THE PREVALENCE OF TRYPANOSOMIASIS IN CATTLE IN AMURU AND NWOYA DISTRICTS

By

TEKKARA ALLAN OBONYOM

(B.Voc. Agric/Educ. KYU)

Reg. No. 09/U/2242/MEP

A dissertation submitted in partial fulfilment of the requirements for the award of the Degree of Master of Science in Applied Tropical Entomology and Parasitology of Gulu University

March, 2016

DECLARATION

I, Tekkara Allan Obonyom, do declare that this dissertation is my original work and has never been submitted before for the award of a degree in any institution of higher learning.

Signature: ..... Date: 15-March-2016

APPROVAL

This research has been submitted for examination with the approval of the undersigned supervisor:

Prof. Elizabeth Auma Opiyo

Director, Institute of Research and Graduate Studies

Gulu University

Signature: ..... Date: 16/3/2016

DEDICATION

This dissertation is dedicated to my late parents, Eng. Oola Desion Obonyom and Mrs. Florence Oola who believed in my education.

ACKNOWLEDGEMENT

I would like to thank Gulu University for the financial and material support through the MSI Trypanosome Diagnostic Project accorded to me in pursuing the Master's degree.

My sincere gratitude and appreciation goes to my wonderful supervisor, Prof. Elizabeth A. Opiyo for her academic and technical guidance from the start of proof reading of the proposal to its completion.

I am also greatly indebted to Dr. Richard Echodu, Senior Lecturer, Faculty of Science, for his effort in handling the materials of research and technical guidance towards the success of this dissertation.

I also wish to thank Ms. Agwech Harriet, and Mr. Opiro Robert and Lecturers, Faculty of Science, for their encouragement and support during the writing of the report.

My gratitude also goes to BeCA-ILRI hub, Nairobi Kenya for providing facilities for PCR analysis of blood samples.

I would also wish to thank my friends, Opoke, Ocan, Opwonya, and Atto many others for moral support accorded to me during my studies.

Last, but not least, my sincere thanks goes to my beloved family for moral and financial support.

ABSTRACT

Trypanosomiasis (AAT) is a parasitic disease of both animals and humans that causes serious economic losses to livestock and productivity in both animals and humans in Northern Uganda. A cross-sectional survey was conducted in the sub-counties of Purongo in Nwoya district and Atiak in Amuru district to determine the prevalence of AAT and the role cattle play in the epidemiology of Human African Trypanosomiasis (HAT). A total of 789 cattle were sampled from ten different parishes in the sub-counties of Purongo and Atiak in 2011. Parasitological techniques of thick smears and buffy coat examinations were used to determine the presence or absence of trypanosomes. Polymerase chain reaction (PCR) assay was used to determine the trypanosome species. The overall prevalence of trypanosomes in the study area was 8.7% (69/789) with 6.7% (26/389) and 10.8% (43/400) in Purongo and Atiak sub-counties, respectively. The highest prevalence for Purongo sub-county was recorded in Patira parish with 20.4 % (20/98) followed by Pabit with 12.5% (4/32). In Atiak sub-county, the highest prevalence was in Pacilo parish with 44% (22/50), followed by Okidi parish with 13.0% (14/108). The most common trypanosome species identified were *Trypanosoma vivax* 87.1% (54/62) followed by *Trypanosoma congolense* 12.9% (8/62) as confirmed by PCR test. None of the cattle tested had the human infective or mixed animal infective trypanosome. The prevalence of trypanosomiasis was significantly associated with location ($p<0.05$) but not with age group or sex ($p>0.05$). In conclusion, the presence of both the *T. vivax* and *T. congolense* highlights the need for increased surveillance and treatment of animals at point of sale to keep the prevalence low. There is also need to carry out prevalence studies in other sub-counties in the two districts to get the actual prevalence of trypanosomiasis in the two districts.

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ABBREVIATIONS, SYMBOLS AND ACRONYMS

AAT	African Animal Trypanosomiasis
BCT	Buffy Coat Technique
CATT	Card Agglutination Test for Trypanosomiasis
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
GIS	Geographical Information System
HAT	Human African Trypanosomiasis
HCT	Haematocrit Centrifuge Technique
IFAT	Indirect Fluorescent Antibody Test
LRA	Lord's Resistance Army
MAAIF	Ministry of Agriculture Animal Industry and Fisheries
NGO	Non-Governmental Organization
OD	Optical density
PCV	Packed cell volume
PCR	Polymerase Chain Reaction
UBOS	Uganda Bureau of Standards
VSG	Variant Surface Glycoprotein
VATs	Variable Antigen Types
WGS84	World Geodetic System 1984
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.0. Background

Trypanosomiasis, a disease of humans and animals is a major constraint to development in 37 countries in Sub-Saharan Africa (WHO, 2013). Human and Bovine trypanosomiasis is an important protozoan disease caused by the genus *Trypanosoma* and is transmitted cyclically through bites of different species of *Glossina* and mechanically by a number of biting flies such as *Tabanus* and *Stomoxys* sp. (Oluwafemi *et al.,* 2009). Human and African Animal trypanosomiasis is found mainly in those regions of Africa where its biological vector, the tsetse fly, exists (Centre for Food Security and Public health, 2009). Tsetse flies occur exclusively in Africa over an area of approximately 10 million km^2, extending on both sides of the equator between 15^0 N and 30^0 S (Ugochukwu, 2008). Uganda continues to remain one of the most tsetse fly infested countries in Sub-Saharan Africa (MAAIF *et al.,* 2010). Like in most African countries, tsetse distribution in Uganda has remained the same and indeed in some areas, the fly has spread to new areas (Okoth, 1999). It is estimated that 70% of Uganda is infested with 11 species of tsetse, and each occupying a different ecological niche (MAAIF *et al.,* 2010). Tsetse flies are of primary importance in the spread and epidemiology of these economically and socially important diseases; Human African Trypanosomiasis (HAT) in humans and African Animal Trypanosomiasis (AAT) in animals (Simmaro *et al.,* 2010).

The disease HAT also called sleeping sickness is caused by the pathogen *Trypanosoma brucei rhodesiense*, which causes an acute form of disease, and *T. b. gambiense*, the chronic form. *Trypanosoma b. rhodesiense* is found in East and Southern Africa, and *T. b. gambiense* is present in central and West Africa (Brun *et al.,* 2013, WHO, 2013). It is only in Uganda that the two forms of HAT are prevalent, *T. b. gambiense* in the North West and *T. b.*

rhodesiense in the South East (Picozzi *et al.*, 2005). The gap between the two forms of HAT in the country is decreasing and is now estimated at 150kms (Picozzi *et al.*, 2005). The acute HAT is spreading rapidly from its traditional disease focus in the south east northwards to new districts, a spread linked to movement of cattle following restocking (von Wismmann *et al.*, 2011, 2014). In the south eastern part of Uganda, cattle remain the principal reservoir of the zoonotic *T. b. rhodesiense* (Fèvre *et al.*, 2005; Picozzi *et al.*, 2005). However, the spread is compounded by the fact that *T. b. gambiense* and *T. b. rhodesiense* have animal reservoirs from which the tsetse fly vectors pick infective trypanosomes (Uilenberg, 1998; Njagu *et al.*, 1999).Very little is known about the actual importance of cattle as reservoirs of the human infective trypanosomes in the two districts of Amuru and Nwoya (Angwech *et al.*, 2015), hence necessitating the study.

In animals, AAT also known as Nagana in cattle manifests as a spectrum of diseases, most commonly caused by the pathogen *T. congolense, T. vivax and T. b. brucei* with the first two species being the most pathogenic (Nakayima *et al.*, 2013; Auty *et al.*, 2012). *Trypanosoma congolense, T. vivax and T. b. brucei* have a wide host range among domestic animals (Auty *et al.*, 2012). However, cattle are the main species affected, due to the feeding preferences of tsetse flies (Simukoko *et al.*, 2007). Cattle provide a shield for other domestic animals from the effects of trypanosomiasis because of higher amount of odour plumes produced attracting tsetse flies (Simukoko *et al.*, 2007). One organism, *T. vivax*, has become established in South America, where it is transmitted by biting flies acting as mechanical vectors (Centre for Food Security and Public health, 2009). In Uganda, the presence of AAT has been reported from different parts of the country. In south-western Uganda, a prevalence of 6.42% and 5.56% was reported in Mbarara and Mubende districts, respectively (Waiswa & Katunguka-Rwakishaya, 2004). In Butalega district in Eastern Uganda, the prevalence of AAT was 8.9% (Jing *et al.*, 2009).

The impact of the two diseases, HAT and AAT is mostly felt by the rural poor (WHO, 2013). In Africa, it is estimated that 69 million people are at a risk of contracting human sleeping sickness. Of the 69 million, 57 million are at risk of infection with *T. b. gambiense*, and 12 million at risk of infection with *T. b. rhodesiense*. During the end of the 20th century, WHO estimated that 300,000 people contracted HAT annually (WHO, 2013). After a global alliance by different international and national institutions including the private sector the number of HAT infections reduced from 37,991 in 1998 to 6,743 in 2011 giving a percentage reduction of 82.3% (Simarro *et al.*, 2011).

In Uganda it is estimated that nine to eleven million people are at risk of contracting the disease. Of the estimated number of Ugandans at risk, approximately 7.9 million people are at a risk of contracting *rhodesiense* HAT and 2.1 million *gambiense* HAT (Simarro *et al.*, 2013).The risk could be due to many of the populations affected by HAT living in remote areas with limited access to adequate health facilities, which hampers the surveillance, diagnosis and treatment of cases leading to death (Odit *et al.*, 2005; FAO, 2012). The costs of treatment of HAT are also substantial as confirmed by studies in Uganda (Odit *et al.*, 2005) and DR Congo (Lutumba *et al.*, 2007). In the country, an estimated 700 new cases are reported each year (Sebuyira, 2010; Kalibbala, 2012).The effect most felt by the communities is the long recovery phase, making the community fear being diagnosed with HAT (Lutumba *et al.*, 2007). A person affected by the disease will not return to normal activities like tilling fields and wood collection for 6 to 12 months (Lutumba *et al.*, 2007). These results in a considerable loss in household income estimated 163.98 US dollars with household income during the recovery period (Gouteux *et al.*, 1987; Lutumba *et al.*, 2007). The cost increases where there are complications and the affected person stays longer or requires a longer recovery period (Lutumba *et al.*, 2007). Families also incur losses in travel, hospital fees and living expenses in the hospital. Living expenses come about because of the treatment

protocols making it a requirement that patients stay in hospital providing their own food and incidentals. People affected by HAT also suffer emotionally and physically. Human African Trypanosomiasis also affects the central nervous system in the late stage causing changes in personality, alteration of the biological clock (the circadian rhythm), confusion, slurred speech, seizures, and difficulties in walking and talking (Reid *et al.,* 2012; Lutumba *et al.,* 2007; WHO, 2013).

In most rural communities in Africa, livestock that includes cattle, goats and sheep among others are the backbone of the socio economic systems. Cattle are important assets to the owners, providing nutritional benefits and contributing to economic growth (Meltzer, 1995). An estimated 45 to 50 million cattle are at risk of infection in Africa. It is estimated that African farmers lose approximately three million livestock annually to trypanosomiasis with direct economic losses estimated at US$ 600 to 1200 million (Kristjanson *et al.,* 1999; Vreysen, 2006; Alemayehu, 2012). In Uganda alone it is estimated that 70% of the national herd is at risk of contracting Nagana, with the prevalence ranging from 5% to 40% (Sebuyira 2010).

