procedures and block treatment of cattle. Treatment of visibly ill cattle with trypanocidal drugs, as practiced at present limits immediate economic losses at the household level but is unlikely to impact on the reservoir of infections and impact on transmission of the parasite, which would be necessary for sustainable control,(Machila, 2005).

The prevalence of HAT parasites in animal blood samples can scientifically be extrapolated to estimate the prevalence human blood samples taking into consideration the vector, hosts, environment characteristics to inform the transmission dynamics. The comparatively low prevalence of *T.b. rhodesiense* detected in cattle and pigs during the current study nevertheless still poses a threat to human health in this area of Western Kenya, as was demonstrated by a case of sleeping sickness reported from Busia District in early 2006 (WHO,2006b) . Only sporadic cases of sleeping sickness cases have been reported from Busia over the last ten years. It has been suggested that anthropogenic changes, especially increased cultivation, control programs such as the Food and Agriculture Organization (FAO) and African Development Bank (ADB) supported PATTEC program now managed by KENTTEC Parastatal have played a role in reducing the tsetse habitat and tsetse densities and thus reducing the overall probability of transmission to humans , (T&T Eradication Strategy, 2010). However, a degree of under detection of human cases, as has been reported for Uganda, (Odiit *et al.,* 2005), plays a key role in the low number of sleeping sickness cases reported from Busia.

4.5 Conclusion

The study was conducted in three different time periods and from 7 different sub counties in Busia County Kenya to establish the period prevalence of HAT causing parasites in domestic animals. Microscopy had very low sensitivity and very high specificity failing to capture

many true positives and PCR was reported to be five to six fold more accurate estimator of prevalence. There was no agreement between microscopy outcome and PCR for all types of trypanosomes among different livestock types ranging from cattle, sheep and goats, and pigs. The choice of the sampling site did not influence the results obtained from PCR and microscopy.

Diagnosis has traditionally been based on parasitological techniques confirmed by microscopy thereby contributing to underreporting the disease. The presence of HAT parasites in animal blood samples and the tsetse vector being present necessitates the occurrence of the disease in humans. Considering that routine HAT surveillance is based on microscopy which is less sensitive than PCR implies that the comparisons of the two tests can shed light on the extent of the prevalence of HAT which can aid in reducing underreporting based on the various prevalence findings.

The prevalence of HAT causing parasites in animal hosts serves as a good indicator of the disease prevalence considering the transmission dynamics. Increased use of molecular techniques such as PCR in diagnostic procedures is essential for improving HAT diagnosis.

CHAPTER FIVE

5.0: A MODEL TO ESTIMATE THE PREVALENCE OF ZOONOTIC AFRICAN TRYPANOSOMIASIS BASED ON THE PREVALENCE OF THE PARASITE IN DOMESTIC ANIMALS TO MITIGATE UNDERREPORTING

5.1 Introduction

A deterministic model has been developed useful in estimating zoonotic Trypanosomiasis prevalence.This can aid in proper disease quantification based on the parasite detection in livestock under non epidemic or endemic situation. The benefit when used shall be to reduce underreporting and mitigate the burden of human African trypanosomiasis (HAT). This is important considering the complexity of HAT transmission that has discouraged the development of mathematical models of the sort that have been available for many decades for other vector borne diseases. The SIRS type epidemiological principle, based on the existence of **S**usceptible (S) hosts who get **I**nfected (I) and later either **R**ecover (R) or die or becomes **S**usceptible for **R**e infection, was used to model the vector and host populations interactions in order to make inference about the prevalence. In such a model, the progression from state S to state I clearly involves disease transmission which is determined by the prevalence of the parasite in the population or source of infection, the underlying susceptible population, the contact structure, the transmission agents or vectors and the probability of transmission.

The compartmental deterministic SIRS model based on the natural history of the infection, accuracy and time period over which model predictions are required has the advantage of enabling analysis of broad disease determinants. Over a wide range of field conditions there is a simple linear relationship between the daily probability of susceptible livestock contracting trypanosomiasis and tsetse fly challenge with the latter being the product of fly infection rate

and apparent density, a crude but acceptable relative measure of tsetse population size ,(Rodgers, 1988). I have developed an explanatory model for the human African trypanosomiasis that incorporates two vertebrate host species thus human and livestock and one tsetse vector species where the equations define the relationship between the hosts and the vectors. The model based on the SIRS premise can be used to quantify prevalence useful in estimating underreporting of HAT given the prevalence of *T. b. rhodesiense* in domestic animal reservoirs, (Trottier, 2000). Most models of infectious disease processes used until now are deterministic since they require less data, are relatively easy to set up and because the computer software is widely available and user friendly. The dynamics of an SIRS based model are now well understood so that deterministic models can be commonly used to explore many aspects of measuring disease determinants.

5.2 Methodology

I developed the model by utilizing the results from chapter 4 on the prevalence of HAT causing parasites in blood from livestock obtained from all the study sites in Busia County.

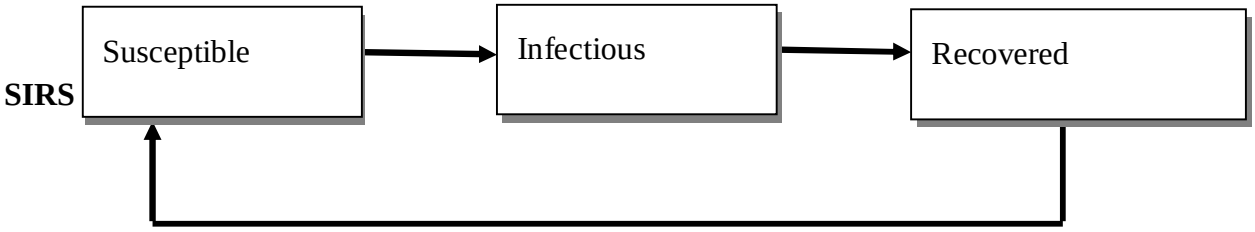
The findings on the prevalence of HAT causing parasites established from all the study sites were used for the purpose of modeling. Prevalence data of the parasites in blood samples from livestock were collected from the nineteen (19) sites within Busia County. As reported in chapter 4, a total of 3799 animal blood samples were analysed comprising of 2288 from cattle, 917 from shoats (sheep and goats) and 594 from pigs. It was reported that 320 blood samples were positive for all forms of trypanosomes under PCR comprising 211 from cattle, 75 from shoats and 34 from pigs. Under microscopy a total of 51 blood samples were positive for all forms of trypanosomes under microscopy comprising 40 from cattle, 7 from shoats and 4 from pigs. While for *T.b.rhodesiense* 47 blood samples were positive under PCR comprising of 33 from cattle, 6 from shoats and 8 from pigs. Under parasitological techniques

and microscopy (confirmed by PCR) for *T.b.rhodesiense* a total of 9 samples were positive comprising 6, 1 and 2 from cattle, shoats and pigs respectively.

Dataset used for modeling purposes, containing counts of the positive and negative samples under PCR and microscopy were recorded and kept in a Microsoft Excel spread sheet (Microsoft Corporation, Redmond, USA). I developed the model through the Software for Model Development in R- Statistics as shown in appendix V. The model incorporated dynamic transmission based on contact between the vector, the human and the livestock hosts with the force of infection being dependent upon the number of infectious individuals dictated by the HAT parasite prevalence and the vector population, (Trottier, 2000). The desired outcome was what happens on average in the population of human and livestock hosts and the vector population. Input parameters were estimated based on existing literature and the model population was stratified into broad sub groups such as those who are susceptible, infectious or recovered. The model described the transmission of infection using the total number of individuals in these compartments or population categories based on difference equations or differential equations in built in the R-software.

The SIR type deterministic compartmental model was deemed suitable to answer the research question since such models are capable of defining the rates of change in different populations that are interacting such as livestock, human and vector populations, (Rodgers, 1988; Matt and Pejman, 2008). The model is also suitable for estimating population dynamics when data sets are not available, once an optimal choice of parameters and satisfactory initial conditions are defined. The SIR model can be constructed following the steps outlined in Figure 5.1 but since HAT infections do not confer immunity and after recovery an individual remains susceptible it is then modified as SIRS, (Matt and Pejman, 2008).

Figure 5.1: Susceptible Infectious Recovered Susceptible model flow chart



5.3 Data collection

The combined results of the prevalence of HAT causing parasites in blood samples from domestic animals obtained from all the study sites were used for the purpose of modeling. The standard SIR model has four parameters consisting of the birth rate, the natural death rate, the average infectious period or conversely the rate of recovery and the transmission rate that were based on results from previous studies for a successful model development.

5.3.1 Parameters identified for data analysis

I utilized the Greek letters to define the parameters in the system as seen in Table 5.1.

Table 5.1: Model parameters

Parameter	**Characteristic**	**Reference**
alpha_1=0.212	Rate at which vector (tsetse fly) bite livestock, which corresponds to 7 bites per 33 tsetse fly lifespan days assuming 1 bite in 3 days for a fly	Bett, 2008
alpha_2=0.09	Rate at which tsetse fly bite Humans, which is 3 bites per 33 tsetse fly lifespan days	Bett, 2008
mu_c=0.0009	Natural mortality rate of livestock (including slaughter) per day. Livestock life span is about 3 to 10 years. If 5 years (average) , then 1/mu_c= 1825 days	Assumption as natural average lifespan based on experience
mu_v=0.03	Natural mortality rate of vector per day. We define the tsetse fly vector life span of 1/0.03=33 days	Hargrove, *et al.*, 2012, McDermont and Coleman 2001

mu_h=0.0000527	Natural mortality rate of Humans per day. We take the human life span of 1/0.0000527=52*365 days=52 years	KDHS, 2010
k_c=0.0006	Disease induced death rate in livestock per day, which is 6 per 10000 livestock per day	Assumption as an Inverse of calving rate
k_h=0.0001	Disease induced death rate in human per day, which is 1 per 10,000 people per day as assumed to die of sleeping sickness on average. This is arguably large, hence k_h is close to zero should be used in sensitivity analysis	KDHS , 2010 and death rate and contribution of HAT morbidity
tau_1=0.62	Transmission probability from vector to livestock. 62 out of 100 bites per day lead to livestock infection from vector	Rodgers,2000, Hargrove, *et al.*, 2012
tau_2=0.065	Transmission probability from livestock to vector. 65 out of 1000 bites per day lead to vector infection from livestock	Rodgers,2000, Hargrove, *et al.*, 2012
tau_3=0.065	Transmission probability from human to vector. 65 out of 1000 bites per day lead to human infection from vector	Rodgers,2000, Hargrove, *et al.*, 2012
tau_4=0.05	Transmission probability from vector to human. 5 out of 100 bites per day lead to vector infection from humans	Rodgers.,2000, Hargrove, *et al.*, 2012
epsilon=0.2	Survival rate of vector. 20% vector survival rate	Stephen *et al.*,2011
Lambda_c=0.00055	Livestock recruitment rate. 55 per 100,000 new livestock births per day	Stephen *et al.*,2011
Lambda_v=0.015	Vector recruitment rate (Range 0.0075, 0.015) 14 per 100 new adults tsetse fly recruited per day	Hargrove *et al.*,2003
Lambda_h=0.000126	Human recruitment rate (we consider an estimate equal to death rate). 45 per 1,000 per year = 46 per 365000 new human births per day	KNBS,2010 Census Report
psi_h=0.02	Treatment rate, humans at 2% per day	Stephen *et al.*,2011
psi_c=0.09	Treatment rate, livestock at 9% per day	Stephen *et al.*,2011
gamma_v = 0.08	Vector control measures at 8% per day	Stephen *et al.*,2011

Picture of the biology of the disease covering the duration of the period of infectivity, incubation period, transmission vectors are important. Further, data on the demographic, epidemiology and biologic characteristics of the infection (transition rates) and the population (birth and death rates) are essential parameters, (Stephen *et al.*, 2011).

5.3.2 Initial conditions

Using the Kenya Demographic and Health Survey (KDHS,2012) demographic data for Kenya, particularly for Busia County, I noted the following information for the year 2009 from the KNBS,2010 Census report and as extrapolated to 2012 is shown in Table 5.2 below. The total area of Busia is 1694.5kilometres and the water mass is 215 square kilometers. From the above data, I used the following initial condition values in the model simulation for the base year 2009.

