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# BOVINE TRYPANOSOMOSIS: EPIDEMIOLOGY AND DRUG RESISTANCE STUDY IN SELECTED SITES OF KELLEM WOLLEGA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

PhD Dissertation

By

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Department of Veterinary Pathology and Parasitology

PhD Program in Tropical Veterinary Parasitology

Feberuary, 2019

Bishoftu, Ethiopia

# BOVINE TRYPANOSOMOSIS: EPIDEMIOLOGY AND DRUG RESISTANCE STUDY IN SELECTED SITES OF KELLEM WOLLEGA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

A dissertation submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Veterinary Parasitology

By

Efrem Degneh Bijiga

Feberuary, 2019

Bishoftu, Ethiopia

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College of Veterinary Medicine and Agriculture

Department of Veterinary Pathology and Parasitology

As members of the examining board of the final PhD open defense, we certify that we have read and evaluated the dissertation prepared by **Efrem Degneh Bijiga** entitled: **"Bovine Trypanosomosis: Epidemiology and Drug resistance Study in Selected Sites of Kellem Wollega Zone, Oromia Regional State, Ethiopia"** and recommend it to be accepted as fulfilling the dissertation requirement for the degree of Doctor of Philosophy in **Veterinary Parasitology**.

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## DEDICATION

I dedicate this dissertation to my family and friends who gave me support that enabled me to undertake this degree course.

Addis Ababa University
College of Veterinary Medicine and Agriculture
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## STATEMENT OF THE AUTHOR

I declare that this dissertation is my authentic and real work and that all sources material used for this dissertation has been properly acknowledged. This dissertation has been duly submitted in partial fulfillment of the requirements for a PhD degree Addis Abeba University, College of Veterinary Medicine and Agriculture and is deposited at the Univesity/College library to be made available to borrowers under rules of the library. I sincerely declare that this dissertation is not submitted to any other institution any where for the award of any academic degree, diploma or certificate and allow access to users.

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## BIOGRAPHICAL SKETCH

Efrem Degneh Bijiga was born 15th of September, 1966 in Ghimbi District, West Wollega Zone, Oromia Regional State, Ethiopia. He attended his elementary and junior education at Haroji Agemsa, Olika Dingil and Ghimbi Comprensive High Schools respectively. After successful completion of High School education, he joined the then Debre-zeit Animal Health Institute in September 1981 and graduated with diploma in Animal Health science in July 1983. Soon after graduation, he was employed by the Ministry of Agriculture (MoA) and served until 1986 in Gidami and Nole Kabba Districts as Animal Health Assistant for three years. Later he joined Moscow Veterinary Academy in 1986 and graduated with DVM degree in 1991. Since then he served for the ministry of agriculture and rural development under extension services in different capacities for a couple of years. He also completed his study leading to Masters of Science in Tropical Veterinary Parasitology from Addis Ababa University, College of Veterinary Medicine in 2007. Following, he again served for Ministry of Agriculture as Zonal veterinary officer and Zonal animal health team leader in west Wollega Zone. He joined Wollega University in November 2009 and served as parasitology instructor for five years at the academic rank of Assistant Professor. In October 2013/14 he joined the School of Graduate Studies of the College of Veterinary Medicine and Agriculture of Addis Ababa University to pursue his PhD study in Veterinary Parasitology. He is authored and co-authored 6 scientific publications of which 2 are parts of this dissertation work. Efrem has married and a father of two sons and two daughters.

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## LIST OF ABBREVIATITIONS

| | |
|---|---|
| AAT | African Animal Trypanosomosis |
| AW-IPM | Area-wide Integrated Pest Management |
| BCT | Buffy Coat Technique |
| Bw | Body Weight |
| CATT | Card Agglutination Test for Trypanosomosis |
| CD | Curative dose |
| CDC | Centers for Disease Control and Prevention |
| CFSPH | Center for food Security and Public Health |
| CI | Confidence Interval |
| CSA | Central Statistics Authority |
| DA | Diminazene Aceturate |
| DDT | Dichloro-Diphényl Trichloro Ethane |
| DNA | Deoxyribonucleic Acid |
| ED | Effective dose |
| ELISA | Enzyme Linked Immuno Sorbent Assay |
| EtBr | Ethidium Bromide |
| FAO | Food and Agriculture Organization |
| GDP | Gross Domestic Production |
| GPS | Global Positioning System |
| HAT | Human African Trypanosomosis |
| HCT | Haematocrite centrifuge technique |
| IAEA | International Atomic Energy Agency |
| IFAT | Indirect fluorescent antibody test |
| IgG | Immunoglobulin G |
| IM | Intramususcular |
| ISM | Isometamedium Chloride |
| LAMP | Loop-mediated Isothermal Amplification |
| M.a.s.l. | Meters above sea level |
| NTTICC | National Tsetse-Trypanosomosis Investigation and Control |

## LIST OF ABBREVIATIONS (Continued)

| | |
|---|---|
| PCR | Polymerase Chain Reaction |
| PCV | Packed Cell Volume |
| RFLP | Restricted Fragment Length Polymorphism |
| rpm | Revolution Per Minute |
| SAT | Sequential Aerosol Technique |
| SIT | Sterile Insect Technique |
| SPSS | Statistical Package for the Social Sciences |
| SSA | Sub Saharan Africa |
| TDR | Trypanocidal Drug Resistance |
| VSG | Variant Surface Glycoprotein Coat |
| WHO | World Health Organization |

## TABLE OF CONTETNTS

BOVINE TRYPANOSOMOSIS: EPIDEMIOLOGY AND DRUG RESISTANCE STUDY IN SELECTED SITES OF KELLEM WOLLEGA ZONE, WESTEN OROMIA

Efrem Degneh Bijiga
PhD Dissertation
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## ABSTRACT

*African Animal Trypanosomosis remains one of the most important disease constraints to livestock and mixed crop-livestock farming in tropical Africa. Trypanosomosis is responsible for the death of 3 million heads of cattle yearly, with 50 million animals at risk in sub-Saharan Africa. The problem of trypanosomosis is still far from being solved due to the fact that trypanosomes affect multiple hosts, widespread trypanocidal drug resistance and antigenic variation displayed by the trypanosomes. Trypanocidal drugs: Isometamidium chloride (ISM) and Diminazene aceturate (DA) are the most widely used drugs for control of animal trypanosomosis in Ethiopia. In Kellem Wollega, trypanosomosis is one of the main livestock diseases. In the area, a number of brands of trypanocidal drugs from various sources are routinely used by veterinary professionals as well as farmers, which may result in rampant misuse and under-dosage of the medications, actions which might contribute for the emergence of trypanocidal drug resistance. However, study on trypanocidal drug resistance is not yet carried out despite of serious complains of drug failure from professionals and farmers. Therefore, the present study in Gidami and Sayo selected districts of Kellem Wollega Zone of Oromia Regional State; Ethiopia was designed to investigate the problem of bovine trypanosomosis with special emphasis on epidemiology of the disease and assessment of*

*trypanocidal drug resistance. Accordingly, questionnaire survey, cross-sectional studies and field trypanocidal drug resistance trial on naturally infected cattle were used to assess the epidemiological picture of the disease and the utilization of trypanocidal drug practices and evaluate the current status of trypancidal drug resistance trypanosomes. In the questionnaire survey, a total of 100 farmers were interviewed, of which 50 from Gidami and 50 from Sayo districts to assess the knowledge, attitude and practices of farmers towards the control of bovine trypanosomosis. A cross-sectional vector survey was conducted in purposively selected eight villages of Gidami and Sayo districts from November, 2015 to June, 2017 in early dry and early rainy seasons. A total of 160 traps (80 per district) were deployed at approximate intervals of 100 to 200 meters for 48 hours at watering and grazing points of animals in two seasons. For cross-sectional study of bovine trypanosomosis a total of 1790 blood samples were collected from systematically selected cattle and examined by buffy coat blood examination technique during early dry and early rainy seasons in Gidami and Sayo districts. Based on the outcome of the cross-sectional study Kellem and Gry Sonka villages from Gidami district were selected for field trypanocidal drug resistance study. An abbreviated 28-day field prototudycol was used to estimate resistance to 1 mg/kg b.w ISM and 7.0 mg/kg b.w DA trypanosome-positive cattle. For this purpose a total of 100 parasitaemic cattle from Kellem and Giray Sonka villages were selected and treated with Isometamidium chloride and Diminazene aceturate and monitored for trypanosomes and packed cell volume (PCV) levels on days 14 and 28 post treatment. Based on the questionnaire survey, 100% of the respondents from Gidami and Sayo district indicated trypanosomiasis as the major important disease of livestock causing considerable direct and indirect economic losses. Isometamidium chloride and Diminazene aceturate are the commonly used drugs to treat*

*bovine trypanosomosis in both study districts. Sixty percent of respondents from Gidami and only 9% from Sayo districts disclosed that they treat their animals nine to fifteen times per year per animal. Trypanocidal treatment failures were reported by 98% and 76% of the respondents from Gidami and Sayo district, respectively. Among 3289 flies trapped during the study period 1739 (52.88%) were Stomoxys, 1158 (35.21%) Glossina, 252 (7.66%) Tabanus and 140 (4.25%) were Haematopota. The overall apparent fly density was 10.28 flies per trap per days. The apparent density of Glossina, Stomoxys, Tabanus and Haematopota were 3.62, 5.43, 0.79 and 0.44 flies per trap per day, respectively. Four Glossina species: G. tachinoides, G.m.sub-morsitans, G. palidipes and G. fuscipes were recovered in Sayo district. However, only G.m.submorsitans and G. palidipes were caught in Gidami district. Out of the total 1158 tsetse flies caught, 51.81% were G. pallidipes; the remaining 25.65%, 16.58% and 5.96% were G. m. submorsistans, G.fuscipes and G. tachnoides, respectively. The overall prevalence of bovine trypanosomosis was 14.08% (95% CI: 12 -16%) and 11.16 % (95% CI: 9-13%) in Gidami and Sayo districts, respectively. However, there was no statistically significant difference (P > 0.05) between these two districts in the prevalence of bovine trypanosomosis. In the present study, the trypanosome prevalence was higher in low altitude (18.09, 13.80%) compared to mid altitude areas (9.13, 8.5%) in Gidami and Sayo districts respectively. The observed difference was found to be statistically significant (P<0.05). Trypanosoma congolense being the predominant species (57.30%) followed by T. vivax (29.10%), T.b.bruci (9.70%) and mixed infection of T. vivax and T. congolense (3.90%) were the prevailing trypanosome species in the area. The overall bovine trypanosomosis prevalence was siginificantly varied between poor (25.37%) compared to both medium (10.02%) and good (2.66%) body conditioned animals and*

*between adult (14.16%) and young (9.38%) age categories. There was no statistically significant difference (P>0.05) in the prevalence of bovine trypanosomosis between sexes of the examined animals. There was a statistically significant difference (P<0.05) in the mean packed cell volume (PCV) between parasitaemic (21.53%) and aparasitaemic cattle (26.11%) regardless of district and season of sampling. The results of field trypanocidal drug resistance study confirmed the presence of drug resistance to the maximum recommended doses of ISM and DA in Giray Sonka and Kellem villages of Gidami district. From 50 trypanosome positive cattle treated with ISM 2% solution of 1 mg/kg bw, 68% had persistent infections at day 28 post treatment. Similarly, of the 50 trypanosome positive cattle treated with DA 7% solution of 7 mg/kg bw, 36% had persistent trypanosomes at day 14 post treatment. In conclusion, bovine trypanosomosis is a major disease constraint of livestock health and agricultural activity in Gidami and Sayo districts of Kellem Wollega Zone of Oromia Regional State, Ethiopia. Trypanocidal drug resistance is a threat in Kellem Wollega and thus sustainable and integrated tsetse and trypanosomosis control practices should be implemented to ensure productivity of cattle and foster agricultural development. Furthermore, extensive data on trypanocidal drug sensitivity tests using advanced molecular techniques is essential.*

**Key words**: *Bovine, Trypanosomosis, Trypanocidal drug resistance, Prevalence, Kellem Wollega, Gidami, Sayo, Oromia, Ethiopia*

# 1. GENERAL INTRODUCTION

African animal trypanosomosis (AAT), also called Nagana is the most important disease constraint to livestock and mixed crop-livestock farming in tropical Africa (Courtin *et al*., 2008). The distribution of the disease is parallels the distribution of tsetse flies and comprises of an area approximately 10 million $Km^2$ (O'Gorman *et al*., 2006) in 37 sub-Saharan African countries (Sachs, 2010). Nagana puts 55 million cattle at risk and leads to the death of three million animals every year, inflicting a direct annual loss of US$ 1.0-1.2 billion in cattle production (Chitanga *et al*., 2011; Cecchi *et al*., 2014).

Although, most species of domestic animals are to some degree susceptible to AAT, but it is most important in cattle and small ruminants, as they are the most frequently reared animals in sub-Saharan Africa (Hotez *et al*., 2009; Spickler, 2010; Namangala and Odongo, 2014). Many wild animal species in Africa also host one or more trypanosome species and can serve as reservoirs for both human and domestic animals (Auty *et al*., 2012). However, certain breeds of African cattle have been shown to exhibit a level of tolerance to trypanosome infection (Naessens, 2006).

Trypanosomosis is a haemoprotozoan disease of animals and humans caused by several species of parasites of the genus *Trypanosoma* (CFSPH, 2009). The most common pathogenic trypanosome species affecting cattle in Africa are *T. congolense, T. vivax* and to a lesser extent, *T. b. brucei* (Simukoko *et al*., 2007; O'Gorman *et al*., 2009). In areas where more than one trypanosome species is present, mixed infections in domestic animals are often encountered (Takeet *et al*., 2013; Moti *et al*., 2015).

Trypanosomosis is a vector-borne disease known to be transmitted cyclically by tsetse flies (Krafsur, 2009; WHO, 2012) and mechanically by a number of biting flies of genus diptera such as *Tabanus, Hematopota, Chrysops* and *Stomoxys* (Desquesnes *et al*., 2009; Kone *et al*., 2011). The main tsetse-transmitted trypanosomes include *T. congolense, T. vivax, T. b. brucei* and *T. simiae* (Namangala, 2011)**.** Mechanical transmission of *T. congolense* has been shown under experimental conditions and can therefore not be

excluded from contributing to its spread in Africa (Desquesnes *et al*., 2009). In addition, *T. equiperdum* is transmitted sexually (Hagos, 2010; Namangala, 2012). Moreover latrogenic transmission could also occur when using the same needle or surgical instrument on more than one animal, at sufficiently short intervals, that the blood on the needle or instrument does not dry (Desquesnes and Dia, 2003).

Ethiopia is believed to have the largest livestock population in Africa. An estimate indicates that the country is a home for about: 59.50 million cattle, 30.70 million sheep, 30.02 million goats, 11.01 million equines, 1.21 million camels and 56.53 million chickens (CSA, 2017). However, Ethiopia is one of the countries suffering from trypanosomosis with approximately 220,000 $Km^2$ of arable land is infested with five species of tsetse flies: namely *Glossina pallidipes, G. m. submorsitans, G. fuscipes, G. tachinoides* and *G. longipennis* (NTTICC, 2004). According to Leta *et al*. (2016), the prevalence of bovine trypanosomosis in Ethiopia range from 1.38 to 17.15 %. The most important trypanosome species affecting livestock in Ethiopia are *T. congolense, T. vivax* and *T. brucei* in cattle, sheep and goats, *T. evansi* in camels and *T. equiperdum* in horses (Getachew, 2005; Hagos, 2010). In Ethiopia, the direct loss (mortality) due to trypanosomosis is estimated to amount 1.5 to 2 billion Birr per year (FAO, 2010).

In trypanosome endemic areas, trypanocidal drugs: ISM , DA and homidium are the main drugs currently available to control AAT (Clausen *et al.*, 2010; Sahin *et al*., 2014; Giordani *et al*., 2016). However, these drugs are fraught with a number of problems including cheaper generic forms (Geerts *et al*., 2010), lower standards of quality control (Chitanga *et al*., 2011), misuse and under dosage (Onono *et al*., 2013), spread of trypanocidal drug resistance (Clausen *et al*., 2010) and lack of new trypanocidal drugs development for field use (Delespaux *et al*., 2008). In addition, the use of Homidium salts is no longer advisable due to their mutagenic effects (Sutcliffe *et al*., 2014).

The problem of trypanosomosis is still far from being solved due to variation in parasites host factors, trypanocidal drug resistance and antigenic variation displayed by the

trypanosomes. Till nowdays, there is no control method that can fully eradicate AAT in spite of many attempts (Steverding, 2008; Achukwi and Musongong 2009). The development of a vaccine against trypanosomes has failed so far because of the different variant surface glycoprotein coats displayed by parasite population during infection (Hill *et al*., 2005).

Trypanocidal drugs: ISM and DA have been in use for control of animal trypanosomosis over 50 years (Giordani *et al*., 2016). However, currently there are increasing evidences of ISM and DA resistance and treatment failures reported (Sow *et al*., 2012; Dagnachew *et al*., 2015a; Moti *et al*., 2015). At present, there are twenty-one African countries in which trypanocidal drug resistance has been reported (Delespaux *et al.,* 2008; Chitanga *et al.,* 2011) including Ethiopia (Moti *et al*., 2012; Hagos *et al*., 2014). More worryingly, strains of *T. congolense* resistant to both ISM and DA have been detected in several locations, Sinyangwe *et al*. (2004) Zambia; Mamoudou *et al*. (2008) Cameroon and Moti *et al*. (2012) Ethiopia. Multiple drug resistance to ISM, DA and Homidium has been reported in trypanosome populations in ten African countries (Delespaux *et al.*, 2008). It is suspected that in several other African countries, resistance is present but is yet to be demonstrated (Delespaux *et al*., 2008).

### 1.1. Statement of the problem

In Kellem Wollega, trypanosomosis is one of the most important livestock diseases, which poses a serious threat to the lives and livelihood of entire communities and constitutes the greatest single disease constraint to livestock production. *Trypanosoma congolense*, *T. vivax*, and *T. brucei* species as well as four species of tsetse flies, *G. m. submorsitans, G. pallidipes*, G. *tachinoides* and *G. f. fuscipes* and biting flies (*Stomoxys, Tabanus* and *Haematopota*) have been demonsrated in western Oromia (Efrem *et al*., 2010; Tasew and Duguma, 2012). Several brands and diverse sources of trypanocidal drugs are routinely used by veterinary professionals as well as by farmers to control trypanosomosis in Oromia Regional State, Ethiopia. As a result, there is less efficient

disease control, a decline in animal health and widespread misuse of drugs. These preventive measures and treatment are used inappropriately which leads to trypanocidal drug resistance. Although a number of studies have reported the prevalence of the disease in Western Oromia (Lelisa *et al*., 2014; Siyoum *et al*., 2014; Kassaye, 2015), there is no comphrensive study conducted to address the epidemiology of bovine trypansomosis and the status of the trypanocidal drug resistance in Kellem Wollega Zone of Western Oromia, Ethiopia.

Therefore, this study is designed with the following objectives:

## 1.2. Objectives

### *1.2.1.1. General objectives*

The present study was undertaken with general objectives of assessing the epidemiology of bovine trypanosomosis and field trial of trypanocidal drug resistance on naturally infected cattle in identified hot spots in selected study districts of KellemWollega Zone, Oromia Regional State, Ethiopia.

### *1.2.1.2. Specific objectives*

To achieve the above general objectives, the study included the following specific objectives:

- To assess the knowledge, attitude and practices of farmers towards the control of bovine trypanosomosis in Gidami and Sayo districts using questionnaire survey.
- To assess seasonal dynamics of vectors of bovine trypanosomosis in both study areas.
- To determine the prevalence of bovine trypanosomosis in Gidami and Sayo districts.
- To conduct trypanocidal drug resistance survey in the field in identified hot spot villages.

## 1.3. Research Questions

This research work attempted to answer to the following research questions which could provide information to strengthen and support the development of control strategies and to improve the utilization and effectiveness of the available trypanocides.

- ✓ What is the prevalence of bovine trypanosomosis and apparent dencities of its vectors in Gidami and Sayo districts?
- ✓ What species of bovine trypanosomosis and tsetse flies are present in Gidami and Sayo districts?
- ✓ How effective are the treatment of ISM and DA on bovine trypanosomosis in the study areas?
- ✓ Does treatment failure occur in cattle treated with ISM and DA in Gidami and Sayo district?

# 2. LITERATURE REVIEW: BOVINE TRYPANOSOMOSIS; EPIDEMIOLOGY AND TRYPANOCIDAL DRUG RESISTANCE

African Animal trypanosomosis (AAT) or Nagana is a complex chronic, debilitating and often fatal diseases of animals caused by different species of flagellated unicellular parasites belong to genus *Trypanosoma* and found in the blood and other tissues fluids of vertebrates including livestock and wild animals (Singh and Singla, 2012). The main species recognised as causing AAT in cattle are *T. congolense*, *T. vivax* and *T. b. brucei* (O'Gorman *et al*., 2009).

