TRYPANOSOMOSIS AND TRYPANOCIDAL DRUGS: DISEASE PREVALENCE, DRUG QUALITY, EFFICACY AND UTILIZATION PRACTICES, IN CATTLE IN SELECTED HOT SPOTS OF SOUTH-WESTERN ETHIOPA

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

BY

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DEPARTMENT OF VETERINARY PATHOLOGY AND PARASITOLOGY

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TRYPANOSOMOSIS AND TRYPANOCIDAL DRUGS: DISEASE PREVALENCE, DRUG QUALITY, EFFICACY AND UTILIZATION PRACTICES IN CATTLE IN SELECTED HOT SPOTS OF SOUTH-WESTERN ETHIOPA

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PhD Dissertation

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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 thesis have been properly acknowledged. This thesis 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 thesis is not submitted to any other Institution any where for the award of any academic degree, Diploma or certificate. and allow access to users. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgement of source is made. In all other instances, however, permission must be obtained from the author and/or the University.

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DEDICATION

First to GOD ALMIGHT and My family

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LIST OF ABBREVIATITIONS

AAT	African Animal Trypanosomosis
Ab	Antibody
BCE	Buffy coat examination
BCT	Buffy coat technique
bp	Base pair
CD	Curative dose
CI	Confidence Interval
CSA	Central statistics authority
CVMA	College of Veterinary Medicine and Agriculture
DA	Diminazene aceturate
EC	European Commission
ED	Effective dose
EDTA	Ethylene diamine tetraacetatic acid
ELISA	Enzyme Linked Immuno Sorbent Assay
FAO	Food and Agriculture Organization
GALVmed	Global Alliance for Livestock Veterinary Medicines
GDP	Gross Domestic production
GEB	Guanidine ethylene buffer
GPS	Global positioning system
HCT	Haematocrite centrifuge technique
HOM	Homidium
HPLC	High Performance Liquid Chromatography
IAEA	International Atomic Energy Agency
IFAH	The International Federation of Animal health
IFAT	Indirect fluorescent antibody test
ILRAD	International Laboratory for Research on Animal Disease
IM	Intramususcular
ISM	Isometamedium chloride
Mabs	Monoclonal antibodies

MoA	Ministry of Agriculture
NAHDIC	National Animal Health Diagnostic and Investigation Center
OIE	Office International des Epizooties
PCR	Polymerase chain reaction
PCV	Packed cell volume
RFLP	Restricted Fragment Length Polymorphism
rpm	Revolution per minute
SADs	Stationary attractive devices
SAT	Sequential aerosol technique
SIT	Sterile Insect Technique
SNNPRS	Southern Nations and Nationalities Peoples Regional State
SSA	Sub Saharan Africa
TAE	Tris/acetic acid
TBE	Tris boric acid
TRYRAC	Trypanosomosis Rational Chemotherapy programme
UV	Ultraviolet
Vmax	Maximal uptake rates

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SUMMARY

Ethiopia has the largest livestock population in Africa and livestock accounts 15-17% of total GDP and 35-49% of agricultural GDP. Unfortunately, the development and intensification of livestock production is hampered by diseases such as African animal trypanosomosis (AAT). Drug treatments often complemented with vector control will continue to be the main tool available to smallholder livestock producers to control the disease. However, due to various reasons, the increasing development of trypanocidal resistance is posing a major threat that is seriously undermining control efforts. A number of brands of trypanocidal drugs are on the local market and control over their circulation and use appear to be very loose in rural communities such as the west and south-western part of the country.

Therefore, this PhD thesis research work hypothesized that owing to their persistent use and the continued threat posed by the disease; the quality and efficacy of such drugs circulating in the local market is low and hence development of drug resistance is inevitable in the study area. The objective of the study was therefore to assess the prevalence of bovine trypanosomosis, determine extent of the occurrence of trypanocide drug resistance in identified hot spots and assess the utilization practice and quality of trypanocides currently in use in south western Ethiopia. Although extensive studies have been reported on the distribution of the disease in many places of the country, only few indicators are available on prevalence of drug resistant to trypanosomes and attention has not given to assess the quality of trypanocidal brands currently in use and the practice of their utilization.