In the tsetse-infested areas as a whole, trypanosomiasis reduces the off take of meat and milk by at least 50%. And by generally constraining farmers from the overall benefits of livestock to farming; less efficient nutrient cycling, less access to animal traction, reduced incomes from milk and meat sales, and less access to liquid capital. Trypanosomiasis reduces yields, area cultivated, and the efficiency of resource allocation (Brent, 1999; Krisjanson *et al.,* 1999; Oluwafemi *et al.,* 2009). In the end trypanosomiasis constitutes the greatest single constraint to livestock and crop production, thereby contributing directly to hunger, protein malnutrition and suffering of entire communities that hamper agricultural and rural development (Okoth, 1999; Alemayehu, 2012). The considerable economic and social

repercussions make control of this disease a priority operation for the development of a large part of the African continent (Finelle, 1983). However, before this is done it is important to know the prevalence of the trypanosomiasis in our livestock.

In Northern Uganda, the risk of infection with trypanosomiasis for both humans and livestock has been amplified. Northern Uganda has been in civil conflict for the past 20 years by the Lord's Resistance Army (LRA) (Nampindo *et al.*, 2005). Conflicts play a role in the introduction and spread of the disease across borders (Ford, 2007). This is so because of the displacement of people with their property (livestock) to or from areas endemic to trypanosomiasis, destruction of health infrastructure, and increase in poverty levels. All these factors mentioned lead to increased transmission and this alters the distribution of the disease. In the end conflicts prevent the processes required in the prevention and control of the disease making it an important determinant of both HAT and AAT transmission.

In Northern, North western and Eastern regions of Uganda, transmission of animal trypanosomiasis (Lawino & Eriku, 2010) and human trypanosomiasis (Picozzi *et al.*, 2005) still remains active. Communities in the Acholi Sub-region in Northern Uganda and their domestic animals are under constant threat of trypanosomiasis. This is because of the continued trade of animals, in particular cattle from areas endemic to the trypanosomiasis like Soroti, Lira, Kaberamaido and Butaleja districts (Fèvre *et al.*, 2001; Jing *et al.*, 2009; Selby *et al.*, 2013). This is further compounded by the fact that farmers may be choosing to sell unproductive (Fèvre *et al.*, 2005) and diseased animals (Picozzi *et al.*, 2005). Given the deliberate re-stocking programs in the Acholi-sub region as part of the poverty eradication plan by both the Government and Non-Governmental Organizations (NGOs), it is postulated that Nagana and HAT could spread in the region as a result of cattle movements. This study

therefore aimed at determining the prevalence of trypanosomiasis and the role of cattle in the epidemiology of HAT in Amuru and Nwoya districts.

1.1. Problem Statement

The insurgence in northern Uganda due to LRA and West Nile Bank Front that lasted for decades affected a number of districts that includes Gulu, Amuru, Oyam, Lira, Pader, Kitgum, Adjumani and some parts of Eastern Uganda like Soroti (Nampindo *et al.*, 2005). This forced people to move to internally displaced peoples' camps abandoning their homesteads that became infested by tsetse flies, with infrastructures destroyed and during this period the disease Nagana has been fluctuating with the political and economic situation in the country (Mbulamberi, 1990). This has left very little published information (Agwech *et al.*, 2015) on the current prevalence of the African Animal Trypanosomiasis in the affected areas of Amuru and Nwoya district.

Deliberate restocking programmes by Government agencies and various Non-Governmental Organisations (NGOs) has increased movement of animals from endemic areas of Soroti, Lira, Kaberamaido and Butaleja districts posing a great risk of introducing infected animals that could lead to resurgence of animal trypanosomiasis. Fevre *et al.*(2001) implicated cattle restocking in the outbreak of *T. b. rhodesiense* disease in 2000 in Teso region. A New Vision article (Lawino & Eriku, 2010) reports that Nagana is quickly spreading outside the traditional known districts. Treatment of animals at the point of origin or before sale forms part of Uganda's national policy for trypanosomiasis control. However, it has been difficult to implement this strategy at local level (Wendo, 2001).With the continued trade and movement of animals from the areas endemic to trypanosomiasis chances of spread of trypanosomiasis is heightened (Fevre *et al.*2005). Pollock (1982) and Finelle (1983) identify a number of animal species both domestic and wild as hosts of trypanosomes. Taken as a

group, the trypanosomiasis of livestock is responsible for severe losses in the agricultural sector (Kristjanson *et al.*, 1999).

This study assessed the prevalence of animal trypanosomiasis and the role cattle plays in the epidemiology of Human African Trypanosomaisis in the post conflict area of Amuru and Nwoya district.

1.2. General objective

This study aimed at determining the prevalence of different species of trypanosomes in cattle in Amuru and Nwoya district.

1.2.1. Specific objectives

- To determine the prevalence of different species of trypanosome parasites in cattle population in Amuru and Nwoya districts.
- To determine the relationship between location, age, and sex of cattle and prevalence rates.
- To determine the role of cattle in the epidemiology of Human African Trypanosomiasis.

1.3. Research questions

- What is the level of prevalence of trypanosome species in cattle in the sub-counties of Atiak and Purongo in Amuru and Nwoya districts, respectively?
- Is there a relationship between location, age, and sex of cattle in determining the prevalence rates?
- What is the role of cattle in the epidemiology of Human African Trypanosomiasis?

1.4. Justification of the study

The people of Northern Uganda in general including those in Adjumani and Moyo districts in particular have been living in internally displaced people's camps and have suffered for 20

years (1987 to 2006) due to persistent rebel activities in the region. Internally displaced people fled areas of intense conflict and abandoned their homes and properties including livestock (Nampindo *et al.*, 2005). The insecurity has now subsided and the population has now resettled in their villages. In their absence, the homesteads were overgrown by new vegetation. The presence of overgrown vegetation results into movement of tsetse flies into peri-domestic environments leading to an increased risk of infection with trypanosomiasis in humans and domestic animals.

It was reported that Nagana, a deadly disease struck the western part of Amuru district killing an unspecified number of animals (Lawino & Eriku, 2010). The reports from District Veterinary Officers in Gulu district indicate the continued presence of trypanosomes in animals despite treatment (Aliro, per.com; Aliker, per. Com).

Despite the reports, not much is known on the potential threat the disease will have on livestock and humans. With the continuous deliberate effort by Government and Non-Governmental Organisations (NGOs) to eradicate poverty in northern Uganda through restocking programmes, trypanosomiasis may increase as livestock are bought from *T. b. rhodesiense* endemic areas of Soroti, Lira, Kaberamaido and Butaleja districts (Fèvre *et al.*, 2001; Jing *et al.*, 2009). Because of the threat and risk of infection from trypanosomes and the role cattle play as reservoirs (Waiswa *et al.*, 2003), there is need to carry out research to provide an accurate situation of the disease, quantify the risk and prevalence, in the end providing a reference point when planning an intervention.

1.5. Scope of the study

The research was limited to cattle in the areas of Amuru and Nwoya districts. The blood from the sampled cattle was viewed under microscopy to determine the prevalence of AAT. Samples positive under microscopy were further analysed using PCR to determine the species

of trypanosome. The percentage prevalence were linked to district of cattle sampling, age, and sex.

1.6. Expected outcomes

- Establish the prevalence rate of AAT in cattle and their role as reservoirs of HAT so that appropriate interventions could be instituted.
- Increased awareness of the community on the impact of trypanosomiasis on livestock.
- A trained Entomologist/ Parasitologist with an award of a Master of Science degree in Applied Tropical Entomology and Parasitology.

CHAPTER TWO

LITERATURE REVIEW

2.0. The Trypanosome

The trypanosome is classified as a Flagellate Protozoa from the genus *Trypanosoma* of the Family Trypanosomatidae which belongs to the Order Kinetoplastida of the class Zoomastigophora. The Zoomastigophora is classified under the Phylum *Sarcomastigophora* (Levine *et al.*, 1980). The genus *Trypanosoma can* belong to two groups; the salivarian group that is injected to the recipient in the saliva via the bite of a tsetse fly and the stercorarian that is transmitted through the faeces of insect from the sub-family *Triatominae* (WHO, 2013). The Salivaria contains the subgenera, *Duttonella, Nannomonas, Pycnomonas, Tejeria, and Trypanozoon.* The Stercoraria include three subgenera, *Megatrypanum, Herpetsoma* and *Schizotrypanum.* Below is a summarized classification (Soulsby, 1982).

Table 2.1: Classification of trypanosomes

Phylum	Sarcomastigophora	Honigberg and Balmuth 1963
Sub phylum	Mastigophora	Diesing, 1986
Class	Zoomastigophora	Calkins, 1909
Order	Kinetoplastida	Honigberg 1963
Sub-order	Trypanomastina	Kent, 1880
Family	Trypanomastidae	Ement and Grobben, 1905
Genus	Trypanosoma	Kent. 1880
1. Group: Salivaria	Anterior station Group	Hoare, 1964
Sub genus	Dutonella	Calmers, 1918
Species	*T. (D) vivax* *T. (D) uniforme*	Zieman, 1905 Bruce *et al.*, 1911
Sub genus	Nannomonas	Hoare, 1964
Species	*T. congolense* *T. simae*	Borden, 1964 Bruce *et al.*, 1911
Sub genus	Pycnomonas	Hoare, 1964
Species	*T. suis*	Ochmann, 1905
Sub genus	Trypanozoon	Luhe, 1906
Species	*T. brucei and sub species* • *T. brucei brucei* • *T. brucei gambiense* • *T. brucei rhodesiense* • *T. evansi* • *T. equiperdum* • *T. equinum*	Plimmer and Bradford, 1899 Dutton, 1902 Stephen and Fatham, 1910 Steel, 1885 Doflein, 1901 Voges, 1901
2. Group: Sterocoraria	Posterior station Group	Hoare, 1964
Sub genus	Megatrypanum	Hoare, 1964
Species	*T. theileri* *T. iragilaphi* *T. ingens*	Leveran, 1902
Subgenus	Herpetsoma	Doflein, 1901
Species	*T. lewski*	
	T. musculi	
Sub genus	*Schizotrypanum*	Chagas, 1909
Species	*T. cruzi*	

Recent studies based on isoenzymatic differences and molecular techniques, resulted in new subdivisions of the species of trypanosomes into several types; *T. congolense* has been divided into four types *T. congolense* savannah type, *T. congolense* Tsavo type, *T. congolense* forest type and *T. congolense* Kilifi type(Uilenberg, 1998).*Trypanosoma godferyi* has been separated lately from *T. congolense* in Gambia on isoenzymatic and molecular bases; although it has the same morphology as *T. congolense* and causes more chronic disease in

pigs (Uilenberg, 1998). Non-pathogenic trypanosomes comprise *T. uniforme, T. melophagium and T. theileri.* Pathogenic trypanosomes that affect animals include *T. b. brucei, T. vivax, T. congolense, T. simiae* and *T. suis.*

2.1. The African Animal Trypanosomiasis

The African animal trypanosomiasis (AAT) is a disease caused by the species of genus *Trypanosoma (T. congolense, T. b. brucei, T. simiae and T. vivax)* and is mainly transmitted cyclically by infected tsetse flies. However, *T. vivax* and *T. congolense* can be transmitted mechanically by tabanid flies (Uilenberg, 1998; Desquesnes & Dia, 2003a, 2003b) whereas mechanical transmission of *T. b. brucei* can only be confirmed by experimental studies and not in natural conditions (Desquesnes and Dia, 2003a). The transmission of *Trypanosoma spp* results into sub-acute, acute or chronic diseases characterised by intermittent fever, anaemia, occasionally diarrhoea and rapid loss of body condition, and often terminates in death (Mare, 1998).