Table 5.2 Initial conditions data

Characteristic	**Class**	**Quantity**
Human Population Estimate as at 2012	Males=387,693 Females=422,295	809,988 (KNBS,2013)
Urban Population (Not susceptible to HAT) in 2009	Males 37,418 Females 40,008	77,426
Rural Population (Susceptible to HAT) in 2009	Males 323,415 Females 353,820	677,325
Livestock Population as at 2009 Census	Cattle 163,795 Sheep 31,741 Goats 85,773 Pigs 48,788	328,557 (KNBS,2010)
Susceptible human population estimated by the model		780,000
Tsetse population (PATTEC,2011)	Based on fly catches	20 (Flies per Trap per Day)

5.4 Data analysis

Model development was done using the R Statistics software. Dataset used for modeling purposes, containing counts of the positive and negative samples under PCR and microscopy were recorded and kept in a Microsoft Excel spread sheet (Microsoft Corporation, Redmond, USA). Test results were appended to this spreadsheet and samples classified as trypanosome positive if they were positive for any of the detectable trypanosome species by microscopy or PCR SRA for *T.brucei rhodesiense.*

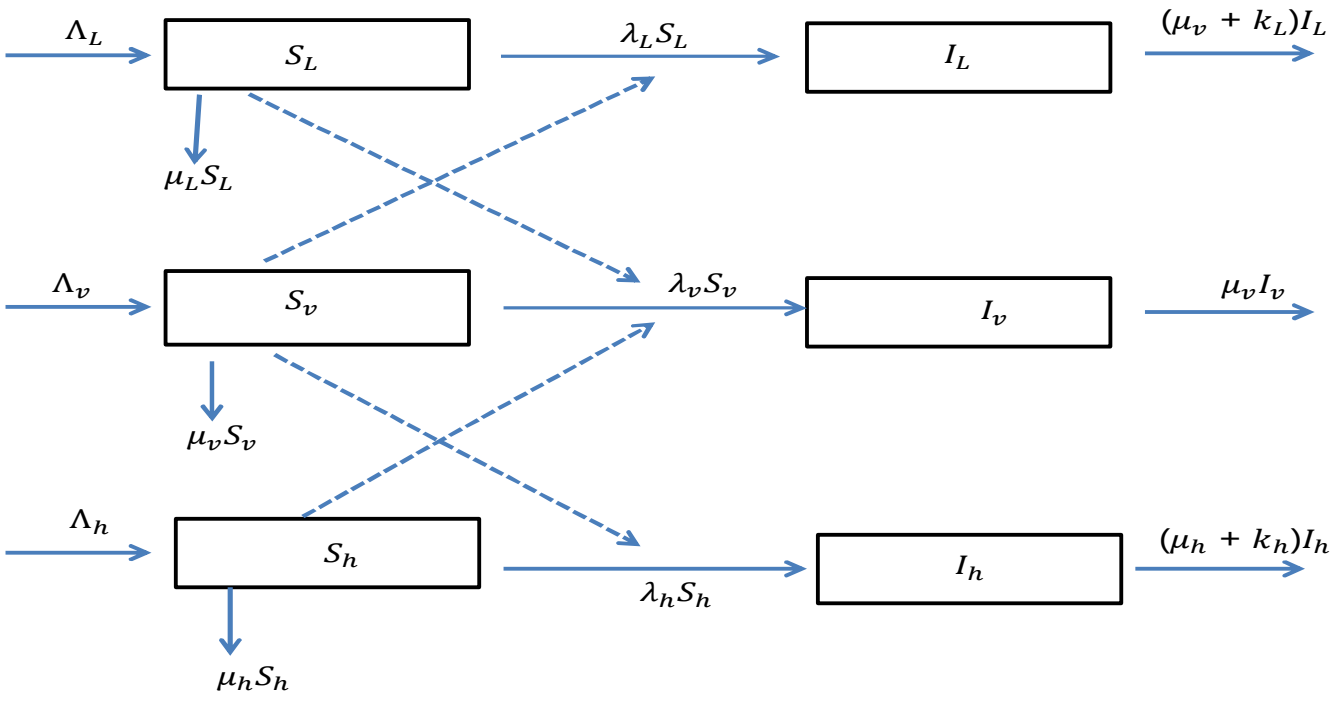
From the initial conditions above, the analysis utilized values in our model simulation, starting from the base year 2009.There was 1 case of HAT reported in 2009, assuming that the prevalence was very low as at only 1 out of 1000 individuals, one can hypothesize 780 HAT cases in the community in 2009 out of a population of 780,000 estimated by the model. This was an estimate on the upper side and a confidence region of (0 to 780) could be used in sensitivity analysis for simulation. This is corroborated since the neighbouring Tororo and Busia Uganda Districts have a much higher HAT prevalence, (Odiit *et al.,* 2005). The livestock population in Busia County was estimated at 328,557 livestock in 2009 and based on a prevalence rate of 1% as reported by Von Wissmann (2011) it was assumed there were about 3286 carrier livestock in 2009. Based on Flies Trapped per Day (FTD) , there were reports of over 50 FTD's in 2008/9 dropping to 10FTD's in 2010/11 period, (PATTEC ,2011) necessitating working with very small numbers for the vector population. The assumption made for the model was that there were only 20 tsetse flies the average of the two FTD figures down from 50 due to the ongoing vector control initiatives and half of them numbering 10 were considered to be carriers of the HAT parasite, (Stephen *et al.,* 2011).

5.5: Results

5.5.1: Model structure

The structure of the underreporting model is as shown in Figure 5.2 below where the lines joining the compartments signify the rates of movement from one compartment to another. The dotted lines show the interaction between the vector and the human/livestock populations. Infected vectors bite and infect the susceptible human and livestock population, while susceptible vector also get infected when they bite an infected human or livestock. For transmission of HAT to occur it is important to have the presence of domestic animals as reservoirs, tsetse as the vector and humans as definitive hosts.

Figure 5.2: The Structure of the model showing compartments of the human, vector and livestock populations.



The interactions where we denote the compartments of the susceptible populations by:

S_L: for the susceptible livestock;

I_L: for the infected livestock population,

S_V: for the susceptible vector (tsetse fly);

I_V: for the infected vector population and

S_h: for the susceptible human population;

I_h: for the infected human population.

The transmission of *T. b. rhodesiense* between the 3 host types namely livestock, the vector and humans is captured by the proposed model. The elements in the first 2 columns represent transmission from tsetse to humans or livestock and those in the first 2 rows account for transmission from vertebrate hosts to tsetse .Arrows show the rate of movement/interaction across compartments.

5.5.2 Infectivity of the human African trypanosomiasis

This is the overall probability of a contact between the vector and the hosts in this case livestock and humans to be able to cause an infection. Considering the force of infection as the per capita rate at which susceptible individuals become infected with a pathogen I consider the equations for livestock and human infections in the following manner:

For livestock infection from vectors,

$$\lambda_L = \alpha_1 \tau_1 \epsilon \frac{I_v}{S_L + I_L}$$

For human infection from the vector

$$\lambda_h = \alpha_2 \tau_4 \epsilon \frac{I_v}{S_h + I_h}$$

For the vector infection from both human and livestock population,

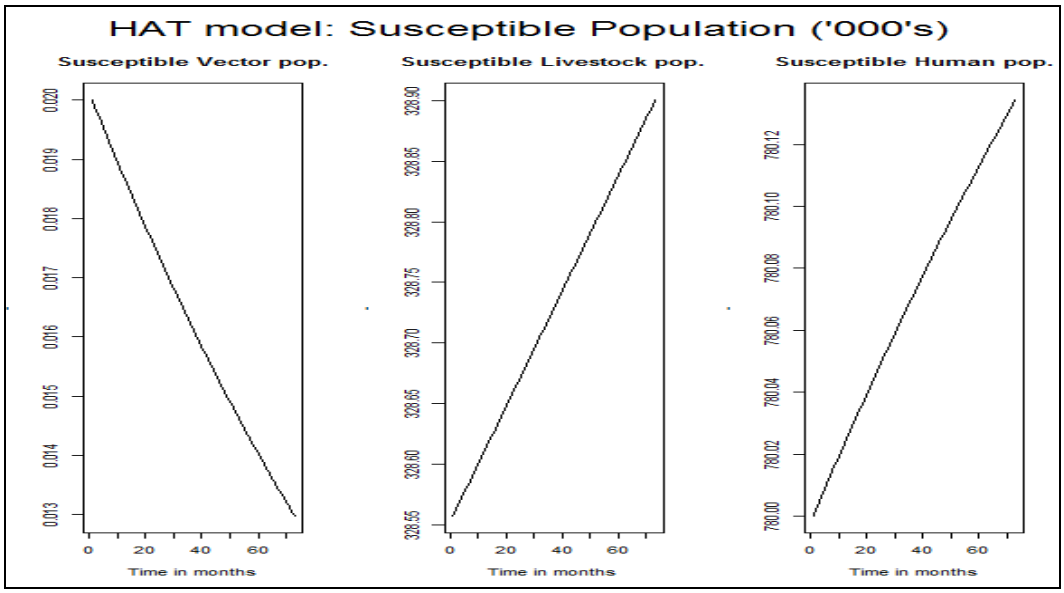
$$\lambda_v = \alpha_1 \tau_2 \frac{I_L}{S_L + I_L} + \alpha_2 \tau_3 \frac{I_h}{S_h + I_h}$$

The model dynamics between the vector, humans and livestock is shown in Figure 5.2.

5.5.3 Human African trypanosomiasis prevalence model

Figures 5.3, 5.4 and 5.5 shows the underreporting model based on the model structure shown in Figure 5.6 and 5.2. The R Statistics software codes used in the simulation of this model are included here in Appendix V. The model helps to understand how HAT spreads in the real world, the complexities affecting the dynamics and actually, to estimate the true population of infected human, livestock and tsetse fly populations.

Figure 5.3: Susceptible population for all the population compartments.



The initial time along the x-axis is 2009 January. The time steps are given in months, until year 2015 January and the simulation is run annually for six years. The vector population decreases due to vector control measures that include deforestation, chemical control, among others). The rate of control is assumed to be about 9% per day on the vector population, (Stephen *et al.*, 2011) hence the steady decline of vector populations to very low levels. Both the susceptible human and livestock population are allowed to increase following the natural demographics.

Figure 5.4: Infected populations in each compartment

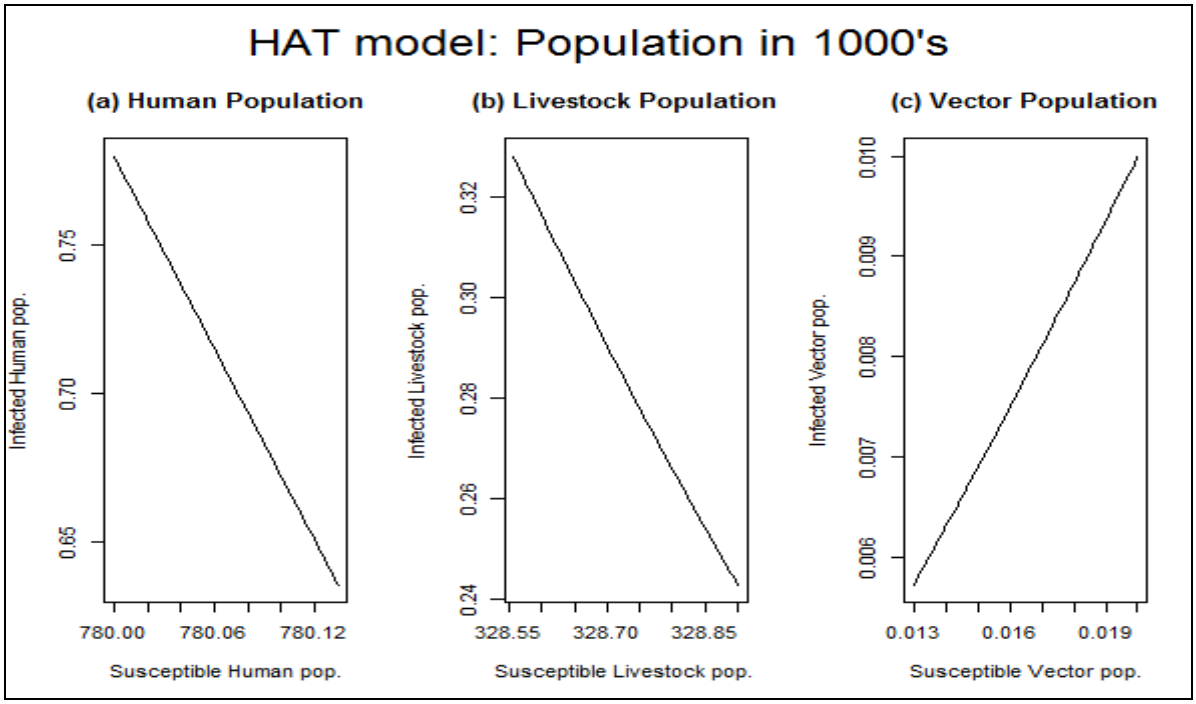
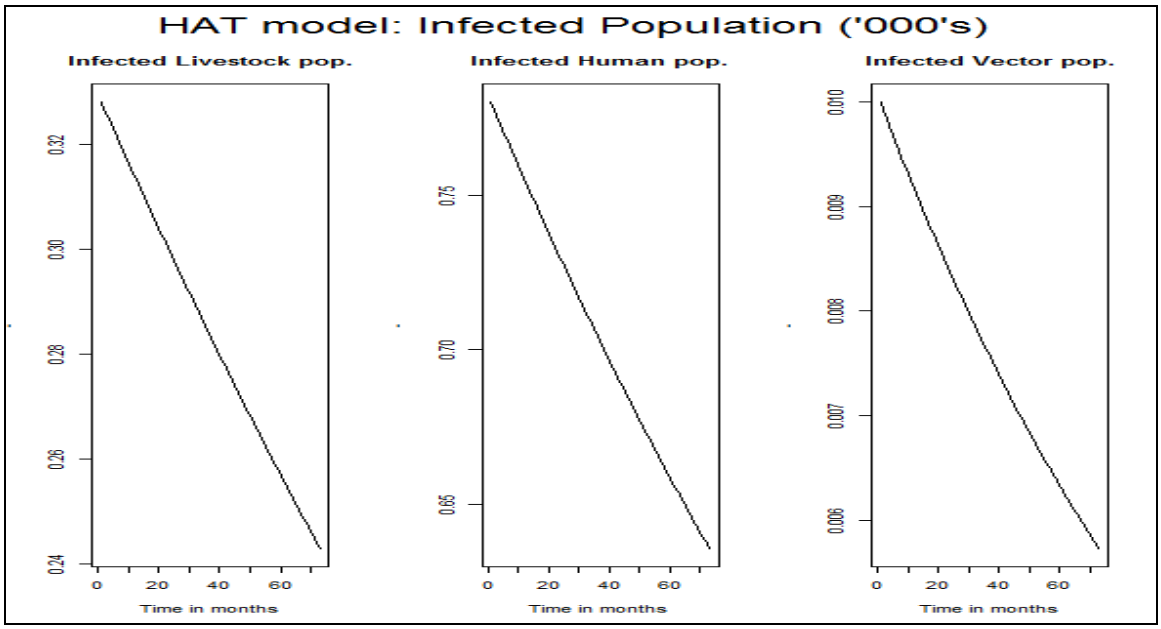


Figure 5.5: Summary of simulated populations in each compartment



5.5.4 Using the HAT model for estimating underreporting

Underreporting masks the true magnitude of disease incidence and reduces the efficiency of the notification system and surveillance potential. Burden of Disease estimates have to be adjusted to take care of underreporting by utilization of techniques such as modeling. Table 5.3 gives the indicative prevalence rates estimated by the model upto to the end of 2014 in livestock and human populations.

Table 5.3: Simulated model populations in 1000's by end of year 2014.