The study has attempted to demonstrate the current situation of bovine trypanosomosis in the study area by using parasitological (woo) and molecular (PCR-RFLP) techniques, assess trypanocidal drug utilization practices of farmers through a structured questionnaire survey, determine the quality of trypanocidal drugs circulating in the local markets and show the prevalence of drug resistant trypanosomes by using a field trial method. For the drug quality testing, a total of 50 samples were collected from different sources (legal and illegal) and the drugs were subjected to high performance liquid chromatography (HPLC) test. For drug susceptibility/resistance trial, 80 parasitaemic animals were selected and studied for a period of

28 days by alternate treatment with Diminazene aceturate and isometamidium chloride. Post-treatment parasitaemia was detected on days 14 and 28. Cross-sectional study to estimate trypanosomosis prevalence was done (December/2013-February2014) on systematically selected 2000 cattle whereas, 100 farmers were randomly selected for the interview on trypanocidal drug utilization practices.

The study indicated that the overall prevalence of trypanosome infection by buffy coat was 4.5% (CI = 3.4% to 5.4%) with significant variation between the study districts; being higher in Abeshege and Cheha (p=0.000). *T. congolense* (61.1%) followed by *T. vivax* (17.8%) mixed infection of the two (10%) and *T. theileri* (11.1%) were the species demonstrated by parasitological technique. One hundred fifty one samples that were considered negative by Woo technique were further subjected to PCR-RFLP. The PCR detected 19.2% additional positive cases (76% of them *T. congolense*) suggesting that parasitologcal techniques are seriously underestimating the magnitude of the problem. The PCR further ascertained that by Woo technique, it was easier to identify *T. congolense* much better than *T. vivax* or mixed infection of the two species. *T. theileri* was often confused with *T. vivax* under Woo technique. The overall mean PCV was significantly reduced in parasitaemic animals (22.6%= CI: 21.46-23.73) compared to aparasitaemic (25.6%= CI: 25.41-25.78) animals (P=0.000).

The questionnaire survey result revealed that trypanosomosis was a significant animal health constraint for all farmers interviewed in the hot spot villages. The majority of respondents of all villages (56%; 95% CI= 46.3-65.7%) get trypanocidal drugs from unauthorized/illegal markets. Significant numbers of farmers in the study villages (59%) administer trypanocidal drugs by themselves or through family members and 85% of the respondents treat their animals with more than six times injections/animal per year. Altogether, this suggests that risk factors for the development of trypanocidal drug resistance to trypanosomes are there.

In addition, trypanocidal drug quality assessment by using HPLC has unequivocally demonstrated that 28% of the drugs tested had substandard quality and hence did not comply with the active ingredient content described by the manufacturers. In this regards, clients of authorized and non-authorized markets are at similar risk of purchasing non-compliant trypanocides. This study has also shown that although some European companies are not

immune from the problem, most non-compliant drugs were produced in Asian pharmaceutical companies ($P< 0.05$) suggesting that some of the drugs were defective right from their origin.

The observation that trypnocidal drugs are inappropriately utilized and some of the drug types circulating in the local market are reported to have poor quality are the basis to undertake field trial to assess the efficacy of known brands of diminazene aceturate and isometamidium chloride on trypanosome positive cattle selected from the study areas using an abbreviated 28 days field protocol. The overall findings revealed multiple drug resistance to recommended doses of Diminazine aceturate (7.5%) and Isometamidium chloride (6.25%) whereas relapse to single treatment with recommended doses (3.5 mg/kg body weight for DA or 0.5 mg/kg body weight for ISM) was 47.5% in case of DA and 27.5% for ISM.