2.2. Host range

Trypanosomes can infect all domesticated animals. Clinical cases have been described in cattle, water buffalo, sheep, goats, camels, horses, donkeys, alpacas, llamas, pigs, dogs, cats and other species. In parts of Africa, cattle are the main species affected, due to the feeding preferences of tsetse flies. In effect, they can shield other domesticated animals such as goats and pigs from the effects of trypanosomiasis. More than 30 species of animals in the wild or zoos, including ruminants such as white-tailed deer, duikers, antelope and African buffalo, as well as wild Equidae, lions, leopards, warthogs, capybaras, elephants, non-human primates and various rodents are also known to be susceptible to infection (Auty *et al.*, 2012). *Trypanosoma vivax* DNA has been found by PCR in crocodiles and monitor lizards (*Varanus ornatus*) in Africa, but whether this organism can become established in reptiles or it is

merely inoculated transiently by insect's remains to be determined. Experimental infections can be established in laboratory animals including mice, rats, guinea pigs and rabbits (Centre for Food and Public Health, 2009).

2.3. Epidemiology of African Trypanosomiasis

The African trypanosomiasis is an infectious disease of both humans and animals that share a similar manner of causation of the disease, which is the protozoan parasites of the genus *Trypanosoma* transmitted by the vector Tsetse fly (Uilenberg, 1998; Steverding, 2008). The distribution of the disease matches the range of tsetse flies in the African continent.

Tsetse flies exist between latitude 15° N and 29° S, from the southern edge of the Sahara desert to Zimbabwe, Angola and Mozambique. Trypanosomes, particularly *T. vivax*, can spread beyond the "tsetse fly belt" and the fact that the disease is transmitted mechanically as well as cyclically has definitely expanded the disease distribution out of the tsetse belt (Centre for Food and Public Health, 2009). The disease has a wide host range both domestic and wild animals including cattle, water buffalo, sheep, goats, camels, horses, donkeys, alpacas, llamas, wild pigs, dogs, cats and other species (Auty *et al.*,2012).

Uganda is the only country to have endemic foci of both HAT causing parasites, *T. b. gambiense* in the north-west and *T. b. rhodesiense* in the south-east of the country (Fèvre *et al.*, 2004). In the parts of South Western and Eastern, North western Uganda, the presence of the AAT causing parasites has been well documented (Okoth, 1999; Fèvre *et al.*, 2001, 2004, 2005; Waiswa & Katunguka-Rwakishaya, 2004; Berrang-Ford *et al.*, 2010, Balyeidhusa *et al.*, 2012). Transmission of trypanosomiasis to new areas in the country has largely been attributed to domestic livestock as the only important reservoirs of epidemiological significance (Duke, 1916; Fevre *et al.*, 2001; von Wissman *et al.*, 2011).

The role of cattle in the epidemiology of HAT is largely determined by the interactions between tsetse flies, cattle and humans. The levels of interaction are distinguished into four situations, first being areas where cattle are absent. Secondly, where cattle have been introduced to game areas but where game is still abundant and constitutes the major source of food for tsetse. Thirdly, areas where because of human interference, the density of game animals is low and cattle constitute the major source of food for tsetse and finally, areas where cattle occur at the edge of tsetse infested zones (Van den Bossche 2001). In all regions of Uganda, cattle are the most important livestock species kept by most communities (UBOS, 2008) and this increases risk of infection through the interactions between the tsetse, cattle and humans. The role of cattle in the epidemiology is heightened where cattle are the major source of food. Accordingly, it was determined that animals are crucial for maintenance of the disease (Funk *et al.,* 2013) suggesting that a risk of re-introduction from animal into human populations would remain even if the disease were eliminated from those human populations (Van den Bossche 2001) resulting in the continued recurrence of HAT (Rogers 1988).However, in the counties of East Moyo, Koboko and Moyo in North western Uganda animals tested showed absence of HAT causing parasites (Balyeidhusa *et al.,* 2012).

The sub-counties of Atiak in Amuru and Purongo in Nwoya district provide a scenario that puts people and cattle at the risk of infection with trypanosomiasis. Wild animals, mainly those kept in protected areas are main parasite reservoirs (Simarro *et al.,* 2013), in this case the Murchison National Park that is partly in Purongo. The people put mostly at risk are those living within or around the park. This risk arises due to the movement of wild animals and tsetse in and out of the park, particularly during the dry season. This is because of the flush growth of grass that comes as a result of bush burning making game animals move out in need of fresh grass (Observation, December 2010). Also at risk, are tourists (Allsopp, 1972), poachers, herdsmen, rangers, guides and persons collecting firewood in the parks (Simarro *et*

al., 2013*)*. In Atiak cattle are the major livestock species in the community, making cattle the main species affected, due to the feeding preferences of tsetse where studies indicate 62.8% preference by Glossina for Bovidea (Clausen *et al.*, 1998). The presence of these animals in close proximity to homesteads brings the disease closer to the people, putting them at some level of risk (Fevre *et al.*, 2001).Also, Atiak being a major hub for cattle moving into South Sudan poses a great risk where animals brought from endemic areas of Soroti, Kaberamaido, Dokolo may be reservoirs of trypanosomes that may be picked by tsetse flies when feeding (Selby *et al.*, 2013).

Inclusive, Government and NGOs restocking programmes, essentially to provide oxen that are used in land opening with the aim of increasing agricultural productivity poses a continuous threat to humans and livestock. This is so because these animals are bought from trypanosomiasis endemic areas of Soroti, Kaberamaido, Dokolo (Selby *et al.*, 2013) and may be reservoirs of both the human and animal infective trypanosomes (Fèvre *et al.*, 2005; Picozzi *et al.*, 2005; Selby *et al.*, 2013).

The government directive to have animals treated with trypanocidal drugs at the point of sale is not being adhered to fully (Selby *et al.*, 2013). This is so because through interviews and discussion indications show that government restocking programmes treated their animals with trypanocidal drugs of either diminazene aceturate or isometamidium chloride, whereas private contractors used by NGOs and a number of private buyers tended to avoid any health inspections unless the animals were ill (Selby *et al.*, 2013). Private contractors and traders considered paying for treatment for a disease that an animal was not perceived to be suffering from as unnecessary and this was supported by the fact that *T. b. rhodesiense is* asymptomatic in cattle (Selby *et al.*, 2013).

Animals that are bought for slaughter do not receive treatment 21 days prior to consumption and yet some buyers tend to abuse this requirement claiming their animals are destined for slaughter to avoid costs of treatment (Selby *et al.*, 2013). In other instances animals are also bought directly from farmers in their villages and movement permits obtained directly from the district veterinary offices avoiding inspection and treatment in the animal markets. Animals bought are between the ages of 2 to 3 years mainly for restocking as private contractors are able to attain a profit at these ages. Animals with a carcass weight of at least 150 kilogrammes and above are preferred and most animals with ages above 2 ½ years have attained this weight (Personal communication from Wokorach Samuel, Veterinary Officer, Gulu district). In the end the disease will be moved from disease endemic districts into pathogen free districts (Selby *et al.*, 2013).

Lastly, in all these areas humans are moving back to areas they had left because of the insurgence, these areas became bushy and are likely to have been re-infested by tsetse. All the scenarios mentioned play a major part in the animal-tsetse-human transmission cycle (Njiokou *et al.*, 2010; Selby *et al.*, 2013; Simarro *et al.*, 2013).

2.4. Economic impacts of Animal trypanosomiasis

The impact of the tsetse-associated disease extends in sub-Saharan Africa over some 10 million km^2 (a third of the continent). Of these 10 million km^2 some 3 million are covered by equatorial rain forest; the remaining area contains some very good grazing areas, which perhaps fortunately have been protected so far by the tsetse fly against overgrazing (Uilenberg, 1998).The disease threatens enormous numbers of cattle and other livestock (Uilenberg, 1998).

Livestock are of enormous importance in Africa, economically, for nutritional and agricultural purposes. The problem of AAT, also called "Nagana", was recognized by

African stockmen long before the cause of the disease was known, and many pastoralists associated the disease with the presence of tsetse flies. By experience and folk memory, methods of husbandry were evolved whereby domestic livestock could be maintained in strictly defined areas. By a process of trial and error it became known that certain zones were safe from the ravages of the disease, some were death traps and others were seasonally affected and fluctuated between good and bad years (Hide, 1999; Sterverding, 2008).

Throughout the rest of the world, progress has been attained by the increasing use of harnessed power, and the use of draught animals. For centuries trained oxen provided traction for cart and plough, while cattle also provided meat, milk and manure. Their absence over a great part of the African continent meant a constraint to the progress of development of huge proportions. Modernisation of Agriculture with the sole aim of mechanising agriculture has brought some relief; however its use remains limited with the majority of farmers practicing small-scale farming and below the poverty line (Uilenberg, 1998).

According to Uganda National Household survey 2009/2010 survey data, 24.5 % of Ugandans are poor, corresponding to nearly 7.5 million persons in 1.2 million households with the incidence of poverty remaining higher in rural areas than in urban, with the poor in rural areas representing 27.2% of the population but only 9.1 percent in the urban areas. The incidence of poverty remains highest in the Northern region that includes districts of Gulu, Amuru, Kitgum, Pader, Apac, Oyam, Lira, Amolatar and Dokolo. On average, poverty incidence in Northern region of 46.2% remains higher than the national average of 24.5% (UBOS, 2010). With 50.4 % of the rural poor relying on subsistence farming as the main source of livelihood income, necessitates the need for livestock to reduce poverty and improve health. Livestock provide meat and milk, help in crop production and a source of income for the rural poor (Delgado *et al.*, 1999; UBOS, 2010). Additionally, if nutritional

requirements are compromised in populations, morbidity and mortality from other types of infectious diseases increases (WHO, 2005).

Accordingly, AAT is one of the major contributors to poverty, food insecurity, and nutritional deficiencies in rural areas across sub-Saharan Africa (Grady *et al.*, 2011). Animal African Trypanosomiasis takes land out of agricultural production and diseased livestock leads to livestock morbidity and mortality, contributing to poverty and nutritional deficiencies. If AAT remains unchecked the economic losses associated with the disease will be immense (Ocaido *et al.*, 2009) and this necessitates the need for information on the prevalence of trypanosomiasis and the role cattle are playing in the epidemiology of trypanosomiasis for planning appropriate intervention.

2.5. Pathology of the AAT

The incubation period for African animal trypanosomiasis ranges from 4 days to approximately 8 weeks. Infections with more virulent isolates have a shorter incubation period (Center for Food Security and Public health, 2009). The first sign of trypanosomiasis may be a localized swelling at the site of the fly bite, but this usually remains unnoticed (Naessens *et al.*, 2003).The parasites thereafter spread to the lymph nodes and blood then continue to replicate. *Trypanosoma congolense* localizes in the endothelial cells of small blood vessels and capillaries. *Trypanosoma b. brucei* and *T. vivax* localize in tissues like lymph nodes (Uilenberg, 1998). Anaemia is one of the major effects of the AAT pathogens. The Packed Cell Volume (PCV) of the affected animals falls rapidly due to erythrophagocytosis, but an equal rapid recovery takes place following trypanocidal drug treatment.

The AAT affects the productivity of animals not only by increasing the mortality and the abortion rates but it affects directly the reproductive system and fertility of the infected

animals. Infection of cattle with *T. vivax* or *T. congolense* causes lesions in the male reproductive organs of cattle. *Trypanosoma congolense* causes more severe effects than *T. vivax* and causes highly significant and drastic decrease in sperm concentration and volume and also increases in sperm morphological defects which results in complete infertility of bulls in the late stages of infection (Sekoni *et al.*, 2004).