## 2.1. The Trypanosomes

Trypanosomes are single celled flagellated protozoan parasites that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and transmitted by the bite of vector flies (FAO, 2012; OIE, 2013). The name Trypanosoma is derived from Greek word *trypano*- (borer) and *soma* (body) because of their cork screw-like motion (Hamilton *et al*., 2004). The trypanosome consists of a single cell varying in size from 8 to 50 μm. The different trypanosome species differ in morphological characteristics as described by in appearance, shape and size between the various species, allowing specific identification (Maudlin *et al*., 2004).

## 2.2. Taxonomy and Classification of Trypanosomes

Trypanosomes are unicellular protozoan parasites of the phylum Sarcomastigophora, order Kinetoplastida (Cox, 2004) due to the presence of a kinetoplast at the base of the flagellum (Eloy and Lucheis, 2009), family Trypanosomatidae and genus *Trypanosoma* (Vaughan and Gull, 2003). Genus *Trypanosoma* presents flagella and an organelle recognized by its kinetoplast (Eloy and Lucheis, 2009). On the basis of the site of development in the insect vector, the genus *Trypanosoma* is divided into two sections:

stercoraria and salivaria. In the stercoraria section the metacyclic trypanosomes develop in the hindgut of the vector and are thus transmitted to the mammalian host via faeces (Benvenuti and Gutierrez, 2007). In contrast, salivarian trypanosomes develop in the anterior portion of the fly's digestive tract, in the salivary glands (*T. brucei*) or in the proboscis (*T. congolense* and *T. vivax*) and are transmitted via the saliva (Peacock *et al*, 2012).

The main pathogens in Salivaria group categorized into four sub genera: Duttonella (*T. vivax; T. uniformes*); Nanomonas (*T. congolense; T. simae*); Pycnomonas (*T. suis*) and Trypanozoon (*T. brucei; T. rhodesiense*; *T.gambiense; T. evansi; T. equiperdum*) (Hamilton *et al*., 2008). However, the major pathogenic species in African cattle are *T. congolense*, *T. vivax*, and, to a lesser extent, *T. b. brucei* (Gibson, 2003; Taylor and Authié 2004) (Figure 1). Salivarian species are the only trypanosomes to exhibit antigenic variation, which allows the parasite to escape the host immune reaction (Stevens and Brisse, 2004). The other trypanosome species of economic importance are *T. evansi* of camels and *T. equiperdum* of horses (Getachew, 2005).

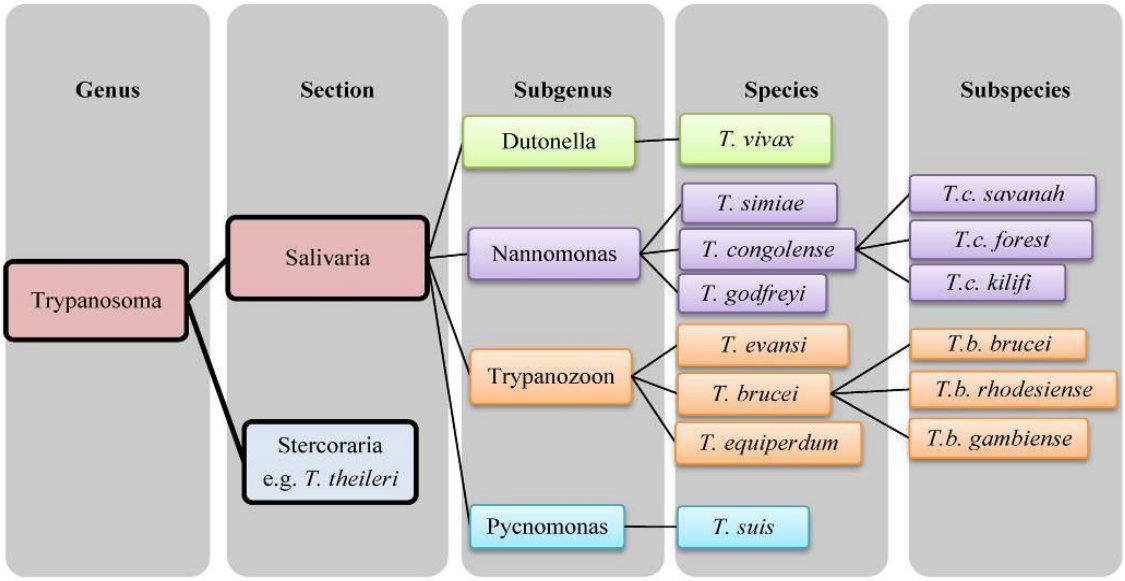



Figure 1: Schematic representation of the taxonomy of trypanosomes. Adapted from Gibson (2003).

*Trypanosoma congolense* species are divided into four main subgroups (Savannah, Forest, Tsavo and Kilifi) based on molecular markers (Nthiwa, 2013; Auty *et al*., 2015). *Trypanosoma congolense* Savannah, the most widespread subgroup geographically, is found over much of sub-Saharan Africa and is considered the main pathogen of cattle (Bengaly *et al*., 2002). The Savannah group can be further clustered into East and West African groups on the basis of genetic similarity. Apart from *T. congolense,* other members of sub-genus *Nannomonas* causing AAT include *T. simiae* (causing fatal disease in domestic suids) and *T. godfreyi* (causes a chronic disease in pigs) (Van den Bossche *et al*., 2011).

Genetic analysis using isoenzymes and a variety of DNA markers indicate that *T. vivax* can be separated in to two groups, which largely correspond to geographic location, i.e. an East African group and a West African group (Nakayima *et al*., 2013). There is some evidence to suggest that West African *T. vivax* is more pathogenic to cattle than East African *T. vivax* (Gardiner, 1989). There have also been several sporadic reports, mainly from East Africa, of *T. vivax* infections that result in a severe haemorrhagic syndrome in cattle (Magona *et al*., 2008).

*Trypanosoma brucei* species comprises morphologically identical subspecies: both animal (*T. b. brucei, T. b. evansi, T. b. equiperdum*) and human (*T. b. rhodesiense*, and *T. b. gambiense). Trypanosoma b.brucei* causes chronic and sub-patent infection, with minimal impact on the health of the infected cattle; wich may play an important role in the epidemiology of human sleeping sickness (Fevre *et al*., 2001) ; while *T. equiperdum* and *T. evansi* cause diseases to equines and camels, the other subspecies cause sleeping sickness in East and West Africa (OIE, 2013). Horses and donkeys are the only natural hosts of *T. equiperdum* whereby it causes a more severe disease in horses than in donkeys (Claes *et al*., 2005). *Trypanosoma brucei* group trypanosomes are found in both the vascular system and in other tissues, and can parasitize the brain in experimental infections (Grab and Kennedy, 2008; Coles *et al*., 2015).

## 2.3. Pathogenesis of African Animal Trypanosomosis

The pathogenesis of AAT depends on several factors, including parasite-related aspects (species and virulence), host (species, breed, age, and nutritional status, presence of co-infection and physical condition), vector (species, density, and infection rate and host preference) and the environment (the availability of food and water and the season (Van den Bossche and Delespaux, 2011). In the pathogenesis of African animal trypanosomosis four features: chancre, anaemia, tissue damage, and immunosuppression are prominent (FAO, 2000).

### *2.3.1. Chancer*

During a blood meal on a mammalian host, an infected tse-tse fly injects metacyclic tryponosomes in to the skin tissues (Hunt, 2010). Following inoculation, trypanosomes then continue to proliferate by binary fission for a few days (Giddings *et al*., 2006), leading to a local inflammatory response called a chancre (WHO, 2013). The size of chancre is determined by the animal immune status, the virulence of the infecting trypanosoma species and the inoculation dose (Nwoha, 2013). The chancre not only forms a site for the establishment of the infection but also is a focus for multiplication and persistence of trypanosomes before their dissemination in to blood stream (Elnasri, 2005). The ‘chancre’ disappears 3 to 15 days post-infection and trypanosomes enter to the lymph nodes and the blood stream and transform in to blood stream trypomastigotes, which are carried to other tissues (CDC, 2012).

### *2.3.2. Anaemia*

Tsetse transmitted trypanosomes can be grouped as haematinic *(T. vivax, T. congolense, T. simiae)* and cause anemia, or humoral (*T. b. brucei*) found in intra and extra vascular spaces (Ngure *et al*., 2008). Anemia is a cardinal pathological feature and cause of death in animals suffering trypanosomosis (Abenga and Vuza, 2005). The mechanism of

development of anemia is complex and multifactorial in origin (Naessens *et al*., 2005). Anemia is largly atributed to an increased rate of erythrophagocytosis in the early phase of infection and may be observed as pallor of the mucous membrane (Eloy and Lucheis, 2009; Nwoha and Anene, 2011a). Indeed, as a response to the trypanosomal infections both IgM and IgG antibodies are produced. While the IgM appear to be directed mainly against VSG antigens, IgGs are oriented against both the somatic trypanosomal antigens and the host's own cells (Connor and Van den Bossche, 2004).

Living and dead trypanosomes can produce various forms of active chemical substances, which can elicit erythrocyte injury (Naessens *et al*., 2005). In addition, trypanosomes affect other body components such as the serum biochemical constituents (Eloy and Lucheis, 2009; Nwoha *et al.*, 2013) and the immune system (Vincendeau and Bouteille, 2006). The virulence of infecting parasite population, age, nutritional status and breed of the host influence the severity of anemia (Bengaly *et al.*, 2002).

### *2.3.3. Immunosuppression*

One of the characteristic features of African animal trypanosomiasis is the profound suppression of the host immune response, which greatly complicates both the clinical and pathological features of trypanosomosis (Barrett *et al.*, 2003). It has been suggested that immunosuppression is mediated by both the macrophages and the T cells (Tabe *et al*., 2008). The immunosuppression caused by trypanosomes can affect animal health by interfering with vaccination against other diseases (Magez *et al*., 2010; Singla *et al*., 2010), or by increasing susceptibility of the host to other infections. As a result of this, antigenic variation and immunosuppression, conventional vaccination strategies against AAT are not effective (Baral, 2010; Magez *et al*., 2010).

### *2.3.4. Tissue damage*

The trypanosome species affecting domestic animals have been subdivided into two groups, the haematinic group (*T. congolense* and *T. vivax*) which remains in the plasma and the tissue invading group (*T. brucei, T. evansi, T. gambiense, T. rhodesiense* and *T. equiperdum*) found in extra and intra vascular spaces (Ngure *et al*., 2008). *Trypanosoma brucei brucei* and *T. evansi* localize also in tissues aside blood vessels (Langousis and Hill, 2014). Thus, *T. congolense* and to a lesser extent *T. vivax*, cause changes in the endothelium of capillaries, and indirectly provoke damage to adjacent tissues. Indeed, in *T. congolense* infections a generalized dilatation of capillary beds is observed, which alters the haemodynamics. In contrast, *T.vivax* infections commonly cause disseminated intravascular coagulation (Connor and Van den Bossche, 2004).

Although *T. vivax* has been considered typically to remain confined to the vascular system of the host, some strains may, especially in late infections, also reach extravascular locations (lymph nodes, eyes and cerebrospinal fluid) where they may directly damage tissues and where they are less accessible to drug treatment (Osorio *et al*., 2008; D'Archivio *et al*., 2013).

## 2.4. Life cycle of Trypanosomes

The life cycle of pathogenic trypanosomes involve mammalian and arthropod hosts (Chappuis *et al*., 2005; Brun *et al*., 2009). Although mechanical transmission through other insects occurs, the tsetse fly is the only cyclical vector of the African trypanosomes (OIE, 2009; Baral, 2010; WHO, 2013). The African trypanosomes have four major life cycle stages. The procyclic form (PF), epimastigote form (EMF) and metacyclic form (MCF) all develop in tsetse while the blood stream form (BSF) is found in the mammalian host (Peacock *et al*., 2012). Tsetse flies ingest infective blood stream trypomastigotes when they feed on infected hosts and may remain infected, acting as a continual source of infection (WHO, 2012). The BSF trypanosomes enter the tsetse mid-gut where most will perish (Peacock *et al*., 2012). In the insect body the slender forms are

rapidly killed by proteases or change in to stumpies (Sbicego *et al*., 1999). Only the stumpy, non-replicative trypomastigotes lose their glycoprotein surface coat and multiply through several developmental stages (procyclics, epimastigotes and metacyclic trypanosomes) in a period of three to four weeks before they become infective (Baral, 2010; Peacock *et al*., 2012).

The sites and the duration of development depend on the sub-genus or species of infecting trypanosomes (Roditi and Lehane, 2008). Development of *T. congolense* occurs in the midgut and proboscis while development of *T. brucei* takes place in the mid gut and salivary glands (Peacock *et al*., 2012). *Trypanosoma vivax* completes cyclical development exclusively within the mouthparts of tsetse flies (Peacock *et al*., 2012). The cycle takes about three weeks and the fly remains infective for life (Chappuis *et al*., 2005).

Trypanosomes are transmitted through tsetse saliva when the fly feeds on an animal host (Van Den Abbeele *et al*., 2010). Inside the host, metacyclic forms undergo multiplication by binary longitudinal fission at the site of inoculation, causing a local skin reaction called a chancre (Kennedy, 2008). This 'chancre' disappears after 3 to 15 days and the trypanosomes transform in to replicative, slender, and non-replicative, stumpy trypomastigotes forms that are covered by the Variant Surface Glycoprotein coat (VSG) and enter the blood stream directly or through the lymphatics (Baral, 2010; Peacock *et al*., 2012). The slender forms are adapted to the vertebrate host, and thus maintain the infection in the blood. Their behaviour thereafter depends on the species of trypanosome transmitted (Peacock *et al*., 2012; Franco *et al*., 2014).

*Trypanosoma vivax* usually multiplies rapidly in blood and is evenly dispersed throughout the cardiovascular system, whereas *T. congolense* tends to aggregate in small blood vessels and capillaries of the heart, brain and skeletal muscle from where a small proportion of parasites enter the blood circulation (Stuart *et al.*, 2008). *Trypanosoma brucei brucei* localize in tissues aside blood vessels (Langousis and Hill, 2014). The

parasites are then transmitted to a subsequent host at the next blood meal (Brun *et al.*, 2010).

In addition to tsetse flies, it has been reported that *T. vivax*, *T. evansi* as well as *T. congolense* can also be mechanically transmitted by various blood feeding dipteras (Desquesnes *et al*., 2009),which occurs in the early stage of the blood feeding, when the fly injects some saliva before sucking the blood of its host (CFSPH, 2009). Trypanosomes can also pass through the placenta and in to the fetus in pregnant animals, as a result some cows abort and cause premature and still birth and sometimes via mother to offspring transmission (WHO, 2010). Other potential modes of transmission include latrogenic routes when using the same needle or surgical instrument on more than one animal at sufficiently short intervals that the blood does not dry or via alternative, as yet unidentified vectors (Osorio *et al*., 2008). *Trypanosoma equiperdum* is transmitted sexually (Namangala, 2012).

## 2.5. Epidemiology

The distribution of trypanosomes of veterinary importance varies with locality and depends on the interaction between tsetse flies, the parasite, domestic and wild animals (Simukoko *et al*., 2007; Moore *et al*., 2012).

### *2.5.1. The Parasite*

Animal African trypanosomiasis is caused by different trypanosome species with *T. congolense* and *T. vivax* being the major pathogens for livestock, especially cattle and other ruminants (Eyford *et al*., 2011, Rotureau and Van Den Abbeele, 2013). Other species that infect livestock include *T. b. brucei, T. simiae, T. godfreyi*, *T. uniforme* and *T. suis* (Rotureau and Van Den Abbeele, 2013). The distribution of the AAT is parallels the distribution of the vector flies (Chappuis *et al*., 2005; O'Gorman *et al*., 2006).

Although host preferences of each *Trypanosoma* species may vary, *T. congolense, T. vivax* and *T. b. brucei* have a wider range of hosts among domesticated animals (Morrison and Macleod, 2011). *Trypanosoma vivax* is the most frequent cause of trypanosomosis among cattle in West Africa (Yaman *et al.,* 2003). On the other hand, *T.congolense* is considered to be the most pathogenic species in domestic ruminants in Southern and East Africa (Marcotty *et al.,* 2007). *Trypanosoma congolense* and *T. vivax* species also have been shown to cause serious infections in horses and asses (Dhollander *et al.,* 2006).

Parasite virulence is also an important factor influencing the epidemiology of AAT. The pathogenicity appears to vary depending on which type or strain of *Trypanosome* is involved (Bengaly *et al.,* 2002). Within *T. congolense,* different types exist (savannah, forest, kilifi and Tsavo) that have a different pathogenicity (Desquesnes *et al.,* 2012). *Trypanosoma congolense* Savannah type is the most pathogenic and is responsible for acute infection and death of diseased animal. However, *T. congolense* Forest and Kilifi types cause mild infections (Bengaly *et al*., 2002). Apart from *T. congolense,* other members of sub genus *Nannomonas* causing AAT include *T. simiae* (affecting domestic suids) and *T. Godfrey*i (Van Den Bossche *et al*., 2011).

*Trypanosoma vivax* shows variable levels of virulence and distinct pathogenicity. In East Africa, there are two types of *T. vivax* isolates: the haemorrhagic *T. vivax* that causes an acute haemorrhagic syndrome and the mild strain (Magona *et al*., 2008). Cattle infected with the haemorrhagic *T. vivax* produce auto-antibodies to red blood cells, a phenomenon that is not observed in the non-haemorrhagic *T. vivax* (Bett *et al*., 2004). Similar variability in the virulence profiles between *T. vivax* strains has also been reported in South America where some stocks induce severe infections and others don't (Davila *et al.,* 2003).

Despite being more likely to invade other tissues, like the central nervous system, *T. b. brucei* is generally less pathogenic than *T. vivax* or *T. congolense* (Taylor and Authie,

2004). In areas where more than one trypanosome species is present, mixed infections in domestic animals are often encountered (Takeet *et al.* 2013; Moti et *al*., 2015). Stress, such as poor nutrition or concurrent disease, plays a prominent role in the disease process (Taylor and Authie, 2004).

### *2.5.2. Vectors of African trypanosomes*

African animal trypanosomosis is found mainly in those regions of Africa where, tsetse fly exists (CFSPH, 2009). Tsetse flies are of primary importance in the spread and epidemiology of trypanosomosis (OIE, 2013). In Africa, 37 countries are infested with tsetse flies covering an estimated 10 million $km^2$ of land (O'Gorman *et al*., 2006), extending on both sides of the equator from $15^0$N to $30^0$S. The presence of a wide range of different types of host animals is essential component of tsetse fly distribution (Eloy and Lucheis, 2009). Some pockets of *G. morsitans* and *G. fuscipes* have been described in south western Saudi Arabia (Franco *et al.,* 2014).

Tsetse flies belong to, phylum Arthropoda, class Insect, and order Diptera, family Glossinidae and genus *Glossina*. Thirty-one species and subspecies have been described and classified in to three subgenera (WHO, 2013). Based on ecological preference and morphological differences in the structure of adult male genitalia, tsetses are classified in to i) savannah (*Gilossina morsitans),* (ii) riverine (G. *Palpalis)* and (iii) forest groups (G. f*usca*) respectively (Eloy and Lucheis, 2009; WHO, 2013). This classification was confirmed by comparative gene sequence analysis and geometric wing morphometry (Leak *et al*., 2008). However, because of distinct morphological features and habitat preference, *G. austeni,* which was formerly placed in the *morsitans* group, has recently been assigned in a separate subgenus called *Machadomyia* (Goodings and Krafsur, 2005).

The morsitans groups is largely found in open woodland savannah best suited for grazing livestock and are the most important in the transmission of animal trypanosomiasis (Taylor *et al*., 2013). *Glossina fusca* group mainly found in thick forests, and are not

important in trypanosomosis transmission since cattle are not kept in thick forests (Kaare *et al*., 2007). Eastern Africa has only *G. longipennis* as a fusca group species and is found in drier areas (Ford and Katondo, 1997). The *palpalis* group is found mainly in the riverine galleries of West and Central Africa, but sometimes extends in to savannah regions between the river systems (Hendrickx *et al.*, 2004). The *palpalis* fly species are less mobile than the *morsitans* group, often relying on sight rather than smell to locate their hosts and are less efficient vectors compared to *G. morsitans* group (Ajibade and Agbede, 2011).

### *2.5.3. The host*

Trypanosomes have wide range of vertebrate hosts including reptiles, birds, amphibian, fish and mammals (Hamilton *et al*., 2004). Among the mammals, livestock exhibit a range of susceptibility to trypanosomosis, from refractory to highly vulnerable (Bishop and Woolliams, 2014). African animal trypanosomosis is most important in cattle but can cause serious losses in pigs, camels, goats, and sheep (Hotez *et al*., 2009; Spickler, 2010). More than 30 species of animals including ruminants such as Antelopes, Buffalos and wild equidae are susceptible and may serve as reservoir hosts (Brun *et al*., 2010; Morrison and Macleod, 2011). Ruminants are extensively known to be active reservoirs of the trypanosomes (Desquesnes *et al.,* 2009).

Susceptibility of cattle to trypanosomosis is based on breed, age, habitat, previous exposure and health status (Orenge *et al.,* 2012). The West African taurine breeds, like the N'Dama, Baoule, Muturu and their crosses with zebu and certain zebu cattle in East Africa are trypanotolerant (Freeman *et al*., 2004). These animals have evolved a superior mechanism that controls trypanosome proliferation and anemia (Yoshihara *et al*., 2007). Exotic imported ruminants are more severely affected than local ones (Taylor and Authie, 2004). Suckling calves are also known not to suffer from serious attacks of trypanosomosis, possibly because of the influence of maternal antibodies in their systems. There is also evidence from studies that tsetse get attracted mostly to larger cattle from which they feed rather than smaller ones (Vale and Torr, 2004).