In conclusion, *T.congolense* and *T. vivax* are the dominant trypanosome species posing significant losses in the study areas as demonstrated by the drop in PCV. Parasitological techniques seem to undermine the magnitude of the disease problem leading to wrong diagnosis and failure to treat sick animals on time. This is being exacerbated by the inappropriate use of available trypanocidal drugs by untrained farmers and the widespread circulation of fake drugs in the local market altogether facilitating the development of drug resistance. This assumption was validated by the demonstration of single and multiple resistances to recommended doses of both diminazene aceturate and isometamidium chloride. Existence of multiple drug resistance has seriously threatened the use of sanative pair. This necessitates intensification of vector control strategies, rational utilization of the few available trypanocidal drugs, effective implementation of regulatory and legislative frameworks for Veterinary drug supply, distribution and utilization, improving veterinary extension programs and veterinary services to educate livestock owners on development of drug resistance and its economic impacts.

1. INTRODUCTION

As in many countries in developing world, agriculture is the backbone of the economies of several countries in East Africa. However, hunger, the most extreme manifestation of poverty, remains acute in rural Sub-Saharan Africa (SSA), with 34% of the population being undernourished (Reichard, 2002). Alleviation of poverty can only start with reduction of hunger and this will be achieved through the development of sustainable agricultural systems, in which livestock plays a key role (Vreysen, 2006). In Ethiopia approximately 85% of the population is engaged in the agricultural sector. Agricultural production contributes 35-49% to the Gross Domestic Products (GDPs), of which livestock production contributes 15-17% (CSA, 2013).

Ruminant livestock products mainly meat and milk, are important components in the diet of most people. Due to an increase in human population and projected urbanization, growth in demand for livestock products will outstrip that for crop-based food (FAO, 2005). Most of these increases in demand will have to be met from indigenous production using local animals. Despite the central role which livestock agriculture plays as an engine for rural development and sustainable food and for nutritional security for the rural and peri-urban households, animal disease stresses, feed inadequacy and low genetic potential still prevail to be factors that constrain the productivity of this sub-sector in SSA. Of these, infectious diseases and in particular tsetse transmitted animal trypanosomosis is arguably the single most important constraint to animal agriculture in the semi-arid and sub-humid parts of SSA (FAO, 2005).

It was estimated that 48 million cattle are continuously at risk of infection in Africa with trypanosomes (Ilemobade, 2009). In cattle, *Trypanosoma congolense*, *T. vivax* and *T. brucei* are the main species responsible for the disease. Most African trypanosomes are transmitted to their mammalian host by tsetse flies which inhabit many parts of the African continent extending 15^0N and 20^0 south of the equator (Abebe, 2005). In sub-Saharan Africa, tsetse fly-transmitted African animal trypanosomosis (AAT) is estimated to cause annual losses that run into billions of dollars in attempts to protect and treat animals against the disease (Swallow, 2000). Drug treatments are and, for the foreseeable future, will continue to be the main tool available to smallholder livestock producers to control the trypanosomosis. Vector control requires donor

support for sustainability while trypanotolerant cattle are a minority limited to the cotton zone of West Africa (Delespaux *et al*., 2008). Therefore, throughout tsetse-infested sub-Saharan Africa, the control of trypanosomosis depends largely on the effectiveness of the currently available trypanocidal drugs. However, only a small group of chemo-prophylactic and chemo-therapeutic compounds are currently in use and no new tryanocidal compounds or anti-trypanosome vaccines is on the horizon. This has led to the rapid development of resistance to the available drugs and consequently become an obstacle to undertake trypanosomosis control and poses a serious threat to cattle productivity in affected countries (Geerts and Holmes, 1998).

According to Delespaux *et al*. (2008) and Chitanga *et al*. (2011), trypanocidal drug resistance has been reported from 21 African countries. In 10 of the 21 African countries, the occurrence of multiple drug resistance to Diminazine aceturate (DA), Isometamidium chloride (ISM) and Homodium bromide (HOM) has been established (Delespaux *et al*., 2008). In Ethiopia, ISM and DA have been in use persistently for a number of years. Trypanocidal resistance particularly against *T. congolense* infection was reported from Ghibe valley as early as in 1989 (Codija *et al*., 1993; Mulgeta *et al*., 1997). It was found that 11 of the 12 isolates tested were resistant in cattle to recommended doses of isometamidium (Samorin®) and homidium (Novidium®). Since then, several other reports have emerged substantiating the widespread occurrence of trypanocidal drug resistance in many parts of the country (Afework *et al*., 2000; Tewelde *et al*., 2004; Shimelis *et al*., 2008; Moti *et al*., 2012, Shimelis *et al*., 2015).