In response to inoculation from an infected tsetse fly into the skin of the animal, antibodies are developed against the particular trypanosome variable surface protein. Each trypanosome is covered by a replaceable surface coat composed of the variant surface glycoprotein (VSG), which specifies the variable antigen type (VAT) of the trypanosome. However, only one VSG is expressed at a time despite the potential of the trypanosome to express hundreds of different VSGs that is not induced by antibodies but spontaneously. In the end there is a persistent infection that results in a continuous trypanosome replication, antibodies production, immune complex development and changing surface coat glycoprotein (Müller *et al.*, 1996; Robinson *et al.*, 1999).

It has been suggested that many of the immunologic lesions significant in AAT e.g. anaemia and glomerulonephritis in these diseases may be the result of the deposit of the immune complexes that interfere with circumventricular organs in the brain or prevent normal neural functions (Aagaard-Hansen & Chaignat, 2010; Kristensson *et al.*, 2010). The most significant and complicated factor in pathogeneses of the AAT is the profound immunosuppression that occurs following the infection with these parasites. This marked immunosuppression lowers the host resistance to other infections thus resulting in secondary diseases, which greatly complicate both the clinical and pathological features of the AAT (Mare', 1998).

2.6. Clinical signs

It is very common for one animal to be infected with more than one species of trypanosomes and also infected simultaneously with other blood parasites (*Babesia* spp., *Theileria* spp., *Anaplasma* spp. and *Ehlrichia* spp). This makes it difficult to conclude which clinical signs are attributed to a given parasite. Few adequately controlled studies have been made, and thus a typical clinical response to any trypanosome is difficult to construct. What follows is a summation of syndromes in field and experimental cases of AAT caused by each of the three trypanosomes (Mare', 1998).

The cardinal sign observed in the AAT is anaemia. Within a week of infection with the haematic trypanosome (*T. congolense* and *T. vivax*) there is usually pronounced decrease in packed cell volume, haemoglobin, red blood cells, and white blood cells level and within 2 months these may drop to below 50% of their pre-infection values (Mulligan& Porr, 1970). The severity of clinical response depends on the species and the breed of the affected animals and the dose and virulence of the infecting trypanosome. Stress such as poor nutrition and concurrent disease plays a prominent role in the disease process and in experimental conditions where stress may be markedly reduced, it is difficult to elicit clinical diseases (Suliman *et. al.*, 1989).

Trypanosoma congolense is a haematic trypanosome found only in the blood vessels of the infected animal. It does not localize or multiply outside the blood vessels. Infection with *T. congolense* may result in per acute, acute or chronic disease in cattle, sheep, goats, horses and camels. Pigs often develop a milder disease; chronic disease is common in dogs. The incubation period is followed by intermittent febrile episodes, depression, lethargy and weakness, loss of condition, anaemia, salivation, lacrimation, and nasal discharges. As the disease progresses there is loss of condition and the hair colour changes, looking rough and

dull (Uilenberg, 1998). The pulse and jugular pulsation increases and breathing are difficult. Anaemia is a prominent sign. Early in the infection, trypanosomes are readily demonstrated in lymph node smears (Mare', 1998).

Trypanosoma vivax has a marked variation in virulence of the different strains and mortality rate of over 50% can occur. It causes mild disease in horses and chronic disease in dogs but remains the most important causes of the AAT of cattle, sheep and goats in West Africa where the strains are believed to be more pathogenic compared to East African strains (Nakayima *et al.,* 2013). However, severe haemorrhagic outbreaks, with high mortality levels, have been periodically reported in East Africa (Magona *et al.,* 2008). *Trypanosoma vivax* is often difficult to find in blood smears and can also be demonstrated in lymph node smears. Ayrshire cattle in Galana, Kenya infected with *T. vivax* resulted in an acute disease characterized by profound anaemia and haemorrhage, which reached maximum severity between 3 and 5 weeks after infection (Gardiner *et al.,* 1989; Wellde *et al.,* 1983). Symptoms ranged from bleeding from the ears, nose and rectum (Gardiner *et al.,* 1989). Farmers and field veterinarians in Mbale reported an outbreak of a strange disease causing considerable death of cattle, manifesting with anaemia, bleeding through the skin and ears, before death and, petechial haemorrhages on the tongue and enlarged spleen at post-mortem similar to trypanosomiasis. According to the findings, clinical signs and high mortality, the outbreak was confirmed to be haemorrhagic *T. vivax* (Magona, 1997).

Trypanosoma brucei brucei causes severe to fatal infection in horses, camels, dogs and cats. It usually causes mild, chronic or sub-clinical disease in cattle, sheep, goat and pigs. A febrile response occurs in horse 4 – 14 days after infection, followed by recurrent febrile reactions. The heart beat and respiration may be accelerated, and the loss of condition and weakness are seen, whereas the appetite remains good. Progressive anaemia, icterus (Jaundice: a yellow

pigmentation of the skin), and oedema of the ventral regions, specially the male genitalia, are characteristic (Suliman & Feldmann, 1989). The trypanosomes are not always easily detected in blood smears and are best demonstrated in tissue smears and sections (e.g. lymph nodes). Infected animals die in a few weeks or several months, depending on the virulence of the strain of *T. b. brucei* (Suliman & Feldmann, 1989).

2.7. Lesions

No pathognomonic change is seen in AAT. Anaemia, oedema, and serous atrophy of fat are commonly observed. Subcutaneous oedema is particularly prominent and is usually accompanied by ascites, hydropericardium, and hydrothorax. The liver may be enlarged, and oedema of lymph nodes is often seen in the acute disease, but they may be reduced in size in the chronic disease (Ikede and Losos, 1975).

The spleen and lymph nodes may be swollen, normal, or atrophic. Necrosis of the kidneys and heart muscle and sub serous petechial haemorrhages commonly occur. Gastroenteritis is common, and focal polioencephalomalacia may be seen. The anaemic blood changes are anisocytosis, poikilocytosis, polychromasia, and punctate basophilia. All, some, or none of the above may be seen (Losos *et.al.*, 1972). The lesions caused by the trypanosomes in susceptible host species vary considerably, depending on the species and strain of trypanosome and the species and breed of host animal affected. The haematic trypanosomes (*T. congolense* and *T. vivax*) cause injury to the host mainly by the production of severe anaemia, which is accompanied in the early stages of the disease by leukopenia and thrombocytopenia. In the terminal stages of the disease caused by the haematic trypanosomes there is focal polioencephalomalacia probably resulting from ischemia due to massive accumulation of the parasites in the terminal capillaries of the brain.

The lesions resulting from *T. b. brucei* (a tissue parasite) are remarkably different from those seen with the haematic trypanosomes. Anaemia is an important lesion, but much more dramatic are the inflammation, degeneration, and necrosis resulting from cellular invasion of various organs. Marked proliferative changes reflecting immunologic response are observed in most body tissues.

2.8. Diagnostic techniques

The diagnosis of *Trypanosoma* infection is based on clinical signs and on the demonstration of the parasites by direct methods that include; wet blood films, stained thin smears, haematocrit centrifugation technique, animal inoculation (Uilenberg,1998; Mitashi *et al.*, 2012) or indirect methods which includes; Indirect fluorescent antibody test, enzyme-linked Immunosorbent assay (ELISA) and Card agglutination tests (CATT). The indirect tests used for examination of both animal and human trypanosomes vary from species to species depending on level of sensitivity, and level of expertise required in carrying out the tests (Chappuis *et al.*, 2005; Mitashi *et al.*, 2012). The clinical signs of the AAT are indicative but are not sufficiently pathognomonic and diagnosis must be confirmed by laboratory methods. The classical direct method for the diagnosis of trypanosomiasis led to the original discovery of the parasite (OIE, 2012). The method is still employed for examining blood or lymph node material, but rarely with extracts of other tissues. In the Tsetse belt where many *Trypanosoma* species are found, species specific identification on blood smears by microscopy is very difficult due to limited magnification.

2.8.1. Direct methods

Direct methods involve the direct examination of blood by light microscopy and can easily be applied in the field. The basic technique of examination of fresh or stained blood films has been modified to improve diagnostic sensitivity by concentrating the blood through

centrifugation in a haematocrit tube namely; Haematocrit Centrifuge Technique (HCT) (Uilenberg, 1998) or the Dark Ground buffy coat technique (DG)(OIE, 2012; Uilenberg, 1998). The actual specificity and sensitivity of these techniques is directly dependent on the volume of blood actually examined and the skill and experience of the Microscopist.

2.8.1.1. Wet blood films

A small drop of blood is placed on to a clean microscope slide and covered with a cover-slip to spread the blood as a monolayer of cells. This is examined by light microscopy (x200) to detect any motile trypanosomes. This method is simple, inexpensive and gives immediate results. On the other hand, the diagnostic sensitivity of this method is generally low and depends on both the examiners experience and the level of parasitaemia. The species of trypanosome cannot be identified by this method although *T. vivax* can often be strongly suspected if the parasites move quickly forward through the microscope field but this is still inconclusive.

2.8.1.2. Stained thin smears

Stained smears are made by placing a small drop of blood (about 5 µl), e.g. from a haematocrit capillary tube, on a clean microscope slide approximately 20 mm from one end and spreading with the edge of another slide. The slide is dried quickly by waving in the air and protected from dust, flies and other insects. The slide is fixed for 3 minutes in methanol, and stained with Giemsa. After staining, the slide is washed gently under tap water and allowed to dry. The slides are then examined, with a ×50 or ×100 oil-immersion objective lens (OIE, 2012; Uilenberg, 1998). The sharp extremity of the smear must be extensively explored because of the capillary properties of blood and trypanosomes may be concentrated at the extremity (especially true for large species like *T. brucei* and *T. vivax*). The technique can also be used for biopsy samples of lymph obtained from punctured lymph nodes.

Use of thin stained smears allows for *Trypanosoma* species identification basing on their morphological characteristics though sensitivity is extremely low, and the main use is in fact the specific identification of trypanosomes found in thick or wet smears, but when only a few parasites have been seen in a fresh preparation or a thick film, the thin smear may well be negative. In addition, where different subgroups exist and have different pathogenicity, trypanosomes can only be distinguished using PCR.

2.8.1.3. Haematocrit Centrifugation Technique (HCT)

In the mild clinical or sub clinical cases also known as carriers, with low parasitaemia in which it is difficult to demonstrate the parasites, concentration methods become necessary. Blood is collected (70 µl) into heparinised capillary tubes (75 x 1.5 mm), which are then sealed at the dry end and centrifuged, sealed end down. Then the capillary tube is placed on a microscope slide and the buffy coat junction where the trypanosomes will be concentrated is microscopically examined for trypanosomes (Uilenberg, 1998). The capillary can also be cut and the buffy coat placed on a slide and checked under dark field microscope. This method has a disadvantage that no specific identification of parasites is possible (Uilenberg, 1998).

2.8.1.4. Animal inoculation

Laboratory animals may be used to reveal subclinical (non-patent) infections in domesticated animals. *Trypanosoma* spp. has a broad spectrum of infectivity for small rodents, and so rats and mice are often used (Uilenberg, 1998). In studies of *T. evansi* infections in camels, comparisons have been made between thick blood film examinations and rat or mouse inoculation methods; animal inoculation gave 15.2% and 17%, respectively. More positive results were obtained with animal inoculation than thick smears alone. Animal inoculation is highly sensitive and definitive when experimental animals susceptible to the particular trypanosome species and strain are used, it can thus be of great value as a research tool, but

not for all species. However, differences may arise in susceptibility to a given trypanosome species or strain between inbred laboratory strains of an experimental animal species (Uilenberg, 1998). Sensitivity of this *in-vivo* culture system may perhaps be increased by use of immunosuppressed laboratory animals. Drugs such as cyclophosphamide or hydrocortisone acetate and X-ray irradiation or splenectomy are used for this purpose (Monzón *et al.*, 1990; Monzón, 2006). This method is not suitable for routine diagnosis because suitable rodents have to be maintained and monitored for long periods, not forgetting the costs and logistics to and from the field (Uilenberg, 1998).