Ser.No.	Year	Month	S_human	S_vector	S_livestock	I_human	I_vector	I_livestock
67	…	…	…	…	…	…	…	…
68	2014	Aug	780.127	0.0133	328.876	0.644	0.005	0.248
69	2014	Sep	780.129	0.0132	328.881	0.642	0.005	0.247
70	2014	Oct	780.134	0.0132	328.886	0.640	0.005	0.246
71	2014	Nov	780.131	0.0131	328.891	0.638	0.005	0.245
72	2014	Dec	780.132	0.0130	328.895	0.637	0.005	0.244

The red shaded value of 637 individuals shows the number of infected humans by December 2014, while the green shaded value of 244 livestock shows the predicted livestock population that is HAT infected. The estimated HAT prevalence by December 2014, i.e., human population with infection is about 637 individuals, i.e,

HAT Prevalence = (637/780132) x 100

= 0.0816%

≈ 0.1% of the entire population

5.6 Discussion

The prevalence model developed in the study predicted a HAT prevalence of 637 people out of the total estimated population by the end of the year 2014 of 780,132 which contradicted the WHO Reports that indicated that there were no HAT cases in Kenya moreso Western Kenya (Busia) since 2009. The prevalence was modeled from the data collected in the 2011/12 period. Within the livestock population, the model estimates approximately 244 livestock as being carriers of HAT parasites out of the total livestock population of 328,895 at the end of 2014, a prevalence rate of 0.074%.

This model estimates under detection for *T. b. rhodesiense* SS in humans based on data from domestic livestock blood samples analyzed for prevalence of HAT parasites. This model was developed based on the framework models for the vector borne diseases involving two vertebrate host species and one insect vector species previously applied to the animal African trypanosomiasis. The model allows for incubation and immune periods in the two host species and for variable efficiency of transmission of different trypanosome species from the vertebrates to the vectors and vice versa. Health and veterinary professionals and decision makers can use this model to estimate HAT prevalence after validating its robustness and help define which regions in the country need prioritization and funding for SS control

activities. The model can be applied to SS endemic areas to improve the quantification of HAT which has traditionally relied on the calculation of the disability adjusted life years (DALYs) during epidemic situations. Critical parameters for estimation of prevalence of *T. b. rhodesiense SS* is the proportion of flies that remain refractory to the parasite, followed by population parameters determining the abundance, composition and life span of tsetse flies a key challenge that requires close monitoring for successful use of this model.

Neglected tropical diseases (NTD's) such as HAT are by definition under observed, often because of limited access to health care or lack of diagnostic or recording capabilities. This position is compounded by the difficulties due to indirect measures of infection which leads to reported cases which do not reflect the true dynamics which can be effectively be addressed through modelling . More investigation leads to better detection leading to more cases while a reduction of reported cases can be a consequence of either effective control or a breakdown in surveillance. Based on the WHO estimates that assumes zero HAT prevalence in Kenya in the year 2015, the model helps to estimate the HAT prevalence since the predicted numbers are slightly above zero figures. The study utilizes data from the prevalence of the HAT parasite in domestic livestock to estimate human prevalence.

The basic reproduction number R_0 is a key quantity routinely defined for single host pathogens as the expected number of secondary cases arising from a primary case in a wholly susceptible population while in the case of multi host pathogens, the definition is less clear, but an extrapolation can be made. This takes the form of a next generation matrix that describes the growth of multiple types of infected as there is more than one type of host where the dominant eigen value of this matrix gives an R_0 with the same properties and a similar interpretation as in the single host species case, (Stephen *et.al*,2011) . The biological parameters to which R_0 is sensitive provide hypotheses about what factors determine the

distribution of a parasite since if R_0 is less than 1, then the parasite will be absent or only sporadically present via introductions and if R_0 is greater than 1 then epidemics are possible, (Stephen *et al.*,2011) . Many of the challenges which face modellers of directly transmitted pathogens are compounded when modeling the epidemiology with indirect transmission whether through environmental stages, vectors, intermediate hosts or multiple hosts. When the R_0 is less than 1, the disease may progressively disappear from the population because the exploding potential of the epidemic reverses especially for directly transmitted diseases with one host.

The exploding potential of an epidemic is based on a large number of contacts between infectious and susceptibles, thereby generating many secondary cases. In the reversed situation, the frequency of these contacts is rare because herd immunity level in high, consequently, susceptibles can hardly turn over to exposeds and, later, to infectious because most contacts occur among non-infectious. In other words, when R<1 one infected cannot make sure that, on average, the parasite an animal or human being carries will successfully be transmitted to at least one susceptible. In this situation, an epidemic cannot thrive. The low value of the basic reproductive rate of HAT in the human population means that infection levels in humans are determined almost entirely by characteristics of the non human reservoir or hosts thus thus the animals . Ideally, humans do not contribute many new infections in the ecosystem. This implies that a greater reduction in human disease may be achieved by treating the livestock reservoir rather than the humans. It is on this basis that that this study has focused on utilizing the prevalence of HAT causing parasites in domestic animals because this is what sustains the disease considering a very low R_0 in humans unable to sustain infection.The focus on domestic animals as presented in this model is critical in successful HAT surveillance because as reported in previous studies contribution to the basic rate of reproduction (R_0) of the human infective *T. brucei* is only 0-11 from the human hosts and 2-

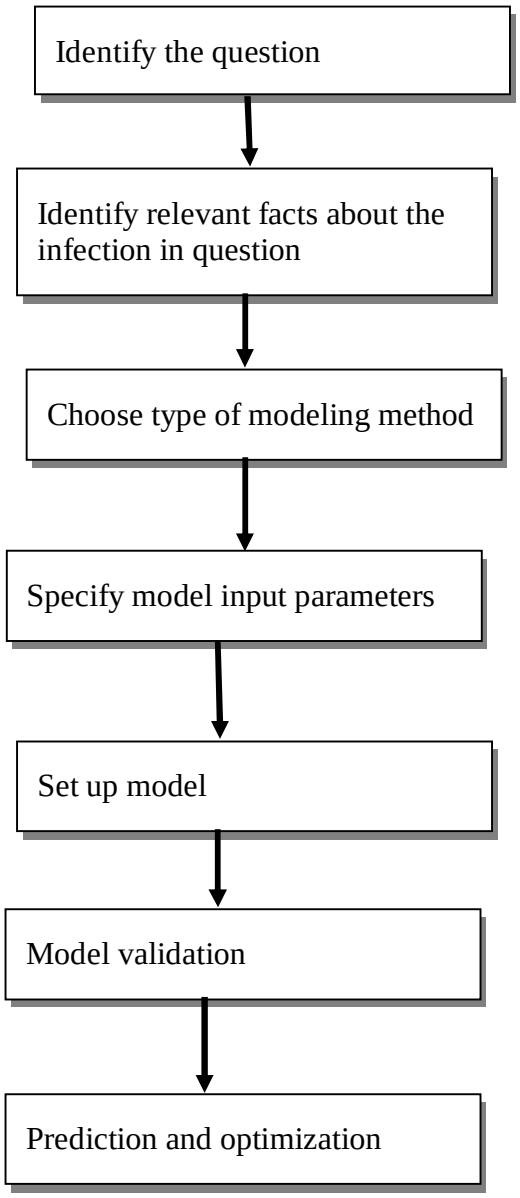
54 from the livestock hosts . Based on this, it is clear that human sleeping sickness cannot be maintained in the human hosts alone, (Rogers, 1988). The livestock reservoir is therefore crucial in determining not only the continued occurrence of the disease in humans, but its prevalence in these hosts as well. Identifying the precise role of the livestock reservoir may suggest that treating such livestock will achieve a greater reduction of human sleeping sickness than direct treatment of the humans alone. The model recognized only one vector although Busia is reported to be infested with both G. *fuscipes* and *G. pallidipes* since the latter has rarely been trapped, (Waiswa *et al.,* 2006, PATTEC, 2011).

The major problem with all the infectious disease models is that the contact pattern in the population is often unknown and difficult to measure however the current study has utilized the vector behavioral characteristics reported in previous studies. Notwithstanding the models with their current limitations can be used to better the understanding of dynamics of infection spread in a population. The complexity of modeling parasitic diseases such as HAT is associated with the complex life cycles in various hosts with some not experiencing direct reproduction in certain hosts and therefore the burden of infection can increase with re-infection, (Hollingsworth *et al.,* 2015).

This study has quantified contributions of host and vector species for vector borne infections such as HAT with complex reservoirs, the risk of cross species transmission being determined by the spillover force of infection. The utilization of vector measurements and prevalence of HAT parasites to estimate prevalence and infection risk in humans is an important risk assessment tool. This is despite the fact that the relationships between entomological measures of infection and human risk complicate the interpretation of such surveillance data where only models can be used to formalize and test this data. The traditional model

development that follows the steps as outlined in Figure 5.6 based on Matt and Pejman (2008) have now been simplified through the use of the R-software.

Figure: 5.6 Steps in model development



The modeling challenges that were experienced in this study included the difficulty in exploiting the impacts of combinations of control measures and whether there are epidemiological and or evolutionary synergies to using multiple control measures. These findings were similar to what was documented in a similar study in Eastern Uganda, (Hollingsworth *et al.*, 2015). In related underreporting studies, the case fatality rate on admission of *T. b. rhodesiense* SS to LIRI hospital was approximately 6% with 40 reported

deaths compared to an estimated 500 undetected deaths during the 1988–1990 epidemic peak similar to that estimated through modeling. Therefore, for every one patient died during the epidemic on admission approximately 12 were dying unseen (Odiit *et al.*, 1997). Humans and livestock are not as mixed as the model assumes complicating accurate estimation of the opportunities for tsetse flies to take feeds from both livestock and humans. While *T. b. rhodesiense* have a wide range of vertebrate host types including humans, livestock and wildlife, the latter plays a negligible small role in Busia, (Fevre *et al.*, 2008).

The model depicted a declining vector population of the tsetse flies indicating an effective control program being in place as the livestock and human populations grew. Sustained tsetse control that lowers the mean survival rate of flies over a period of years should have strong effects on sleeping sickness control. The human data based under detection models have estimated the undiagnosed deaths that had sought health care in a past outbreak (2000–2002) resurgence of *T. b. rhodesiense* SS around LIRI hospital in Tororo district Uganda. During the outbreak it was estimated that approximately 85% of the patients who died undiagnosed entered the health system at some stage and that one third of those that did enter the health system died undiagnosed, (Fevre *et al.*, 2008).

Currently, the major burden of SS is because of morbidity and mortality, but if a greater proportion of cases could be detected, almost all of the deaths would be preventable. There is a lack of reliable statistics on under detection of disease in sub Saharan Africa because of amongst other reasons, infrastructural and costs constraints, (Snow *et al.*, 1999). This model can be used to generate this important data. As a result, disease estimates are often guesses based on a clinical diagnosis and occassional parasitological or other diagnostic tests. While relative disease burdens during periods of endemic and epidemic periods in themselves are

important, it would be a mistake to calculate a DALYs for an epidemic and assume that this was then the more general baseline level of burden for longer term planning even under endemic conditions, (Fevre *et al.,* 2008). The study has endavoured to develop a model serve as a tool for estimating HAT prevalence under endemic conditions. When HAT burden is determined for epidemics or outbreaks this should be explicit so that generalization is avoided to non epidemic situations. The utilization of this compartment model shall aid in obtaining the actual prevalence of HAT instead of blanket reports of recent decreases in the number of reported HAT cases could lead to a reduction in effort in detecting future cases, a situation which can lead to disease resurgence.

The model output can justify the costs arising from treating cattle and other livestock to restore their health since the trypanocidal drugs used help to clear infection and they are effective against the trypanosomes that are pathogenic to cattle as well as the zoonotic *T. b. rhodesiense*. Lowering the incidence of sleeping sickness by treating the animal reservoir will reduce future costs of treating human patients. Sleeping sickness tends to affect the poorest and most disenfranchised rural communities with the least access to health care. Public health messaging and extension services are urgently needed to improve knowledge and reporting of these diseases.Cattle are a major reservoir of HAT parasites and therefore the risk of transmission increases with large cattle populations. Cattle often graze around villages and farming areas and occasionally, they are moved to local markets for trading. Thus, people at risk of contracting HAT in these areas are mainly cattle keepers, although all villagers are also exposed to a certain level of risk. The treatment of cattle with antitrypanosomal drugs combined with their elective insecticide impregnation has been shown to be effective for Rhodesiense HAT control by reducing the domestic animal reservoir and the vector density.

5.7 Conclusion

The proposed model, based on a measure of prevalence of HAT parasites in the blood of livestock, provides a practical method that may be used across sub Saharan Africa in specific foci to estimate the prevalence of HAT in humans based on the determined prevalence of the parasites in domestic animals. The data obtained after extrapolating prevalence of HAT parasites from domestic animals can be a useful estimate of the disease occurrence.This can therefore can help determine the level of under detection of *T. b. rhodesiense* and the consequent burden of the disease. The modeling is an important method to gauge the effects of interventions aimed at increasing SS diagnosis and treatment coverage. The underreporting model of *T. b. rhodesiense* SS developed utilizes the prevalence of HAT causing parasites in livestock to estimate the prevalence in Humans under non epidemic conditions which is critical in quantifying the extent of underreporting of HAT in the Busia foci and beyond. This should help in data and information generation. The model is beneficial in assisting researchers and planners to gain insight into the factors controlling the persistence and stability of HAT parasites within large communities.

Accurate disease burden estimates are crucial to predict the likely impact of and resources needed for control efforts where models need to be developed that can combine patchy data to fill the gaps and produce reliable burden estimates in the absence of routine surveillance

After correction for Underestimation (UE), preferably by species, age and sex, surveillance and notification data become a better estimate for evidence based and comparable disease burden estimations. However, since adjusting for UE results in higher disease burden estimates and can result in diseases with differing ranks of public health importance compared with unadjusted surveillance data, care should be taken to clearly communicate both the need for such adjustment and the methodologies applied to adjust the raw data.

Research effort should therefore be concentrated in developing tools that improve the level of estimating the extent of the HAT burden. Finally it is hoped that a model such as the one described here will contribute to future studies aimed at improving quantification of African trypanosomiasis.

CHAPTER SIX

6.0 SUMMARY AND RECOMMENDATIONS

6.1 Summary

The study intended to analyze factors contributing to underreporting of human African trypanosomiasis (HAT) and further quantify underreporting through utilization of prevalence data of the causative parasite in the blood of domestic animals and feedback from medical practitioners under a non outbreak or endemic conditions in Busia County. This was achieved by determining the knowledge and practices of medical practitioners in identification and management of human African trypanosomiasis (HAT). The study also assessed the level of agreement between routine field diagnostic tests and the Polymerase Chain Reaction (PCR) test in establishing prevalence of human African trypanosomiasis. Finally utilizing data from the livestock blood samples, a model useful in estimating the prevalence of HAT parasites in humans given that in livestock was developed. This model can be important in approximating the prevalence of HAT based on the results in livestock contributing information important for measuring the extent of underreporting human African trypanosomiasis (HAT) based on the parasite detection in livestock under non epidemic or endemic situation.