Development of resistnace to trypanocidal drugs could be due to inappropriate handling and frequent use of the compounds and /or circulation of substandard products (Geerts and Holmes., 1998). Therefore, the effectiveness of trypanocidal drugs and the speed with which trypanocide resistance develops and the type of resistance (single or multiple) depend on a multi-factorial process driven by (i) the trypanocidal drug use practices, (ii) the quality of trypanocidal drugs on the local market, (iii) the ability to detect resistance and (iv) the availability of strategies to minimize and control resistance at the smallholder level (Afework *et al*., 2000; Moti *et al*., 2012). Detection of drug resistance could be maximized by the use of a more accurate diagnostic technique such as the polymerase chain reaction (PCR). The PCR allows the identification of parasites at levels far below the detection limit of the commonly used parasitological techniques

and greatly helped a more accurate species identification such as by ITS1 PCR (Desquesnes *et al*. 2002).

In this regard while prevalence of resistance has been declared in many places, none of the previous studies have attempted to assess the quality of trypanocidal drugs circulating in Ethiopia and the factors associated with drug resistance. A number of brands of both DA and ISM are on the local market and control over their circulation and use appear to be very loose in rural communities such as the west and south-western part of the country.

Therefore, this PhD thesis research work hypothesizes that owing to the persistent use of trypanocidal drugs and the continued threat posed by the disease; the quality and efficacy of such drugs available in the local market are doubtful and may contribute and lead to the development of resistance.

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

- What is the prevalence of bovine trypanosomosis problem in the study areas?
- How is the practice of trypanocidal drug utilization in the study area?
- How is the quality of the different brands of trypanocidal drugs circulating in the study area?
- What is the level of efficacy of the common trypanocidal drugs circulating in the area? Is there trypanocidal drug resistance?

To respond to the above key questions, the following objectives were set:

General objective

The general objective of this study was to assess the prevalence of bovine trypanosomosis, determine extent of trypanocide drug resistance in identified hot spots and assess the utilization practice and quality of trypanocides (DA and ISM) currently in use in South western Ethiopia.

The specific objectives of the study were to:

I. Estimate the prevalence of bovine trypanosomosis using parasitological (buffy coat) and molecular (PCR-RFLP) detection methods in four districts (Abeshege, Cheha, Enemor and Ener and Ameya) of southwestern Ethiopia
II. Describe the trypanocidal drug utilization practices by using a structured questionnaire in the selected five hot spot areas (Borer-4, Borer-5, Wolaita, Wuhalimat and Misreta) in Abeshege district of the Gurage zone
III. Evaluate the quality of diminazene aceturate and isometamidium chloride-based brands collected from in and around the five hot spot areas
IV. Assess the presence and extent of trypanocidal drug resistance in cattle in the selected hot spot villages of Abeshege district

2. LITERATURE REVIEW

2.1.Epidemiology of bovine trypanosomosis

2.1.1. Trypansomes

Trypanosomosis is a disease complex caused by several species of protozoan parasites of the genus Trypanosoma. Trypanosomes are unicellular protozoan parasites of the phylum Sarcomastigophora, order Kinetoplastida, family Trypanosomatidae, and genus Trypanosoma (Levine *et al*., 1980). Three principal parasites namely, *T. congolense, T. vivax* and *T. brucei brucei* are known to cause trypanosomosis in bovines normally via the bite of an infected tsetse. *Trypanosoma vivax* and *T.evansi* are also transmitted mechanically by biting insects, such as tabanids and stomoxys, in areas outside the tsetse belt as well as in South and Central America and Asia (Nantulia, 1990; Osorio *et al*., 2008), while *T. equiperdum* is transmitted sexually and has a wider geographic distribution (Brun *et al*., 1998; Hagos 2010).