In the end, these direct methods are impractical for routine veterinary purposes. The application of the various tests in veterinary medicine is based upon cost effectiveness and the availability of suitably trained manpower. Although at the village level a veterinary auxiliary may be competent to measure the haematocrit and examine the buffy coat, it is prohibitively expensive to provide the necessary equipment widely for use under such conditions (Connor, 1992).

2.8.2. Indirect methods

The indirect tests are divided into serological and molecular tests. Indirect tests are normally carried out where direct examination of parasites fails or if the parasite cannot be directly demonstrated due to the life cycle in the host (Ambrosio and De Waal, 1990). Serological tests detect specific antibodies developed by the host against the infection, whereas molecular tests target or demonstrate the occurrence of a sequence on nucleotides, which are specific for a trypanosome subgenus, species, species or even type or strain (Uilenberg, 1998). Serological includes; Indirect Fluorescent Antibody (IFAT), Enzyme Linked Immunosorbent Assay (ELISA) and Card Agglutination Tests for Trypanosomiasis (CATT) that are regularly

employed. Recently, diagnostic molecular tests include DNA probes and PCR analysis (OIE, 2012; Uilenberg, 1998).

2.8.2.1. Indirect fluorescent antibody test

The Indirect Fluorescent Antibody Test (IFAT) detects specific antibodies produced by the host against an infection. The test is sensitive and the antigens to be used in the detection can be produced easily and in large quantities. However, the IFAT test has a number of drawbacks. It can only be carried out in a laboratory with qualified personnel. Also the procedure to detect antibodies of the trypanosome is rather long, complicated and is subjective with the possibility of different operators giving different results after titration. In addition, commercial conjugates are expensive and each batch has to be tested as to which dilution is optimal. Lastly, the species specific conjugates are commercially available only for most domesticated animals and therefore may not be applicable where tests on wild animals are involved (Uilenberg, 1998).

2.8.2.2. Enzyme-Linked Immunosorbent Assay (ELISA)

The principle of this test is very similar to that of IFAT test. The binding of anti-trypanosomal antibodies to the antigen is shown by a conjugate of serum immunoglobulins labeled with an enzyme, which can be visualized by adding an appropriate chromogenic substrate, the interaction between the enzyme and substrate (Rebeski *et al.*, 1999). ELISA test uses a reading instrument which will quickly give the optical density (OD) of each well (showing quantitatively the intensity of the interaction between the enzyme and the substrate), thus helping to speed up the processing of large numbers of sera. The ELISA test therefore lends itself to automation (Uilenberg, 1998). The results can also be viewed visually. Problems arise with the ELISA test as it lends itself to subjectivity because results are viewed visually. The challenges of this method are that it can not be conducted in the

field; an adequately equipped laboratory is needed. The reagents are comparatively expensive, antigens are not easy to produce, and where antigens of whole trypanosomes are used, the test is not species specific (OIE, 2012; Uilenberg, 1998).

2.8.2.3. Card agglutination test

It is well known that certain predominant variable antigen types (VATs) are expressed in common by different strains of salivarian trypanosomes from different areas (OIE, 2012). On this basis, a field test for the diagnosis of Gambian sleeping sickness, the card agglutination test – CATT/*T. brucei gambiense* – was developed at the Laboratory of Serology, Institute of Tropical Medicine, Antwerp in Belgium. For the diagnosis of *T. evansi* infections, a similar test system has also been developed, CATT/*T. evansi* (Nantulya, 1995).

The test, which is simple to carry out even in the field, has been used for diagnosis of *T. evansi* (Nantulya, 1990; Uilenberg, 1998; Chappuis *et al.*, 2004) but is unlikely to be of use for *T. vivax* or *T. Congolense*. This is because of the difficulty of identifying suitable variable antigens of these species (Nantulya, 1990; Luckins, 1992). Lastly, its specificity and sensitivity needs further evaluation. However CATT is being used as an initial screen in Field diagnosis of HAT due to infection of *T.b.gambiense* (Chappuis *et al.*, 2004; Mitashi *et al.*, 2012).

2.8.2.4. Detection of trypanosomal DNA

During the past few years, several research centres have been working on the development of PCR procedures for the detection of minute amounts of trypanosomal DNA sequences. This is so because parasite DNA can be amplified into a detectable quantity if the number of cycles is high making it extremely sensitive. It can also be highly specific depending on the primers available for the reaction (Uilenberg 1998; Aslam *et al.*, 2010; OIE, 2012). A comparative study of two methods of diagnosis for the presence of trypanosomes was carried

out on 80 apparently healthy animals in contact with 51 animals which were clinically positive for *T. evansi.* Blood samples collected from the 80 animals were each subjected to two tests that is microscopic examination of blood films and PCR analysis. Results obtained from blood showed a percentage infection rate of 6.3% (5/80) for microscopic examination and 43.6% (35/80) for PCR analysis.Consequently, PCR was considered the most suitable for early diagnosis and a confirmatory test of Trypanosoma (Radwan & El Madawy, 2010).

2.9. Observations of previous studies done

Thumbi *et al.* (2010), in western Kenya observed the trypanosome prevalence of 41% and 29% in Suba and Teso districts, respectively. *Trypanosoma vivax* infections were most prevalent in both areas. Higher proportions of *T. brucei* infections (12%) were observed in Suba, a known sleeping sickness foci compared with 2% in Teso. Average nearest neighbour analysis showed the pattern of trypanosome infections as random. An overlay with tsetse maps showed cases lying outside the tsetse infested areas, mostly being cases of *T. vivax* which is known to be transmitted both biologically by tsetse and mechanically by biting flies.

Magona *et al.*, (2011) revealed that cattle kept under open grazing (n = 1612) had a lower mean packed cell volume (PCV) (28.6% 95% CI 28.4-28.6) and a higher prevalence of trypanosome infection (7.3%) than those kept under tethering (n = 451): mean PCV-30.2% (95% CI 29.7-30.7) and prevalence-6.7%. Overall, Nkedi Zebu had the highest prevalence (7.2%), followed by Ankole (7%) and Crosses (0%) due to the difference in management systems of open grazing and tethering and level of management. There was no difference between the mean PCV of Nkedi Zebu (29.0% 95% CI 28.8-29.2) and Ankole (28.8% 95% CI 28.0-29.6), which were higher than that of Crosses (27.7% 95% CI 24.5-30.8).

Magona *et al.*, (2011) observed differences in the proportion of trypanosome species affecting animals in the different grazing systems. In the open grazing system proportions of

T. vivax was the dominant species (59%), followed by *T. congolense* with 21%, mixed infections with 21% and finally *T. brucei* with 5%. In the tethering system the proportion of *T. vivax* was 73%, followed by *T. congolense* with 20%, and then mixed infection with 7% and finally *T. brucei* having no infections.

The major differences were probably because tethering restricts free movement of animals, thereby reducing chances of animals being bitten. Increased exposure to tsetse bites increases the chances of infection by a wider range of trypanosome species (Magona *et al.*, 2011).

Research carried out in Ogun state Nigeria revealed that of the 39.1% positive cattle examined in Ogunola community, 23.9% were positive for *T. vivax*, 8.7% were positive for *T. congolense*, 2.2% were positive for *T. b. brucei* while 4.3% were cases of mixed infection (Olufemi *et al.*,2010). Selby *et al.*, (2013) in the period 2008 and 2009 conducted a study that involved a mixture of biomedical and social research. The study was conducted in 10 livestock markets in the districts of Apac, Lira Soroti, Kamuli, Pallisa, Kumi and Bukedea. Polymerase Chain Reaction analysis of blood samples revealed 8 out of the 10 markets had animals infected with *T. b. rhodesiense.* The prevalence ranged from 3.8% in Adograo market in Apac to 0% in both Arapai market and Bukedea market in Soroti and Bukedea districts, respectively. Selby *et al.*, (2013) on interaction with District Veterinary Officers form 7 districts in Acholi and West Nile sub-regions revealed a total of 18 different organizations, 3 Government and 15 Non-Governmental that were involved in cattle restocking. These animals are bought from the 10 markets and it was observed that minimal regular trypanocidal treatment was given at the point of sales while most of the treatments given were antibiotics (Selby *et al.*, 2013).

2.10. Treatment of animals and drug resistance

The field control of AAT has, over the years, relied on two broad strategies: using chemotherapeutic agents on infected animals and vector control. In general, however, the chemotherapeutic approach is used much more than vector control because it is easier to kill the trypanosomes than the flies (Ilemobade, 2009). Drug control of animal trypanosomiasis relies essentially on three drugs, namely: Homidium (Homidium chloride – Novidium; and Homidium bromide – Ethidium), Diminazine aceturate (Berenil) and Isometamidium chloride (Samorin, Trypamidium) (Geerts & Holmes, 1998). Quinapyramine sulphate (Antrycide) has been reintroduced because of the need to especially combat camel trypanosomiasis (Geerts & Holmes, 1998).

The three trypanocides used to control tsetse-transmitted trypanosomiasis in domestic animals in Africa have been in use for over 35 years in 1998 (Geerts & Holmes, 1998), now 52 years in 2015 and, not surprisingly, resistance of trypanosomes to these drugs has emerged (Geerts & Holmes, 1998). The first reports on resistance to Isometamidium chloride, Homidium and Diminazine aceturate date from the 1960s (Jones-Davies, 1967; Na'Isa, 1967). Currently, there are 17 African countries in which trypanocidal drug resistance has been reported (Delespaux *et al.*, 2008). And this was a greater number than the 13 countries mentioned by Geerts and Holmes (1998). In addition, drug resistance to Diminazene aceturate was also reported in Kibaha Tanzania (Mbwambo *et al.*, 1988), Mozambique (Jamal, 2005), Mali, Guinea and Cameroon (Mamoudou, 2006). In several African countries trypanocidal drug resistance is suspected to be present but has not been demonstrated using standardized tests.

However, there are 10 countries (Burkina Faso, Mali, Cameroon, Ethiopia, Kenya, Nigeria, Tanzania, Zambia, Uganda and Zimbabwe) in which area-wide drug resistance was

demonstrated in at least one region of the country (Delespaux *et al.*, 2008). These studies showed that drug sensitivity and resistance varied considerably between individual trypanosomes. In most of the countries *T. congolense* showed resistance to mostly Isometamidium and in a few cases to Diminazene and Homidium. *Trypanosoma brucei* showed resistance to Homidium, Diminazene and Isometamidium. *Trypanosoma vivax* showed resistance to mainly Diminazene and Isometamidium though resistance to Homidium was also reported (Geerts *et al.*, 2001). In Uganda in particular, *T. brucei* showed resistance to Diminazene and Isometamidium (Matovu *et al.*, 2001). In general drug resistance to Isometamidium chloride is more widespread than to Diminazine aceturate (Geerts *et al.*, 2001).

Besides, international pharmaceutical companies have shown little interest in the development of new trypanocides for use in either animals or humans. This little interest is because of the relatively limited market in Africa and the high costs of developing and licensing new drugs (Geerts *et al.*, 2001). Therefore, the current challenge is to achieve optimal use of the relatively old existing drugs, and it is in this context that the problem of drug resistance has to be quantified. It is estimated that about 35 million doses of trypanocides are administered annually in Africa (Geerts *et al.*, 2001) with fake or substandard drugs accounting for up to 60% of the drug market in most developing countries (Feldmann, 2001).