This study emphasized the need to improve surveillance of rhodesiense SS. Strategies that improve both passive and active case findings should be provided. These will include increased awareness among the affected communities, the health workers and a commitment of more resources for health services at the local level. HAT has a high mortality when untreated and therefore preventive measures such as chemotherapy of the livestock reservoir for trypanosomiasis can greatly reduce the risk of outbreaks occurring in rhodesiense SS endemic areas like Busia whose costs considering the savings given the prevalence rate predicted by the model. The model designed in this study provides an insight into the prevalence aiding data for use in assessing the cost effectiveness of these interventions by

quantifying the deaths that can potentially be averted. Such an explanatory and analytical model provide much greater insight into equilibrium and stability conditions and the threshold phenomena which are important in determining disease control strategies . Translation of knowledge into proper care of patients is among the critical areas in health care delivery which is only possible if health service providers have the right knowledge of health problems they are dealing with.

As we put emphasis on increasing the diagnostic capacity of diseases such as HAT, efforts should be made to equip the practitioners and the general communities with adequate knowledge of zoonoses to enable them seek these services. There is also an urgent need for accurate tools for the diagnosis of HAT. The existing tests for diagnosis especially for routine field use are not sensitive and specific enough due to the characteristically low number of parasites found in the blood of sleeping sickness patients. Diagnosis has traditionally been based on microscopy and although the microscopic techniques used under field conditions have limitations based on their sensitivity, they provide an important basis for investigations into the importance of trypanosomiasis in livestock and humans. The presence of HAT parasites in animal blood samples and the tsetse vector being present provides a basis for suspecting the occurrence of the disease in humans. Considering that routine HAT surveillance is based on microscopy which is less sensitive than PCR implies that the comparisons of the two tests can shed light on the extent of the prevalence of HAT which can aid in reducing underreporting based on the various prevalence findings.

6.2 Recommendations

PCR has improved the sensitivity and accuracy of diagnosing trypanosome infections when compared to the direct microscopic observation of parasites within infected blood. Large differences in prevalence as may be obtained by PCR and parasitological methods could have

a significant impact on the control strategies selected. Effective diagnostic tools need to be utilized during routine diagnoses in hospitals and health facilities to reduce the extent of under diagnosing and subsequent underreporting of HAT.

There is need to review the accuracy of available tsetse and trypanosomiasis maps. Attempts to control trypanosomiasis are based on large scale epidemiological studies, active detection and treatment of confirmed cases, combined with tsetse control programmes. The accuracy of these studies depends on the quality of the study design, and on the sensitivity and specificity of diagnostic methods used.

Knowledge and practices of medical practitioners of HAT and other zoonotic diseases is a critical factor that requires to be addressed as an important contributing factor to their under diagnosis and under reporting in Kenya. Refresher courses and continuing professional development programs on HAT and other zoonotic diseases should be conducted to medical practitioners. Emphasis should be put on zoonotic diseases especially HAT in teaching curricula of medical practitioners' training institutions in Kenya to improve the diagnosis, reporting and control of zoonotic diseases.

Under the one health initiative focus, veterinary and medical intersectoral collaboration should be strengthened to enable more effective control of zoonotic diseases in Kenya including sharing of information. Innovative educational approaches and incentives need to be addressed in targeting specific groups of medical practitioners to facilitate effective diagnosis and management of HAT.

The most effective approach in tackling rhodesiense HAT would be the reinforcement of a HAT diagnosis and enhancing the treatment capabilities of existing health care facilities in

endemic areas. This would ensure the rapid identification and treatment of cases, which coupled with vector control, would decrease vector density and subsequently diminish human vector contact.

Continuous research is needed to improve the current weaknesses of the control tools currently used, mainly to engage health services in HAT elimination. In the process of eliminating HAT, criteria and indicators must be established to monitor and evaluate the actions implemented. The contributions of different hosts to HAT parasites transmission remains a key knowledge gap in our understanding of these pathogens. There are many mathematical and statistical tools for estimating and analyzing transmission trees or infection processes for directly transmitted pathogens, but these are yet to be effectively adapted to vector borne modeling.

While more finances, material and human resources can be mobilized one should focus on increasing resources through training, integration of programmes, focused planning, motivation, facilitation, supervision and feedback, welfare, assignment, reassignment and delegation. Equipment availability can be improved by optimization and sharing procurements (purchase or donations/partnerships) of e.g. microscopes, centrifuges and consumables.

The involvement of other sectors is mandatory in order to eliminate rhodesiense sleeping sickness, it is therefore necessary to identify the sectors or stakeholders, conduct a needs assessment propose remedies. The main sectors involved in surveillance and control of sleeping sickness are livestock and agriculture, tourism, county government, finance, information, environment, wildlife, health, research and partners who require mobilization.

Underreporting is associated with under diagnosis due to lack of patient access in remote rural areas to services for HAT diagnosis and treatment. Problems with capturing data routinely that can be improved through capacity building to improve the index of suspicion, streamlining sleeping sickness within routine surveillance and establishing sentinel surveillance to complement routine data needs to be addressed to cost effectively control AT.

REFERENCES

Abel P.M., Kiala G., Loa, V., Behrend M. and **Musolf J. (2004).** Retaking Sleeping Sickness control in Angola. *Tropical Medicine & International Health* **9**: 141–148.

Aron J.L. and **May R.M. (1982).** The population dynamics of malaria. In: The Population Dynamics of Infectious Diseases, Theory and Applications, R.M. Anderson (Ed.), Chapman and Hall, London. pp. 139-179.

Artzrouni M. and **Gouteux J. P. (2001).** Population dynamics of sleeping sickness: A microsimulation. *Simulation and Gaming,* 32 (**2)** pp. 215-227.

ASDS, 2010. Agricultural Sector Development Strategy 2010–2020. Government of Kenya.

Asano K., Suzuki K., Asano, R. and **Sakai, T. (2003).** Investigation on zoonoses among veterinary technicians. *Kansenshogaku Zasshi*. 77 (**5**), 323-330.

Ashford R. W. (2000). The leishmaniases as emerging and reemerging zoonoses. *International Journal of Parasitology* **30:** 1269–1281.

AU, 2014: http://rea.au.int/en/RO/PATTEC Retrieved on 11th December 2014.

Barrett M.P. and **Okali R. (2006).** The rise and fall of sleeping sickness. *Lancet*. 9520 **(367)** :1377-1378.

Bell B.S., Hoskins R.E., Pickle L.W. and **Wartenberg D. (2006).** Current practices in spatial analysis of cancer data mapping health statistics to inform policymakers and the public. *International Journal of Health Geographics* **5**:49.

Bett B. (2008). An analysis of the effect of a tsetse repellant technology on the incidence of trypanosomiasis on the pastoral cattle and the potential use of technology in tsetse and trypanosomiasis control. Phd Thesis, University of Nairobi.

Bhalla N. (2002). Pan African group takes lead against the tsetse fly. *Lancet,* **359**:686.

Bossert T.J. and **Beauvais J.C.(2002)**. Decentralization of health systems in Ghana, Zambia, Uganda and the Philippines a comparative analysis of decision space. Health Policy Plan **17**: 14–31.

Budd L.T. (1999). DFID funded tsetse and trypanosome research and development since 1980. *Volume 2. Economic analysis.* London, UK, DFID.

Cattand P., Jannin J. and **Lucas P. (2001)**. Sleeping sickness surveillance an essential step towards elimination. *Tropical Medicine and International Health*, 5(**6**):348-361.

Chappuis F., Louis L., Simarro P., Veerle L. and **Philippe B. (2005).**Options for Field Diagnosis of Human African Trypanosomiasis . *Clinical Microbiological Reviews* 18**(1**):133.

Cheryl L. G., Marie-Josée J. M. , Dietrich P., Arie, H. H., Russell J. B., Piotr K., Karen L., Peterson I, Anke L Stuurman A., Eric M. F. and **Mirjam, E.E.K.(2014) .** Measuring underreporting and under ascertainment in infectious disease datasets: a comparison of methods: *BMC Public Health* 2014, **14**:147

Clausen P.H., Wiemann A., Patzel, R., Kakaire D. and **Poetzsch C. (1998).**Use of a PCR assay for the specific and sensitive detection of *trypanosoma spp* in naturally infected dairy cattle in peri urban Kampala, Uganda. *Annals of the New York Academy of Sciences* **849**: 21–31.

Cochran G.W. (1977). Sampling Techniques, Third Edition, John Wiley and Sons Publishers. 72-86

CIDP (2014). County Integrated Development Plan 2013-2017.

County Fact Sheet, (2015): http//www.opendata.go.ke , Retrieved on 17th April 2015.

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

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

Desquesnes M., McLaughlin G., Zoungrana A. and **Davila A.M.R. (2001).** Detection and identification of Trypanosoma of African livestock through a single PCR based on internal transcribed spacer of rDNA. *International Journal of Parasitolology,* **31**:610–614.

Desquesnes M., Ravel S., Deschamps J.Y., Polack B. and **Roux F. (2012).** Atypical hyperpachymorph Trypanosoma (Nannomonas) congolense forest-type in a dog returning from Senegal. *Parasite*, **19:** 239–247.

DFID (2006). Research Sleeping Sickness waking up to reality. Department for International Development, United Kingdom, Case study.

Dohoo I., Martin, W. and **Stryhn H. (2010).** Veterinary Epidemiologic Research. AVC Inc. Charlotteton, Prince Edward Island, Canada.

Doyle T.J., Glynn M.K. and **Groseclose S.L. (2002)**. Completeness of notifiable infectious disease reporting in the United States: an analytical literature review. *American Journal of Epidemiology* , 155 **(9)**:866-874.

ECDC, (2010). European Centre for Disease Prevention and Control (ECDC): Report: Surveillance and Prevention of Hepatitis B and C in Europe. Stockholm, Sweden.

FAO, (2012). Global geospatial datasets for African Trypanosomiasis Management a review. Food and Agriculture Organization of the United Nations, Animal Health Service.

Fevre E.M., Coleman P.G., Odiit M., Magona J.W., Welburn S.C. and **Woolhouse M.E.J. (2001).** The origins of a new Trypanosoma brucei sleeping sickness outbreak in eastern Uganda. *Lance*t **358:** 625–628.

Fevre E.M., Picozzi K., Fyfe J., Waiswa C. and **Odiit, M. (2005a).** A burgeoning epidemic of sleeping sickness in Uganda. *Lancet* **366:** 745–747.

Fevre E.M., Kaboyo R.W., Persson V., Edelsten M. and **Coleman P.G. (2005b).** The epidemiology of animal bite injuries in Uganda and projections of the burden of Rabies. *Tropical Medicine and International Health* **10:** 790–798.

Fevre E. M., Wissmann B. V., Welburn S. C. and **Lutumba P. (2008a).** The Burden of Human African Trypanosomiasis. *PLoS Neglected Tropical Diseases 2(12):**10**.1371*

Fevre E.M., Odiit M., Coleman P.G., Welburn S.C. and **Woolhouse M.E.J. (2008b).** Estimating the burden of rhodesiense sleeping sickness during an outbreak in Serere, eastern Uganda. *BMC Public Health* **8**: 96-102.

FITCA, (2005). Farming in Tsetse Controlled Areas. Kenya Project 1999–2006. Lessons Learned.

Fox R.J.A. and **Hanson K. (2001).** Calculating and presenting disability adjusted life years (DALYs) in cost effectiveness analysis. *Health Policy Plan* **16**: 326–331.

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

Gibson W. (2009). Species specific probes for the identification of the African tsetse-transmitted trypanosomes. *Parasitology*. **136**:1501–1507.

Gibson W., Backhouse T. and **Griffiths A. (2002).** The human serum resistance associated gene is ubiquitous and conserved in Trypanosoma brucei rhodesiense throughout East Africa. *Infection, Genetics and Evolution*. **1**: 207–214.

Gombe S.(2003). Sleeping Sickness. Epidemic, what epidemic? Medecine Tropicale: Revue du Corps de Sante Colonial Issue 362.

Goodman C.A., Coleman P.G. and **Mills A.J. (1999).** Cost effectiveness of malaria control in sub Saharan Africa. *Lance*t **354**: 378–385.

Gouteux J.P., Bansimba P., Noireau F. and **Frezil J.L. (1987).** Le couˆt du traitement individuel de la trypanosomiase a` T. b. gambiense dans le foyer du Niari (Congo). *Medecine tropicale revue du Corps de sante colonial's* **47**: 61-63

GOK, (2001): Effective management for sustainable economic growth and poverty reduction. Busia District Development Plan 2002–2008, Ministry of Finance and Planning Government of Kenya.

GOK, (2010): Income and Expenditure Estimates, Budget 2010/11

Gubler D.J. (1998). Resurgent vector borne diseases as a global health problem. *Emerging Infectious Diseases, **4**:* 442-450.

Hardnett F.P., Hoekstra R.M., Kennedy M., Charles L. and **Angulo F.J.(2004)**.Emerging Infections Program FoodNet Working Group: Epidemiologic issues in study design and data analysis related to FoodNet activities. *Clinical Infectios Diseases*, **38** (Supplement 3):S121-S126.

Hardy A. (2003). Animals, disease, and man making connections. *Perspectives in Biology and Medicine,* 2(**46**):200-215.

Hargrove J.W., Torr S.J. and **Kindness H. (2003**). Simulation of tsetse control using baits.

Optimized simulation of the control of tsetse flies *Glossina pallidipes* and *G. m. morsitans* using odour-baited targets in Zimbabwe. *Bulletin of Entomological Research* **93** issue 1:19-29

Hargrove J.W., Ouifki R., Kajunguri D., Vale G.A. and **Torr S.J. (2012).** Modeling the control of Trypanosomiasis using trypanocides or insecticide-treated livestock, *PLOS Neglected Tropical Diseases*, **6**:1615.

Hay S.I., Omumbo J.A., Craig M.H. and **Snow R.W.2000.** Earth observation, geographic information systems and Plasmodium falciparum malaria in sub-Saharan Africa. *Advanced Parasitology* , **47**:173-215.