The species of trypanosomes causing Nagana were discovered by different scientists in the beginning of the 20th century; Bruce (1895) discovered *T.brucei* as the cause of cattle trypanosomosis and in the mean time, the two other animal pathogenic trypanosome species *T. congolense* and *T. vivax* were discovered in 1904 and 1905, respectively by Broden and Ziemann (Steverding, 2008). *T. congolense, T.vivax and T. brucei* are the major causative agents of bovine trypanosomosis in sub-Saharan Africa (Stephen, 1986; Duggan, 1977). The different trypanosome species differ in morphological characteristics as described by Maudlin *et al*., (2004).

Trypanosoma congolense (Figure 1) is smaller in size, usually without free-flagellum, but has marginally located medium sized kinetoplast (Seifert, 1996). It is divided into four subtypes, with different distributions and pathogenicity: savannah type, forest type, Tsavo type, and Kilifi type (Majiwa *et al*., 1993). *Trypanosoma congolense* savannah type is the most pathogenic of the four and is capable of causing severe anaemia and even death of infected cattle (Bengaly *et al*., 2002). Other *T. congolense* types cause mild disease that in certain instances does self-cure.

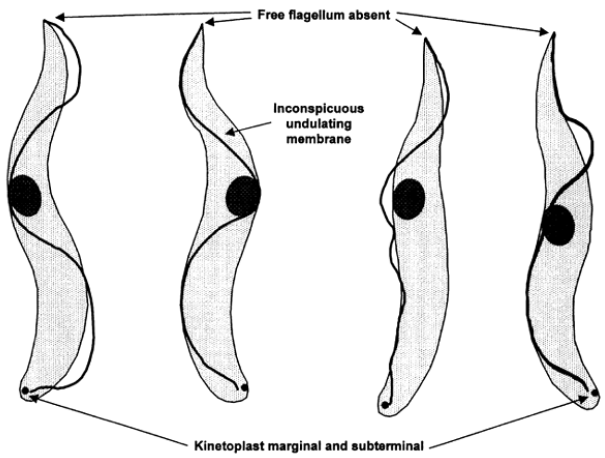

Figure 1. *Trypanosoma congolense* blood stream forms (Source: Uilenberg, 1998).

Trypanosoma vivax is a monomorphic parasite with distinct free flagellum and larger and terminal kinetoplast (Figure 2). It shows variable levels of virulence and distinct pathogenicity in West African isolates, causing an acute disease in cattle often accompanied by weight loss, reduced milk yield, abortions and mortality, whereas the East African isolates largely cause chronic infection (Gardiner and Mahmoud, 1992). 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 Bett *et al*., 2004; 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).

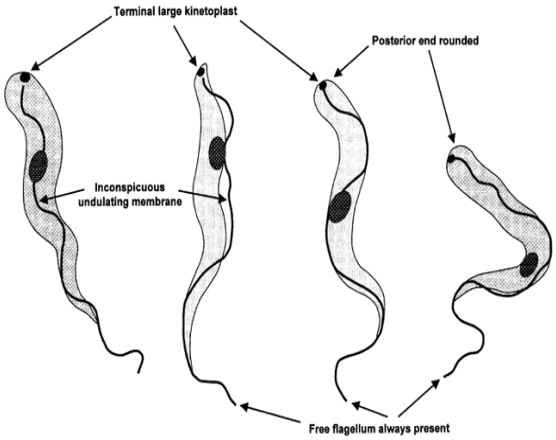

Figure 2. *Trypanosoma vivax* blood stream forms (Source: Uilenberg, 1998).