Many wild animals like warthog, bush pig, bush buck, buffalo, wild equidae, lions… etc, in Africa also host one or more trypanosome species and can serve as reservoirs for both human and domestic animal (Maudlin, 2006; Brun *et al*., 2010; Auty *et al*., 2012). In these animals, African trypanosomes become established but do not cause any disease because both the trypanosome and host have evolved for many years that a balanced relationship exits (Namangala, 2011). In domestic animals relationship with the parasite has not fully developed; leading to development of the disease (Namangala, 2011). Consequently, tsetse flies in these areas may remain infected, acting as a continual source of infection for domestic species (WHO, 2012).

### *2.5.4. Climate and environment*

Tsetse flies are a class of insects that are very sensitive to environmental changes and ecological instability (Terblanche, 2008). Climate strongly influences temporal and spatial distribution of vectors and vector-borne diseases (Rogers and Randolph, 2006; Gage *et al*., 2008). The eco-distribution of tsetse is determined by climate; presence of vegetation, water and presence of blood meals, but tsetse are mostly found in rural areas (Lutje *et al*., 2010). Both average climatic conditions and day-to-day variation in temperature are known to be important for vector and parasite development (Lukaw *et al*., 2014).

Temperature has a strong influence on tsetse population dynamics (Hargrove, 2004), and can be one of the strongest abiotic determinants of tsetse distributions (Rogers and Robinson, 2004). The tsetse flies only live in regions where the average annual temperature is above 20°C of which 25°C is the optimum temperature for their survival (Radostits *et al*., 2007). Unlike high temperatures, low temperatures slow down tsetse activity and general physiology (Terblanche, 2008). Under extreme temperatures of below $10^{o}$C, tsetse will die within 3 hours of exposure (Rogers and Robinson, 2004).

In trypanosomosis, changes in climatic factors can impact on i) survival and reproduction rates of the vectors, thus influence their distribution and abundance, ii) intensity of activity of the vectors and iii) cycle, survival and reproduction rates of the trypanosomes (Van den Bossche *et al*., 2010). The bovine trypanosome species like the *T.congolense*, *T, brusei* and *T.vivax* are normally associated with the humid and sub-humid areas of Africa ($15^{o}$N and $25^{o}$ S), that is inhabited by their intermediate hosts the *Glossina* (Sow, 2013).

## 2.6. Diagnosis

The available laboratory diagnosis of trypanosomes is divided into three main methods parasitological, serological and molecular (Wastling and Welburn, 2011), which have different advantages and are used for different objectives. However, diagnostic procedures vary not only according to the tools available, but often even more to what one wishes to know (Sinshaw *et al*., 2006). However, in many tsetse infested areas, conventional diagnostic facilities are not available, and clinical signs of trypanosomosis are usually used for making a tentative diagnosis (Sinshaw *et al*., 2006).

### *2.6.1. Clinical diagnosis*

The clinical manifestation of bovine trypanosomosis is influenced by the host as well as the trypanosomes species and "strain" (Bezie *et al*., 2014). Infection of cattle by one or more of the three African animal trypanosomes results in subacute, acute, or chronic disease. However, under natural challenge, disease manifestation may be more complex (Taylor and Authié, 2004). *Trypanosoma congolense* causes chronic disease, *T. vivax* a more acute disease and *T. b. brucei* a mild to chronic disease in cattle (Radostits *et al*, 2007).

Many of the clinical and pathological manifestations of trypanosomosis are common to domestic animals, irrespective of the host and the species of trypanosomes involved

(Taylor *et al*., 2004). The first sign of infection is the swelling of the skin (chancre) where the parasites are multiplying (Maudlin *et al*., 2004). The swelling of the skin is followed by fever (Wolkmer *et al*., 2009), anaemia, emaciation, hair loss and often terminates in death (Goodwin, 2009). Sheep and goats infected with *T.b. brucei* species may also show neurological signs such as staggering and paralysis (Taylor and Authie, 2004). Within a week of infection there is usually pronounced decrease in packed cell volume, hemoglobin, red blood cells, and white blood cells levels (Mersha *et al*., 2012). Sudden deaths have been reported in small ruminants infected with *T. vivax.* Trypanosomes can cause immunosuppression and concurrent infections may complicate the disease (CFSPH, 2009). However, these clinical signs are only indicative but are not sufficiently pathognomonic and diagnosis of trypanosome infection based on clinical signs alone is unreliable (Wastling and Welburn, 2011).

Clinical signs are important for tentative diagnosis of trypanosomosis in areas where this disease is endemic and laboratory services are not available (Steverding, 2008). However, severity of clinical response is dependent on breed and species of affected animals, as well as the dose and virulence of the infecting trypanosome. Stress due to poor nutrition or a concurrent disease, also plays a prominent role in the disease process (Taylor and Authié, 2004). For example, *T. b.brucei* is very well tolerated by cattle but is rapidly life threatening for horses and dogs (Allsopp *et al*., 2004). Even within the same species virulence might vary a lot as in the case for *T. congolense* (Masumu *et al*., 2006). However, trypanosomosis should be suspected when an animal in an endemic area is febrile, anemic and in poor condition or when animal lags behind the herd (Masumu *et al*., 2006).

### *2.6.2. Parasitological diagnosis*

Visualization of trypanosomes in body fluids by microscopy is currently the gold standard of AAT diagnosis in endemic regions (Chappuis *et al*., 2005; Bronsvoort *et al.,* 2010). The actual specificity and sensitivity of these techniques is directly dependent on

the volume of blood examined, the skill and experience of the microscopist (OIE, 2013). Freshly collected blood can also be inoculated in to laboratory rodents which can then be examined for periods of up to 30 to 60 days to determine if trypanosome infections develop in them (Balows *et al*., 2012).

#### 2.6.2.1. Wet blood smear

In the early phases of infection, especially with *T. vivax* and *T. congolense*, blood can be directly examined as wet smears for trypanosome presence by light microscopy (Chappuis *et al*., 2005; WHO, 2006). Although, this technique has a very low detection power, it is the most widely used method for the diagnosis of animal trypanosomosis in endemic regions (François *et al*., 2005; Cecchi *et al*., 2014). Trypanosomes were recognised by their movement among the red blood cells **(**OIE, 2013). Microscopic examination of fresh blood films is quick, simple and inexpensive, however, the sensitivity of wet blood smear is limited as less than 50% (Sivajothi *et al*., 2016), but it can be improved significantly by lysing the Red Blood Cells (RBCs) before examination using a haemolytic agent such as Sodium Dodecyl Sulphate (SDS), (OIE, 2013).

#### 2.6.2.2. Thin and thick stained blood films

Thin and thick blood films are the most widely used, even if examination of each slide can take 10 to 20 minutes and must be carried out by an expert microscopist (Chappuis *et al*., 2005). Stained thin and thick smear techniques permit detailed morphological studies and identification of different trypanosoma species by light microscopy (Borden, 2005), but trypanosomes may be damaged during the staining process, which may make it difficult to identify the species (OIE, 2013). Thin and thick blood films virtually 100% specificity, but suffers from a very low sensitivity, failing to confirm 20-30% of infections, because of low peripheral parasitemia in infected animals (Chappuis *et al*., 2005; Lutumba *et al*., 2005).

Thick smears contain more blood than thin smears and, hence, have a higher diagnostic sensitivity. Thin smears on the other hand preserve the morphology of trypanosomes and hence allow trypanosome species identification (WHO, 2006; Buscher *et al*., 2009; OIE, 2013). Sensitivity of blood smears may be also improved by centrifugation of blood and microscopic examination of the resultant buffy coat (De Rissio *et al.*, 2010).

#### 2.6.2.3. Parasite concentration techniques

A more effective and reliable method of detecting parasitaemia is to concentrate the parasites before microscopic detection. In this procedure, heparinzed capillary tubes are three-quarters filled with the suspected blood sample containing an anticoagulant. The dry ends of the capillary tubes are sealed with cristaseal (OIE, 2008) and centrifuged at 12,000 rpm for 5 minutes (HCT) (Woo, 1970). After centrifugation the buffy-coat/plasma junction is located between the plasma and the red blood cells and contains white blood cells as well as the parasites. The capillary tubes are then mounted on a woo chamber and can then be directly viewed at low magnification for mobile parasites (Chappuis et *al.*, 2005).

Microhematocrit Centrifugation Technique is a more sensitive technique than wet mount, and the sensitivity of mHCT is increased to fewer than 500 trypanosomes/ml with the number of tubes examined (OIE, 2008). This haematocrit centrifugation technique was subsequently improved in the buffy coat technique by cutting the capillary tube, expressing the buffy coat/plasma interface on a microscope slide and examining under a light microscope for presence of motile trypanosomes (FAO, 2000).

Compared with the microhaematocrit centrifugation technique, the buffy coat technique has the added advantage that preparations can be fixed and stained for more accurate identification of species and for retention as a permanent record, (OIE, 2013). The centrifugation techniques have the added advantage that the packed cell volume and

hence the level of anaemia, can be determined at the individual animal and/or herd level, (OIE, 2013).

#### 2.6.2.4. Miniature anion-exchange centrifugation technique (mAECT)

Another parasite concentration method that can be used is the mini anion exchange centrifugation technique (Maudlin *et al.,* 2004). This technique is based on the ability of the negatively charged RBCs to be held back in the anion column, and the less negatively charged trypanosomes to pass through with the solution (Souto-Padron, 2002). The trypanosomes are concentrated in the solution by low-speed centrifugation (François *et al.,* 2005), after which, the tip of the tube is mounted on a special holder and examined for the presence of trypanosomes using light microscopy at low magnification.

The mAECT is highly sensitive parasitological technique for detection of trypanosomes in blood, because of large blood volume (300 µl) used, which enables the detection of less than 100 trypanosomes/m l (Lutumba *et al*., 2006). Although the preparation is time consuming, the mAECT is currently the gold standard for trypanosome detection (Chappuis *et al*., 2005).

### *2.6.3. Serological diagnosis*

Serological methods are indirect methods for diagnosis of trypanosomosis based on detection of antibodies or circulating antigens of parasites. The most commonly used serological tests include, indirect haemaglutination test (IHT), precipitation tests, indirect fluorescent antibody test (IFAT), and ELISA (Borden, 2005). However, these serological tests are used as tools for research, monitoring and control surveys (Chappuis *et al*., 2005; Ezeani *et al*., 2008). The one exception to this generalization is in the application of the CFT for the diagnosis of dourine in horses (Cauchard *et al*., 2014).

#### 2.6.3.1. Antibody detection tests

Several antibody detection techniques have been developed to detect trypanosomal antibodies for the diagnosis of African animal trypanosomiasis (OIE, 2013). The most commonly used antibody tests in cattle are the Indirect Fluorescent Antibody Test (IFAT), Antibody Ab-ELISA and the CardAgglutination Test for *T. evansi* (Monzon *et al*., 2003). However, the disadvantages of serodiagnosis are (i) antibody ELISAs are not species-specific because of strong cross reactions between the pathogenic trypanosome species (Desquesnes *et al.,* 2001) and (ii) persistence of antibodies after a curative treatment or a self-cure is estimated to be on the average of 3-4 months (Desquesnes *et al.,* 2012).

#### 2.6.3.2. Antigen detection tests

These assays detect the circulating antigens of *T. congolense, T. vivax* and *T.b. brucei* in blood of infected animals. ELISA and field-oriented latex agglutination test for surra diagnosis have been developed and used in Africa and some other countries. Antigen-detecting ELISA (Ag-ELISA) would have allowed the detection of circulating trypanosomal antigens and therefore confirm the occurrence of an active infection (Eisler *et al*., 2004).

### *2.6.4. Molecular diagnosis*

The method that is currently recommended for trypanosome diagnosis is molecular identification, due to the high sensitivity and specivitivity (Wastling and Welburn, 2011). The principle of molecular methods is to detect DNA sequences that are specific for trypanosome subgenus, species, subspecies, type or strain. There are a number of PCR based techniques that have been developed for identification of trypanosome species (Cox *et al.,* 2005; Thumbi *et al.,* 2008). These molecular techniques rapidly detect and identify trypanosome species in both mammalian hosts and the tsetse vector with high

sensitivity and specificity (Cox *et al*., 2005; Mwandiringana *et al.,* 2012), even in cases of low parasitaemia; however, none have been validated for routine diagnostic purposes (Deborggraeve and Buscher, 2010).

#### 2.6.4.1. Polymerase chain reaction (PCR)

The polymerase chain reaction is one of the most sensitive and specific diagnostic method of AAT (Marcotty *et al*., 2008; OIE, 2013), and has overcome the constraints of parasitological and serological techniques (Gonzales *et al.,* 2003). PCR is based on the use of an enzyme, DNA polymerase, which amplifies sequences of DNA bases until sufficient material is produced to be detected (Geysen *et al.*,2003). The PCR has been widely used for research purposes to identify infecting trypanosome DNA in animal and human blood (Brun and Balmer, 2006).

Several studies have shown that PCR is a specific and more sensitive method in the diagnosis of trypanosomosis in experimental as well as natural infections (Bengaly *et al*., 2002; Gall *et al*., 2004), identifying parasites at the species level (Gutierrez *et al.,* 2004). It was observed that PCR can detect the DNA equivalent of one trypanosome/10 ml of host blood (Masake *et al*., 2002). Currently, several PCR based diagnostic assays have been developed to improve the detection of pathogenic trypanosomes (Thumbi *et al*., 2008). The two internal transcribed spacer PCR (ITS-PCR) is used as universal primers as it can detect all the pathogenic trypanosome species (Thumbi *et al*, 2008; Nakayima *et al.* 2012). The test detects many *Trypanosoma* species in a single PCR, thereby reducing the number of reactions per sample (Nakayima *et al*., 2012).

The main disadvantage of PCR is that, it is very expensive in terms of cost, require skilled manpower, need for precision instruments and elaborate visualisation methods, (Njiru *et al*., 2008). Moreover, a positive PCR does not necessarily mean the presence of live trypanosomes and this makes it difficult to accurately assess treatment outcome (Deborggraeve *et al*., 2011). Furthermore, PCR can be relatively slow and suffers a

significant risk of sample contamination (Mugasa *et al*., 2008). However, in order to detect trypanosomes and avoid false positive results; it is possible to combine PCR and the DNA probes technology (Desquesnes *et al.,* 2001).

#### 2.6.4.2. Loop-mediated isothermal amplification (lamp)

The Loop-mediated isothermal DNA amplification method is another molecular technique that is simple, sensitive, rapid and highly specific (Parida *et al*., 2008). LAMP is a novel strategy which amplifies DNA under isothermal conditions (60-65°C), producing large quantities of DNA within 30-60 minutes (Thekisoe *et al*., 2007). It requires simple equipment (water bath or block heater) for amplification reaction and is cost effective (Njiru, 2012).

The Loop-mediated isothermal DNA amplification allows visual detection of amplicons by naked eyes or through measurement of turbidity or fluorescence (Wastling *et al*., 2010). The LAMP method has several advantages over PCR in that: (i) LAMP amplification can be achieved using partially or non-processed template there fore DNA extraction may not be necessary, (ii) reactions are rapid and require shorter time and (iii) sensitivity is equal or higher than that of PCR. These characteristics make LAMP strategy ideal for AAT/HAT diagnosis in resource poor endemic regions (Njiru *et al*., 2008). An added benefit of LAMP over PCR-based methods is that DNA amplification can be monitored spectrophotometrically and/or with the naked eye without the use of dyes (Kuboki *et al*., 2003).

### *2.6.5. Sub-inoculation methods*

The sub-inoculation of blood and other body fluids into susceptible experimental animals is another diagnostic technique for trypanosomosis, especially when parasitaemia is scanty (FAO, 2000). These methods can be used to detect some *T. congolense* and *T. brucei* complex (but not the non-rodent-adapted *T. vivax*) infections (Gathuo *et al*., 2007) and have the advantage to conserve (stabilate) isolated trypanosomes for further

investigations (Eisler *et al*., 2004). However, its disadvantage is that not all trypanosome species, including some strains of *T. congolense* and *T. vivax* become established in rodents (Duleu *et al.,* 2004). Therefore, sub-inoculation using domestic small ruminants may be recommended for the isolation of *T. vivax* (Eisler *et al*., 2004). Artificial immuno-suppression of recipient animals by irradiation or drug treatment (cyclophosphamide) greatly increases the chances of isolating the parasite (Eisler *et al*., 2004).

### *2.6.6. Xenodiagnosis*

Xenodiagnosis is the feeding of a clean susceptible vector species on a suspected case of trypanosomosis, after which it is either dissected and examined for trypanosomes or allowed to feed on a clean animal which is itself examined for the development of infection (Eisler *et al*., 2004). Although this method is found to be sensitive, it is rarely used due to the scarcity of laboratory reared *Glossina* species. This method is also time consuming and not likely to be a practical field technique (Eisler *et al*., 2004). This method is extremely sensitive and may be used if the presence of a trypanosome infection in a particular animal or cattle population is suspected on the basis of surrogate tests (tests that do not conclusively demonstrate the presence of a parasite, such as antigen ELISA) but cannot be conclusively demonstrated by other means (Eisler *et al*., 2004).

## 2.7. Economic Importance of Animal Trypanosomosis

African Animal trypanosomiasis continues to be a serious health concern across large areas despite several decades of research, limiting agricultural and economic growth in sub-Saharan Africa (Kubi *et al.,* 2006; O'Gorman *et al.,* 2009). In domestic animals, trypanosomosis is a disease with a great economic impact, affecting not only the well-being of the livestock population, but also efficient food production in crop-livestock production systems (Shaw *et al*., 2014). It is estimated that about 37% of the African continent or approximately 10 million km² is infested by tsetse flies (Mattioli *et al*., 2004). African animal trypanosomiasis puts 50 million cattle at risk and leads to the death

of three million animals every year, inflicting a direct annual loss of US$ 1.0-1.2 billion in cattle production (Cecchi *et al*., 2014).

The main economic losses attributed to AAT are related to cattle mortality and morbidity, diagnosis and treatment costs, the reduction in meat and milk production and the reduction of livestock production areas (Oluwafemi *et al.*, 2007) and limiting the optimal utilization of land for agricultural production (Mahama *et al*., 2003). African animal trypanosomosis results in high mortality rate in acute cases and in a severe loss of production in chronic cases (Zewdu *et al.*, 2013). Livestock kept under trypanosomosis challenge have 6-20 % higher annual calf mortality, a 6-19 % lower calving rate and a 20% decrease in milk yield and up to 38% weight loss (Shaw, 2004). Thirty five million doses of trypanocides are used annually in sub-Saharan Africa (Holmes, 2013) and farmers lose 3 million cattle and other valuable livestock (Vreysen, 2006).

In addition, trypanosmosis reduces work efficiency of animals, thus hindering crop production (Adams, *et al.,* 2006). African animal trypanosomiasis also discourages the introduction of drought animals in to crop farming (Omotainse *et al*., 2004). The disease is characterised by a reduction in fertility, weight lose and milk and meat off take by at least 50% per year (Feldmann *et al*., 2005). All impacts of the disease combined results are an estimated economic loss of US$ 4.5 billion per annum across the continent (DFID 2010). In affected rural areas, tsetse and trypanosomiasis have prohibited human occupation of some areas and precluding agricultural and livestock keeping activities (Ilemobade, 2009; Malele, 2011).

### 2.8. Control of Bovine Trypanosomosis

There has been a long history of tsetse and trypanosomosis control in Africa (Hoppe, 2003), but today the problem is still far from being solved and there is no control method that can fully eradicate AAT and the incidence of both animal and human trypanosomosis remained high with occasional endemic outbreak (Steverding, 2008; Achukwi and

Musongong, 2009). A range of methods have been developed and put into practice to keep the disease under control, some of them less good than others (Grev, 2002).

Despite extraordinary research efforts directed at the development of vaccines against trypanosomes, no vaccine has so far has been developed in the near future (Magez and Radwanska, 2009; Magez *et al*., 2010). Hence, control of animal trypanosomosis relies primarily on control of the vector (Bouyer *et al*., 2010), farming of trypanotolerant breeds (Courtin *et al.*, 2008), use of trypanocidal drugs (Holmes, 2013), or a combination of them (Delespaux *et al*, 2008). Prevention of successful establishment and/or maturation of trypanosomes within the tsetse fly have been proposed as possible future control method (Aksoy *et al*., 2003).

### *2.8.1. Control of the vector*

Controlling the vector of trypanosomosis remains theoretically the most desirable way of containing the disease (Bouyer *et al*., 2010). The absence of eggs and a free larval stage in nature and the fact that the pupal development occurs in the soil makes the adult fly the only phase easily accessible for control purposes (Vreysen *et al*., 2000). A wide variety of tsetse control techniques have been developed and have undergone trial. These control techniques include: ecological methods, use of insecticides (Allsopp and Hursey, 2004), use of traps and targets (Esterhuizen *et al*., 2011), use of insecticide treated cattle (Shaw *et al*., 2015), use of sterile insect technique (Vreysen, 2006; Bouyer *et al*., 2014) or reducing the risk of exposure through changes in livestock management (Holt *et al*., 2016).