Hide G., Tait A., Maudlin I. and **Welburn S.C.(1996).** The origins, dynamics and generation of Trypanosoma brucei rhodesiense epidemics in East Africa. *Parasitology Today* **12**: 50–55.

Hide G. (1999). History of sleeping sickness in East Africa. *Clinical Microbiology Review.* **12:**112-125.

Holden S.(1999). The economics of the delivery of veterinary services. *Revue et Scientifique et Technique.* **18**(2):425-439.

Hollingsworth T.D., Pulliam, J.R., Funk, S., Truscott, J.E., Isham, V. and **Lloyd, A.L.2015**. Seven challenges for modelling indirect transmission: vector-borne diseases, macro parasites and neglected tropical diseases. *Journal of Epidemiology* **10**:16-20

Hyder A., A. and **Morrow R.H.(2006).** Measures of health and disease in populations. Merson MH, Black RE, Mills A, Essentials of International health. 1–42.

IGAD, LPI, (2012). Intergovernmental Authority on Development, Livestock Policy Initiative

Irungu P., Nyamwaro S.O. and **Masiga D.K.(2002).** Financial implication of rearing sheep and goats under natural trypanosomosis challenge at Galana ranch Kenya. *Tropical Animal Health and Production*, **34**:503-513.

James K.Bartlett Joe, W., Chadwick, C. and **Chadwick, H. (2001).**Organizational Research: Determining appropriate sample size in Survey Research. *Information Technology, Learning, and Performance* Journal, **19,** No. 1, 43-49.

Jeppsson A., Okuonzi S.A., Ostergren P.O. and **Hagstrom B. (2004).** Using burden of disease/cost-effectiveness as an instrument for district health planning experiences from Uganda. *Health Policy* 70:261-270.

Kabasa J.D.(2007). Public-private partnership works to stamp out sleeping sickness in Uganda. *Trends in Parasitology* **23**: 191–192.

Kabayo J.P. (2002). Aiming to eliminate tsetse from Africa. *Trends Parasitology* **18:**473-475.

Katunguka R. E.(1996). The prevalence of trypanosomosis in small ruminants and pigs in a sleeping sickness endemic area of Buikwe country Mukono district, Uganda. Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux 49:56-58.

Kapiriri L., Norheim O. F. and **Heggenhougen, K. (2003).** Using burden of disease information for health planning in developing countries experiences from Uganda. *Social Science Medicine* **56:** 2433–2441.

Kennedy P.G.E.(2004). Human African Trypanosomiasis of the CNS current issues and challenges. *Science in Medicine*. **113**, 496-503.

KDHS, (2010). Kenya National Demographic and Health Survey: Kenya National Bureau of Statistics publication.

KDHS, (2012). Kenya National Demographic and Health Survey: Kenya National Bureau of Statistics publication.

Kilonzo B.S. and **Komba, E.K. (1993).** The current epidemiology and control of Trypanosomiasis and other zoonoses in Tanzania. *Central African Journal of Medicine*, **39**(1):10-20.

Kinung'hi S.M., Malele I.I., Kibona S.N., Matemba L.E. and Sahani J.K. (2006). Knowledge, attitudes and practices on tsetse and sleeping sickness among communities living in and around Serengeti National Park, Tanzania. *Tanzania Health Research Journal* **8:** 168–172.

KNBS, (2010): Kenya National Bureau of Statistics, Livestock Census Results

KNBS, (2013): Kenya National Bureau of Statistics, Projected Human Population

Knobel D.L., Cleaveland S., Coleman P.G., Fevre E.M. and Meltzer, M.I. (2005). Re evaluating the burden of Rabies in Africa and Asia. Bulletin of the World Health Organization **85**: 360–368.

Kothari C.R.(2004)**. Research Methodology, Methods and Techniques, New Age International Publishers 2nd Ed: 55-69.**

Kristjanson P.M., Swallow, B.M., Rowlands G.J., Kruska R.L.and **de Leeuw P.N. (1999).** Measuring the costs of African animal trypanosomosis, the potential benefits of control and returns to research. Agricultural Systems, **59**: 79–98.

Kunda J., Rudovic K. and **Mfinanga G.S.(2008).** Knowledge of causes, clinical features and diagnosis of common zoonoses among medical practitioners in Tanzania. *BMC Infectious Diseases*, **8**:162 -168.

Luckins A.G. (1977). Detection of antibodies in trypanoasome infected cattle by means of microplate enzyme linked immunosorbant assay. *Tropical Animal Health and Production*, **9**:53–62.

Lutumba P., Robays J., Bilenge M.C., Kande B.M.V. and **Molisho D.(2005):** Trypanosomiasis control, Democratic Republic of Congo, 1993–2003. *Emerging Infectious Disease* **11:** 1382–1388.

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.

MacDougall L., Majowicz S., Dore K., Flint J., Thomas K., Kovacs S. and **Sockett, P. (2008).** Underreporting of infectious gastrointestinal illness in British Columbia, Canada: who is counted in provincial communicable disease statistics? *Epidemiology and Infection* 2008, **136** (02):248 -256.

Machila E. N.2005. Improved targetting and appropriate use of trypanocidal drugs for the control of African Bovine Trypanosomiasis in tsetse endemic areas of western and coastal Kenya within the context of primary veterinary care .PhD Thesis, University of Edinburgh.

Martin E, Hugh-Jones (1995). Zoonoses: recognition, control, and prevention. 1st edition. Ames: Iowa state university press;

Masake R.A, Njuguna J.T., Brown C.C. and **Majiwa P.A. (2002**). The application of PCR-ELISA to the detection of *Trypanosoma brucei* and *T. vivax* infections in livestock. Veterinary Parasitology, **105**:179–189.

Masiga D.K., Irungu P. and **Nyamwaro S.O. (2002).** Financial implications of rearing sheep and goats under natural trypanosomosis challenge at Galana Ranch, Kenya. *Tropical Animal Health and Production*, **34**(6):503-513.

Mathers C.D., Ezzati M. and **Lopez A.D., (2007**). Measuring the burden of neglected tropical diseases the Global Burden of Disease framework. PLoS Neglected Tropical Diseases **1:**114,1371-1375.

Matt K. and Pejman R. (2008). Modelling Infectious Diseases in Humans and Animals. Princeton University Press.15-52.

Maudlin I., Holmes P.H. and **Miles A.M., (2004).** The Trypanosomiases. CABI Publishing 1st Edition. 203-253.

Mc Dermont J.J. and **Coleman P.G.(2001).** Comparing apples and oranges – Model based assessment of different tsetse transmitted control strategies. *International Journal for Parasitology* **31**:603-609.

Milligan P.J. and **Baker R. D. (1988).** A model of tsetse transmitted animal trypanosomiasis. *Parasitology*. **96** (Pt 1):211-39.

Milligan P. (1990). Modelling Trypanosomiasis Transmission. International Journal of *Tropical Insect Science*, **11**/Issue 3/1990 : 301-307

Molyneux D.H., Pentreath V. and **Doua F.(1996)** . African Trypanosomiasis in man**.** In Manson's Tropical Diseases 20th edition, Edited by Cook GC. London W.B. Saunders Company Ltd; 1171-1196.

Monath T.P. (2006). Yellow fever as an endemic/epidemic disease and priorities for vaccination. Bulletin de la Société de pathologie exotique **99**: 341–347.

Murray M., Murray P.K. and **Mclntyre W.M.(1977).** An improved parasitological technique for the diagnosis of African Trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* , **71**:325-326.

Murray C.J.L. and **Lopez A.D. (1996a)** . The Global Burden of Disease: a comprehensive assessment of mortality and disability from diseases, injuries and risk factors in 1990 and projected to 2020. Cambridge: Harvard University Press: 990.

Murray C.J.L. and Lopez A.D. (1996b) Global health statistics: a compendium of incidence, prevalence and mortality estimates for over 200 conditions. Cambridge: Harvard University Press. pp 990.

NLP, (2008). .National Livestock Policy of the Government of Kenya

Ng'ayo M.O., Njiru Z.K., Kenya E.U., Muluvi G.M.and **Osir E.O.(2005).** Detection of trypanosomes in small ruminants and pigs in western Kenya: important reservoirs in the epidemiology of sleeping sickness? *Kinetoplastid Biology and Disease* **4**: 5.

Njiru Z.K., Constantine C.C., Guya S., Crowther J., Kiragu J.M., Thompson R.C. and **Davila, A.M.(2005).** The use of ITS1 PCR in detecting pathogenic African trypanosomes. *Prasitology Research*, **95** (3):186-192.

Njiokou F., Simo G., Nkinin S.W., Laveissière C. and **Herder S. (2004).** Infection rate of Trypanosoma brucei s.l , T. vivax, T. congolense "forest type", and T. simiae in small wild vertebrates in south Cameroon. *Acta Tropica*. ;**92** (2):139-146.

Odiit M., Kansiime, F. and **Enyaru, J.C.K.(1997).**Duration of symptoms and case fatality of sleeping sickness caused by Trypanosoma brucei rhodesiense sleeping sickness in Tororo, Uganda. *East African Medical Journal* **74**:792–795.

Odiit M., Shaw A., Welburn S.C., Fevre E.M., Coleman P.G. and **McDermott J.J. (2004a).** Assessing the patterns of health seeking behaviour and awareness among sleeping sickness patients in Eastern Uganda. *Annals of Tropical Medicine and Parasitology*, **4**(98):339-348.

Odiit M., Coleman P.G., McDermott J.J., Fèvre E.M., Welburn S.C. and **Woolhouse M.E. (2004b) .** Spatial and temporal risk factors for the early detection of Trypanosoma brucei rhodesiense sleeping sickness patients in Tororo and Busia districts, Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **98**(10):569-76.

Odiit M., Coleman P.G., Liu C.W., McDermott J.J., Fevre E.M., Welburn S.C. and **Woolhouse, M.E.J. (2005).** Quantifying under detection of *T. b. rhodesiense* the early; detection of T. b. rhodesiense sleeping sickness patients in Tororo and Busia districts, Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **98**: 569–576.

OIE, (2009). African Animal Trypanosomiasis, World Animal Health Organization (OIE) Terrestrial Animal Health Code.

OIE, (2013). The Terrestrial Code on Trypanosomiasis. World Animal Health Organization, Paris.

Paalman M., Bekedam H., Hawken L. and **Nyheim D. (1998).** A critical review of priority setting in the health sector the methodology of the 1993 World Development Report. *Health Policy Plan* **13:** 13–31.

Palmer S.R., Soulby E.J.L. and **Simpson D.I.H.(1998).** Zoonoses: biology, clinical practice, and public health control. New York: Oxford University Press; 266.

PATTEC, (2011). Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) Kenya, Annual Report.

Pawlowski Z.S.(2008). Control of neurocysticercosis by routine medical and veterinary services. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **102**: 228–232.

Perry B.D., Randolf T.F., McDermott J.J., Sones K.R. and **Thornton P.K. (2002).** Investing in animal health research to alleviate poverty. International Livestock Research Institute (ILRI), Nairobi, Kenya,:133.

Pearce F.(2002). An atomic powered plan to end sleeping sickness. *The Boston Globe,* Retrieved March 7, 2007, from Lexis-Nexis Academic database.

Picard A. (2000). A legendary killer allowed to get away, once almost extinct, Africa's dreaded sleeping sickness will claim 350,000 lives this year. The Globe and Mail*, retrieved* March 7, 2014, from Lexis-Nexis Academic database.

Picozzi K., Fevre E., M., Odiit M., Carrington M. and **Eisler M. (2005).** Sleeping sickness in Uganda a thin line between two fatal diseases. *BMJ* **331**: 1238–1241.

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

Picozzi K., Carrington M. and **Welburn S.C.(2008)**. A multiplex PCR that discriminates between *Trypanosoma brucei brucei* and zoonotic *T. b. rhodesiense*. *Experimental Parasitology* **118**: 41–46.

Politi C., Carrin G., Evans D., Kuzoe F.A.S. and Cattand P.D.(1995). Cost effectiveness analysis of alternative treatments of African gambiense Trypanosomiasis in Uganda. ***Health Economics,*** Volume **4,** Issue 4: **273–287**

Radwanska M., Chamekh M., Vanhamme L., Claes F., Magez S., Magnus E., de Baetselier P., Buscher P. and **Pays E. (2002).** The serum resistance associated gene as a diagnostic tool for the detection of Trypanosoma brucei rhodesiense. *American Journal of Tropical Medicine and Hygiene* **67**: 684–690.

Rodgers D.J. (1988). A general model for the African Trypanosomiases. *Journal of Parasitology* **97:** 193-212.

Rodgers J.D. (2000). Satellites, space, time and the African trypanosomiases. *Advances in Parasitology* 47:129–171.

Rushton J., (2009). The Economics of Animal Health and Production Second Edition. CABI International, 1-105.

Simarro P.P., Jannin J. and **Cattand P. (2008).** Eliminating human African Trypanosomiasis: where do we stand and what comes next? *PLoS Medicine* **(5)** 55:11-14.

Simarro P.P., Franco J.R., Diarra A., Ruiz P.J.A. and Jannin J. (2013). Diversity of human African Trypanosomiasis epidemiological settings requires fine tuning control strategies to facilitate disease elimination. Research and Reports in Tropical Medicine **4**: 1–6.

Simo G., Asonganyi T., Nkinin S.W., Njiokou F. and Herder S. (2006). High prevalence of *Trypanosoma brucei gambiense* group 1 in pigs from the Fontem sleeping sickness focus in Cameroon. *Veterinary Parasitology* ,**139**:57-66.

Snow R. W., Craig M. H. Deichmann U. and **Marsh K. (1999)**. Estimating Mortality, Morbidity and Disability Due to Malaria among Africa's Non-Pregnant Population. Bulletin of the World Health Organization, **77:**624–640 .

Steverding D.(2006). A new initiative for the development of new diagnostic tests for human African Trypanosomiasis. *Kinetoplastid Biology & Disease* , **5:**(1) 12-15

Stephen D., Serap A. and **Galvani A. (2011).** A global sensitivity analysis for African sleeping sickness . *Journal of Parasitology***, 138**(4): 516–526

Stephen L.E.(1986). Clinical manifestations of the Trypanosomiasis in livestock and other domestic animals. The African Trypanosomiases ,Ministry of overseas Development, United Kingdom 774-794.