Parasites in Trypanosoma brucei group show pleomorphism (Figure 3) with slender, intermediate or stumpy forms. They have small sub-terminal kinetoplast, undulating membrane with conspicuous posterior end taper to a point except in stumpy forms. During the course of the infection, there is a change in the trypanosome population from the long thin forms, through the intermediate, to the short stumpy, and this altered appearance is accompanied by a change in the type of respiration, as the trypanosome prepares for its period within the tsetse fly. The short stumpy forms are adapted to living and developing in the tsetse, while long thin forms are the true mature blood forms which die in the gut of the insect. (Similar metabolic changes also occur in other trypanosome species, but there are no such obvious morphological changes associated with them as in *T. brucei*.) Maudlin *et al*. (2004)

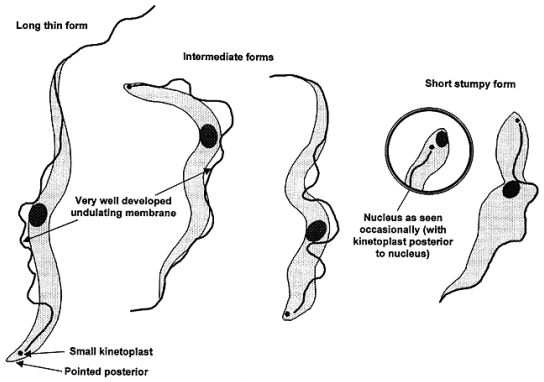

Figure 3. *Trypanosoma brucei* blood stream forms (Source: Uilenberg, 1998).

2.1.2. Vector of African animal trypanosomosis

Tsetse flies, the vectors for AAT, belong to the family *Glossinidae*, order Diptera – the two winged flies. There are 31 recognized Glossina species and sub-species, divided into three groups (morsitans, palpalis and fusca) which have been given sub-generic status (Solano *et al*., 2010). Recently, comparative gene sequence analysis and geometric wing morphometry have been proposed to help in the Glossina group identification (Patterson and Schofield, 2005). The morsitans group that includes *G. morsitans morsitans, G. m. submorsitans, G. pallidipes, G. longipalis* and *G. austeni* is found mainly in the savannah ecosystems. They are the most important vectors of bovine trypanosomosis (Leak *et al*., 1999). The *palpalis* group is found mainly in the riverine galleries of West and Central Africa but sometimes extends into 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 (Leak, 1999). In West Africa, important bovine trypanosomosis vectors among the *palpalis* group include *G. palpalis palpalis, G. p. gambiensis and G. tachinoides* (Hendrickx *et al*., 2004; Raiyaisse *et al*., 2010; Solano *et al*., 2010). The fusca group flies settle mainly in forests and are therefore less important vectors of bovine trypanosomosis. *G. longipennis* and *G. brevipalpis* found in the drier areas of Kenya are exceptions among the fusca group, as they have been demonstrated to effectively transmit trypanosomes (Makumi *et al*., 2000). Other than tsetse flies, other haematophagous insects like tananids and Stomoxys species also transmit trypanosomosis mechanically as has been demonstrated by Desquesnes and Dia (2003).

2.1.3. Hosts

Trypanosomosis is known to affect a number of mammalian vertebrates, either as African animal trypanosomosis (AAT) and or as human African trypanosomosis (Leak, 1999). *Trypanosoma vivax, T. congolense* and *T. brucei,* for example, affect various ungulates including cattle, sheep, goats, horses, pigs and camels (Maudlin *et al*., 2004). Other animals like dogs, cats and the wild carnidae are also affected (Hoare, 1972). *Trypanosoma evansi*, principally a parasite of camels and equines, also infects other animals like water buffaloes, sheep, goats, cattle and deers.

Trypanosoma vivax and *T. evansi* by virtue of their transmission by haematophagous biting flies occur in SSA, Asia, Central and North and Southern America (Hoare, 1972). The silvatic cycle that involves wild animals is known to greatly influence the epidemiology of trypanosomosis since wild animals serve as reservoirs for both human and animal trypanosomosis (Taylor and Authié, 2004).