The report of resistance presents a major area of concern to the farmers. Where farmers treat their animals based on disease symptoms and with no laboratory diagnosis increases the occurrence of trypanosome resistance. In the end this results in increased prevalence of trypanosomiasis due to failure of drugs to eliminate the parasites. And with the reluctance of new companies to produce new drugs it may lead to severe losses in the livestock industry.

The reports of resistance present a major problem to farmers in that in-spite of these treatments being administered. This also means that there will be an increased likelihood of animals being carriers or infected with trypanosomes as treatment failure creates reservoirs of infection.

From the literature, it is evident that very little information (Agwech *et al.*, 2015) is available on the prevalence of trypanosomiasis affecting cattle and the role of cattle in the epidemiology of HAT in Amuru and Nwoya districts. Hence, there was need to undertake this crucial study to determine the prevalence of trypanosomes in cattle in the study areas of Amuru and Nwoya districts.

CHAPTER THREE

MATERIALS AND METHODS

3.0. Study area

The research was carried out in the trypanosomiasis endemic areas of Nwoya and Amuru districts which were purposively sampled because of the livestock-wildlife interface with Murchison National Park, and cross border movement of animals to South Sudan from endemic areas of Uganda putting animals at a risk of infection (Selby *et al.*,2013).Both Nwoya and Amuru were formally part of Gulu district.

Amuru district is bordered by a number of districts; Adjumani district to the North, South Sudan and Lamwo district to the Northeast, Gulu district to the East, Nwoya district to the South, Nebbi district to the Southwest and Arua district to the West. Nwoya district is bordered by Amuru district to the North, Gulu district to the Northeast, Oyam district to the south, Bullisa district to the Southwest, Kiryandongo district to the Southeast, Masindi district to the Southwest and Nebbi district to the West of Nwoya. Amuru district covers a total area of 3,625.9 km^2 with the district headquarters located at coordinates at $02^0$50'N $33^0$05'E. Nwoya district covers a total area of 4,736.2 km^2 with the district headquarters located at coordinates $02^0$38'N $32^0$00E.

The climatic conditions in Nwoya and Amuru districts are equally characterized by dry and wet seasons. The average total annual rainfall precipitation is 1,500 mm per year (Matthias, 2009; Zanchi *et al.*, 2013) with a monthly average varying between 14 mm in January and 230 mm in August. However, measurements taken in 2005 and 2007 in Aswa-Lolim suggest average rainfall could be lower in some areas of Amuru district, from 900 to 1000 mm per year. The wet season has a span of eight months (April to November), with the highest peaks during May, August and September. Subsequently, the dry season starts from November and

extends to March. The average annual temperature of 22 °C is slightly lower than that of Gulu (16 °C–36 °C) (Zanchi *et al.*, 2013). Amuru district has the highest forest cover, occupying about3.3% of the total land size and open savannah grassland with 90% of the land suitable for farming. On the other hand, Purongo sub-county in Nwoya district shares part of its land with Murchison National Park, being characterised by woody open savannah grassland.

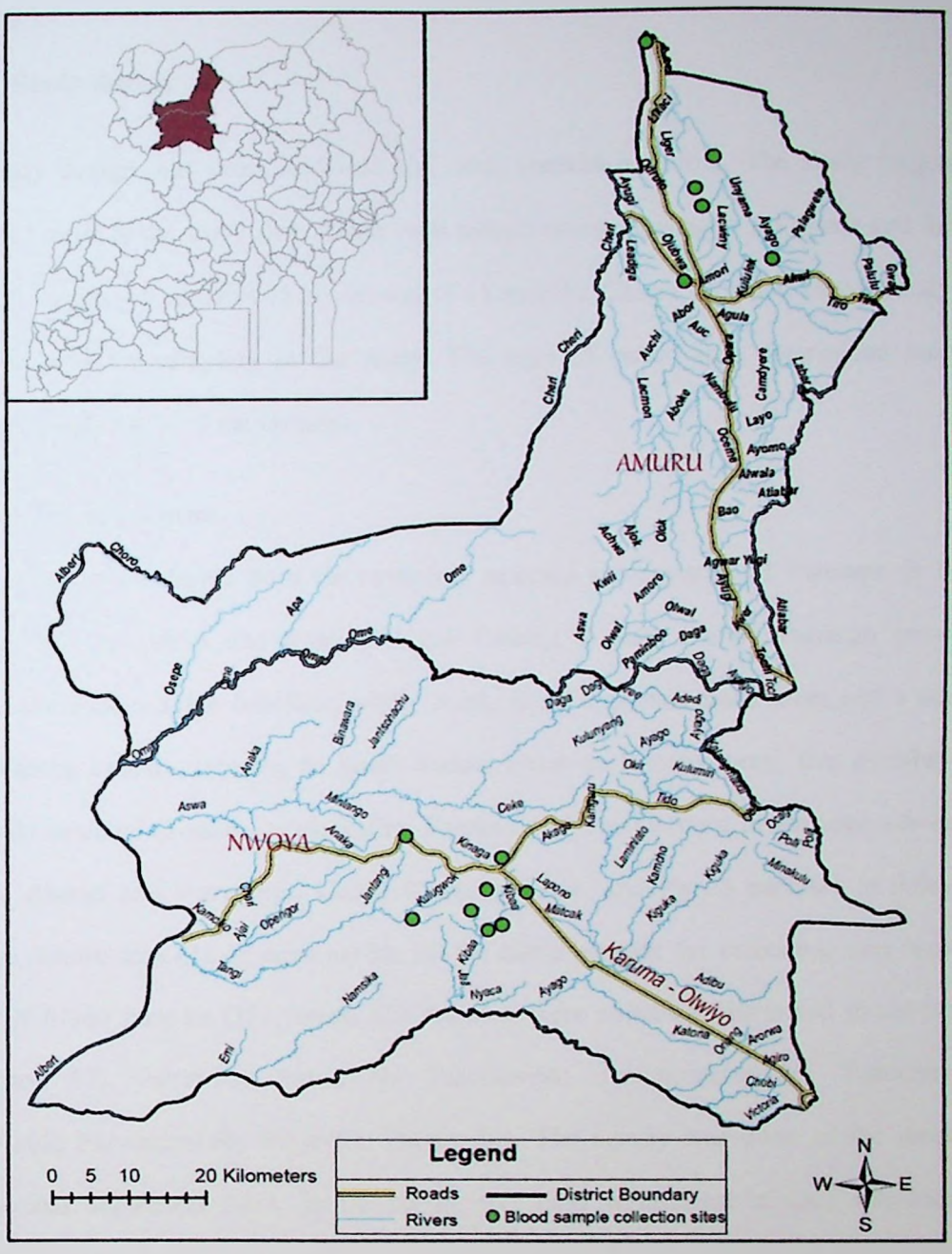

Figure 3.1: Location of study sites in Amuru and Nwoya districts showing sampled parishes and blood sample collection sites

3.1. Study design

The study design was cross-sectional and used quantitative tools. The study targeted all breeds of cattle in the study area. Cattle were categorized according to sex (male and female), age (< 9 month, >9 month< 18 month and > 18 month). Calves included in this study were those brought for sampling in the study. The ages of cattle were determined based on information provided by the farmers.

3.2. Sampling Frame

The samples were drawn from the randomly selected sub-counties of Purongo in Nwoya County, Nwoya district and Atiak in Kilak County, Amuru district. Purongo provides a unique livestock-wildlife interface, while, Atiak, is an important trade route and a stopover for domestic animals crossing to South Sudan. From each sub-county, five parishes were randomly selected (Todora, Pabit, Patira, Paromo, and Pawatomeru in Purongo sub-county, Nwoya district and Pupwonya, Okidi, Parwaca, Bibia, and Pacilo parishes in Atiak sub-county, Amuru district). In each parish, all the cattle brought for screening were tested. In total 789 blood samples (357 males, 432 females) were collected distributed in ten parishes (Todora= 57; Pabit=32; Patira=98; Paromo=66; Pawatomeru=136; Pupwonya=50; Okidi=108; Parwaca=140; Bibia=52; Pacilo=50). The survey was done in the months of August and September 2011. In the survey five days were spent in each sub-county of Purongo and Atiak.

3.2.1. Study population

The survey covered only cattle in the study area. All breeds of cattle were sampled from the two districts of Amuru and Nwoya. The breeds included the Ankole, Zebu and cross breeds of Ankole and Zebu (Rege *et al.*, 1999; Magona *et al.*, 2011).

3.2.2. Sample size determination

The expected sample size for the number of cattle to estimate the prevalence of different trypanosome species in Purongo (Nwoya) and Atiak(Amuru) was computed using the formula provided by Uilenberg (1998),basing on an expected prevalence of 20%, an accepted error of 5% and thus 95% confidence interval.

$n = P(100 - P)Z^2/d^2$

Where,

P is the anticipated prevalence

Z is the appropriate value from the standard normal distribution, at 5% error level

d is the desired precision

To calculate the number of cattle to be sampled the study

Sample size is thus given by; 20 *(100-20)1.960^2/5^2= 246 *samples*

The number of animals sampled depended on the number of animals present at the time of sampling in the different parishes.

The expected minimum number of animals sampled was 246.

3.3. Blood sample collections, examination and storage

Whole blood was collected from bovine in EDTA tubes following jugular vein puncture. The collected blood was analysed microscopically using HCT, complemented with direct smears to examine for presence of parasites. In the HCT, two heparinized capillary tubes were filled ¾ with blood and centrifuged at a speed of 1000 rpm for five minutes in a Haematocrit centrifuge, and the buffy coat as seen in the capillary tubes were later observed under the microscope for presence of trypanosomes (Uilenberg, 1998). Where the buffy coat was negative, the capillary tube was cut using a diamond pencil and wet smear made from the buffy coat and observed under a microscope (Olympus, magnification X40) to detect the

presence or lack of parasites. The blood samples confirmed to contain the parasites by microscopy were spotted on the Whatman FTA cards and kept at room temperature in an airtight container with silica gel for further PCR analysis. Animals found to be parasitologically positive were treated with diminazene aceturate by the District Veterinary personnel.

A hand held global positioning system was used to geo-reference the locations within the study area. Coordinates were taken in the World Geodetic System 1984 (WGS84) geographical coordinate system in decimal degrees. Data was exported to Universal Transverse Mercator for map drawing. Geographical Information System (GIS) map was produced showing the locations of where blood samples were collected.

3.4. DNA extraction from FTA blood spots

Using a 3 mm micro-punch, five sample discs were cut from a dried blood and buffy coat spots from the Whatman FTA cards and placed in a labelled 1.5 ml eppendorf tube. To prevent carry-over contamination between samples, punches were cleaned after each sample using diluted household bleach (Sodium hypochlorite) and 70% ethanol, and then flamed. The punch was then used to cut a clean filter paper, before using on the next sample.

DNA extraction was done using the Invitrogen™ PureLink Genomic DNA kit for purification of genomic DNA (Invitrogen Inc.) following the manufacturer's instructions. 180 µl of PureLink Genomic Digestion buffer and 20 µl of proteinase K were added to the discs / tissues in 1.5 ml tubes and incubated at 55°C for three hours with occasional vortexing. The sample was centrifuged at a maximum speed of 10,000 rpm for three minutes at room temperature to pellet debris. The supernatant was transferred to a clean 1.5 ml tube. 20 µl RNAse A was added to the lysate. The tube was vortexed and incubated at room temperature for two min. 200 µl PureLink Genomic lysis/binding buffer was added, vortexed, and briefly

centrifuged. Then 200 µl of ethanol was added to the lysate and mixed well by vortexing to obtain a homogenous solution. The lysate was added to a PureLink spin column with a collection tube, and centrifuged at 10000rpmfor 1 min. The spin column was washed twice using 500 µl of wash buffers W1 and W2, respectively. The column was then placed in a clean 1.5 ml eppendorf tube and the DNA was eluted in 30 µl elution buffer. The purified DNA sample was stored at -20°C for later use.