Swallow B. (1999). Impacts of trypanosomiasis on African agriculture. Report of the International Livestock Research Nairobi, Kenya.

Taylor,K. and **Authie, E. (2004)**. Pathogenesis of animal Trypanosomiasis, The Trypanosomiases. Wallingford, Oxfordshire,CABI Publishing. 331–353.

Taylor L.H., Latham S.M. and Woolhouse M.E. (2001). Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London Series B, Biological Sciences,* **1411**(356):983-989.

Thumbi S.M., McOdimba F.A., Mosi R.O. and **Junga J.O.(2008).** Comparative evaluation of three PCR base diagnostic assays for the detection of pathogenic trypanosomes in cattle blood. Parasite and Vectors, **1**:46.

Thumbi S.M., Jung'a J.O., Mosi R.O. and **McOdimba F.A.(2010)** . Spatial distribution of African Animal Trypanosomiasis in Suba and Teso districts in Western Kenya . BMC Research Notes.

Trottier H. and **Philippe P. (2000)** . Deterministic Modeling of Infectious Diseases: Theory and Methods. *The Internet Journal of Infectious Diseases*, Volume 1 Number 2:1-15

Uilenberg G. (1998). A field guide for the Diagnosis, Treatment and Prevention of African Animal Trypanosomosis . FAO Publication.

Urquart C.M. and **Urquhart G. M. (1987).** Veterinary Parasitology. Second edition. Longman Scientific & Technical ,New York ,Churchill Livingstone 192-198

Vanhamme L., PaturiauxHanocq F., Poelvoorde P., Nolan D.P., Lins L., Van Den Abbeele J., Pays A., Tebabi P., Van Xong H., Jacquet A., Moguilevsky N., Dieu M., Kane J.P., De Baetselier P., Brasseur R. and **Pays E.(2003).** *Nature* **422**: 83–87.

Waiswa C., Olaho-Mukani W. and **Katunguka R. E. (2003).** Domestic animals as reservoirs for sleeping sickness in three endemic foci in south-eastern Uganda. *Annals of Tropical Medicine and Parasitology* **97**: 149–155.

Waiswa C., Picozzi K., Katunguka R. E., Olaho-Mukani W. and **Musoke R.A.(2006).** Glossina fuscipes fuscipes in the Trypanosomiasis endemic areas of south eastern Uganda: Apparent density, trypanosome infection rates and host feeding preferences. Acta Tropica 99: 23–29.

Wamwiri F.N., Nkwengulila G., Clausen P.H. (2007). Hosts of *Glossina fuscipes fuscipes*and *G. pallidipes* in areas of western Kenya with endemic sleeping sickness, as determined using an egg-yolk (IgY) ELISA. *Annals of Tropical Medicine and Parasitology* **101:** 225–232.

Wanga C. H., Ritho C. and Njeru, A. (2013). Evaluating factors that affect the viability of private animal health practices in Kenya. *International Journal of Social Sciences and Entrepreneurship*, 1 **(7),** 252-266**.**

Wastling S.L.and **Welburn S.C. (2011).** Diagnosis of human sleeping sickness: sense and sensitivity. *Trends in Parasitology***, 27:**394–402.

Welburn S.C. and **Maudlin I. (1999).** Tsetse trypanosome interactions, rites of passage. *Parasitology Today*, **15**(10):399-403

Welburn S.C., Picozzi K., Fevre E.M., Coleman P.G., Odiit M., Carrington M. and **Maudlin I., (2001):** Identification of human infective trypanosomes in animal reservoir of sleeping sickness in Uganda by means of serum resistance associated (SRA) gene. *Lancet* , **358**:2017– 2019.

Welburn S.C. and Odiit M. (2002). Recent developments in Human African Trypanosomiasis. Current Opinion in Infectious Disease, **15**: 477–484.

Welburn S.C., Fevre E.M., Odiit M., Maudlin I. and **Eisler M.C. (2006)**. Crisis, what crisis? Control of rhodesian sleeping sickness. *Trends in Parasitology* **22**: 123–128.

Winkle S. (2005). *Geißeln der Menschheit. Kulturgeschichte der Seuchen.* Düsseldorf: Artemis & Winkler.

WHO, (1998) . Control and surveillance of African trypanosomiasis. Report of a WHO Expert Committee. WHO technical report series 881. Geneva, World Health Organization.

WHO, (2003). The world health report 2003. Shaping the future. World Health Organization (WHO), Geneva.

WHO, (2005). The history of sleeping sickness. World Health Organization Report.

WHO, (2006a). Human African Trypanosomiasis (sleeping sickness). World Health Organization Fact Sheet.

WHO, (2006b). Human African Trypanosomiasis (sleeping sickness): World Health Organization epidemiological update, Weekly Epidemiol Rec 81: 71–80.

WHO, (2006c). The control of neglected zoonotic diseases a route to poverty alleviation. World Health Organization Geneva: WHO. 65.

WHO, (2008) Global Burden of Disease. World Health Organization: http:// www.who.int/healthinfo/global_burden_disease/en/index.html. Accessed 21 November 2012

WHO, (2012). World Health Statistics 2012, World Health Organization Fact Sheet 290

WHO, (2015). Trypanosomiasis, human African (sleeping sickness), Fact sheet N°259. The World Health Organization.

Wissmann V., B., Machila N., Picozzi K., Fevre E.M. and **Bronsvoort B.M. (2011).** Factors Associated with Acquisition of Human Infective and Animal Infective Trypanosome Infections in Domestic Livestock in Western Kenya. *PLoS Neglected Tropical Diseases* **5**(1): 941.

Woo P.T.K. (1970). The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Tropica* **27**: 384–386.

Woolhouse M. E. J. and **Hargrove, J. W. (1998).** On the interpretation of age-prevalence curves for trypanosome infections of tsetse flies. *Parasitology*, **166:**149 – 156.

World Bank , (2000). World Bank Development Indicators 2000. World Bank, Washington. (http://www.worldbank.org/data/ countrydata/countrydata.html).

World Bank, (1993). World development report 1993: Investing in health, New York Oxford University Press. pp 329.

Worrall E., Rietveld A. and **Delacollette C.(2004).** The burden of malaria epidemics and cost effectiveness of interventions in epidemic situations in Africa. *American Journal of Tropical Medicine Hygiene* **71:** 136–140.

Xong H.V., Vanhamme L., Chamekh M., Chimfwembe C.E., Van Den Abbeele J., Pays A., Van Meirvenne N., Hamers R. and **De Baetselier P.(1998).** A VSG expression site-associated gene confers resistance to human serum in Trypanosoma rhodesiense. *Cell*, **95**:839–846.

Zuckerman J.N., Rombo L. and Fisch A. (2007). The true burden and risk of Cholera implications for prevention and control. *Lancet Infectious Diseases* **7**: 521–530.

APPENDICES

APPENDIX I: QUESTIONNAIRE SEEKING INFORMATION ON KNOWLEDGE, ATTITUDE AND PRACTICE BY MEDICAL PRACTITIONERS OF CAUSES, DIAGNOSES AND MANAGEMENT OF TRYPANOSOMIASIS AND OTHER ZOONOSES

THIS QUESTIONNARE IS ANONYMOUS AND RESPONDENTS ARE REQUESTED TO BE AS TRUTHFUL AS POSSIBLE IN THEIR RESPONSES AFTER SIGNING THE CONSENT FORM

NUMBER_____

1. Age of respondent: _____
2. Sex of respondent : a) Male b) Female
3. Specialty a) Consultant b) Medical Officer c) Clinical Officer d) Nurse e) Others
4. How many years' experience do you have? _____
5. How many of the years' mentioned in 4 above have you spent at your current station? _____
6. At your current work station in your opinion identify four commonest or important diseases and rank them based on the criteria set;
   a. Disease 1_____
      i. Very Important
      ii. Important
      iii. Average
      iv. Unimportant
      v. Very unimportant

   b. Disease 2_____
      i. Very Important
      ii. Important
      iii. Average
      iv. Unimportant
      v. Very Unimportant

   c. Disease 3_____

i. Very Important
ii. Important
iii. Average
iv. Unimportant
v. Very Unimportant

d. List any five zoonotic diseases not listed in 6a, b, or c above and rate them in order of importance of occurrence in your workstation.

Disease	Rating			
	Very important	Important	Average	Unimportant

7. How often do you use the laboratory to confirm your clinical diagnosis?

a) Always
b) Sometimes
c) Rarely
d) Never (Please Specify why)_____

8. What in your opinion, would improve diagnosis?

a) Proper laboratory facilities
b) Increasing medical personnel
c) Continuous professional development programs
d) All of the above
e) others, state_____

9. In your opinion, when are the following ailments diagnosed?

a) Rabies

i. First consultation
ii. Second consultation

iii. Laboratory diagnosis

iv. Post mortem

v. Hardly

b) Trypanosomiasis (Sleeping Sickness)

i. First consultation

ii. Second consultation

iii. Laboratory diagnosis

iv. Post mortem

v. Hardly

c) Echinococcus (Hydatidosis)

i. First consultation

ii. Second consultation

iii. Laboratory diagnosis

iv. Post mortem

v. Hardly

d) Anthrax

i. First consultation

ii. Second consultation

iii. Laboratory diagnosis

iv. Post mortem

v. Hardly

10. List the common clinical diagnostic features for

a) Malaria

b) Rabies

c) Human Sleeping Sickness

d) Typhoid

11. What sample is necessary for the diagnosis of

a) Malaria

i. Blood ii. Stool iii. Urine

b) HIV/AIDS

i. Blood ii. Vaginal Fluid iii. Semen iv. Saliva

c) Human Sleeping Sickness

i. Blood ii. Stool iii. Urine

d) Typhoid

i. Blood ii. Stool iii. Urine

12. In your opinion, list the two zoonotic diseases that are most neglected by clinicians during diagnosis?

a) Most neglected disease 1

b) Most neglected disease 2

13. In your opinion, list the two zoonotic diseases that are most neglected by the government through poor resource allocation?

a) Most neglected disease 1

b) Most neglected disease 2

14. What is the average number of patients attended to in a day at the outpatient department in your workstation? _____

15. Of the number stated in 14 above, what is the average proportion of patients diagnosed after laboratory results?

a) Less than 1 out of 10

b) Less than 3 out of 10

c) Less than 5 out of 10

d) Greater than 5 out of 10

16. At your current work station what is the most diagnosed disease (please rank them from most diagnosed '1' to least diagnosed)

a) Malaria

b) Cholera

c) Typhoid

d) HIV/AIDS

e) Tuberculosis

f) Sleeping Sickness

g) Other

(specify)_____

17. At what stage is the disease mentioned in 16 above diagnosed?

a) Malaria

i. 1st visit

ii. 2nd visit

iii. Laboratory diagnosis

iv. Postmortem

v. Other,

state_____

b) Cholera

i. 1st visit

ii. 2nd visit

iii. Laboratory diagnosis

iv. Postmortem

v. Other,

state_____

c) Typhoid

i. 1st visit

ii. 2nd visit

iii. Laboratory diagnosis

iv. Postmortem

v. Other,

state_____

d) HIV/AIDS

i. 1st visit

ii. 2nd visit

iii. Laboratory diagnosis

iv. Postmortem

v. Other,

state_____

e)Tuberculosis

i. 1st visit

ii. 2nd visit

iii. Laboratory diagnosis

iv. Postmortem

v. Other,

state_____

f) Sleeping Sickness

i. 1st visit

ii. 2nd visit

iii. Laboratory diagnosis

iv. Postmortem

v. Other,

state_____

18. Which are the longest average periods, patients at your current work station are hospitalized?

a) Less than 30 days

b) Between 1-2 months

c) Between 2-6 months

d) Between 6 -12 months

e) Greater than 1 year

19. Which is the disease commonly associated with prolonged stay in hospitals?

_____

20. If the answer to 19 above is HIV/AIDS what are the most common secondary complicating infections?

_____

21. Which zoonotic diseases are rare to encounter at your current work station? (List)

_____

22. Have you ever encountered mixed zoonotic disease infections?

a) Yes b) No

23. If answer to 22 above is 'Yes' what was the diagnostic challenge?

_____

24. What is the capacity of the laboratory at your current work station?

a) Can carry out all tests

b) Can carry out both serology and microscopy tests

c) Can carry out microscopy tests only

d) Other,

state_____

25. Have you ever managed any case of sleeping sickness?

a) Yes b) No

26. If your response to 25 above is 'Yes', how did you identify it ?

a) Clinical features

b) Laboratory tests

c) Postmortem

d) Referral from other doctor/hospital

e) Other, state_____

END, THANK YOU

APPENDIX II: FOCUSSED GROUP DISCUSSION DISCUSSION SEEKING INFORMATION ON KNOWLEDGE, ATTITUDE AND PRACTICE BY MEDICAL PRACTITIONERS OF CAUSES, DIAGNOSES AND MANAGEMENT OF TRYPANOSOMIASIS AND OTHER ZOONOSES

Introduction:

1. Welcome

I thanked the participants for agreeing to participate. I introduced myself and the notetaker who was my research assistant and handed the participants the attendance sheet with demographic questions on age, gender, cadre and years at this facility to the focus group (FG). I stated that we were very interested to hear your valuable opinion on the knowledge, attitude and practice by medical practitioners on diagnosing and managing zoonoses especially Human African Trypanosomiasis (HAT).I volunteered the following information to the FG:

- Who we are and what we're trying to do
- What will be done with this information
- Why we asked you to participate

2. Consent information

- The information you give us is completely confidential, and we will not associate your name with anything you say in the focus group.
- We would like to tape the focus groups so that we can make sure to capture the thoughts, opinions, and ideas we hear from the group. No names will be attached to the focus groups and the tapes will be destroyed as soon as they are transcribed.
- You may refuse to answer any question or withdraw from the study at anytime.
- We understand how important it is that this information is kept private and confidential. We will ask participants to respect each other's confidentiality.
- We shall give you an attendance form for registration which shall signify your consent to take part in this focus group discussion.

3. Explanation of the process

I asked the group if anyone had participated in a focus group before and explained that focus groups are being used more and more often in health and human services research.

- Focus group will last about one hour
- Feel free to move around

4. Ground Rules

I asked the group to suggest some ground rules. After they brainstormed some, I made sure the following were on the list.