A number of factors contribute to the severity of disease in its various hosts. Exotic animals (dairy cattle) are more severely affected by trypanosomosis than the local genotypes, which exhibit a range of breed and individual animal susceptibility. The West African taurine breeds, like the N'Dama, Baoule and their crosses with zebu (d'Ieteren *et al*., 1998), and certain zebu cattle in East Africa (Njogu *et al*., 1985) can survive and remain productive under trypanosomosis risk. This phenomenon is called trypanotolerance and involves the ability of the animals to control parasitaemia, maintain weight and resist anaemia (Murray *et al*., 1982). Clausen *et al*., (1993) established through experimental work that animals within the trypanotolerant breeds with previous exposure to trypanosomosis suffer less severe trypanosomosis effects as compared to those without previous exposure (naïve animals).

Suckling calves are also known not to suffer from serious attacks of trypanosomosis, possibly because of the influence of maternal antibodies in their systems (Dwinger *et al*., 1992). There is also evidence from studies that tsetse get attracted mostly to larger cattle from which they feed rather than smaller ones (Torr and Mangwiro, 2000; Vale and Torr, 2005). Torr and Mangwiro (2000) estimated that a large ox was bitten ~ 10 times more often by tsetse than a calf. Within herd differences have also been established (Torr *et al*., 2001) where ~ 75% of the tsetse feed from ~ 25% of the herd. The physiological status of the host, as well as nutritional and environmental factors, further play important roles in modulating the severity of the disease (Taylor and Authié, 2004). It would be expected that animals with concomitant infections with other parasites like Haemonchus species would develop serious disease when infected by trypanosomes, particularly *T. congolense*, as was reported in the Gambia (Kaufmann *et al*., 1992).

2.1.4. The environment

Trypanosomosis maintains large areas of Africa (so-called 'fly belts') and it is presumed that wildlife have contributed a lot in the maintenance of the diseases in a relatively defined ecosystem (Reichard, 2002). The environment allows for the interaction between the Glossina species, vertebrate hosts and the trypanosomes in order for trypanososmosis to be produced. In West Africa, tsetse habitats have been sub-divided along distinct north-south climatic gradients, with predominantly riverine tsetse species in the north and a mixture in the south (Hendrickx *et al*., 2004). In the north, arid conditions prevent fly spread and riparian vegetation constitutes suitable niches for the localized, well-demarcated pockets of tsetse populations. Outside these favourable micro-climates, tsetse hardly survives and it would appear that no links exist between pockets, except occasionally and in spatially limited neighboring areas during the rainy seasons. In the intermediary band, climatic conditions and vegetation become gradually more suitable. Distinct fly pockets tend to merge and tsetse distribution patterns become more linear along main streams. Tsetse populations still remain concentrated in pockets during the dry season, but disperse (Bouyer *et al*., 2006) during the rainy season over large parts of the river systems, including important tributaries and savannah buffers. In the humid south, there are no climatic limitations to fly distribution and flies are present along river systems and even the surrounding humid woodlands and forests.

Due to increasing human population and consequently the opening up of more land for crops, the morsitans group is disappearing in most places of Africa (Djiteye *et al*., 1997). Riparian tsetse species on the other hand are more versatile and can co-exist with human development. They are opportunistic feeders; where agricultural intensity is low; they feed on wild reptiles and rarely carry pathogenic trypanosomes (de la Rocque *et al*., 2001).

2.2. Impact of AAT on African agriculture

Infections by trypanosomes affects a wide range of domestic animals such as cattle, horses, donkeys, mules, camels, water buffalo, pigs, goats and dogs (Brun *et al*., 1998; Uilenberg *et al*., 1998; Stevens and Brisse, 2004). African trypanosomes also affect humans, causing Sleeping sickness or Human African trypanosomosis. In domestic animals these parasites cause a severe, often fatal disease while in wild animals the parasites cause relatively mild infection. As the illness progresses the animals weaken more and more and eventually become unfit for work, hence the name of the disease "Nagana" which is a Zulu word that means "powerless/useless" (Steverding, 2008). It is estimated that about 37% of the African continent or approximately 8-11 million km² is infested by tsetse flies (Jordan, 1986; Mattioli *et al*., 2004). About 65% of this area (7 million km2) could be used for livestock or mixed agriculture development without stress to the environment if trypanosomosis was controlled (MacLennan, 1980). Estimates put the number of cattle at risk from trypanosomosis between 50-70 million animals (Geerts and Holmes, 1998; Swallow, 2000).