The choice of the control method to be applied will depend on the target zone, the impact on the environment, the tsetse species, and the possibility of isolation of treated areas, the possibilities of post control land use and the available funding of the operations now and for the future (Sow, 2013). However, ilimination or eradication of tsetse is unlikely to

succeed in the nearfuture because tsetse interventions must be tediously planned at acontinental scale in order to prevent tsetse re-infestation (Schofield and Kabayo, 2008).

#### 2.8.1.1. Ecological methods

Tsetse flies are a class of insects that are very sensitive to environmental changes and ecological instability (Terblanche *et al*., 2008). Ecological control of tsetse flies involves clearing of vegetation and game elimination. Vegetation clearance results in denial of a suitable tsetse habitat while shooting of wild animals deprives the flies of blood meal sources and may lead to their starvation and death (Aksoy *et al.*, 2003).

These environmental changes were efficient, but nowadays, this is not ecologically or politically acceptable because of their negative impacts on biodiversity (Bouyer *et al*., 2010). It has been suggested that the removal of the vegetation at ground level without removing high trees or by cutting only some of the tree or shrub species (partial selective bush clearing) could be very effective to decline the tsetse population (Bouyer *et al*., 2010).

#### 2.8.1.2. Stationary attractive devices

Traps and targets attract and either kill or the flies are guided and trapped in a non-return cage (Reichard, 2002). The use of traps and screens impregnated with synthetic pyrethroids insecticides (Hargrove *et al.,* 2003) are techniques currently employed to control tsetse flies. Biconical and Nitse traps have been extensively used for tsetse sampling and control (Dede *et al.,* 2005). At a density of 1 to 4 targets per $Km^2$ certain tsetse fly populations can be suppressed to low numbers in a short time period. The method exerts an additional daily mortality of 2-3% of the female fly population (Leak, 1999).

The effectiveness of traps and targets can be greatly enhanced by addition of appropriate odor bait which are either attractants or repellants and have been isolated from host animals (Omolo *et al*., 2009). The technique is more efficient when the targets/traps are impregnated with insecticides like pyrethroids, as only 20% of the attracted flies are generally entering the device (Rayaisse *et al*., 2010). The shape, colour, and odour attractants (octenol, acetone and phenol), play a role in the attraction of tsetse flies to traps (FAO, 1989). Blue colour attracts tsetse, while black and ultraviolet, reflecting white, attract them to land on a device or enter the trap (FAO, 1989).

Because of the relatively low cost of the materials and the apparently unsophisticated technology, the use of traps and targets has been promoted as the most sustainable method of tsetse control, especially in the context of community participation (Vreysen, 2001). The application of these techniques is necessary prior to the implementation of SIT, because the tsetse population has to be drastically reduced before the release of irradiated sterile males (Vreysen *et al*., 2000). However, the disadvantage of this method is that it is labor intensive, can be destroyed by flood, strong wind, wild animals, and fire and are damaged or stolen by humans (Vale and Torr, 2004). In addition, reinvasion remains a reoccurring problem (Hargrove, 2002).

#### 2.8.1.3. Use of insecticides

Tsetses are exceptionally sensitive to insecticides and have been controlled or eliminated from extensive areas of Africa by widespread application from the ground or air (Allsopp and Hursey, 2004). The use of insecticides as control methods against tsetse fly commenced in the mid 1940s and is still today a major technique used in large scale (Grev, 2002). Organochlorines, notably DDT and dieldrin, and more recently the synthetic pyrethroids, are the two chemical groups that have enjoyed the wider success in the control of tsetse flies (Allsopp *et al*., 2004). The application of residual insecticides to tsetse resting sites was very widely used. However, both chemicals fall victim of

international ban due to their environmental side effect (Allsopp and Hursey, 2004) and effects on non-target organisms (Barret *et al*., 2003).

Delivery methods of residual insecticide include (i) depositing either on to the resting and breeding sites of tsetse by ground spraying or by treating the entire habitat from the air (Kgori, 2006) (ii) repeated spraying of non-residual insecticide, either over large areas using aircraft (sequential aerosol technique-SAT) (Allsopp and Phillemon-Motsu., 2002). An extensive area of tsetse control or elimination is achieved by the application of Deltamethrin insecticidal aerosols from air-crafts over large tracts of land (Kgori, 2006). However, there are increasing demands to limit the use of insecticides because of their detrimental effect on naturally occurring fauna and flora (Allsopp *et al*., 2004) and lack of funds to manage such large scale programmes (Barrett and Okali, 2006).

#### 2.8.1.4. Live bait technique

Live bait technique involves treating cattle with appropriate insecticide formulations, usually by means of cattle dips, or as pour-on, spot-on, or spray-on,which can be very effective in tsetse control (Ndeledje *et al*., 2013). An important means of reducing the risk of contracting bovine trypanosomosis is the control of tsetse (Maudlin *et al*., 2004), and the simplest and cheapest method of achieving this is the application of pyrethroids to cattle (Shaw *et al*., 2013). This exploits the blood sucking behavior of tsetse flies. The tsetse attempting to feed on treated livestock are killed by picking up a lethal deposit of insecticide (Leak, 1999). These insecticides have low mammalian toxicty and they degrade quickly in tropical environments, with halflives of ~1 week for deltamethrin on vegetation or in soil (Rosendahl *et al*., 2009).

Continuous uses of insecticide-treated livestock is highly effective against tsetse and have the additional advantage of controlling other flies and cattle ticks (Ndeledje *et al*., 2013) and appears to be cost effective (Eisler *et al*., 2003). Currently it is the most common method which has been easily adopted by farmers to control tsetse flies (Hargrove *et al*.,

2003). Considerable success has been achieved by directly applying insecticides on animals (Okiria *et al*., 2002). In the Eastern Province of Zambia, the insecticide-treated cattle method (cyfluthrin in pour on) resulted in a drastic decline in the incidence of trypanosomosis (Van den Bossche *et al*., 2004).

Recently, it has been proposed to limit the application of the insecticide to certain areas of the host that are preferred biting sites (lower parts of cattle) for riverine and savannah species to reduce the cost and environmental impact (Bouyer *et al*., 2007; Torr, *et al*., 2007; Bouyer *et al*., 2009). The disadvantages of live bait treatment are the high treatment frequency, the high cost of the insecticides, insecticide residues in cattle dung, and the potential development of resistance to the insecticides in ticks and insects with a high reproductive rate (Vale and Grant, 2002) and the profound effect of interfering with the enzootic stability that is manifested in young indigenous cattle when exposed to tick challenge (Bourn *et al*., 2005).

#### 2.8.1.5. Use of sterile insect technique (SIT)

It has been known that insect pests can be controlled or eradicated through a method based on Sterile Insect Technique (Klassen and Curtis, 2005). The principle of SIT consists of the mass production of the target *Glossina* in specialized production centers, the sterilization of the males by gamma irradiation (Okhoya, 2003) and the sustained and systematic release of the sterile males over the target area in an estimated ratio of 50 sterile males for every 1 wild male. The released sterile males in the target area do out-compete the wild male population for wild females (Vreysen, 2006). Mating between sterile and fertile insects causes sterility in their offspring (Dyck *et al*., 2005), because female tsetse usually mate only once in their life (Feldmann, 2004).

The Sterile Insect Technique as part of area-wide integrated pest management approaches (AW-IPM) has been successfully used to suppress or eradicate several dipteran pests, including fruit and tsetse flies (Dyck *et al*., 2005). It is generally agreed that SIT makes

only sense as part of area-wide integrated pest management (AW-IPM) of tsetse. The AW-IPM approach aims at the sustainable removal of an entire tsetse fly population within a delimited geographical area and uses a combination of various techniques including SIT (Klassen, 2005).

An example of success of SIT was the eradication of *G. austeni* from the Island of Unguja, Zanzibar (Vreysen *et al*., 2000). Contrarily to the theoretical 10:1 ratio of sterile males to wild males a ratio of more than 100:1 was needed on Unguja. This can be explained by the fact that -contrary to the common belief-some tsetse flies mate more than once (Bonomi *et al*., 2011). The success in Zanzibar demonstrated the technical feasibility of fighting the disease through the sterile insect technique approach (Kabayo, 2003).

As opposed to insecticides, the SIT is environmentally non polluting method, has no adverse effects on non-target organisms, species specific and can easily be integrated with other biological control methods (Vreysen, 2006), but it is only effective when the population density of the target flies is very low which implies prior suppression of the flies using other techniques. One way this could be achieved is by suppressing the native population through other means before SIT (Bett, 2008).

The obvious constraints of sterile insect technique are the high cost associated with mass rearing, low competitiveness of released sterile males (Whitten and Mahon, 2005) and low reproductive rate (Feldmann, 2004). In addition, the SIT necessitates efficient release and monitoring methods, which have to be applied on an area-wide basis (Vreysen, 2005). However, sterile insect technique still remains an exceptionally promising pest control method in terms of efficacy and environmental compatibility (Nagel and Peverling, 2005).

### *2.8.2. Use of trypanotolerant animals*

Trypanotolerance is the characteristic of an animal that enables it to remain productive under tsetse and trypanosomosis challenge (Murray *et al.*, 2004; Courtin *et al.*, 2008). The most well known trypanotolerant breeds are West African short-horned cattle (Muturu, Baoulé and N'dama), (Naessens, 2006). In East Africa, the *Maasai Zebu* and *Orma Boran* breeds show reduced susceptibility. Apart from cattle, Djallonke sheep and dwarf goats breeds in West Africa are also relatively trypanotolerant (Geerts *et al*., 2009). Trypanotolerance has also been found in other species of livestock such pigs, horses and donkeys (Murray *et al.*, 2004). Although the trypanotolerant trait is not absolute, cross breeding indigenous cattle with these breeds improves resistance to disease (Franco *et al.*, 2014).

The mechanism of trypanotolerance has a genetic basis (Naessens, 2006). It is thought to involve a strong immune response towards the parasite antigens as well as cytokine synthesis with lethal effects on trypanosome proliferation (Yoshihara *et al*., 2007). Several studies reported that there is a difference in cytokine production between resistant and susceptible hosts (Namangala *et al*., 2009). Magez *et al*. (2006) and Baral *et al*. (2007) showed that trypanotorelant hosts elicit a stronger antibody production than the susceptible ones.

A major factor enabling these animals to cope with trypanosome infections is a better capacity to limit both anaemia and parasitaemia (Naessens, 2006). Under natural conditions of tsetse challenge, trypanotolerant cattle had lower mortality, lower trypanosome levels, less severe anaemia, superior weight gain and better reproductive performance than more susceptible breeds. However, Trypanotolerance is severely affected by heavy challenge, malnutrition, stress, age, season, and concurrent disease (Feldmann and Hendrichs, 2001) and their utilization is often supplemented by the use of trypanocidal drugs in areas of heavy tsetse challenge, (Bett, 2008).

Trypanotolerant breeds represent a small proportion (6%) of the cattle population of Africa and 17% of the cattle in the tsetse challenged areas, with the largest population in West Africa (Agyemang, 2005). One limitations to the use trypanotolerant cattle breeds in husbandry practice is their small size, productivity and traction power compared with the large zebu breeds (Chater, 2002), and also their low price fetched in markets (Kamuanga *et al.*, 2001).

### *2.8.3. Chemotherapy and chemoprophylaxis*

In trypanosome endemic areas trypanocidal drugs are the most widely used methods of trypanosomosis control, ensuring maximum effects at relatively little cost (Grace *et al*., 2009; Clausen *et al.*, 2010; Van den Bossche and Delespaux, 2011). This approach of controlling the parasite in the host relies on limited and extensively used trypanocidal drugs with little or no regular monitoring (Stein *et al*., 2011; Mungube *et al*., 2012 ; Sow *et al.*, 2012).

In African countries chemotherapy for trypanosomosis in cattle, sheep and goats mainly relies on use ISM and DA (Giordani *et al*., 2016), which have been and are still in use for more than 50 (Wilkinson and Kelly, 2009; Geerts *et al*., 2010). These drugs are grouped either as curative or prophylactic or both depending on their use. Diminazene aceturate has curative properties whereas Isometamidium chloride and the Homidium salts have both curative and prophylactic activities (Holmes *et al.*, 2004). The use of preventive drugs can help to prevent susceptible animals from contracting the disease for a period of two to four months (Sow, 2013).

In sub-Saharan Africa 35 million doses of trypanocides are used annually (Holmes, 2013). However, the effectiveness of these drugs is threatened by drug resistance (Delespaux *et al*., 2008). In addition, all of these drugs have toxic effects on animals and cross-resistance of isometamidium with homidium and, to a lesser extent, with diminazene, limits their efficacy (Delespaux and de Koning, 2007). Unfortunately, drug

research and development for AAT is a minor activity due to lack of profit perspectives for pharmaceutical companies (Trouiller *et al*., 2002).

#### 2.8.3.1. Curative drugs

Diminazene aceturate (DA) is the main therapeutic drug used in the management of clinical trypanosomosis in animals (Holmes *et al*., 2004). Diminazene aceturate belongs to the diamidine class of compounds (Steverding, 2010). Diminazene is the most commonly used trypanocide in cattle, sheep and goats, due to its activity against both *T. congolense* and *T. vivax* and its relatively low toxic side effects. It is administered i.m. at a dose rate of 3.5 mg/kg b.w. At these dose rates, DA, in addition to its curative uses, also offers short term protection of up to 2 weeks (Geerts *et al*., 2001).

However, Diminazene is relatively toxic to horses (Taylor *et al*., 2013) and contraindicated in dogs (Irwin, 2010; Han *et al*., 2014) and also is not recommended for dromedaries (Taj Eldinl *et al*., 2005). This class of drugs has tendency to accumulate in tissue, therefore half life is very long, which may lead to residual problems in food producing animals (Riviere and Popich, 2009).

#### 2.8.3.2. Prophylactic drugs

Prophylactic treatments target all animals in a herd or a particular group of valuable or `at risk' animals (Holmes *et al*., 2004). Isometamidium (ISM) administered intramuscularly (i.m.) at a dose rate of 0.5-1mg/kg b.w is used as a curative and prophylactic drug up to six months of protection in cattle against *T. vivax* and *T. congolense* (Delespaux *et al*., 2010) as well as *T.b. brucei* in equidae and *T. evansi* in camels (Geerts *et al*., 2001), but this period may be shorter (two to four months) when challenge is higher (Magona *et al*., 2004; Sow, 2013).

Moreover, studies have shown that ISM can kill the trypanosomes developing in tsetse flies (Van den Bossche *et al*., 2006). During SIT campaigns, the incorporation of ISM in the first blood meal of sterile males will significantly reduce the ability of the released males to transmit trypanosomes (Bouyer *et al*., 2010). The preventive effect of ISM is conferred by the slow diffusion of the drug owing to the tissue binding at the intramuscular injection site. When trypanosomes are resistant to ISM, EtBr will be ineffective as cross-resistance is observed between the two drugs (Delespaux *et al*., 2002).

Homidium is better known by its chloride salt or Novidium® and its bromide salt or Ethidium bromide. Homidium is recommended for i.m use in 1 or 2.5% solutions at a dose of 1 mg/kg (Sinyangwe *et al*., 2004). Homidium was widely used in Africa to treat *T. congolense* and *T. vivax* infections in cattle, sheep and goats, in spite of its proven mutagenic and possible carcinogenic properties as DNA intercalator (Sutcliffe *et al*. 2014). Additional studies indicate that homidium bromide can be toxic at high concentrations (Sutcliffe *et al.,* 2014) .Contrary to ISM; homidium is spread much more diffuse throughout the trypanosome (Boibessot *et al*., 2002). Although used as a curative drug, homidium also possesses chemoprophylactic properties, but these are less pronounced than those of ISM. However, the use of homidium salts is no longer advisable due to their mutagenic activity (Sutcliffe *et al*., 2014), but are still on use in Africa (Geerts *et al*., 2010).

#### 2.8.3.3. Vaccination

All of the important livestock trypanosomes are extracellular parasites in mammals and evade the host immune defences by continuously changing their surface coat (Horn, 2014). The almost unlimited antigenic variation during infection by one single strain of trypanosome and the antigenic strain diversity within each of the several trypanosome species and types are the main obstacles preventing vaccine development against trypanosomes (Magez *et al*., 2010; La Greca and Magez, 2011; Cnops *et al*., 2015).

The one area where there has been some progress has been in the development of an anti-disease vaccine. Some attempts are still being made at vaccine development using internal non-variable antigens or at immunizing against proteins causing pathogenic effects, instead of against the parasite itself (Silva *et al*., 2009). Promising results were obtained using low molecular weight VSG-specific trypanolytic nanobodies that impede endocytosis (Stijlemans *et al*., 2011). There are also better prospects of success in developing transmission blocking vaccines (reactive oxygen species (ROS) that interfere with the maturation of the insect stages of the trypanosome (Macleod *et al*., 2007).

### 2.9. Trypanocidal Drug Resistance

Over most of sub-Saharan Africa, bovine trypanosomosis continues to be controlled primarily by trypanocidal drugs (Holmes *et al*., 2004). This is essential because without treatment, the outcome of African trypanosomosis is almost always fatal (Legros *et al*., 2002). However, the effectiveness of these drugs is threatened by the development of widespread drug resistance (TDR) (Clausen *et al*., 2010). Cases of resistance to veterinary trypanocides started to be reported in the field soon after their introduction, and their numbers have been increasing ever since (Delespaux *et al*., 2008). Nowdays, the development, the spread and the control of TDR have been the subject of various researches (Grace, 2005; Clausen *et al*., 2010) and considered a serious problem in trypanosomiasis control particularly for the resource poor, atrisk populations and farmers in Africa (Kagira and Maina, 2007).

Trypanocidal drug resistance is increasingly reported all over Africa and is now present in 21 sub-Saharan countries (Geerts *et al*., 2010; Chitanga *et al*., 2011). It is suspected that in several other African countries, resistance is present but is yet to be demonstrated (Delespaux *et al*., 2008). Trypanocidal drug resistance has also been reported in South America, where the compound is the first line drug to treat these infections (Cadioli *et al*., 2012). Furthermore, in some instances, multiple drug resistance has been reported by (Holmes *et al.*, 2004). The origin of multiple resistances to trypanocides by trypanosomes

in the field is unclear, though cross-resistance between the different compounds probably due to their closely related molecular structures was implicated (Tsegaye *et al.*, 2015), but it can also occur between very different drugs (Delespaux and Koning, 2007).

Recent case surveys conducted in some African countries (Chitanga *et al*., 2011; Mungube *et al*., 2012) and in Ethiopia (Moti *et al*., 2012; Hagos *et al*., 2014), revealed that almost all of the commercially available trypanocidal drugs are gradually losing their efficacy due to the development of multiple drug resistance. For instance trypanocidal drug resistance was observed for ISM and DA against *T. congolense*, *T. vivax* and *T. evansi* (Kumar *et al*., 2012; Mungube *et al*., 2012; Sow *et al*., 2012). The situation has become more worrying are the recent reports of multiple drug resistance to ISM and DA (Mamoudou *et al*., 2008).

The drug resistant parasites can be partially resistant, reappearing a few days post-treatment, or fully resistant, where they do not respond to the treatment at all (Prudhomme *et al.*, 2006). Resistance appears to be stable with the trypanosomes remaining resistant for several years, even when transmitted between animals or cultivated over a long period of time in the absence of trypanocidal drugs (Chitanga *et al*., 2011). Unfortunately, drug research and development for AAT is a minor activity due to lack of profit perspectives for pharmaceutical companies (Trouiller *et al*., 2002; Shiferaw *et al*., 2015).

### *2.9.1. Factors contributing to trypanocidal drug resistance*

Drug resistance in trypanosomes is likely to be promoted by the same factors that cause bacterial resistance to antibiotics, such as large-scale drug use and sub-curetive doses (Holmes *et al*., 2004) and by using correct dosing with drugs that are slowly eliminated from the body (Holmes *et al*., 2004). Dose that are too small only kill some trypanosomes; other survive and becomes resistances to drugs (Taylor *et al*., 2007). In addition the release of preparations that contain low quantities of active drug provokes

ideal conditions for the selection of drug resistance as well as leading directly to therapeutic failure (Barrett *et al*., 2004).

It is believed that the emergence of drug resistance is linked to bad handling and utilization practices as well as to poor drug quality (Holmes *et al*.,2004), which severely reduces the effectiveness of chemotherapy. Recent studies on the quality of the trypanocides DA and ISM sold in sub-Saharan Africa, showed that a great majority of these products do not respect the standards established by the original producers (Schad *et al*., 2008; Vitouley *et al*., 2012).

In addition to these, the lack of proper diagnosis is considered as the major causes of increasing development of drug resistance throughout Africa. Furthermore, some trypanocidal drugs such as ethidium are well-known mutagenic compounds and might induce mutations, the most resistant of which might be selected under drug pressure (Holmes *et al*., 2004). Mutations that confer drug resistance are thought to arise randomly, independent of the presence of the drug, with a positive selection in parasite strains exposed to the drug (Prudhomme *et al*., 2006).