3.5. DNA amplification

ITS PCR using the protocol of Njiru *et al.,* (2005) was employed for the assays in this study. The ITS1 CF and ITS1 BR primers previously designed to amplify the internal transcribed spacer (ITS1) of rDNA, was used for the tests. Primers for the ITS1 PCR were: ITS1 CF, 5'CCGGAAGTTCACCGATATTG-3' and ITS1 BR, 5'TTGCTGCGTTCTTCAACGAA-3' used as the forward and reverse primers respectively (Njiru *et al.,* 2005). All primers were supplied by Bioneer Corporation.

PCR was carried out in a GeneAmp PCR System 9700 thermo cycler in a total volume of 20 µl containing 10pmol of each primer, 10 mM Tris-Cl, pH 8.3 and 50 mM KCl, 1.5 mM $MgCl_2$,2.5 mM dNTPs, 2µl of the DNA template and 1unit Taq DNA Polymerase. The conditions were as follows: initial denaturation at 94°C for 5 mins, followed by 35 cycles of denaturation at 94 °C for 40 seconds, annealing at 58°C for 40s, extension at 72 °C for 90seconds, and a final extension at 72°C for 5 minutes.

Positive and negative controls were included in each PCR assay. The positive controls were obtained from trypanosome DNA stocks preserved at BeCA-ILRI Hub Research laboratories where this work was carried out. Negative controls consisted of the Master Mix for each PCR and fresh double distilled water instead of parasite DNA. A 4 3µl of the amplified products were then loaded into a casted Gel red-stained 1.8% agarose gel with a 75bp gene marker,

positive controls and negative controls. These were run in a Mupid-exu Sub-marine electrophoresis (Helix Technologies Inc. MEXO 0800137) gel tank for 45 minutes at 100V in 0.5X TBE(0.045 M Tris-Borate and 0.001 M EDTA) buffer. The amplified products were then visualized under ultra-violet illumination and a picture of the gel taken using the gel documentation and analysis systems. The bands were then sized and used to infer the species or sub-species of the trypanosomes based on the expected and obtained sizes as presented in Njiru *et al.* (2005).

3.6. Data analysis

A Pearson chi-square test and logistic regression were used to determine the association between age, sex, location and prevalence of trypanosomiasis in the study area. A p value of 0.05 was considered significant. All analyses were carried out using the statistical package Stata version 12.

3.7. Limitation of the study

The study was sponsored by the MSI Trypanosome Diagnostic project (MSI/WAI/3/29/2010) of Gulu University. However, the project came to an end before completion of the study. Due to limitation of funds, PCR analysis was only done on the parasitologically positive blood samples spotted on Whatman FTA cards and not on all the samples that were collected.

3.8. Ethical considerations

To conduct this study, the approval by the Gulu University Institutional Review Committee (Clearance no. GU/IRB/05/06/11) and the Uganda National Council for Science and Technology (UNCST) was obtained. The study communities were briefed on the objectives of the study and hence the importance of their participation in the study. Permission was sought from respondents before blood samples were obtained from their domestic animals.

CHAPTER FOUR

RESULTS

4.1. Prevalence of Trypanosomiasis

Examination of blood samples collected from 789 cattle using microscopy revealed an overall prevalence of 8.7% (69/789) with 91.3% (720/789) of the total number of cattle testing negative. Microscopic examination of blood samples showed Amuru district with a prevalence of 10.8% (43/400) and 6.7% (26/389) for Nwoya district (Table 4.1).

Table 4.1: Prevalence of Trypanosomosis in Amuru and Nwoya districts

District	Parish	Negative	Positive	Total	Prevalence (%) [(Number infected / Number examined)*100]
Nwoya	Todora	57	0	57	0%
	Pabit	28	4	32	12.5%
	Patira	78	20	98	20.4%
	Paromo	66	0	66	0%
	Pawatomeru	134	2	136	1.5%
Sub-total Nwoya		**363**	**26**	**389**	**6.7%**
Amuru	Pupwonya	50	0	50	0%
	Okidi	94	14	108	13%
	Parwaca	133	7	140	5%
	Bibia	52	0	52	0%
	Pacilo	28	22	50	44%
Sub-total Amuru		**357**	**43**	**400**	**10.8**
Total		**720**	**69**	**789**	**8.7%**

Microscopic examination found female cattle to have a prevalence of 9% (39/432*100) and males 8.4% (30/357*100) (Figure 4.3). Cattle older than 18 months had a prevalence of 9.4% (59/567*100) followed by cattle aged between 9 and 18 months with 7.9% (8/93*100) and

lastly animals less than 9 month of age with the least infection prevalence of 3.2% (2/60*100) (Figure 4.1).

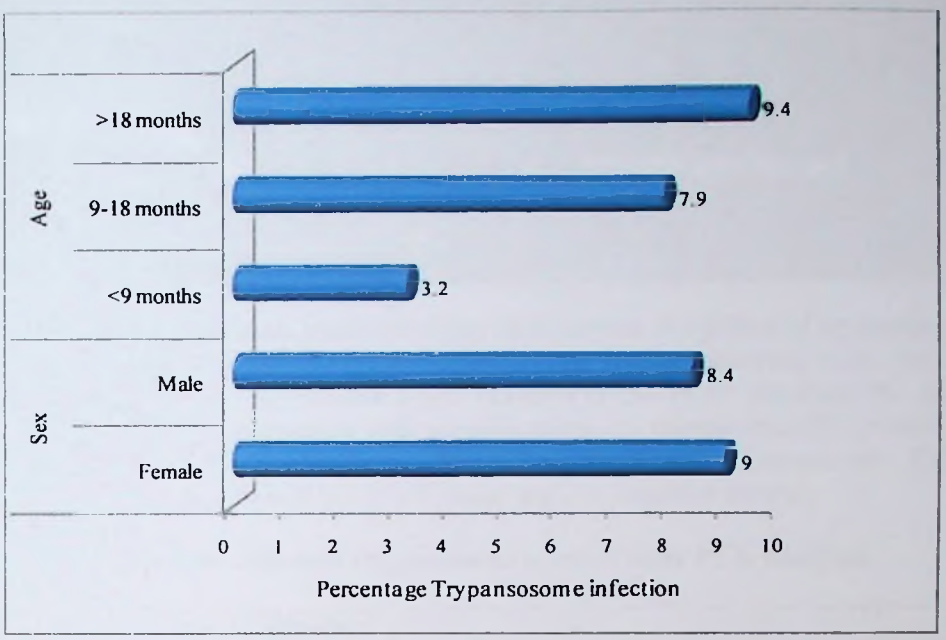

Figure 4.1: Prevalence of trypanosomiasis in the different ages and sexes of cattle

4.2. Identification of trypanosome species

Through PCR analysis of the 69 HCT microscopic positive samples, 62 were positive for different trypanosome species with 11.6 % (8/69) found to be infected with *T. congolense* and 78.2% (54/69) with *T. vivax* while 7(10.2%) failed to amplify or could not be detected (Figure 4.2, Table 4.2). No Trypanozoon group trypanosome was detected by PCR test. At district level Nwoya had 30.8% (8/26) of the samples positive for *T. congolense* and 50% (13/26) for *T. vivax*. In Amuru 95.3% (41/400) of the animals tested positive for *T. vivax* species and none of the samples tested positive for *T. congolense* (Table 4.2).

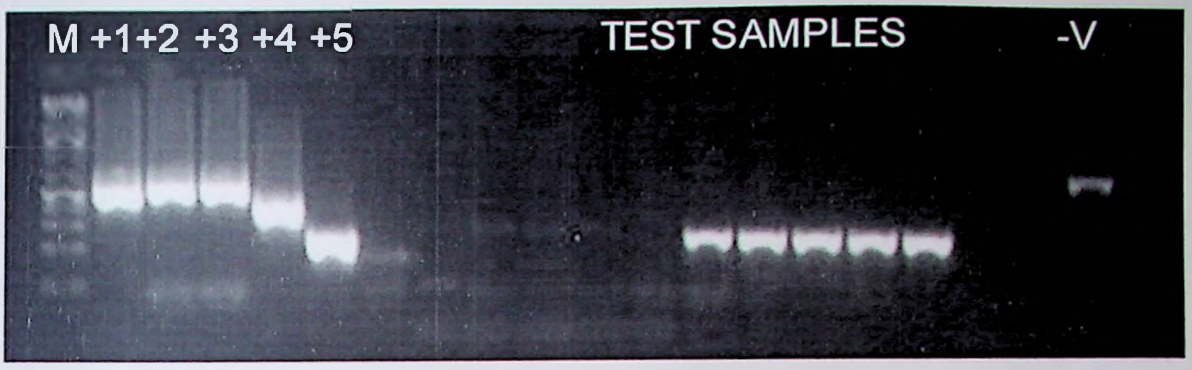

Figure 4.2: Gel electrophoresis photo showing clear bands of species of trypanosomes
Key: Agarose gel electrophoresis photo showing the results obtained with the internal transcribed spacer 1 (ITS1) polymerase chain reaction (ITS1-PCR) showing the species of trypanosomes after DNA extraction and amplification of microscopically positive cattle blood spotted on FTA cards+1: *T.congolense Savannah*; +2: *T.congolense Kilifi*; +3: *T.congolense Forest;* +4: *T.brucei s.l;* +5: *T. vivax* and –v: negative control.

Table 4.2: Distribution of different trypanosome species after PCR analysis

Variable	District				
	Nwoya		Amuru		
PCR analysis	Number	Percentage	Number	Percentage	Total
Trypanosoma congolense	8	30.8	0	0	8 (11.6%)
Trypanosoma vivax	13	50	41	95.3	54 (78.2%)
No detection/amplification	5	19.2	2	4.7	7 (10.2%)
Total	**26**	**100**	**43**	**100**	**69(100%)**

There was a significant association between district location and the prevalence of trypanosomiasis (Chi-square value ($\chi 2$) =4.08, df =1, p-value < 0.05). Amuru district had a higher risk of infection with trypanosomiasis (62.3%) than Nwoya district (37.7%; OR 1.7; 95% CL: 1.0–2.8; P = 0.043). Furthermore, the prevalence of infections was significantly associated with the parish location ($\chi 2$=130.6, df=9, p< 0.001).Pacilo parish in Nwoya district has higher prevalence than Patira parish in Amuru district. Animals in Pacilo had a higher

odds of testing positive trypanosomiasis (44%) than Patira parish (20.4%; OR 0.3; 95% CL: 0.2-0.7; P=0.003). There was however no significant association between sex, age and prevalence of trypanosomiasis respectively (Table 4.3, Table 4.4).

Table 4.3: The association between different variables and microscopic prevalence of Trypanosomiasis

Variable	Negative (%)	Positive (%)	P-value*(χ2,df)
District			
Nwoya	363 (50.4)	26 (37.7)	0.043*(4.08, 1)
Amuru	357 (49.6)	43 (62.3)	
Sex			
Female	393 (54.6)	39 (56.5)	0.757 (0.09, 1)
Male	327 (45.4)	30 (43.5)	
Age			
<9 months	60 (8.3)	2 (2.9)	0.245 (2.81, 2)
9 to 18 months	93 (12.9)	8 (11.6)	
>18 months	567 (78.8)	59 (85.5)	
* Significant chi-squared p-value			

Table 4.4: Logistic Regression results for different predictors of infection

Variable	Univariate Odds ratio(95% CI)	P-Value
District		
Nwoya	1	
Amuru	1.7 (1.0178 - 2.8)	0.043
Sex		
Female	1	
Male	0.9 (0.6-1.5)	0.757
Age in months		
<9	1	
9-18	2.6 (0.5-12.6)	0.241
>18	3.1(0.7-13.1)	0.120

4.3. Role of cattle in the epidemiology of human trypanosomiasis

None of the animals tested positive for the *T. brucei* group of trypanosomes after analysis using PCR diagnostic tools.PCR analysis showed animals infected with *T. vivax* and *T. congolense* (Table 4.5).