- Everyone should participate.
- Information provided in the focus group must be kept confidential
- Stay with the group and please don't have side conversations
- Turn off cell phones if possible
- Have fun

Turned on the Tape Recorder

I asked the group if there were any questions before we got started so that I address the questions.

Discussion began, I made sure I gave people time to think before answering the questions and didn't move too quickly. I used the probes to make sure that all issues are addressed, but moved on when I felt I was I was starting to hear repetitive information.

Questions:

1. Let's start the discussion by talking about what makes this hospital a good place to work. What are some of the positive aspects of working here at this facility?

2. What are some things that aren't so good about this as a place to work?

3. Did your training prepare you well to diagnose and manage zoonoses especially HAT? If not so, why? What factors can lead to better preparation to manage these zoonoses after graduation?

4. What are the routine diagnostic procedures you are involved in? What suggestions do you have to improve the management and diagnosis of HAT?

5. What is the cause of HAT? How is the disease transmitted? How do you control the disease? Do you have capacity to diagnose and manage the disease?

6. Do you meet animal health practitioners to discuss the management of zoonoses? If so how often? Are the meetings structured? If not then what is your view?

Probes for Discussion:

- Curriculum
- Resources
- Culture of patients and colleagues

    - Relationships, camaraderie and customer care
- Safety & Health protection
    - Protective measures (e.g., gloves)
    - Abuse issues on the job
- Working conditions
    - Access to supplies, equipment, drugs
- Respect/recognition from management or others
- Opportunity, training, achievement, growth
    - Advancement, further education, responsibility
- Referral system
- Is there a sense of ownership of the outcomes here?
    - Work content, responsibility
- Feedback
    - Common diseases managed
    - Overall hospital challenges
- Any important issue of relevance that was raised by the discussants.

That concludes our focus group. Thank you so much for coming and sharing your thoughts and opinions with us. We have a short evaluation form that we would like you to fill out if you time. If you have additional information that you did not get to say in the focus group, please feel free to write it on this evaluation form.

Materials and supplies for focus groups

- Sign-in sheet that also served as a consent forms
- Evaluation sheets, one for each participant
- Pads & pencils for each participant
- Focus group discussion guide for facilitator
- 1 recording device
- Batteries for recording device
- Extra tapes for recording device
- Notebook for note-taking
- Refreshments

APPENDIX III: DATA SHEET USED FOR TRYPANOSOMIASIS

SURVEILLANCE IN BLOOD OF DOMESTIC ANIMALS

Farmer _____ Village _____ Date _____

ID No.	Weight Condition Score	Packed Cell Volume (PCV) Results	Buffy Coat Technique (PCV) Results	Treatment	Comments

APPENDIX IV: RESULTS FROM ANIMAL BLOOD SAMPLES ON

MICROSCOPY AND PCR

Table i a Results from Chakol

Field Visit for Data Collection	Animal Species	Total Number of Blood Samples Tested	ITS1-PCR samples positive for trypanosomes out of the total tested	TBR-PCR samples positive for *Trypanosoma brucei* out of the total tested	SRA-PCR samples positive for *Trypanosoma brucei* rhodesiense out of the total tested	Blood sample results from Parasitological examination under Microscopy
Initial Jan- Mar 2011	Cattle	49	4	1	0	0
	Shoats	19	1	1	0	0
	Pigs	12	1	0	0	0
Second Jun-Aug 2011	Cattle	47	4	2	1	1 *T.brucei, T.congolense*
	Shoats	16	0	0	0	0
	Pigs	14	1	0	0	0
Third Jan-Mar 2012	Cattle	45	2	2	1	1*T.brucei*
	Shoats	18	2	1	1	0
	Pigs	11	0	0	0	0
Total	Cattle	141	10	4	2	3
	Shoats	53	3	2	1	0
	Pigs	34	2	0	0	0

Table i b: Summary results from Chakol

Animal species and number of blood samples tested	Positive samples for Trypanosomiasis tested by PCR	Positive samples for Trypanosomiasis tested by microscopy	Positive samples for Human African Trypanosomiasis (PCR)
Cattle -141	10	3	2
Shoats -53	3	0	1
Pigs -34	2	0	0

Table ii a: Results from Apatit

As in Table ia						
Initial Jan- Mar 2011	Cattle	44	6	2	1	2 *T.congolense*
	Shoats	22	2	0	0	1 *T. brucei*
	Pigs	10	0	0	0	0
Second Jun-Aug 2011	Cattle	41	5	1	1	1 *T.vivax*
	Shoats	17	3	0	0	0
	Pigs	11	1	1	0	0
Third Jan-Mar 2012	Cattle	40	3	1	1	*1 T.brucei*
	Shoats	16	0	0	0	0
	Pigs	12	2	1	0	0
Total	Cattle	125	14	7	2	4
	Shoats	55	5	1	1	1
	Pigs	33	3	1	0	0

Table ii b Summary results from Apatit

As in Table ib			
Cattle -125	14	4	2
Shoats -55	5	1	1
Pigs -33	3	0	0

Table iii a: Results from Obechun

As in Table ia						
Initial Jan- Mar 2011	Cattle	39	5	2	1	1*T.vivax*
	Shoats	18	2	1	1	1 *T.bucei*
	Pigs	10	3	1	1	0
Second Jun-Aug 2011	Cattle	41	3	1	1	1 *T.brucei*
	Shoats	15	1	0	0	0
	Pigs	12	0	0	0	0
Third Jan-Mar 2012	Cattle	44	7	3	1	0
	Shoats	15	2	0	0	0
	Pigs	09	0	1	0	0
Total	Cattle	124	15	5	3	2
	Shoats	48	5	1	1	1
	Pigs	31	3	2	1	0

Table iii b Summary results from Obechun

As in Table ib			
Cattle -124	15	2	3
Shoats -48	5	1	1
Pigs -31	3	0	1

Table iv a Results from Obekai

As in Table ia						
Initial Jan- Mar 2011	Cattle	38	5	2	1	0
	Shoats	15	2	0	0	0
	Pigs	13	3	1	1	0
Second Jun-Aug 2011	Cattle	43	5	3	0	1 *T.brucei*
	Shoats	18	3	1	0	0
	Pigs	15	1	0	0	0
Third Jan-Mar 2012	Cattle	41	4	1	1	0
	Shoats	21	3	2	0	0
	Pigs	07	0	0	0	0
Total	Cattle	122	14	6	2	1
	Shoats	54	8	3	0	0
	Pigs	35	4	1	1	0

Table iv b: Summary results from Obekai

As in Table i b			
Cattle -122	14	1	2
Shoats -54	8	0	0
Pigs -35	4	0	1

Table v a: Results from Kolanya

As in Table i a						
Initial Jan- Mar 2011	Cattle	38	6	5	1	1 *T.viax*
	Shoats	17	2	1	0	0
	Pigs	13	1	0	0	0
Second Jun-Aug 2011	Cattle	46	4	1	0	1 *T.brucei*
	Shoats	15	3	1	0	0
	Pigs	12	1	0	0	0
Third Jan-Mar 2012	Cattle	42	4	2	1	2 *T.congolense*
	Shoats	16	3	1	1	0
	Pigs	10	0	0	0	0
Total	Cattle	126	14	8	2	4
	Shoats	48	8	3	1	0
	Pigs	35	2	0	0	0

Table v b: Summary results from Kolanya

As in Table i b			
Cattle -126	14	4	2
Shoats -48	8	0	1
Pigs -35	2	0	0

Table vi a: Results from Katelenyang'

As in Table i a						
Initial Jan- Mar 2011	Cattle	46	8	3	1	0
	Shoats	18	1	1	1	1 *T.vivax*
	Pigs	08	1	1	1	0
Second Jun-Aug 2011	Cattle	41	6	1	0	0
	Shoats	15	3	1	1	1 *T.brucei*
	Pigs	14	1	0	0	0
Third Jan-Mar 2012	Cattle	42	5	2	1	1 *T.congolense* 2*T.brucei*
	Shoats	12	0	0	0	0
	Pigs	12	1	0	0	0
Total	Cattle	129	19	6	2	4
	Shoats	45	4	1	1	1
	Pigs	34	3	1	1	0

Table vi b: Summary results from Katelenyang'

As in Table i b			
Cattle -129	19	4	2
Shoats -45	4	1	1
Pigs -34	3	0	1

Table vii a: Results from Matayos

As in Table i a						
Initial Jan- Mar 2011	Cattle	40	4	1	0	0
	Shoats	21	2	0	0	0
	Pigs	13	0	0	0	0
Second Jun-Aug 2011	Cattle	42	5	2	0	0
	Shoats	13	2	1	1	1 *T.brucei*
	Pigs	09	1	1	0	0
Third Jan-Mar 2012	Cattle	45	3	0	0	0
	Shoats	15	1	1	1	1 *T.brucei*
	Pigs	08	1	0	0	0
Total	Cattle	127	12	3	1	0
	Shoats	49	5	1	2	2
	Pigs	30	2	0	0	0

Table vii b: Summary results from Matayos

As in Table i b			
Cattle -127	12	0	1
Shoats -49	5	2	2
Pigs -30	2	0	0

Table viii a: Results from Mayenje

As in Table i a						
Initial Jan- Mar 2011	Cattle	28	2	1	0	0
	Shoats	14	1	0	0	0
	Pigs	16	1	0	0	0
Second Jun-Aug 2011	Cattle	30	4	2	1	0
	Shoats	16	3	1	1	0
	Pigs	06	0	0	0	0
Third Jan-Mar 2012	Cattle	25	2	0	0	0
	Shoats	15	1	1	0	0
	Pigs	10	0	0	0	0
Total	Cattle	83	8	3	1	0
	Shoats	45	5	2	1	0
	Pigs	30	1	0	0	0

Table viii b: Summary of results from Mayenje

As in Table i b			
Cattle -83	8	0	1
Shoats -45	5	0	1
Pigs -30	1	0	0

Table ix a: Results from Nambale

As in Table i a						
Initial Jan- Mar 2011	Cattle	38	3	1	0	1 *T.vivax*
	Shoats	17	1	0	0	0
	Pigs	15	1	0	0	0
Second Jun-Aug 2011	Cattle	41	5	2	0	0
	Shoats	13	0	0	0	0
	Pigs	09	1	0	0	0
Third Jan-Mar 2012	Cattle	40	2	0	0	*0*
	Shoats	19	0	0	0	0
	Pigs	10	0	0	0	1 *T.brucei*
Total	Cattle	119	10	3	1	1
	Shoats	49	1	0	0	0
	Pigs	34	2	2	1	1

Table ix b: Summary results from Nambale

As in Table i b			
Cattle -119	10	1	1
Shoats -49	1	0	0
Pigs -34	2	1	1

Table x a: Results from Namahindi

As in Table i a						
Initial Jan- Mar 2011	Cattle	40	3	1	0	1 *T.brucei*
	Shoats	16	2	1	0	0
	Pigs	07	0	0	0	0
Second Jun-Aug 2011	Cattle	45	6	2	0	*1 T.brucei*
	Shoats	19	0	0	0	0
	Pigs	11	1	0	0	0
Third Jan-Mar 2012	Cattle	41	4	0	0	1 *T.congolense*
	Shoats	17	1	0	0	0
	Pigs	10	1	0	0	0
Total	Cattle	126	13	4	1	3
	Shoats	50	3	1	0	0
	Pigs	28	2	0	0	0

Table x b: Summary results from Namahindi

As in Table i b			
Cattle -126	13	3	1
Shoats -50	3	0	0
Pigs -28	2	0	0

Table xi a: Results from Rumbiye

As in Table i a						
Initial Jan- Mar 2011	Cattle	43	4	1	0	1 *T.vivax*
	Shoats	21	1	0	0	0
	Pigs	08	1	0	0	0
Second Jun-Aug 2011	Cattle	33	1	0	0	0
	Shoats	12	0	0	0	0
	Pigs	12	1	0	0	0
Third Jan-Mar 2012	Cattle	45	5	1	1	1 *T.congolense*
	Shoats	18	2	1	1	0
	Pigs	10	0	0	0	0
Total	Cattle	121	10	2	1	2
	Shoats	51	3	1	1	0
	Pigs	30	2	0	0	0

Table xi b: Summary of results from Rumbiye

As in Table i b			
Cattle -121	10	2	1
Shoats -51	3	0	1
Pigs -30	2	0	0

Table xii a: Results from Odiado

As in Table i a						
Initial Jan- Mar 2011	Cattle	44	3	1	0	0
	Shoats	15	2	0	0	0
	Pigs	08	0	0	0	0
Second Jun-Aug 2011	Cattle	42	4	1	1	1 *T.congolense*
	Shoats	13	1	1	1	0
	Pigs	10	1	0	0	0
Third Jan-Mar 2012	Cattle	35	1	0	0	1 *T.brucei*
	Shoats	18	2	0	0	0
	Pigs	13	1	0	0	0
Total	Cattle	121	10	2	1	2
	Shoats	46	4	1	1	0
	Pigs	31	2	0	0	0

Table xii b: Summary of results from Odiado

As in Table i b			
Cattle -121	8	2	1
Shoats -46	5	0	1
Pigs -31	2	0	0

Table xiii a: Results from Sibinga

As in Table i a						
Initial Jan- Mar 2011	Cattle	34	2	1	0	0
	Shoats	11	1	0	0	0
	Pigs	12	1	0	0	1 *T. simiae*
Second Jun-Aug 2011	Cattle	50	6	2	1	1 *T.vivax*
	Shoats	18	2	0	0	0
	Pigs	11	0	0	0	0
Third Jan-Mar 2012	Cattle	44	2	0	0	0
	Shoats	19	1	0	0	0
	Pigs	09	1	1	1	0
Total	Cattle	128	10	3	1	1
	Shoats	48	4	0	0	0
	Pigs	32	2	1	1	1

Table xiii b: Summary results from Sibinga

As in Table i b			
Cattle -128	10	1	1
Shoats -48	4	0	0
Pigs -32	2	1	1

Table xiv a: Results from Budumbusi

As in Table i a						
Initial Jan- Mar 2011	Cattle	36	3	1	0	0
	Shoats	15	1	0	0	0
	Pigs	12	0	0	0	1 *T.brucei*
Second Jun-Aug 2011	Cattle	42	1	0	0	1*T.brucei*
	Shoats	16	2	1	0	0
	Pigs	10	1	0	0	0
Third Jan-Mar 2012	Cattle	41	4	1	1	0
	Shoats	16	2	2	1	1 *T.brucei*
	Pigs	12	0	1	1	0
Total	Cattle	119	8	2	1	1
	Shoats	47	4	3	1	1
	Pigs	34	1	1	1	1