### *2.9.2. Mode of action of trypanocidal drugs*

Isomethamidium and Diminazene aceturate accumulate inside the cytoplasm and mitochondrion of trypanosomes and have a common target: the mitochondrial DNA. Isomethamidium is actively transported into the kinetoplastic compartment probably due to the mitochondrial electrical potential or through an as yet unidentified energy-consuming transmembrane transporter (Delespaux *et al*., 2005). Isomethamidium also enters the nucleus where it binds to the nuclear DNA (Kaminsky *et al*., 1997). Isomethamidium® selectively inhibits the kDNA type II topoisomerase, a protein involved in the replication of the mitochondrial DNA, which leads to the shrinking and disappearance of the kDNA network, and ultimately to cell death (Roy Chowdhury *et al*., 2010).

The trypanocidal mode of action of diminazene has not been completely elucidated. Diminazene aceturate has high affinity for A-T base pairs and binds to circular double strand DNA to induce complete loss of the kDNA network (Gonzalez *et al*., 1997). Diminazene inhibits synthesis of RNA primers, resulting in accumulation of replicating intermediates, thereby inhibiting kDNA replication (Brack and Delain, 1975). In another work, Shapiro and Englung, (1990), have shown that Diminazene specifically inhibits mitochondrial type II topoisomerase in viable trypanosomes.

### *2.9.3. Mechanism of drug resistance*

Drug resistance is the loss of sensitivity by a strain of an organism to a drug to which it had previously been susceptible and implies failure of treatment and prevention of a disease (Uilenberg, 2017). In trypanosomosis, drug resistance is usually the result of the loss of capacity for drug uptake by the parasite, an alteration in drug-target interaction, a change in the efflux mechanism (Baker *et al*., 2013).

The mechanism by which trypanosomes acquire resistance varies with the drug; resistance to DA is attributed to the alteration of a membrane transporter namely, P2-type purine transporter, TcoAT1 (Mäser *et al*., 2003; Delespaux *et al*., 2008), which is involved in the drug uptake by the parasite. However, this may not be the only mechanism contributing to DA resistance. Indeed, a novel gene TeDR40, the encoded protein appeared to have a ubiquitous cellular localization, is shown to contribute to a high DA resistance in *T. evansi*. It is probable that such a high level of DA resistance is the result of the cumulative effect of two distinct resistance mechanisms (TeDR40 and P2-type purine transporter) (Witola *et al*., 2005).

Resistance to Isomethamidium® is attributed to synergistic combination of reduced uptake and increased efflux of the drug at the level of the mitochondrion i.e. (i) a decrease in transport through the mitochondrial membrane (lowered mitochondrial electrical potential), (ii) the modification of a possible transporter located in the inner

mitochondrion membrane, (iii) an increased efflux of the drug from the cytoplasmic compartment via a yet to be identified transporter or (iv) a combination of these processes (Delespaux *et al*., 2008) into the mitochondria. Trypanosome kinetoplast is the primary site of ISM accumulation and decreased levels of drug accumulation have been observed in drug resistant populations of *T. congolense* (Sutherland *et al*., 2016). Reduction of net drug uptake could be as a result of either decreased drug import or increased drug export (Mäser *et al*, 2003). Sutherland and Holmes, (2004) found indirect evidence of an increased efflux of drug from resistant trypanosomes.

An alternative mechanism of ISM resistance could include the alteration or modification of the targeting site of the drug in the trypanosome. The modification of the topoisomerase gene as possible mechanism of resistance to ISM was explored in *T. congolense* (Delespaux and Koning, 2007). The resistance to Homidium is still unknown even if some studies suggested that it is similar to Isomethamidium® (Peregrine *et al*., 1997).

### *2.9.4. Detection of drug resistance*

Currently, four types of laboratory techniques are commonly used to identify drug resistance: field tests (Holmes *et al*., 2004), *in vivo* and *in vitro* tests, ISM-ELISA and more recently molecular tools (Delespaux *et al*., 2008). The commonly used field techniques include field tests and in vivo tests (Vitouley *et al*., 2011).

#### 2.9.4.1. Field tests

Several authors reported the application of field tests to assess trypanocidal drug resistance (McDermott *et al*., 2003; Tewelde *et al*., 2004; Mungube *et al*., 2012). Isomethamidium chloride treatment failure can be assessed in the field, under natural *Trypanosoma* challenge, by using the "block treatment" approach (Eisler *et al*., 2000). It consists of two groups of infected cattle (30 to 80 animals) either treated with 1mg/kg

ISM or untreated (control group). The animals are exposed to natural challenge and tested for the presence of trypanosomes in the blood by using the phase contrast buffy coat technique (Murray *et al*., 1977) every two weeks during two to three months. Drug resistance will be strongly suspected if more than 25% of ISM-treated animals become infected within eight weeks of exposure (McDermott *et al*., 2003).

A variant of the "block treatment" technique is also used to assess suspected ISM and DA treatments failures by treating both the two groups with ISM and DA respectively at the start of the experiment, and checking for the presence of the parasites two weeks after treatment (McDermott *et al*., 2003; Mamoudou *et al*., 2006; Mungube *et al*., 2012). This variant is effective in areas where trypanomosis risk is high (prevalence is >10%) as has been demonstrated in the cotton zone of West Africa (Grace, 2005). Analysis of data on frequency of infections after block prophylactic or curative drug treatment allows identifying suspected cases of drug resistance. If more than 25% of ISM-treated cattle become infected within four weeks of exposure or for DA after two weeks treatment drug resistance is strongly suspected (McDermott *et al*., 2003).

#### 2.9.4.2. Tests in ruminants

The common *in vivo* tests used to identify drug resistance are tests in ruminants and tests in mice. The tests in ruminants consists of infecting a group of cattle or small ruminants with the isolate under investigation and later, when they are parasitaemic, treating them with various dosages of trypanocides (Holmes *et al*., 2004). Standardized protocols for the tests in animals have been developed, which should allow better comparisons of data on a temporal and spatial basis (Eisler *et al*., 2001). A minimum of 3 and preferably 6 animals in each group are inoculated with the same trypanosome isolate, as result from one animal is not always reliable (Eisler *et al*., 2001). The experimental animals should be kept in a fly-proof stable to eliminate the risk of re-infection and regularly monitored for 100 days to determine the effective dose (ED), i.e. the dose that clears the parasites from the circulation, and the curative dose (CD), i.e. the dose that provides a permanent

cure (Sones *et al*., 1988). This test is useful because of the difficulty in extrapolating the curative dose in cattle from the results of tests in mice (Sones *et al*., 1988).

#### 2.9.4.3. Tests in mice

Either single-dose or multi-dose tests are conducted in mice to provide information on resistant trypanosome isolates from a given area, as described in the protocol by Eisler *et al*. (2001). After expansion of an isolate in a donor mouse, experimental mice are inoculated with the test trypanosome isolate and treated with a trypanocidal drugs. The parasitemia in the mice is checked 2-3 times per week for parasites up to 60 days. The effective dose that gives temporary clearance of the parasite in 50% (ED50) and 95% (ED95) can be calculated as can the curative dose that gives complete cure in 50% (CD50) and 95% (CD95) curative dose that gives complete cure in 50 and 95% of the animals, respectively (Sones *et al.*, 1988; Eisler *et al*., 2001).

There are however several disadvantages with this method. Firstly, most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice (Holmes *et al*., 2004). Secondly, although there is a reasonable correlation between drug sensitivity between mice and cattle, higher doses of drugs must be used in mice (normally ten times higher) in order to obtain results comparable to those from cattle, because of the vast different metabolic size (Sones *et al.*, 1988). Thus, the curative dose for ruminants cannot be extrapolated from the assay results in mice (Sones *et al*., 1988).

#### 2.9.4.4. In vitro tests

In vitro assays test have been used to detect resistance in *T. brucei* and *T. congolense* (Clausen *et al*., 2000), using bloodstream or metacyclic forms instead of procyclic forms. However, the slow adaptation of trypanosomes to the experimental conditions is one of the major constraints of these tests (Clausen *et al*., 2000). Furthermore, it is difficult to maintain *T. congolense in vitro* (Clausen *et al*., 2000).

Considering the complexity of maintaining *T. congolense in vitro*, two alternative methods have been developed. The Drug Incubation *Glossina* Infectivity Test (DIGIT): it consists in feeding flies with blood containing trypanosomes and different concentrations of drugs. The flies are then dissected 20 days later to check their infection status. This method is limited by the availability of tsetse flies (Clausen *et al*., 1999). In the second method, the Drug Incubation Infectivity Test (DIIT), mice are infected by trypanosomes after drug incubation (Knoppe *et al*., 2006).

#### 2.9.4.5. *Trypanocidal drug ELISA*

The use of ELISA has proved valuable in diagnosing ISM resistance (Murilla *et al*., 2002) and can be combined with field block treatment studies or for individual treatment of ruminants to detect resistant trypanosomes (Eisler *et al*., 1997). The presence of trypanosomes in animals with an ISM serum concentration > 0.4ng/ml suggests that parasites are resistant (Eisler *et al*., 1997). Further, an ELISA has also been developed for the detection of of sub-nanogramme amounts of homidium bromide and diminazene aceturate (Holmes *et al*., 2004).

#### 2.9.4.6. Molecular methods

Molecular methods for the diagnosis of ISM resistance were recently developed (Afework *et al*., 2006; Delespaux *et al.*, 2008). Developments of such tests require the identification of genetic mutations that may be associated with drug resistance in livestock-infective trypanosomes (Vitouley *et al*., 2011). The first method enables discrimination between ISM sensitive and ISM-resistant strains of *T. congolense* by MboII-PCR-RFLP. This test is based on the polymorphism observed in the 381 bp fragment (in sensitive strains) or the 384 bp fragment (in resistant strains) of a putative gene presenting some homologies with an ABC transporter (Delespaux *et al*., 2005).

The second method has been developed to distinguish ISM-resistant from ISM-sensitive strains of *T. brucei* (Afework *et al*., 2006). This SfaNI-PCR-RFLP test is based on the polymorphism of the 677 bp fragment of the TbAT1 gene. The same set of six point mutations could confer resistance to the melarsenoxide cysteamine cymelarsan (an arsenical diamidine) and to ISM (diamidine compound) and the detection of one of these six mutations could enable reliable identification of sensitivity or resistance to ISM (Mäser *et al*., 2003).

### *2.9.5. Control of trypanocidal drug resistance.*

Based on current knowledge in the field of trypanocide resistance and on experience in the control of resistance to insecticides, anthelmintics, antibiotics and other drugs (Geerts *et al*., 1997) the following recommendations are proposed in order to delay the development of resistance.

#### 2.9.5.1. Use of the correct dose

Under dosing is one of the major causes of resistance development. Sub-therapeutic drug concentrations exert a strong selective pressure for the emergence of resistant clones that pre-exist in the trypanosome population. Unfortunately, under dosing occurs very frequently by farmers or unskilled persons in many countries of Africa due to the absence of strict rules about the utilization of veterinary drugs (Geerts and Holmes, 1998). Furthermore, there are an increasing number of generic products available on a somewhat loosely regulated market, and some of these have questionable efficacy and many contain lower doses of drug than the stated amount (Holmes *et al*., 2004).

#### 2.9.5.2. Reduction of the number of treatments

It is widely agreed that the most efficient way to delay the development of drug resistance remains the reduction of drug selection pressure by decreasing the number of

treatments, especially incase of multiple drug-resistance (McDermott *et al*., 2003). Reduction in drug pressure impacts drug resistance evolution in three ways; (i) delays its appearance, (ii) reduces the likelihood of its establishment and (iii) slows its spread (Smith *et al.*, 2010).

It is therefore strongly recommended that control of trypanosomosis should not rely solely on drugs. An integrated approach should be adopted using vector control, to reduce the tsetse challenge, along with reduced frequency of drug dosing. Minimizing drug use by reducing contact between livestock and tsetse vectors and intensifying vector control activities has also been suggested as an effective means of preventing or delaying the emergence of trypanocidal drug resistance (Holmes *et al*., 2004). Furthermore; frequently repeated trypanocidal drug treatments have been associated with toxicity problems (Eisler *et al.*, 1997).

#### 2.9.5.3. Avoiding exposure of the whole parasite population to a drug

In the past mass treatments are commonly used to control animal trypanosomosis. However this form of treatment exerts a strong selection pressure on the trypanosome population. The higher the proportion of trypanosome population exposed to the drug and the lower the proportion in refugia (i.e. the proportion of trypanosomes present in the fly population or in other hosts), the higher the selection pressure. Therefore, in well-monitored situations there is a strong case for limiting treatment to individual clinical cases; this is also desirable on grounds of minimizing drug residues, avoiding potential toxicity and reducing costs (Maudlin *et al*., 2004).

#### 2.9.5.4. Sanative Treatment

The concepts of sanative treatment is the use of a pair of trypanocides which are chemically unrelated and therefore, unlikely to induce cross resistance. DA and ISM constitute a "sanative pair" which means that once resistance develops to one of the

drugs, the other drug should be used to control the infection (Desquesnes *et al*., 2013). However, combination therapy only works when the drugs are unrelated and if instituted early enough before significant resistance to either drug is present in the population (Smith *et al*., 2010). However, the effectiveness of this strategy may be questioned by the increased field reports from many parts of Africa of multiple resistant trypanosomes (Clausen *et al*., 2010; Mungube *et al*., 2012).

#### 2.9.5.5. Banning the use of Quinapyramine in cattle

Quinapyramine are well-known mutagenic compounds and might induce mutations, the most resistant of which are certainly selected under drug pressure. Quinapyramine was withdrawn from sale for cattle use because of the problem with toxicity and resistance development (Maudlin *et al*., 2004). The use of Quinapyramine was the suggested cause of the multiple drug resistance problems in Ghibe Valley of Ethiopia. Therefore, its use as a trypanocide in cattle is completely contraindicated (Maudlin *et al*., 2004). Moreover, general improvement of animal health conditions by deworming and reduction of animal disease risk, and vector control as described by Clausen *et al*. (2010) can also be of great impact.

## 2.10. Status of Tsetse and Trypanosomosis in Ethiopia

Ethiopia is believed to have the largest livestock population in Africa, which is a significant contributor to economic and social development in the country. In Ethiopia, livestock accounts for 15-17% of total GDP and 35-49% of agriculture GDP (CSA, 2017). Unfortunately, the development and intensification of livestock productivity in Ethiopia is hampered among others by cross-border epizootic diseases such as African animal trypanosomosis. Out of the nine regions of Ethiopia (Oromiya, Benishangul Gumuz, Amahara, Gambella. and SNNPR) are infested with more than one species of tsetse flies (Getachew, 2005).

In tsetse infested areas 14 million of cattle, equivalent number of small ruminants and more than 7.5 million equines and 1.2 million of camels are at risk of contracting trypanosomosis (CSA, 2005). The direct and indirect agricultural and livestock production annual losses due to trypanosomosis are estimated US $200 million (FAO, 2010, Taye *et al*., 2012). Trypanosomosis also prevents full use of land and the introduction of highly productive exotic dairy animals and draught oxen to lowland areas (Getachew, 2005).

According to Langridge, (1976), the distribution of trypanosomosis and tsetse flies in Ethiopia are confined to the southern and western regions between longitudes 33°E and 38°E and latitude 5°N and 12°N. Currently, evidences has indicated that tsetse flies are advancing to a new ecological niches, due to which 220,000 $Km^2$ areas of fertile land is infested with five species of tsetse flies: *Glossina pallidipes*, *G .morsitans submorsitans*, *G. fuscipes*, *G. tachinoides* and *G. longipennis* (NTTICC, 2004). The most important trypanosome species affecting livestock in Ethiopia are *T. congolense*, *T. vivax* and *T. brucei* in cattle, sheep and goats, *T. evansi* in camels, and *T. equiperdum* in horses (Getachew, 2005). The prevalence of trypanosomosis in tsetse infested areas range from 11.85-37% (Fikru *et al*., 2012).

There are different efforts to control tsetse and trypanosomosis in the country. In Ethiopia, beside trypanocidal drugs, vector control practices such as odour-baited and insecticide impregnated targets and traps and insecticide treated cattle have been implemented (Tafese *et al*., 2012). Recently, in the southern rift valley tsetse fly suppression for eradication is underway from 25,000 $km^2$ using sterile insect technique as component of the integrated tsetse eradication campaign to eradicate the major vector in the area, i.e. *G. pallidipes*. According to Geja *et al*. (2012), the fly density, trypanosomosis prevalence and mortality due to trypanosomosis have been significantly reduced and the government of Ethiopia has conducted a massive settlement program in early 2000s to the tsetse and trypanosomosis belt.

## 2.11. Control of Bovine Trypanosomosis in Gidami and Sayo districts

In Gidami district, Trypanocidal drugs: diminazene aceturate and isometamidium chloride are the only control strategy used for control of animal trypanosomosis. However, in Sayo district, beside trypanocidal drugs odour-baited and insecticide impregnated targets and insecticide treated cattle has been conducting by the National Tsetse and Trypanosomosis Investigation and Control Center, through using targets impregnated with Deltamethrin 20% (w/v) suspension concentrate diluted with water to a concentration of 0.4% spray liquid. In addition, insecticide treated cattle were also used by spraying Deltamethrin 1% (w/v) pour-on ready-for-use formulation at a dose rate of 10 ml per 100 kg body weightduring early rainy and early dry seasons as the strategy for tsetse suppression (KWZAHFRDO, 2014).

## 2.12. Trypanocidal Drug Practices in Ethiopia

Trypanocidal drugs remain the only widely available control method for trypanosomosis in Ethiopia with DA and ISM is the most commonly used drugs. Similar to the situation in most African countries, the use of these drugs is often unregulated and the widespread presence of all risk factors for resistance development is documented (Dagnachew *et al*., 2008). In addition, several brands and diverse sources of trypanocidal drugs are known to circulate in local markets of Ethiopia (Tekle *et al*., 2018). These drugs have been mainly sourced from public veterinary clinics, private veterinary drug shops and local markets (Zewdu *et al*., 2013).

In Ethiopia, the problems of drug resistance against one or both of the drugs (ISM and DA) have been reported by different researchers (Moti *et al*., 2012; Hagos *et al*., 2014; Dagnachew *et al*., 2015b) (Table 1). The continued use of the same Trypanocides for years has resulted in drug resistance that has been largely responsible for the current chemotherapeutic failures in Ethiopia (Geerts *et al*., 2010; Shiferaw *et al*., 2015). As in other African countries (Holmes *et al*., 2004), the emergence of drug resistance is also

linked to bad handling and utilization practices as well as to poor drug quality (Zewdu *et al*., 2013).

Table 1 : Summary of trypanocidal drug resistance in Ethiopia

| Country | Trypanosome species | Resistance to trypanocidal drug (s) | References |
|---|---|---|---|
| Ethiopia | *T. congolense* | ISM,HOM and DA | Mulugeta *et al*., 1997 |
| | *T. congolense* | ISM and DA | Afework *et al*., 2000 |
| | *Trypanosoma b. brucei* | ISM | Afework *et al*., 2006 |
| | *T. congolense* | ISM | Tewolde *et al*., 2004 |
| | *T. brucei* | | |
| | *T. congolense* | ISM and DA | Dagnachew *et al*., 2008 |
| | *T.vivax* | | Dagnachew *et al*., 2015b |
| | *T. vivax* | ISM and DA | Desalegn *et al*., 2010 |
| | *T. congolense* | ISM and DA | Moti *et al*., 2012 |

# 3. MATERIALS AND METHODS

## 3.1. Study Area

This study was conducted in Gidami and Sayo districts of Kellem Wollega Zone of Oromia Regional State, Ethiopia. Kellem Wollega Zone is located at about 680 Kms west of Addis Ababa (Figure 2). The mean annual temperature range of the area is 15 to 25°C. The area lies within an altitude of 1300-1900 meter above sea level and has mean annual rain fall of 1200-2200 mm (KWZAHFRDO, 2014). Based on altitude the area is sub-divided into three agro-climatic zones: highland (14%), midland (48%) and lowland (38%).