Table 4.5: Animal number and trypanosome species infection

No.	Parish	No. of animals	Trypanosome species		
			T. brucei sp.	*T.vivax*	*T.congolense*
1	Todora	57	0	0	0
2	Pabit	32	0	1	3
3	Patira	98	0	10	5
4	Paromo	66	0	0	0
5	Pawatomeru	136	0	2	0
6	Pupwonya	50	0	0	0
7	Okidi	108	0	13	0
8	Parwaca	140	0	7	0
9	Bibia	52	0	0	0
10	Pacilo	50	0	21	0
Total		**789**	**0**	**54**	**8**

The AAT infections were highest in the Pacilo parish and lowest in the parishes of Bibia, Pupwonya and Paromo (Table 4.5). There were no *T. brucei* human infective trypanosomes in all the Parishes. According to the herd size, each kraal had an average of 56 cattle (789/14) and each farmer had an average of 7 cattle (789/121).

CHAPTER FIVE

DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1. Prevalence of Trypanosomiasis in Nwoya and Amuru districts

The study revealed an overall prevalence of 8.7% in Amuru and Nwoya district. Parasitological prevalence of trypanosomiasis was highest in Atiak sub-county (10.8%) in Amuru district and lowest in Purongo sub-county (6.7%) in Nwoya district. In South-western Uganda, an average prevalence of 6.42% with 5.56% and 7.26% specifically from Mbarara and Mubende districts respectively was reported (Waiswa *et al.*, 2004). In Butalega district in Eastern Uganda, a prevalence of AAT was 8.9% (Jing *et al.*, 2009). At parish level the prevalence rate varied from parish to parish in the different districts with Pacilo parish in Atiak having the highest trypanosomiasis incidence of 44% and Patira in Nwoya having the highest with 20.4%. The differences in infection can be explained by a number of specific factors such as different microhabitats, land use patterns, husbandry practices, season (Majekodunmi *et al.*, 2013), host species, age (von Wissmann *et al.*, 2011), feeding areas, grazing routes (Sam-Wobo *et al.*, 2010), presence and absence of tsetse and tsetse control practices in the different areas (Uilenberg, 1998). Differences in the two sub-counties of Purongo in Nwoya and Atiak in Amuru can be explained by the differences in the micro-habitats. Purongo in Nwoya is most parts an open savannah grassland with a few shrubs whereas Atiak in Amuru being mostly forested with water points on river Achwa that provides a favourable microhabitat for tsetse flies increasing the intensity of contacts between the cattle and infected tsetse flies. In addition, the low prevalence in cattle in Purongo could be explained by the presence of wildlife that could be preferred by tsetse (Clausen *et al.*, 1998), ultimately a higher infection rate in Amuru. In the case of Patira parish in Atiak sub-county, the high prevalence could probably be due to the proximity of the

animal kraal to Achwa River that is an important microhabitat for tsetse, increasing exposure of the cattle to tsetse bites.

The study found no statistically significant association between cattle age group and trypanosome infection rates. This agrees with results of several previous studies (e.g., Ohaeri, 2010, Biyazen *et al.*, 2014, Gebreyohannes *et al.*, 2014 and Bekele and Nasir, 2011). This could be due to the equal chance of exposure to the parasite (Biyazen *et al.*, 2014) and differences in sample size among the age groups (Bekele and Nasir, 2011). However, this is contrast to a study conducted in the same districts by Angwech *et al.* (2015) that reported a significant difference in prevalence among the different age groups. This might be explained by the fact that the cattle were sampled at different periods, parishes, and difference in sample size.

The study found no statistically significant association between cattle sex and trypanosome infection rates. Mulaw *et al.*, (2011) and Rowlands *et al.*, (2001) also reported no significant difference in trypanosome infections between males and females. This was attributed to the similarity in the ecosystems of the study locations that supported proliferation of both tsetse and biting flies that feed on both sexes indiscriminately. In contrast to Mulaw *et al.*, (2011) and Rowlands *et al.* (2001), Angwech *et al.* (2015) working in a similar area reported that males had a statistically significant higher prevalence of trypanosomiasis than females. This contrast could be due to the fact that samples were collected at different times and in different parishes. In addition, Angwech *et al.* (2015) obtained the trypanosomiasis prevalence through PCR analysis of all blood samples collected and not from only parasitologically positive samples.

The major trypanosome species affecting cattle in the districts of Amuru and Nwoya is *T. vivax*. However, *T. congolense* species was also found. A similar study carried out in Amuru

and Nwoya (Angwech *et al.*, 2015), Tororo, Jinja and Rakai districts in Uganda (Magona *et al.*. 2008; Biryomumaisho *et al.*, 2013), Suba and Teso districts in western Kenya (Thumbi *et al.*, 2010), Ogun state in Nigeria (Sam Wobo *et al.*, 2010) revealed a similar trend. The higher percentage of *Trypanosoma vivax* in the study could be explained through its mode of transmission. *Trypanosoma vivax and T. congolense* can be transmitted biologically by the tsetse fly and mechanically by tabanid flies (Uilenberg, 1998; Desquesnes & Dia, 2003a, 2003b). However, studies indicate that mechanical transmission incidence of *T.vivax* under experimental conditions are higher than that of *T. congolense* (Desquesnes & Dia, 2003a, 2003b). Also *Trypanosoma vivax* infections tend to be common at a time when the survival of tsetse flies is lowest favouring the development of *T. vivax* that has a short development cycle (Leak, 1999; Magona *et al.*, 2008). Agwech *et al* (2015) indicated that the predominant tsetse species in the study area as *Glossina fuscipes fuscipes* that has a higher transmission index for *T. vivax* than *T. congolense* (Moloo *et al.*, 1980).

Using Buffy coat technique, 69 animals were found positive, whereas further analysis of these samples using PCR showed 62 samples positive for different trypanosome species. This decrease is surprising since PCR analysis is a more sensitive test (Radwan & El Madawy, 2010).However, the sensitivity of molecular tools depends on degree of parasitaemia in trypanosome-infected animals, because the chance in a less parasitized animal to have detectable trypanosome DNA template is less (Biryomumaisho *et al.*, 2013). Trypanosomiasis in most natural infections takes a chronic course that is characterized by low parasitaemia, making the technique less efficient in detecting natural trypanosome infections. This was evident in the study where animals found positive were not showing any symptoms of the disease.

5.2. Role of cattle in the epidemiology of Human African Trypanosomiasis

A number of animals including cattle have been reported as potential hosts of *T. brucei* group that causes HAT (Auty *et al.*, 2012; FAO, 1992; Welburn *et al.*, 2001b; Selby *et al.*, 2013). However, in this survey none of the cattle in Amuru and Nwoya district sampled tested positive for *T. brucei rhodesiense* and *T. b. gambiense*. A similar study from North-western Uganda, reported that cattle are not infected with *T. b. gambiense* and *T. b. rhodesiense* parasites (Balyeidhusa *et al.*, 2012). The implication of this finding is that the cattle with their vectors, tsetse, are apparently free from the human infective *T. brucei* species or were not carrying infection at the time of study. If the tsetse flies were infected, this would pose a serious risk to the livelihood and well-being of the farmers.

Studies show that the presence of cattle can form a transmission cycle for HAT (Welburn *et al.*, 2001b; Waiswa *et al.*, 2003). However, despite the absence of human infective parasites, animal reservoirs still remain a threat, which means elimination strategies may have to be reconsidered. This is because targeting human cases alone would be insufficient for control and re-introduction from animal reservoirs would remain a threat (Funk *et al.*, 2013). This risk is further augmented by the continued restocking of areas that were under civil strife in Northern Uganda by the Government and NGOs (Jameli *et al.*, 2009; Selby *et al.*, 2013). A number of these animals used in the restocking are bought from livestock markets in Apac, Lira Soroti, Kamuli, Pallisa, Kumi, Kaberamaido and Bukedea, and Dokolo; areas endemic for *T. b. rhodesiense* trypanosomiasis putting the human population at risk (Selby *et al.*, 2013).

Van den Bossche *et al.*, (2010) argued that the distribution, prevalence and impact of vector-borne diseases are often affected by anthropogenic environmental changes that alter the interactions between the host, the parasite and the vector. In the case of tsetse-transmitted

trypanosomiasis these changes are a result of the encroachment of people and their livestock into tsetse-infested areas and animals being kept in kraals in close proximity to homesteads. Herdsmen that take their animals to graze increase the risks of their infection with HAT. Animals kept in close proximity to human settlements may in turn bring infection close to the people exposing them to a certain level of risk (Fevre *et al.*, 2001). The animal reservoir ensures the continued occurrence of the disease in humans, (Rogers, 1988). In some areas, interactions occur where livestock and wildlife coexist, and a mixed transmission pattern is observed like in western Tanzania (Alsopp, 1972). This is a possibility especially in areas of Purongo Sub-County where part of the sub-county is in the Murchison National Park where cattle owners graze their animals and live in the areas that border the park.

Farmers carrying out diagnosis and treatment of animals based on clinical signs or on only visibly diseased animals are unlikely to achieve the sustainable disruption of the animal infective and zoonotic human infective trypanosomiasis. Study by von Wissmann *et al* (2011) showed that most infections in cattle were detected in animals that don't show classical clinical signs of trypanosomiasis. In fact the continued treatment of animals by unqualified farmers may lead to increased resistance to drugs used for trypanosome control due to misuse of drugs (Geerts & Holmes, 1998).

5.3. Conclusions

In general, the results show that *T. vivax* and *T. congolense* are the major species infecting cattle in Amuru and Nwoya. If the disease is left unchecked in the area of study, reductions in cattle productivity are likely to occur. Despite the absence of the human infective trypanosomes in cattle, the possibility of infection is still present. Human African trypanosomiasis may be re-introduced from wild animals that are known reservoirs of human infective trypanosomes, in the case of Purongo in Nwoya district. Animals brought from the

districts of Apac, Lira Soroti, Kamuli, Pallisa, Kumi, Kaberamaido and Bukedea, and Dokolo; areas endemic for *T. b. rhodesiense* (Selby, *et al.,* 2013) to the districts of Amuru and Nwoya may in-turn bring in the animal and human infective trypanosomes. The possibility of introducing *T. b. rhodesiense* creates a fear of a potential merger of *T. b. gambiense* and *T. b. rhodesiense* HAT causing strains. The two human infective trypanosomes each have a different treatment regime that in the end would cause a predicament in treatment of trypanosomiasis.

The results revealed that cattle had a prevalence of 8.7% and a significant association was between district location and prevalence of trypanosomiasis. Amuru district had a higher risk of infection than Nwoya district. No significant relationship was found between the age group and sex of the animal. The study revealed a lower prevalence of trypanosomes in younger animals which is an important finding. Young animals instead of old should be used in restocking to minimize the chances of introducing HAT in the districts of Amuru and Nwoya.

5.4. Recommendations

The study was limited to two sub-counties of Purongo in Nwoya and Atiak in Amuru, this necessitates further research to be carried out in the remaining sub-counties of the two districts.

Further research should be carried out to determine the risk of proximity of domestic animals to wildlife areas with special emphasis on the role of wild animals as reservoirs of the AAT and HAT trypanosomes in the two districts of Nwoya and Amuru. In addition animals used in restocking should be treated at site or point of purchase.

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