Table xiv b: Summary of results from Budumbusi

As in Table i b			
Cattle -119	8	1	1
Shoats -47	4	1	0
Pigs -34	1	1	1

Table xv a: Results from Rugunga

As in Table i a						
Initial Jan- Mar 2011	Cattle	36	2	0	0	0
	Shoats	15	0	0	0	0
	Pigs	10	0	0	0	0
Second Jun-Aug 2011	Cattle	33	4	0	0	0
	Shoats	16	1	0	0	0
	Pigs	09	0	0	0	0
Third Jan-Mar 2012	Cattle	39	5	2	0	0
	Shoats	14	2	1	0	0
	Pigs	08	0	0	0	0
Total	Cattle	108	11	3	1	0
	Shoats	45	3	1	0	0
	Pigs	27	0	0	0	0

Table xv b: Results from Rugunga

Animal species and number of blood samples tested	Positive samples for Trypanosomiasis tested by PCR	Positive samples for Trypanosomiasis tested by microscopy	Positive samples for Human African Trypanosomiasis
Cattle -108	11	0	1
Shoats -45	3	0	0
Pigs -27	0	0	0

Table xvi a: Results from Busangwa

Field Visits for Data Collection	Animal Species	Total Numbers of Blood Sampled Tested	ITS1-PCR samples positive for trypanosomes out of the total tested	TBR-PCR samples positive for *Trypanosoma brucei* out of the total tested	SRA-PCR samples positive for *Trypanosoma brucei* rhodesiense out of the total tested	Blood sample results from Parasitological examination under Microscopy
Initial Jan- Mar 2011	Cattle	37	3	1	0	1*T.vivax*
	Shoats	19	2	0	0	0
	Pigs	08	1	0	0	0
Second Jun-Aug 2011	Cattle	31	3	0	0	0
	Shoats	15	1	0	0	0
	Pigs	08	0	0	0	0
Third Jan-Mar 2012	Cattle	42	5	2	1	1*T.vivax*
	Shoats	17	1	0	0	0
	Pigs	09	2	1	1	1 *T.brucei*
Total	Cattle	110	11	3	1	2
	Shoats	51	4	1	0	0
	Pigs	25	3	1	1	1

Table xvi b: Summary of results from Busangwa

Animal species and number of blood samples tested	Positive samples for Trypanosomiasis tested by PCR	Positive samples for Trypanosomiasis tested by microscopy	Positive samples for Human African Trypanosomiasis
Cattle -110	9	2	1
Shoats -51	4	0	0
Pigs -25	3	1	1

Table xvii a: Results from Bukuyudi

As in Table i a						
Initial Jan- Mar 2011	Cattle	41	5	2	1	1 *T.vivax*
	Shoats	18	2	1	0	0
	Pigs	07	0	0	0	0
Second Jun-Aug 2011	Cattle	36	2	0	0	0
	Shoats	13	1	0	0	0
	Pigs	12	1	1	1	0
Third Jan-Mar 2012	Cattle	42	4	1	0	1 *T.brucei* , 1 *T.congolense*
	Shoats	16	0	0	0	0
	Pigs	12	1	0	0	0
Total	Cattle	119	11	3	1	3
	Shoats	47	3	1	0	0
	Pigs	31	2	1	1	0

Table xvii b: Summary results from Bukuyudi

As in Table i b			
Cattle -119	11	3	1
Shoats -47	3	0	0
Pigs -31	2	0	1

Table xviii a: Results from Bukhalalire

As in Table i a						
Initial Jan- Mar 2011	Cattle	46	3	0	0	0
	Shoats	17	1	0	0	0
	Pigs	12	0	0	0	0
Second Jun-Aug 2011	Cattle	44	1	0	0	1 *T.brucei*
	Shoats	12	2	1	0	0
	Pigs	11	0	0	0	0
Third Jan-Mar 2012	Cattle	35	3	0	0	1 *T.vivax*
	Shoats	15	1	0	0	0
	Pigs	10	1	1	1	0
Total	Cattle	125	7	0	0	2
	Shoats	44	7	1	0	0
	Pigs	33	2	1	1	0

Table xviii b: Summary results from Bukhalalire

As in Table i b			
Cattle -125	7	2	0
Shoats -44	7	0	0
Pigs -33	1	0	1

Table xiii a: Results from Emauko

As in Table i a						
Initial Jan- Mar 2011	Cattle	33	3	1	0	0
	Shoats	12	0	0	0	0
	Pigs	09	0	0	0	0
Second Jun-Aug 2011	Cattle	42	4	1	0	0
	Shoats	14	1	0	0	0
	Pigs	11	1	0	0	0
Third Jan-Mar 2012	Cattle	40	2	0	0	0
	Shoats	16	0	0	0	0
	Pigs	07	0	0	0	0
Total	Cattle	115	9	2	0	3
	Shoats	42	1	0	0	0
	Pigs	27	1	0	0	0

Table xiii b: Summary results from Emauko

As in Table i b			
Cattle -115	9	0	3
Shoats -42	1	0	0
Pigs -27	1	0	0

APPENDIX V: MODEL DEVELOPMENT IN R- STATISTICS SOFTWARE

R-codes

```
#The HAT model#########################
# Parameter and description##############################
# alpha_1=0.212:    Rate at which vector (tsetse fly) bite cattle, 7 bites per 33 lifespan days assuming 1 bite in 3 days.
# alpha_2=0.09: Rate at which tsetse fly bite Humans, #3 bites per 33 lifespan days.
# mu_c=0.0009: Natural mortality rate of cattle (including slaughter) per day
# Cattle life span is 1/0.0009=3*365 days=3 years  (Gaff et al 2007)
# mu_v=0.03: Natural mortality rate of vector per day
#Vector life span of 1/0.03=33 days  (Hargrove, et al 2012, McDermont and Coleman 2001)
# mu_h=0.0000527:   Natural mortality rate - Humans per day, Human lifespan of 1/0.0000527=52*365 days=52 years
# k_c=0.0006:Disease-induced death rate in cattle per day; 6 per 10000 cattle per day
# k_h=0.0001:Disease-induced death rate in human per day; 1 per 10,000 people per day.
# tau_1=0.62: Transmission probability from vector to cattle; 62 out of 100 bites per day lead to cattle infection from vector    (Rodgers, 2000, Hargrove, et al 2012)
# tau_2=0.065: Transmission probability from cattle to vector; 65 out of 1000 bites per day lead to vector infection from cattle    (Rodgers, 2000, Hargrove, et al 2012)
# tau_3=0.065: Transmission probability from human to vector; 65 out of 1000 bites per day lead to human infection from vector  (Rodgers, 2000, Hargrove, et al 2012)
# tau_4=0.05: Transmission probability from vector to human; 5 out of 100 bites per day lead to vector infection from humans (Rodgers , 2000, Hargrove, et al 2012)
# epsilon=0.2: Survival rate of vector; 20% vector survival rate. (estimate)
# Lambda_c=0.00055: Cattle recruitment rate; 55 per 100,000 new cattle births per day
# Lambda_v=0.015:   Vector recruitment rate [Range 0.0075, 0.015] (Hargrove 2003 et al.,); 14 per 100 new adults tsetse fly recruited per day (estimate)
# Lambda_h=0.000126: Human recruitment rate (equal to death rate); 45 per 1,000 per year = 46 per 365000 new human births per day  (2009 census)
#psi_h=0.75: Treatment rate, humans
#psi_c=0.98: Treatment rate, cattle
#gamma_v = 0.75: Vector control measures
#Simulation attempt (see list of parameters ahead).##########################################
#parameters <- c(alpha_1 = 0.0032, alpha_2=0.01, mu_c=0.00055, mu_v = 0.003, mu_h = 0.0000456,
#         k_c = 0.006, k_h = 0.0001, tau_1 = 0.62, tau_2 = 0.065, tau_3 = 0.065, tau_4 = 0.5,
#            epsilon = 0.5, Lambda_c = 0.00055, Lambda_v = 0.142, Lambda_h = 0.0454,
#           psi_h=0.00075,psi_c=0.98, gamma_v=0.75)
#Periodic control
#t<-1:6*365
```

```
#pi<-3.14
#psi_h<-0.02+cos(pi*t)
#plot(psi_h, type="l")
parameters<- c(alpha_1 = 0.212, alpha_2=0.01, mu_c=0.00055, mu_v = 0.003, mu_h =
0.0000527,
k_c = 0.006, k_h = 0.0001, tau_1 = 0.62, tau_2 = 0.065, tau_3 = 0.065, tau_4 = 0.5,
epsilon = 0.5, Lambda_c = 0.00055, Lambda_v = 0.0142, Lambda_h = 0.000126,
psi_h=0.02, psi_c=0.08, gamma_v=0.09)
#Initial conditions (as per year
2009)####################################################
#S_h=780: 780,000 individuals,
#S_v=0.02: 2000 flies
#S_c=328.557; 328,557 Livestock population
#I_h=0.78: 780 infected humans, (1 case per 1000 individuals)
#I_v=0.01: 10 infected flies out of the 2000 flies.
#I_c=0.328: 328 infected cattle (1 in 1000 cattle infected with AAT)
#state <- c(S_h =699.6, S_v =400, S_c = 328, I_h = 40, I_v = 5, I_c = 32.80)
state<- c(S_h =780.0, S_v =0.02, S_c = 328.557, I_h = 0.78, I_v = 0.01, I_c = 0.328)
#Model:#############################################################
HATmodel1<-function(t, state, parameters) {
with(as.list(c(state, parameters)),{
   # rate of change
dS_c<- Lambda_c*(S_c+I_c) -(mu_c + alpha_1*tau_1*epsilon*(I_v/(S_c+I_c)))*S_c
+psi_c*I_c
dS_v<- Lambda_v*(S_v+I_v) -(mu_v +
alpha_1*tau_2*(I_c/(S_c+I_c))+alpha_2*tau_3*(I_h/(S_h+I_h)) )*S_v-gamma_v*S_v
dS_h<- Lambda_h*(S_h+I_h) -(mu_h + alpha_2*tau_4*epsilon*(I_v/(S_h+I_h)))*S_h
dI_c<- (alpha_1*tau_1*epsilon*(I_v/(S_c+I_c)))*S_c - (mu_c + k_c + psi_c)*I_c
dI_v<- (alpha_1*tau_2*(I_c/(S_c+I_c))+alpha_2*tau_3*(I_h/(S_h+I_h)))*S_v - (mu_v +
gamma_v)*I_v
dI_h<- (alpha_2*tau_4*epsilon*(I_v/(S_h+I_h)))*S_h - (mu_h + k_h + psi_h)*I_h
   # return the rate of change
list(c(dS_c, dS_v, dS_h, dI_c, dI_v, dI_h)) # end with(as.list...
  })}#Time
specification#####################################################################

#We run the model for 6 years, 2009 till 2015, times<-seq(2009,2015,by=1/12)
length(times)/12  #6 years#######ODE Model
simulator#################################################################
#install.packages('deSolve') require(deSolve)
out2 <- as.data.frame(ode(y=state,times=times,func=HATmodel1,parms=parameters))
#if ( out2$I_h > 0){out2$I_h == out2$I_h} else {out2$I_h == 0} head(out2); tail(out2)
length(out2); length(times)
```

```
#Return Zero entries for any negative values for(i in 1:length(out2$I_h))
{y[i]<-max(out2$I_h[i],0)} Y   #returns
results#Graphics#####################################################################
par(mfrow=c(1,2), oma=c(0,0,3,0)) plot (1:length(out2$S_v),out2$S_v
type="l",main="Susceptible Vector pop.", xlab="Time in months", ylab="-")
plot (out2$S_c ,type="l",main="Susceptible Cattle pop.", xlab="Time in months", ylab="-")
mtext(outer=TRUE,side=3,"HAT model: Susceptible Population ('000's)",cex=1.5)
par(mfrow=c(1,1), oma=c(0,0,3,0))

par(mfrow=c(1,2), oma=c(0,0,3,0)) plot (1:length(out2$S_h),out2$S_h
,type="l",main="Susceptible Human pop.", xlab="Time in months", ylab="-")
plot (1:length(out2$I_h), out2$I_h , type="l", main="Infected Human pop.", xlab="Time in
months", ylab="-")
mtext(outer=TRUE,side=3,"HAT model: Human Population ('000's)",cex=1.5)
par(mfrow=c(1,1), oma=c(0,0,3,0))

par(mfrow=c(1,3), oma=c(0,0,3,0)) plot (1:length(out2$I_v),out2$I_v
,type="l",main="Infected Vector pop.", xlab="Time in months", ylab="-")
plot (out2$I_c ,type="l",main="Infected Cattle pop.", xlab="Time in months", ylab="-")
mtext(outer=TRUE,side=3,"HAT model: Infected Population ('000's)",cex=1.5)
par(mfrow=c(1,1), oma=c(0,0,3,0))

par(mfrow=c(1,2), oma=c(0,0,3,0)) plot (out2$S_h,out2$I_h, type="l",xlab= "Susceptible
Human pop.", ylab= "Infected Human pop.", main="(a) Human Population")
plot (out2$S_c,out2$I_c, type="l",xlab= "Susceptible Cattle pop.", ylab= "Infected Cattle
pop.", main="(b) Cattle Population")
mtext(outer=TRUE,side=3,"HAT model: Population in 1000's",cex=1.5)
par(mfrow=c(1,1), oma=c(0,0,3,0))

plot (out2$S_v,out2$I_v, type="l",xlab= "Susceptible Vector pop.", ylab= "Infected Vector
pop.", main="(c) Vector Population") plot (out2$I_h,type="l",main="(d) Infected Human
Pop.", xlab="Time in Months from 2009 Jan", ylab="Infected Human pop.")
#Export Data to Ms.
Excel#################################################################
setwd("C:\\Users\\Admin\\Documents\\PhdProject\\write.table(out2$I_h,          file          =

"DrWanga_I_h.csv", append =FALSE, sep=",")write.table (out2, file = "out2.csv", sep="
```