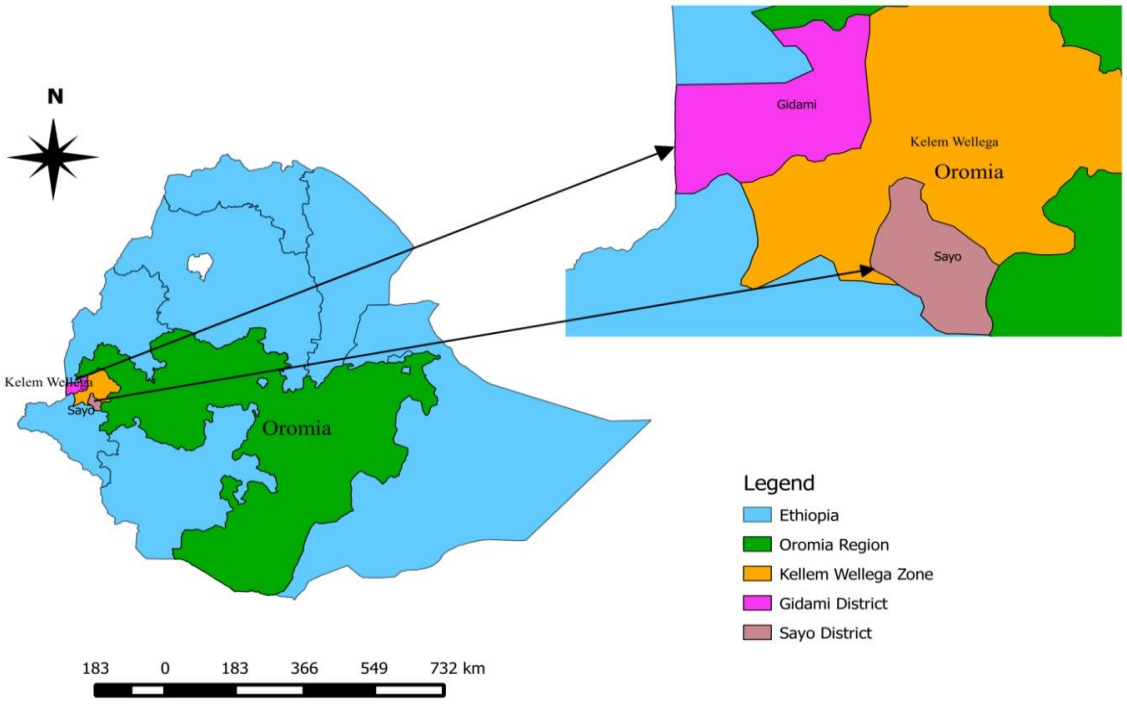



Figure 2: Map of Gidami and Sayo districts, Kellem Wollega Zone, Oromia Regional State, Ethiopia (KWZAHFRDO, 2014).

In Kellem Wollega, mixed crop-livestock farming is the main source of livelihoods. The vegetation cover is dominated by savanna grassland, forest, riverine and bush lands. Gullary forests are also encountered around permanent surface of rivers (CSA, 2017). Wild games like buffalos, bush pig, warthog, antelopes, hyena and monkeys are commonly found in the area. The major cattle breeds found in the study area were Horro breed, but the Abigar cattle breeds are also scantly distributed. Cattle are kept mainly for animal traction, manure, savings and social obligations and only secondrily for milk and meat production. Communal grazing without any supplementary feeding is the major livestock husbandry system throughout the year. As most of the land is intensively cultivated during the long rainy season, cattle are moved to the vicinity of the forests where permanent grassland. According to the data obtained from (CSA, 2017), the total livestock population of the study area is indicated below in the (Table 2). According to the (CSA, 2017), the total human population in the two districts is estimated to be 227,849.

Table 2 : Livestock populations of Gidami and Sayo districts,Kellem Wollega Zone Oromia Regional State, Ethiopia

| **Study area** | **Cattle** | **Sheep** | **Goats** | **Equines** |
|---|---|---|---|---|
| Gidami | 73, 000 | 47, 000 | 24, 000 | 12, 000 |
| Sayo | 59, 593 | 53, 377 | 6, 225 | 10, 714 |
| Kellem Wollega Zone | 569, 399 | 230, 2422 | 119, 528 | 38, 891 |

### 3.2. Study Methodology and Design

The study designs include: questionnaire survey, cross-sectional studies and field assessment of trypanocidal drug resistance. In the present study, thirteen peasant associations were purposively selected for the sake of convenience and accessibility

seven from lowland and the rest six from midland. The cross-sectional study was employed to assess the epidemiology of bovine trypanosomosis and specifically to to estimate tsetse density, trypanosome prevalence and trypanocide use practices. The cross sectional study was performed in the two districts with lowland (≤1600 m.a.s.l) and midland (>1600 m.a.s.l) agro-climatic zones in two seasons of the year. Field assessment of trypanocidal drug resistance trail was conducted on naturally infected cattle using Isometamidium chloride and Diminazene aceturate.

### *3.2.1. Questionnaire survey*

A structured questionnaire was administered to randomly selected cattle owners (Appendix 1), with a major objective of targeting some basic information about livestock management, composition of the herd, perception about trypanosomosis, tsetse flies, seasonality issues, source and type of trypanocidal drugs, practices of trypanocidal drug usage and incidence of relapses experienced after treatment. Based on the formula given by Arsham, (2002) for questionnaire surveys, a total of 100 farmers were randomly selected and interviewed from both districts.

$$N = \frac{0.25}{SE^2}$$

Where:

N=sample size

SE= standard error assuming the standard error of 5% at a precision level of 0.05 and the confidence interval of 95%.

### *3.2.2. Vector survey*

A cross-sectional vector survey was conducted in eight villages of the Gidami and Sayo districts in early dry (November-December, 2015) and early rainy (April-June, 2016) seasons, respectively. A total of 160 (80 per district) monoconical traps baited with

acetone, octenol (1-3-octane) and cow urine filled in separate bottles to increase trapping efficiency (Malele *et al.*, 2003) were deployed (Figure 3). Traps were set at regular intervals of 100 to 200 meters for 48 hours at watering and grazing points of animals. Each trap was supported on a metal pole such that the trap entrance was at a height of about 45 cm above the ground. The lower portions of the supporting pole were smeared with grease in order to prevent the ants climbing up on the pole towards the collecting cage that could damage the tsetse flies. The location of each trap was recorded with a Global Positioning System (GPS). After 48 hours of deployment the tsetse flies in the cages were collected, counted and identified based on their habitat and morphology to the genus level.

Figure 3: Monoconical trap deployed for entomological study, Giray Sonka, Gidami District,Kellem Wollega Zone , Oromia Regional State, Ethiopia

Species of captured flies were determined according to procedures and keys provided in the guidelines for the collection of entomological baseline data for tsetse area-wide integrated pest management programmes (FAO, 2008). Biting flies were identified to

genus level based on their morphological characteristics such as size, color, wing venation structure, and proboscis (Marquardt *et al*., 2000). Sexing was done for tsetse fly by observing hypopygium on the posterior end of the ventral aspect of male flies. The Apparent density of the tsetse fly was calculated as the number of tsetse catch/trap/day (STEP, 2012).

### *3.2.3. Cross-sectional parasitological study*

A cross-sectional study was conducted to determine the prevalence of bovine trypanosomosis and apparent density of the vectors during early dry (November-December, 2015) and early rainy season (Appril –June, 2017) in the Gidami and Sayo districts.

#### 3.2.3.1. Study animals and sampling strategy

The study animals consisted of all Horro and Abigar cattle breeds above one year of age kept under small holder extensive husbandry system. The two destricts were purposively selected to represent tsetse controlled (Sayo) and non-tsetse controlled (Gidami) areas of Kellem Wollega Zone of Oromia Regional State, Ethiopia. The sample size was determined using the formula given by Thrusfield, (2005) with an expected prevalence of 16.9% (Siyoum *et al*., 2014), at 95% confidence interval and 5% desired absolute precision for both districts. Accordingly, 215 animals were required to be sampled from midland and lowland areas from each district in each study season (total=430). Thus, a total of 860 animals were sampled from each district and both seasons. However, due to excess availability of animals in the field the sample size for Gidami district was 930. For Sayo district 860 samples were taken as stipulated above. Potential host related risk factors associated to the disease such as age, sex and body condition score of the studied animals were recorded during sampling. Based on the appearance of ribs and dorsal spines for zebu cattle as per the description given by (Nicholson and Butterworth, 1986), animals were grouped into poor, medium, and good body condition categories. The age

of study animals was estimated by means of their dentition as described by (Pasquini *et al.*, 2003) and conventionally categorized as young (≤3 years) and adult (>3 years).

3.2.3.2. Blood sampling and examination.

A total of 1790 cattle aged one year and above from 13 villages (7 from Gidami and 6 from Sayo districts were sampled by systematic random sampling technique to sample every other individual animal caught at communal grazing points and examined for trypanosomes as per recommendation by (Eisler *et al.*, 2001). Cattle owners were informed to gather their animals at one point one day ahead of sample collection. Blood samples were collected from marginal ear veins using sterile lancet and micro-haematocrit capillary tube and sealed on one side with cristaseal (Figure 4).

Figure 4 : Blood sample collection during early rainy season, Sayo district, Kellem Wollega Zone, Oromia Regional State, Ethiopia

The capillary tubes were then transferred to a haematocrit centrifuge with sealed end outer most and spun for 5 min at 12,000 revolutions per minute. The centrifuged capillary tube was measured for PCV values on the haematocrit reader. In this study, a PCV measurement of 25% and above was considered to be normal (Douglas and Wardrop, 2010). The contents of the capillary tube (including about 1 mm above and below the buffy coat) was expelled by cutting with diamond tipped pen, mixed and spread on a clean glass slide and covered with cover slip. The preparation was then examined under 40x magnifications as a wet preparation and trypanosome species were identified according to their movement as described by OIE (2013).

Thin blood smears were made from trypanosome positive samples and stained with Giemsa for species identification by light microscope (WHO, 2006; Buscher *et al*., 2009). The trypanosome species were distinguished using their size, position of the kinetoplast, presence and degree of development of undulating membranes and length of the free flagellum according to (OIE, 2008) (Figure 5).

Figure 5: Blood and haemathological examination during early rainy season, Gidami District, Kellem Wollega Zone, Oromia Regional State, Ethiopia

### *3.2.4. Assessment of field trypanocidal drug resistance*

Standard laboratory methods to characterize drug resistance in trypanosomes, both *in vivo* and *in vitro* are expensive and time consuming (Eisler *et al*., 2000). The practical application of genetic markers is still limited in many sub-Saharan African countries (SSA) due to the lack of appropriately equipped laboratories and skilled personnel. The drug incubation Glossina infectivity test (DIGIT) and the drug incubation infectivity test (DIIT) used in characterizing *T. congolense* resistance (Knoppe *et al*., 2006) are reliable screening methods but limited by the availability of tsetse flies and time-consuming. Longitudinal studies (McDermott *et al*., 2003; Tewelde *et al*., 2004) are also reliable but take up to 90 days to generate results.

In the present study, an abbreviated 28-day field protocol was used to estimate resistance to 7.0 mg/kg b.w DA and to 1 mg/kg b.w ISM in trypanosome-positive cattle (Diall *et al*., 2005). Based on the outcome of the cross-sectional study (Efrem *et al*., 2017), Giry Sonka and Kellem villages with the highest trypanosome prevalence (28% and 30.85%) were selected from Gidami district. Field trypanocidal drug resistance study was conducted in May 2017 at the beginning of the rainy season on 100 purposively selected trypanosome positive cattle. Equal numbers of study animals were randomly allocated into Isometamidium chloride (ISM) and Diminazine aceturate (DA) groups. Group I (ISM) treated with 2% solution of 1 mg/kg b.w Isometamidium chloride (Trypamidium Samorine®, France ISM) and group II (DA) was treated with 7% solution of 7 mg/kg b.w Diminazene aceturate (Veriben®, Ceva Animal Health INC., France DA) through deep intramuscular injections. The treatment day was considered as day 0 and the treated cattle were monitored for trypanosomes and packed cell volume (PCV) levels on days 14 (for DA and ISM) and 28 (for ISM) post treatment as described in Eisler *et al*. (2000).

A treatment failure rate of 25% of the cattle in the ISM treated group on days 14 and 28 were indicative of resistance (McDermott *et al*., 2003; Laufer *et al*., 2006), but Diminazen aceturate response was considered only 14 days post-treatment due to its short

prophylactic activity (Holmes *et al*., 2004). Trypanosome-positive cattle in ISM group on days 14 and 28 were retreated with DA 7 mg/kg b.w. Similarly, cattle that resisted DA were again treated with 1 mg/kg b.w ISM.

### 3.3. Data Analysis

Raw data collected from questionnaires and laboratory results were coded into appropriate variables and entered in Microsoft excel spread sheet (MESS). The apparent density of fly population was calculated by dividing the number of flies caught by the number of traps deployed and the number of days of deployment and expressed as fly/trap/day (FTD). Trypanosome prevalence was calculated as the number of infected individuals divided by the number of individuals sampled x 100. Bovine trypanosomosis prevalence on the basis of sex, age, and body condition were compared using $\chi 2$ test (chi-square) and odds ratio. The student t-test was used for comparison of mean PCV between infected and non infected groups of animals with different altitudinal range. The block treatment data was summarized by drugs and villages. All statistical analysis was performed using statistical packages (SPSS, version 20) and Stata 12 software. All significant values was set at $P<0.05$ and 95% confidence level.

# 4. RESULTS

## 4.1. Questionnaire Survey

To assess the farmers' knowledge, attitude, and practices towards bovine trypanosomosis and its vectors, a total of 100 respondants were interviewed through a questionnaire (Appendix 1).

### *4.1.1. Livestock farming practices*

All the respondents from Gidami and Sayo districts disclosed that they rear cattle primarily for draught purpose and income generation, small ruminants and chickens for household consumption and/or sale and equines for transportation. Above 98% of the farmers livelihood dependeds on mixed agricultural farming system. The number of cattle owned by each respondent was variable. Communal or free grazing practices are predominant in the study areas. Apart from cattle, the other types of animals kept in the study areas were, goat (16%), sheep (26%), poultry (40%) and equines (18%).

### *4.1.2. Knowledge about the trypanosomosis*

All farmers from both districts were able to describe trypanoscomosis, which is known locally as "Ghendi" or "Soofaa". About 96% of the respondents were aware of the clinical signs suggestive of bovine trypanosomosis, which are used as basis for treatment. Most frequently reported signs of bovine trypanosomosis include rough hair coat, emaciation, weakness and constipation. Only 4% of farmers cited signs inconsistent with bovine trypanosomosis, such as diarhoea, cough, lameness and lacrimation. Overall, 85% of farmers think that the incidence of trypanosomosis is higher in early rainy season, while 15% of them replied that as occurring throughout the year.

### *4.1.3. Livestock constraints*

Based on the findings of the questionnaire survey, the major problems of livestock in Gidami and Sayo districts were: diseases (65%), biting flies (18%), scarcity of veterinary supplies and services (15%) and insufficient pasture (2%). As indicated in (Table, 3), all the respondents of the questionnaire survey ranked trypanosomosis as the most important cattle health problem in the current study.

Table 3 : The Main Livestock Diseases in Gidami and Sayo Districts, Kellem Wollega Zone, Oromia Regional State, Ethiopia

| **Diseases** | **Sayo (%)** | **Gidam (%)** | **Overall (%)** |
|---|---|---|---|
| Trypanosomosis | 100 | 100 | 100 |
| Ectoparasites | 72 | 87 | 79.5 |
| Endo-parasites | 70 | 68 | 69 |
| Pasteurollosis | 40 | 50 | 45 |
| Lumphy skin disease | 20 | 24 | 22 |
| Foot and mouth disease | 15 | 19 | 17 |
| Black leg | 12 | 16 | 14 |
| Anthrax | 8 | 10 | 9 |

### *4.1.4. Means of transmission*

Eighty seven percent of the respondents knew about tsetse flies and locally named as “Titissa gamojjii”. However, 13% of them did not know tsetse flies. Out of 100 respondents, 78% were aware of the causal association between biting flies (called Warannaa) and bovine trypanosomosis. However, 22% of the respondents associated trypanosomosis with tick bite, wild animals and drinking stagnant water. Most respondents from both districts (82%) believed that the tsetse fly population is higher in the early rainy season and in low land areas. On the contrary, 18% of them replied that,

the tsetse fly populations occur throughout the year. Ninty percent of the respondents have identified riverside, forest and grazing areas as the most risky places for tsetse fly and trypanosomes exposure.

### *4.1.5. Control of trypanosomosis*

Trypanocidal drugs were the only way of combating bovine trypanosomosis in Gidami district. In Sayo district, the National Tsetse and Trypanosomosis Investigation and Control Center has been conducting control schemes through integration of pour-on with odor baited and insecticide impregnated target technologies for the last few years. The most frequently cited drug of first choice for bovine trypanosomosis treatment was DA (65%), followed by ISM (35%) in both study districts. When considering the control of trypanosomosis, 97% and 82% of the respondents from Gidami and Sayo districts preferred treatment of their animals with trypanocidal drugs rather than other control options. The study showed that 85% of respondents from Gidami district had not used any control methods against tsetse flies. Very few (15%) respondents indicated that they use deltamethrin pour-on for control of tsetse flies only during early rainy season. Even though 90% of the respondents from Sayo district indicated positive attitude towards tsetse control program, about 10% of them responded negatively. Farmers from Kure Gaib village complained on the pour-on, indicating as, as it causes hair losses and skin damage on the back of cattle.

### *4.1.6. Sources of trypanocidal drugs*

According to the results of the questionnaire survey, the main sources of trypanocidal drugs are depicted in (Figure 6).

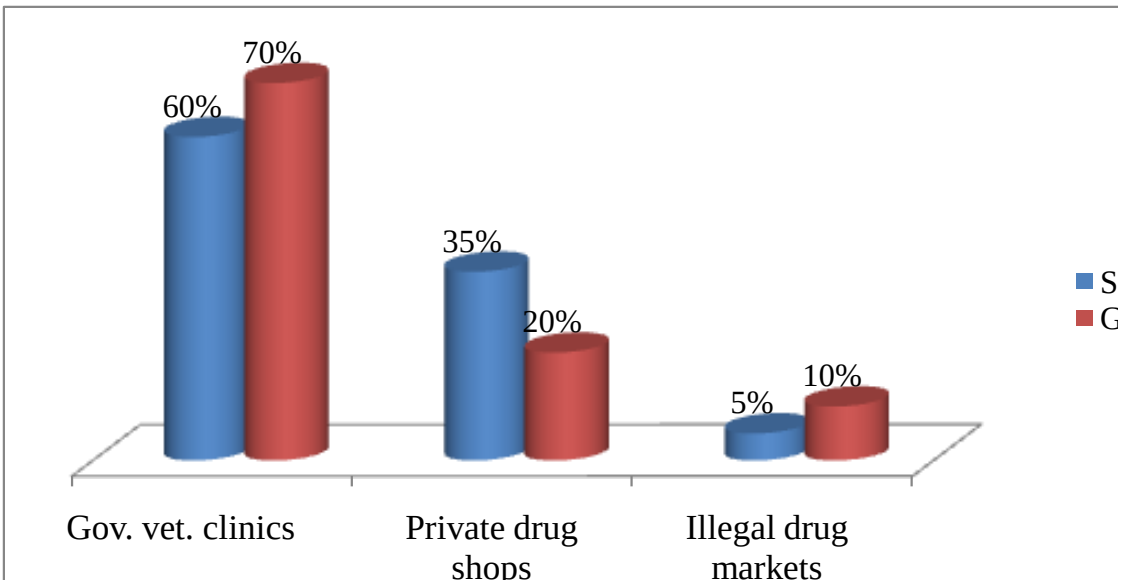



Figure 6: Sources of Trypanocidal Drugs in Sayo and Gidami Districts, Kellem Wollega Zone, Oromia Regional State, Ethiopia

Overall, 86% and 80% of trypanocidal drugs were administrated by animal health personnels, while 14% and 20% of cases were treated by animal owners in Sayo and Gidami districts respectively, thus, favouring the space for rampant missuse and under-dossage of the medications.

### *4.1.7. Decreased efficacy of trypanocidal drugs*

Based on the results of the questionnaire survey trypanocidal treatment failures were reported by 98% and 76% of the respondents from Gidami and Sayo districts, respectively. This revealed statistically significant diffirence ($P<0.05$) between the two districts. Eighty seven percent of the farmers complained that the commonly used drugs became less effective over time, which indicated trypanocidal drug resistance in the area. Responses on intervals of trypanocidal drug treatment showed considerable variation. Sixty percent of respondents in Gidami district treat their animals nine to fifteen times per

animal per year, whereas only 9% of those from Sayo district treat nine to fifteen times per animal per year with staststically significant diffirence (P<0.05) (Figure, 7).

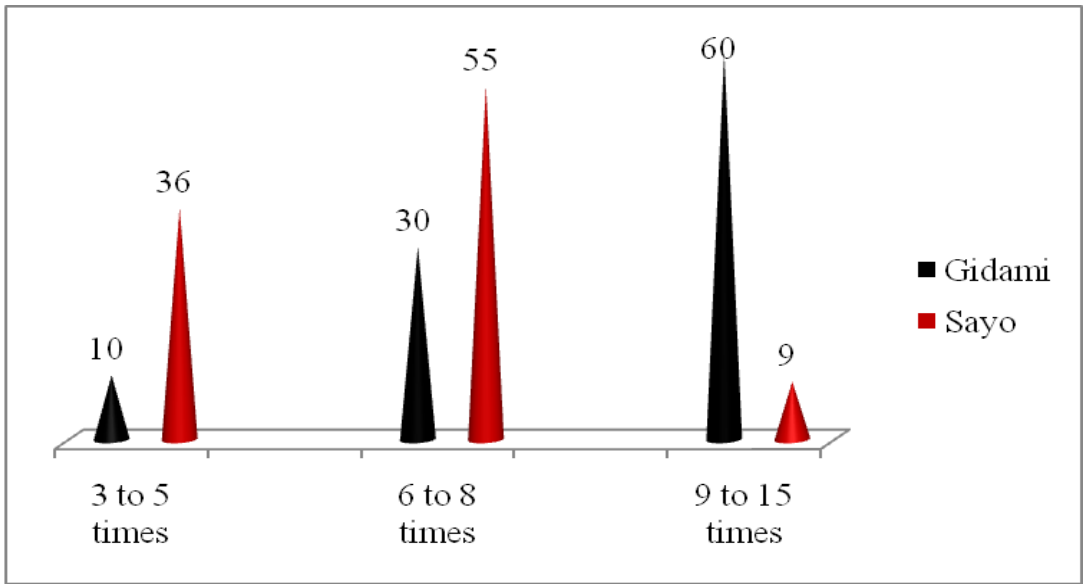



Figure 7: Treatment Frequency of Bovine Trypanosomosis in Gidami and Sayo Districts, Kellem Wollega Zone, Oromia Regional State, Ethiopia

### 4.2. Entomological Findings

A total of 3289 flies were trapped during early dry and early rainy seasons of the study period in Gidami and Sayo districts. Of these, 1158 (35.21%) belong to *Glossina*, 1739 (52.88%) *Stomoxys*, 252 (7.66%) *Tabanus* and the remaining 140 (4.25%) were *Haematopota*. There was no single *Haematopota* species caught in Sayo district. The overall apparent flies' density was 10.28 flies/trap/days (f/t/d). The apparent density of *Glossina*, *Stomoxys*, *Tabanus* and *Haematopota* were (3.62), (5.43), (0.79) and (0.44) f/t/d, respectively (Table 4).

Table 4 : Seasonal Distribution of Vectors of Trypanosomosis in Gidami and Sayo Districts , Kellem Wollega Zone, Oromia Regional State, Ethiopia.

| Genus of flies | Early dry season | | Early rainy season | | Total | % |
|---|---|---|---|---|---|---|
| | Gidami | Sayo | Gidami | Sayo | | |
| *Stomoxys* | 371 | 441 | 561 | 366 | 1739 | 52.88 |
| *Glossina* | 150 | 453 | 341 | 214 | 1158 | 35.21 |
| *Tabanus* | 51 | 79 | 100 | 22 | 252 | 7.66 |
| *Haematopota* | 21 | 0 | 119 | 0 | 140 | 4.25 |
| Overall | 593 | 973 | 1121 | 602 | 3,289 | 100 |

The seasonsl apparent density of flies was (7.67, 14.01) and *(*12.16, 7.52) f/t/d, in early dry and arly rainy seasons in Gidami and Sayo districts, respectively. Out of the total 1158 tsetse flies caught, 51.81% were *G. pallidipes;* the remaining 25.65%, 16.58% and 5.96% were *G. m. sub-morsistans*, *G.fuscipes* and *G. tachnoides*, respectively. The over all apparent tsetse fly density was (3.06) and (4.16) f/t/d in Gidami and Sayo districts, respectively. The seasonal apparent tsetse fly density was (1.87, 5.66) during early dry and (4.26, 2.68) in early rainy season f/t/d in Gidami and Sayo districts, respectively (Table 5).

Table 5: The Distribution and Apparent Densities of Tsetse Flies in Gidami and Sayo Districts, Kellem Wollega Zone , Oromia Regional State, Ethiopia

| District | Traps deployed | *G.m.m.* | *G.p.* | *G. tach.* | *G.f.* | Total | FTD |
|---|---|---|---|---|---|---|---|
| **Gidami** | | 86 | 405 | 0 | 0 | 491 | 3.06 |
| Early dry season | 40 | 24 | 126 | 0 | 0 | 150 | 1.87 |
| Early rainy season | 40 | 62 | 279 | 0 | 0 | 341 | 4.26 |
| **Sayo** | | 211 | 195 | 69 | 192 | 667 | 4.17 |
| Early dry season | 40 | 164 | 155 | 7 | 127 | 453 | 5.66 |
| Early rainy season | 40 | 47 | 40 | 62 | 65 | 214 | 2.68 |
| Total | 160 | 297 | 600 | 69 | 192 | 1158 | 3.61 |

*G.m.m. = G. m. sub-morsistans, G.P. = G. pallidipes, G. tach. = G. tachnoides, G.f. = G.fuscipes.*

Out of the total of 1158 *Glossina* species captured, 676 (58.38%) flies were females and 482 (41.62%) flies belong to males with a sex ratio 1.4:1.

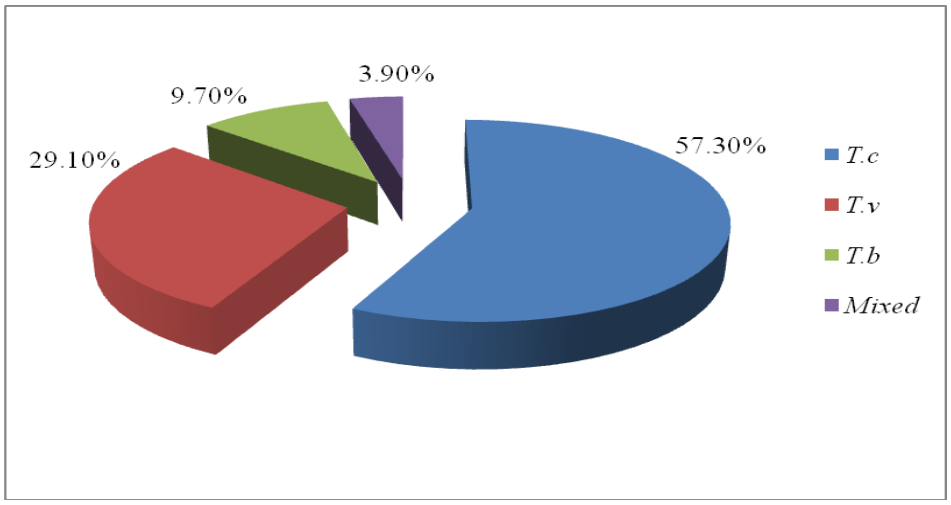
### 4.3. Parasitological and Hematological Findings

Out of 930 and 860 animals examined from Gidami and Sayo districts, 14.08% (95% CI: 12 -16%) and 11.16 % (95% CI: 9-13%) were found positive, respectively. However, there was no statistically significant difference ($P > 0.05$) between these two districts. In the present study, trypanosome prevalence was (9.07 and 7.20%) in early dry and (18.4 and 7.20%) in early rainy seasons, showing a statistically significant difference ($P < 0.05$) between the two seasons in Gidami and Sayo districts, respectively (Table 6).

Table 6: Seasonal Prevalence of Bovine Trypanosomosis in Gidami and Sayo Districts , Kellem Wollega Zone , Oromia Regional State, Ethiopia.

| District | No.examined animals | No.infected animals | Prevalence (%) | 95% CI | $X^2$ test | P-Value |
|---|---|---|---|---|---|---|
| **Gidami** | | | | | | |
| Early dry | 430 | 39 | 9.07 | 6-12 | | |
| Early rainy | 500 | 92 | 18.4 | 6-12 | 16.61 | 0.00 |
| Overall | 930 | 131 | 14.08 | 12 -16 | | |
| **Sayo** | | | | | | |
| Early dry | 430 | 65 | 15.11 | 12-19 | | |
| Early rainy | 430 | 31 | 7.20 | 5-10 | 13.55 | 0.00 |
| Overall | 860 | 96 | 11.16 | 9-13 | | |

Three trypanosome species: *T. congolense, T. vivax* and *T.brucei* were identified in the study animals (Figure, 8).




*T.v.=T.vivax, T.c=T.congolense, T.b.=T.brcei,* Mixed= *T.vivax and T.congolense*

Figure 8: The Proportion of *Trypanosome* Species Identified During in Gidami and Sayo Districts, Kellem Wollega Zone, Oromia Regional State, Ethiopia

In the present study, the trypanosome prevalence was higher in low latitude (18.09%, 13.80%) compared to mid altitude areas (9.13%, 8.50%) in Gidami and Sayo districts, respectively (Table, 7). The trypanosome prevalence was varied significantly between the lowland and midland areas (P<0.05).

Table 7 : Prevalence of Bovine Trypanosomosison the basis of altitudes in Gidami and Sayo districts, Kellem Wollega Zone , Oromia Regional State, Ethiopia

| District | No. examined animals | No. infected animals | Prevalence (%) | $X^2$ test | P- Value |
|---|---|---|---|---|---|
| **Gidami** | | | | | |
| Low land | 514 | 93 | 18.09 | 15.24 | |
| Midland | 416 | 38 | 9.13 | | 0.00 |
| **Sayo** | | | | | |
| Low land | 436 | 60 | 13.8 | 6.02 | |
| Mid land | 424 | 36 | 8.5 | | 0.00 |

The trypanosome prevalence was also observed between body condition scores, sex categories and different age groups. The overall bovine trypanosomosis prevalence was highest in poor (25.37%) followed by medium (10.02%) and lowest in good body conditioned animals (2.66%), with statistically significant differences (P<0.05) among different body conditioned animals. The prevalence of bovine trypanosomosis with the age categories were 9.38% and 14.16% in young (≤3 years) and adult cattle (> 3years), respectively. Accordingly, there was a statistically significant difference (P <0.05) in the prevalence of bovine trypanosomosis between young and adult group of animals. However, the prevalence of bovine trypanosomosis was not statistically significant (P >0.05) between males (13.86%) as compared to female animals (11.44%) (Table 8).

Table 8 : Over all Prevalence of Bovine Trypanosomosis Based on Host Related Risk Factors During the Study Period in Gidami and Sayo Districts, Kellem Wollega Zone, Oromia Regional State, Ethiopia.

| Variables | No. animals examined | No. infected animals | Prevalence (%) | $X^2$ test | P- Value |
|---|---|---|---|---|---|
| **Body condition** | | | | | |
| Poor | 544 | 138 | 25.37 | | |
| Medium | 758 | 76 | 10.02 | 16.12 | 0.00 |
| Good | 488 | 11 | 2.66 | | |
| **Sex** | | | | | |
| Male | 916 | 127 | 13.86 | 2.37 | 0. 07 |
| Female | 874 | 100 | 11.44 | | |
| **Age** | | | | | |
| ≤3 years | 554 | 52 | 9.38 | 7.87 | 0.00 |
| >3 years | 1236 | 175 | 14.16 | | |

Packed cell volumes (PCV) of examined cattle were ranged between 13% and 41%, with an overall mean PCV of 25.53±4.24 (Figure 9). The anemic distribution was higher in infected cattle (81.18%) than in the non-infected (37.62%). From the total of 1790 examined animals, 770 (43.17%) were anemic having PCV < 25%. The mean PCV of parasitaemic cattle (21.53%) was significantly (P< 0.05) lower than that of aparasitaemic cattle (26.11%) irrespective of district and season of sampling (Figure 9).

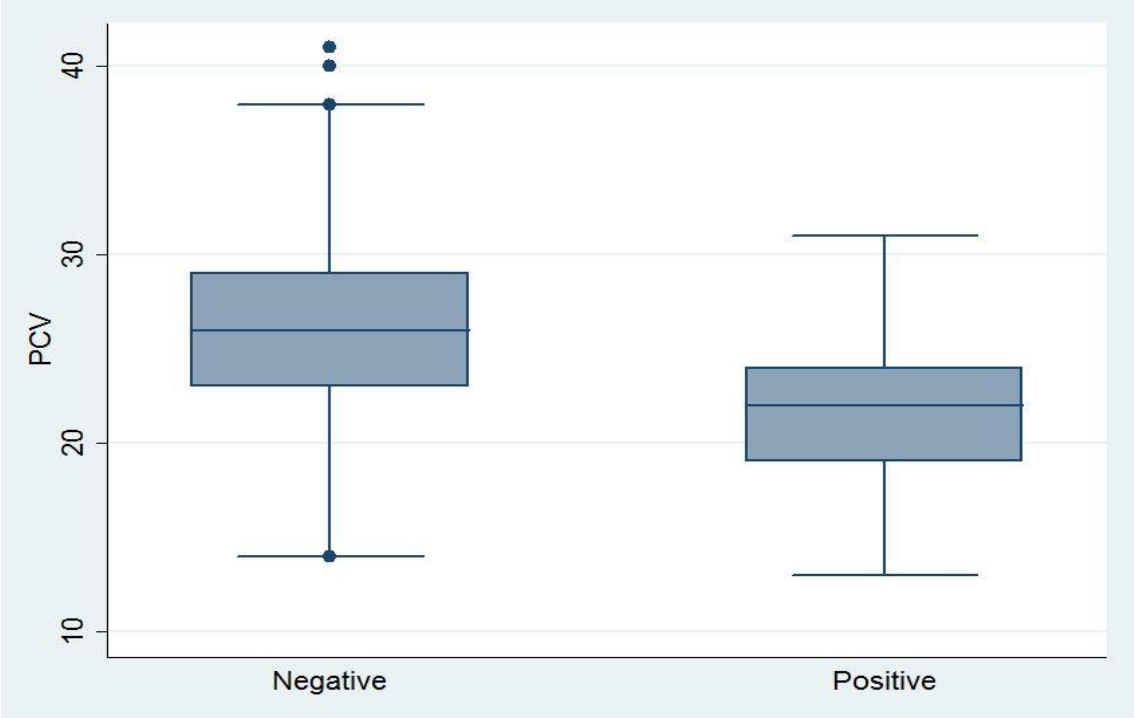



Figure 9 : Overall Mean PCV of parsitemic and aparsitemic cattle, Gidami and Sayo Districts, Kellem Wollega Zone, Oromia Regional State, Ethiopia.

### 4.4. Field Trypanocidal Drug Resistance Trial

According to the 28 days abbreviated field protocol used to assess the field trypanocidal drug resistance, resistance to the double doses of ISM and DA were observed. Out of 50 trypanosome-positive cattle treated with 1 mg/kg b.w. ISM, 42% (21/50) had persistent infections on day 14 post-treatment. Of the 29 cattle aparasitaemic at day 14 post-treatment, 44.83% (13/29) were trypanosome-positive on day 28. The cumulative ISM treatment failure rate (day 14 and 28) was 68% (34/50). Of all ISM treatment failures, *T. congolense* accounted for 70.59%, *T. vivax* for 23.53% and *T. brucei* for 5.88%.

Similarly, from 50 trypanosome positive cattle treated with 7 mg/kg b.w. DA, 36% had persistent trypanosomes on day 14 post treatment. *Trypanosoma congolense* accounted for 66.67% of the failed DA treatments, whereas *T. vivax* and *T.b. brucei* failures were

identified in 27.78% and 5.55% of the cases, respectively. All cattles that showed treatment failure against ISM were subjected to 7 mg/kg b.w. DA, whereas those that have resisted DA were again treated with 1mg/kg b. w. ISM. In addition all cattle were treated with 1% deltamethrin at the end of the study.

Treatment of the trypanosome-positive cattle with DA and ISM significantly ($P<0.05$) increased the PCV % from 20.72 ± 3.40% at day 0 to 24.74% ± 1.86 at day 14 and eventually from 20.74±2.9 to 23.48±2.41 at day 28 (Table 9).

Table 9 : Mean PCVs of Trypanosome-Positive Cattle Post Treatment with DA and ISM in Kellem and Giray Sonka villages of Gidami District, Kellem Wollega Zone, Oromia Regional State, Ethiopia

| Treatment schedule | No. of animals examined | Mean PCV | Std. dev. | 95%CI | P value |
|---|---|---|---|---|---|
| **ISM** | | | | | |
| Day 0 | 50 | 20.74 | 2.90 | 19.91-21.57 | |
| Day 14 post treatment | 50 | 23.48 | 2.31 | 22.42-23.74 | 0.00 |
| Day 28 post treatment | 50 | 23.08 | 2.41 | 22.80-24.16 | |
| **DA** | | | | | |
| Day 0 | 50 | 20.72 | 3.40 | 20.04-21.60 | 0.00 |
| Day 14 post treatment | 50 | 24.74 | 1.86 | 22.52-24.08 | |

# 5. DISCCUSSION

The current study indicated that bovine trypanosomosis to be the leading disease constraint hampering the health and productivity in Gidami and Sayo districts, Kellem Wellega Zone of Oromia Regional State, Ethiopia. This was supported by the findings of the questionnaire survey, where 100% of respondents disclosed that trypanosomosis was ranked as the first important disease of cattle in both districts. Similar observations have been reported by Zewdu *et al*. (2013) from Western Ethiopia and Siyoum *et al*. (2014) from south western Ethiopia, where trypanosomosis was perceived as the number one among other diseases affecting cattle production. Studies from Kenya (Ohaga *et al*., 2007) and Tanzania (Muangirwa *et al*., 2001) reported that farmers consider bovine trypanosomosis as the most important cattle disease. All farmers interviewed from Southern Ethiopia (Riedel *et al*., 2007) and Nigeria (Oluwafemi *et al*., 2007) replied trypanosomosis as the number one important disease in their areas.

Although trypanosomosis does not have pathognomonic signs, most farmers in the present study areas were aware of the clinical signs suggestive of bovine trypanosomosis, which are used as the basis for treatment (Radostits *et al*., 2007). Most frequently reported signs for trypanosomosis were weakness, rough hair coat, constipation and emaciation. Similarly, studies conducted in tsetse infested areas of Ethiopia (Tesfaye *et al*., 2011) and from three West African countries (Grace *et al*., 2009) also revealed that most of the interviewed farmers were able to mention the common symptoms of trypanosomosis.

Most of the interviewed farmers seemed to be well aware of the seasonality of bovine trypanosomosis, indicating the incidences of the disease reach to its peack at the beginning of rainy season (April-June) while infection is persistent throughout the year. Season of peak trypanosomosis challenge identified in the current study is inline with the findings of (Kebede *et al*., 2009; Stein *et al*., 2011) from Ethiopia and (Grace *et al*., 2009) from West Africa. However, Tewelde (2001) reported that incidence of bovine

trypanosomosis was high through out the year. Acorrding to Kebede and Animut, (2009), extreme environmental conditions in the dry season could be unfavorable for the survival and reproduction of tsetse and other biting flies.

In the present study, significant number of respondents knew the causal association between biting flies (Waranaa) and bovine trypanosomosis, indicating that forest and grazing areas as the most risky places for fly exposure. Similar findings were reported by Zewdu *et al*. (2013) in Ethiopia, Magwisha *et al*. (2013) in Tanzania and Gumel *et al*. (2012) in Nigeria, where they found that livestock keepers had a good knowledge on tsetse and bovine trypanosomosis. However, few respondents associated trypanosomosis with tick bite, wild animals and drinking stagnant water. Based on the present finding tsetse flies were known to most of the farmers (87%) and had local name for the flies "Titissa gamojjii". However, 13% of respondents did not know tsetse flies. Acorrding to the observation of Tesfaye *et al*. (2012), farmers new the local name of tsetse flies.

This study revealed that Diminazene aceturate and Isometamidium chloride were commonly used drugs for treatment and control of animal trypanosomosis in both study districts. The average number of treatments per year per cattle as indicated by the respondents was 8 times (range: 3-15). The frequency of treatment reported in the present study is higher than earlier reports from other parts of Ethiopia (Tewelde *et al*., 2004; Dagnachew *et al*., 2011; Zewdu *et al*., 2013). Above 80% of animals were treated by animal health personnels while significant number of cases were reported to be treated by animal owners, yet farmers complained on the decreasing efficacy of these trypanocidal drugs. The drug most reported showing a decreasing efficacy was Isometamidium chloride (78%) followed by Diminazene aceturate (22%). According to Maudlin *et al*. (2004), the number of treatments over a year reflects the magnitude of trypanosomosis and drug resistance presence in the area.

The entomological findings revealed four *Glossina* species (*G. m. submorsitans, G. pallidipes, G. tachinoides* and *G. fuscipes*) and three genera of biting flies (*Stomoxys,*

*Tabanus* and *Haematopota*). These species of *Glossina* have also been reported in the Western and South Western parts of the country (Dayo, *et al*., 2010; Duguma *et al*., 2015; Lelisa *et al*., 2015). The over all tsetse flies apparent density was (3.06) and (4.16) f.t.d. in Gidami and Sayo districts, respectively. Our finding was lower than that of Teka *et al*. (2012), Fentahun and Tekeba, (2013), Lelisa *et al*.(2015), who reported 14.97; 13.01 and 11.9 f.t.d, respectively from western part of Ethiopia. The relative low level of tsetse population in the present study may be due to the control intervention under taken in Sayo district by National Tsetse and Trypanosomosis Investigation and Control Center (NTTICC) and expansion of farm lands leading to the destruction of tsetse habitat and elimination of their wild hosts (Van den Bossche *et al*., 2010). According to Malele (2011), tsetse distribution can be affected by different factors such as changes in the land use and increases in human population and activities.

The seasonsl apparent density of tsetse flies was higher in early rainy season than early dry season in Gidami district. The observed difference can be explained in view of the possible development of optimum vegetation, temperature and humidity favorable for tsetse fly breeding and survival in the early rainy season (Cherenet *et al*., 2006). In contrast, the apparent tsetse fly density was lower in early rainy season than early dry season in Sayo district. During early rainy season farmers use Deltamethrin pour-on more frequently in the rainy period to combat the higher density of tseste flies and minimize the effect of bovine trypanosomosis. Use of impregnated targets and deltamethrin treated cattle has been shown to reduce tsetse populations by 90% and the incidence of bovine trypanosomiasis from over 30% to below 5% in a 6 month period (Bauer *et al*., 1999). Such disparity in tsetse seasonal dynamics between the both seasons was expected (Lehane, 2005). The socio-economic activities of the rural dwellers can also influence the distribution of tsetse flies in an area (Lukaw and Ochi, 2012).

Greater numbers of female tsetse flies 676 (58.37%) were caught than males 483(41.70. %). Similar result was reported by Fentahun and Tekeba, (2013). This could be attributed

to the longer life span of female *Glossina* (Lehane, 2005). In general females live longer than males, being more numerous in natural populations (WHO, 2013).

Results of the parasitological tests indicated that bovine trypanosomosis was widespread in the study areas. The prevalence of bovine trypanosomosis was 14.08% and11.16% in Gidami and Sayo districts, respectively. Relatively, low prevalence of bovine trypanosomosis was recorded in Sayo district owing to the ongoing vector control interventions. Similar observations were noted in the prevalence of bovine trypanosomosis in controlled (9.1%) versus non-controlled (15.1%) areas in Hawa Gelan district of Kellem Wollega zone, Western Ethiopia (Fentahun and Tekeba, 2013). This finding is generally in agreement with reports of studies carried out in western, North western and Southern parts of Ethiopia (Feyissa *et al*., 2011; Mekuria and Gadisa, 2011; Lelisa *et al*., 2015). However, different figures were reported from different parts of tsetse infested areas in Ethiopia: a prevalence of 17.2% in Metekel (Samdi *et al*., 2010), 28.1% in Asosa District (Mulaw *et al*., 2011) and 16.9% in Sayo Nole District (Kassaye, 2015). Such variations may exist because of differences in agro-ecology, sampling season, and practice of trypanocidal drug use and fly control operations (Majekodunmi *et al*., 2013).

In this study three species of bovine trypanosomes: *T. congolense, T. vivax* and *T. brucei* were identified. These species are widespread in most parts of Western and South Western Ethiopia (Mulaw, 2011; Lelisa *et al*., 2014). The dominancy of *T. congolense* (57. 30%) in the present study is in agreement with previous results (Bitew *et al*., 2011; Duguma *et al*., 2015). In several studies conducted in sub-Saharan Africa, *T. congolense* has been found to be the most prevalent trypanosome species in cattle (Ameen *et al*., 2008; Mwandiringana *et al*., 2012; Simo *et al*., 2015). The high ratio of *T. congolense* may be ascribed to the more efficient transmission of this species by tsetse flies than *T. vivax* in tsetse infested areas (McDermott *et al*., 2003). Leak (1999) indicated that *T. vivax* was highly susceptible to treatment while the problems of drug resistance were higher in *T. congolense*.

The prevalence of bovine trypanosomosis in Gidami district was significantly higher at the beginning of the rainy season. This high prevalence of trypanosome infections at the beginning of the rainy season may be explained by the increasing density of tsetse and other biting flies during this time of the year (Van den Bossche and De Deken, 2002). In contrast, the prevalence of bovine trypanosomosis in Sayo district was higher during early dry season than early rainy season ($P<0.05$). During early rainy season it is obvious that the population of flies increases. Due to this farmers inject their animals with trypanocidal drugs and also use insecticide spray in this season better than any other time to minimize the effect of the disease. On the other hand, low plane of nutrition during dry season stress the animals resulting in lowered immunity and increased vulnerability to diseases including bovine trypanosomosis (Elamin *et al*., 1998). This result is inconsistent with the report of (Ameen *et al*., 2008; Lelisa *et al*., 2015), who indicated higher prevalence infection in dry season than in wet season. According to Mahama, (2005), in the dry season, only the permanent rivers remain available for the cattle, allowing contact between vectors and hosts.

The prevalence of bovine trypanosomosis is also known to be affected by agro-ecological conditions such as altitude (Sinshaw *et al*., 2006). In this study, low land areas showed significantly ($P< 0.05$) higher prevalence compared to those in mid-land study sites during early rainy and early dry seasons irrespective of the district. Higher prevalence in lowland areas might be attributed to the fact that animals in lowland areas are more challenged by vectors than animals in mid-altitudes. It is assumed that among many factors that contribute to the distribution of tsetse flies and tsetse transmitted trypanosomosis: altitude with temperature is one of the major biotic factors. This finding is in agreement with many other studies (Mamoudou *et al*., 2006; Mekuria and Gadisa, 2011).

Body condition of the examined cattle was another factor that has showed strong association with trypanosomosis prevalence. The occurrence of infection was 25.37%, 10.02% and 2.66% in cattle with poor, medium and good body conditions, respectively.

This finding is in line with previous reports (Molalegne *et al*., 2010; Feyissa *et al*., 2011), who found the highest prevalence of trypanosomosis in poor body conditioned animals. This might be attributed to reduced resistance of those animals having poor body condition or related to the progressive weight loss arising from debilitating nature of the disease itself (Radostits *et al*., 2007). In contrast, 74.63% of poor body conditioned animals were not infected by trypanosomes. This could be due to malnourishment, internal parasites and other body loss causing diseases (Pereckiene *et al*., 2007; OIE, 2009).

The prevalence of bovine trypanosomosis was slightly higher in male than female animals. The difference was found to be statistically insignificant ($P>0.05$). This result was in agreement with previous reports (Magona *et al*., 2008; Dayo *et al*., 2010; Teka *et al*., 2012). This might be due to the fact that both sexes have virtually similar exposure to biting flies in grazing areas. The slightly higher prevalence in males as compared to females may be attributed to stress factors related to work, where male animals are used for drought purpose (Zecharias and Zerihun, 2012).

In this study, the prevalence of bovine trypanosomosis was higher in older animals (14.16%) as opposed to young groups (9.38%). The difference was found to be statistically significant ($P<0.05$). Previous reports by (Tasew and Duguma, 2012; Singla *et al*., 2013; Terefe *et al*., 2015) also revealed higher prevalence in older than young animals. It could possibly be attributed to the restriction of grazing in the young animals to a nearby homestead (Alemayehu *et al*., 2012). In addition, young animals are protected to some extent by maternal antibodies (Fimmen *et al*., 1999). According to the (Torr *et al*., 2006) tsetse flies are attracted significantly more by odor of older animals. Other reports showed that *T. congolense* is a chronic disease increasing with age of animals and its infection is usually higher in adult animals than in young animals (McDermott *et al*., 2003).

One of the clinical evidences of trypanosomosis in cattle is low mean PCV (Marcotty *et al*., 2008; Mbewe *et al*., 2015). In the present study, trypanosome positive animals had significantly lower PCV ($P<0.05$) than trypanosome negative animals. These lowered PCV of parasitemic animals was previously reported in similar studies from Ethiopia (Bitew *et al*., 2011; Tasew and Duguma, 2012) and in Zambia (Marcotty *et al*., 2008). Furthermore, (81.06%) of the parasitemic cattle were anaemic. Similar results were reported by Afework *et al*. (2006) at Pawe, North-West Ethiopia (90%) and Abraham and Tesfaheywet (2012) at Arba Minch, Southern Ethiopia (85%). On the other hand, the (18.94%) cattle that were trypanosome positive but not anaemic can be explained as new infections that had not progressed to chronicity. The (37.62%) aparasitaemic cattle with PCV ≤25% in this study could be due to the low sensitivity of buffy coat method used (Marcotty *et al*., 2008) or due to other anemia causing diseases or delayed recovery of anaemic situation after recent treatment with trypannocidal drugs (Simukoko *et al*., 2011). Trypanosomes become very difficult to detect when the parasitemia is lower than 60 trypanosomes/ ml blood (Luckins, 2010).

Drug resistance to trypanocides is a dynamic process, and it is spreading and increasing in Africa. In the present study, an abbreviated 28-day field protocol based on treatment of naturally infected cattle was used (Diall *et al*., 2005) over the longer follow-up protocol (Eisler *et al*., 2001). Although this method has the risk of under estimating the resistance situation, it has the advantage that, resistance to ISM and DA could be simultaneously assessed (Knoppe *et al*., 2006). This protocol also uses cattle of known trypanosome infection status and takes only one month to generate results at a reasonably low cost. Additionaly, the drop-out rate of participating herd owners/study animals where the abbreviated protocol is applied is lower than for the long follow-up protocol. This method also does not require sophisticated laboratories like genetic markers (Afework *et al*., 2006). Furthermore, they do not require the isolation of trypanosomes (McDermott *et al*., 2003).

The Field trypanocidal drug treatment trials had revealed that there were wide spread resistance to 1 mg/kg b.w ISM and 7.0 mg/kg b.w DA in Giray Sonka and Kellem villages of Gidami district based on the cut-off point suggested by Eisler *et al*. (2001). In the present study, from 50 trypanosome positive cattle treated with 1 mg/kg b.w ISM, 68% were found to be resistant on day 28 post treatment. This finding is in agreement with the reports of (Chaka and Abebe 2003; Sow *et al*., 2012) who indicated that *T. congolense* and *T. vivax* isolates treated with ISM at a dose of 1mg/kg b.w were found to be resistant. The result concords also with the work of (Qadeer *et al*., 2013); that, resistant occurred even with higher doses of 1.5 mg/kg b.w ISM. The current study revealed that *T. congolense* accounted for 70.59%, *T. vivax* 23.53% and *T. brucei* 5.88% of all ISM treatment failures. Resistance of *T. congolense* to ISM has been reported in Ethiopia (Tewelde *et al*., 2004) and in Burkina Faso (McDermott *et al*., 2003; Knoppe *et al*., 2006). Similarly, Dagnachew *et al*. (2015b) has experimentally demonstrated resistance of *T. vivax* isolates to ISM in Ethiopia.

As was the case with ISM, DA failures also occurred in the treated trypanosome positive cattle in Kellem village. Of the 50 trypanosome positive cattle treated with 7.0 mg/kg b.w DA, 36% had persistent trypanosomes on day 14 post treatment. Similarly Dagnachew *et al*. (2015a) reported diminazene resistant of *T. vivax* isolates from tsetse free areas of the northwest Ethiopia to 7.0 mg/kg b.w DA. The present study demonstrated that *T. congolense* accounted for 66.67% of failed DA treatments, whereas *T. vivax* and *T. brucei* failers were identified in 27.78% and 5.55% of cases, respectively. Treatment failure against *T. congolense* and *T. vivax* infections with either of these drugs have been observed in both East Africa (Dagnachew *et al*., 2015a; Moti *et al*., 2015) and West Africa (Mungube *et al*., 2012).

According to Mungube *et al*. (2012) re-treatment of the cattle positive for persistent *T. congolense* with a double dose (7.0 mg/kg) of DA did not completely clear trypanosomes in re-treated cattle. Codjia *et al*. (1993) reported resistance to the maximum recommended dose of DA (7 mg/kg) for *T. congolense* isolates from cattle bred in the

Ghibe valley western Ethiopia. Higher frequency of treatment, under dosage and mishandling, especially when the drugs were administered by farmers and possible occurrence of poor quality drugs on the market could be the reasons for the development of trypanocidal drug resistance (Vitouley *et al*., 2012; Tchamdja *et al*., 2016; Tekle *et al*., 2018). However, the isolation of drug resistant trypanosomes from wildlife that were never in contact with the drug (Chitanga *et al*., 2011) suggests that trypanocidal drug resistance is existing without any drug pressure and was existing before the discovery of the drugs. This information is fundamental when considering the control of trypanosomosis in the area. A better understanding of the drug resistance in the area will allow defining evidence-based, adapted measures for the progressive control of bovine trypanosomosis (Diall *et al*., 2017).

In the present study, treatment of the trypanosome-positive cattle with ISM and DA significantly ($P<0.05$) increased the PCV of animals from 20.74-23.08 and 20.72-24.74 respectively.This finding is in agreement with previous reports in the country (Tewelde *et al*., 2004; Moti *et al*., 2012) and elsewhere in Africa (Grace, 2005; Knoppe *et al*., 2006). Several studies have established that trypanosome-infected cattle show decreased PCVs (Cherenet *et al*., 2006; Dayo *et al*., 2010), although depending on the trypanosome species.

# 6. CONCLUSIONS AND RECOMMENDATIONS

In Kellem Wollega, trypanosomosis is one of the most important livestock diseases, which poses a serious threat to the lives and livelihood of entire communities and constitutes the greatest single disease constraint to livestock production. Trypanocidal drugs are the main method used in the control of bovine trypanosomosis. However, treatment of trypanosomosis currently facing a number of problems, including poor quality generic formes and development of trypanocidal drug resistance by the parasites. Several brands and diverse sources of trypanocidal drugs are routinely used by veterinary professionals as well as by farmers to control trypanosomosis in Kellem Wollega Zone of Oromia Regional State, Ethiopia. These preventive measures and treatment are used inappropriately which leads to trypanocidal drug resistance. In Kellem Wollega, there are evidences of ISM and DA resistance and treatment failures reported to the recommended doses of these drugs.

The present study evaluated the epidemiology of bovine trypanosomosis and its vectors in Gidami and Sayo districts and assessed trypanocidal drug resistance to the double doses of ISM and DA on naturaly infected cattle in Giray Sonka and Kellem villages of Gidami district, Oromia Regional State, Ethiopia. Based on the questionnaire survey and cross-sectional study, bovine trypanosomosis was a major disease constraint of livestock production and agricultural development affecting animal health and productivity in both study districts. In addition, the study further revealed high trypanocidal drug treatment frequencies and the practice of trypanocidal drugs treatment by farmers themselves, which signals rampant misuse of the medications and possible presence of drug failures due to trypanocidal drug resistance in both districts.

The occurrence of bovine trypanosomosis was associated with high challenges of tsetse flies (*G. m. submorsitans, G. pallidipes*, *G. tachinoides* and *G. fuscipes*) and three genera of biting flies (*Stomoxys, Tabanus* and *Haematopota*), that serve as potential vectors for

trypanosomes in the study areas.The cross-sectional study demonistrated that bovine trypanosomosis was prevalent with different magnitude within the study districts and three trypanosome species, *T. congolense*, *T. vivax* and *T.brucei* were identified in the study animals. It was confirmed that, season, altitude, body condition scores and age of animals are significant risk factors for bovine trypanosomosis distribution in the areas. This study also revealed the negative impact of bovine trypanosomosis on the PCV values of infected animals.

The field trypancidal drug resistance studies undertaken provided valuable information on the widespread presence of trypanocidal drug resistance to high doses of Isomethamidium chloride and Diminazine aceturate in Giray Sonka and Kellem villages of Gidami district. The research work provided vital information on the epidemiological picture of bovine trypanosomosis and its vectors in Gidami and Sayo districts and the occurrence of trypanocidal drug resistance to high dose of Isomethamidium chloride and Diminazine aceturate. The findings of the present study can be used in planning and implementation of bovine trypanosomosis control programme in these areas.

Therefore, to alleviate the problem of bovine trypanosomosis the following recommendations are forwarded:

1. Prompt integrated tsetse control strategy, which combines insecticide treated targets and cattle pour-on has to be implemented in Gidami district to minimize the distribution of tsetse flies.
2. In Sayo district the ongoing tsetse and trypanosomosis control intervention decreased the prevalence of bovine trypanosomosis. In order to sustain the control program, interventions are required from local government bodies in mobilizing and increasing the active participation of the farmers.
3. In addition to tsetse and trypanosomosis control, the use of molecular techniques should be encouraged in view of the poor sensitivity and specifiticity of the

available parasitological tests as well as widespread occurrence of trypanocidal drug resistance.

4. Rational uses of trypanocidal drugs and control of co-infections to exploit self-cure against resistant trypanosome populations are recommended.
5. Policies on the importation of quality trypanocidal drugs, distribution and treatment are essential to avoid misuse.
6. Awareness creation to the farmers about tsetse and trypanosomosis coupled with exisiting control methods is crucial and timely.
7. Efforts should be intensified for the discovery of new trypanocidal drug compounds and or effective vaccines.

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## 8. APPENDICIES

Appendix i : Questionnaire survey on the disease trypanosomosis and trypanocidal drug utilization in Gidami and Sayo district, Kellem Wollega Zone, Oromia Regional State, Ethiopia.

Respondents name_____

District_____Kebele_____Village_____Date_____

1. General Animal Health

1.1. List of animal diseases in your locality in their order of importance:

_____

_____

_____

2. Perception and incidence of trypanosomosis

2.1. Is there trypanosomosis in your locality?

1. Yes ☐ 2. No ☐

2.2. What are the clinical signs to suspect trypanosomosis?

_____

2.3. How is the prevalence of trypanosomosis hitherto?

1. Increased ☐ 2.Decre☐ why?_____

2.4. How is the mechanism of transmission among bovine?

_____

2.5. Is there seasonal variation in prevalence of trypanosomosis?

1. Yes ☐ No ☐

2. If yes, when? _____

3. Therapeutic Practices

3.1. How do you combat trypanosomosis when it exists in your herd?

1. Traditional medicine locally available ☐

2. Buying and adminstration of veterinary drugs by your own ☐

3. Travelling to nearby veterinary clinic ☐

4. All alternatives are employed ☐

3.2. Do you use traditional medicine to treat your animals?

Yes ☐ No ☐

3.2.1. What type of traditional medicine you use for your bovine when affected by trypanosomosis?

_____

3.2.2. How do you prepare and adminster to your animals?_____

3.2.3. Does it cure the disease or give sort of relief? _____

3.3. Do you use veterinary drugs to treat trypanosomosis? _____

3.3.1. Which type of veterinary drugs you know and experienced as treatment for your animals?

1. Diminazene/yellow powder/Bicha ☐

2. Isometamidium/coffee/Buna ☐

3. Ethidium (red tablet) ☐

4. Others (specify)_____

3.3.2. Who adminster drugs to your animals?

1. Yourself ☐ 2. Experienced villager ☐

3.3.3..How do you apply /prepare injectable solutions?

1. Measurement

A. Standard measurement /Gramm Other local measurement ☐

B. Locally adopted measurement/metallic probe ☐

2. Solvent used (water, oil, etc) ☐

3.3.4. How do you administer trypanocidal drugs to your animals?

1. Route of administration ☐

2. Site of adminstraion ☐

3.3.5. What amount of injectable solution do you give for each of your animals with trypanosomosis?

1. For calves ☐ 2. For heifers ☐

3. For adults ☐

3.3.6. How many of your animals get cured after your treatement?

¼ ☐ ½ ☐ ¾ ☐

3.3.7. How many times you treat your animals per year?

☐ ☐ ☐ ☐

3.4. Where do you get trypanocidal drugs?

1. Pharmacy/Vet. Or human ☐

2./Shop/Others ☐

3.5. Can you show one sample drug or its container left?

3.6. What is common price for each drug sample? _____

Appendix ii : Format for cross sectional survey

Region……Zone……District……Kebele……Date of collection……

| S/N | Owner's name | Animal ID | Sex | | Age | | | Body condition | | | Infection type | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | M | F | Calf | young | Adult | Lean | Good | Cachectic | *T.c* | *T.b* | *T.v* | |
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Appendix iii : Format for block treatment study

Region _____Zone _____

District _____Villag _____

Date of collection _____

| No | Owner's name | Animal ID | Sex | | Age | | | Body condition | | | Infection type | | | Treatment group | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | M | F | Calf | young | Adult | Lean | Good | Cachectic | *T.c* | *T.b* | *T.v* | Day 0 | Day 14 | Day 28 | |
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Appendix iv : DA field treatment study, Kellem village, Gidami district, Kellem Wollega Zone, Oromia Regional State, Ethiopia..

Appendix v : ISM field treatment study, Gry Sonka village, Gidami district, Kellem Wollega Zone, Oromia Regional State, Ethiopia.

Appendix vi : Field drug resistance study, Gidami district, Kellem Wollega Zone, Oromia Regional State, Ethiopia.

Appendix vii : An ox after 14th day of ISM treatment, Giray Sonka Village, Gidami District , Kellem Wollega Zone, Oromia Regional State, Ethiopia

## 9. PUBLICATIONS


1. **Efrem**, **D**., H.T. Yacob, A.T. Hagos and A.K. Basu, (2010): Bovine trypanosomosis in Gimbi district of Western Oromia, Ethiopia. *Animal Biol*., **60**: 123-131.
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3. **Efrem**, **D**., Workineh S., Getachew, T.,Kaleab, A. and Hagos, A. (2017): Bovine trypanosomosis:changes in parasitemia and packed cell volume in dry and wet seasons at Gidami District, Oromia Regional State, western Ethiopia. *Acta*. *Vet*. *Scand*. **59***:* 59.
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5. Mezene, W., Ahimedine B., Moti Y., **Efrem D**. and Kumela L. (2014): Bovine Trypanosomosis and Tsetse Fly Survey in Bure District, Western Ethiopia. *Acta*. *Parasitol. Glob.* **5** (2), 91-97.
6. **Efrem D**.,Hagos A., Getachew T., Tesfu K., Nigatu K., Workineh S.and Kaleab A.,(2018): Knowledge, Attitude and Practices of Smallholder Farmers Towards the Control of Bovine Trypanosomosis in Gidami and Sayo Districts, Kellem Wollega Zone, Westen Oromia. *Res. Vet. Sci.* doi.org/10.1016/j.prevetmed.2019.03.017.
7. **Efrem D**.,Hagos A., Getachew T., Tesfu K., Nigatu K., Workineh S.and Kaleab A. (2018): Trypanocidal Drug Resistance: A Threat to Animal Health and Production in Gidami District of Kellem Wollega Zone, Oromia Regional State, Western Ethiopia. *Prev. Vet. Med.* (Submitted for publication).