The economic impacts of trypanosomosis in Africa are diverse and complex, with direct effects on animal production and human health, as well as indirect effects on settlement patterns, land use, draught power use, animal husbandry and farming (Swallow, 2000; Bourn *et al*., 2005). Quantifying these wide-ranging effects has proven to be difficult, but a considerable body of evidence has been gathered through numerous studies of specific situations (Swallow, 2000; Shaw 2004). The impact of trypanosomes in African agriculture is most obviously seen in the birth and mortality rate of young animals (Erkelens *et al*., 2000). In susceptible cattle breeds, the disease reduces caving by up to 20% and causes the death of another 16-20% of young stock (Shaw, 2004; Geja *et al*., 2012). Direct aggregate losses due to animal trypanosomosis are estimated to exceed US$ 1.3 billion annually (Kristjanson *et al.*, 1999), which excludes losses from reduced efficiency of draught oxen and manure use. These authors estimated that the potential benefits of improved trypanosomosis control in terms of meat and milk productivity alone amount to US$ 0.7 billion a year in Africa. To this figure, the expenditure on trypanocides, estimated at around US$ 30 million per annum for some 35 million doses, needs to be added for

a more encompassing assessment (Holmes *et al*., 2004). Cattle mortality associated with trypanosomosis is another example of direct loss. It was, for instance, estimated through a questionnaire survey that the high trypanosomosis risk in the pastoral area of Yalé in Burkina Faso caused herd mortality of between 75% and 85% (Kamuanga *et al*., 2001).

Each year in SSA, milk and meat off take are 10-40% lower due to trypanosomosis infections (Swallow, 1997). Likewise, cattle numbers would increase by 37% in the sub-humid and 70% in humid zones if trypanosomes were to be eradicated. The indirect impact of AAT on agriculture in sub-Saharan Africa (SSA) is believed to exceed the amount estimated for direct losses. The overall impact extends to the restricted access to fertile and cultivable areas, imbalances inland use and exploitation of natural resources and compromised growth and diversification of crop-livestock production system (Mattiolli *et al*., 2004). Trypanosomosis reduces total agricultural production by between 2% and 10% (the crude relationship is that a 50% increase in livestock numbers would increase total agricultural output by 10%) (Swallow, 1997). Furthermore, about 50 million people in Africa are exposed to the risk of contracting sleeping sickness (Franco *et al.*, 2014).

2.3.Diagnosis of African animal trypanosomosis

2.3.1. Parasitological tests

The examination of wet blood films and Giemsa-stained thick and thin fixed blood films with the aid of a light microscope have been used as diagnostic methods ever since they were first used to identify the etiological agents of trypanosomosis. With a wet smear, a drop of blood can be examined next to the animal, provided that a microscope is available. Thin and thick blood smears fixed in methanol or acetone and stained with Giemsa may be used in the laboratory to detect blood parasites and determine trypanosome species involved, respectively. However, these techniques are not sensitive enough to detect low parasite levels, characteristic of the disease in large animals (Eisler *et al*., 2004).

Parasite concentration techniques such as the haematocrit centrifugation technique (HCT) described by Woo (1970) and its improved version, the buffy coat technique (BCT) described by Murray *et al*. (1977) were developed to improve the detection capacity of parasitological technique. The two methods are associated with increased diagnostic sensitivity following concentration of parasites in the buffy coat after centrifugation. The packed cell volume (PCV %) can be determined simultaneously as a measure of anaemia. With the BCT, the three most important trypanosome species in ruminants can be identified by their characteristic movement patterns, with a possibility for estimation of parasitaemia through a scoring system (Paris *et al*., 1982). The analytical sensitivity of BCT depends on the species of trypanosome as has been demonstrated by Paris *et al*. (1982), with the smallest numbers detectable per milliliter of blood being $2.5x10^2$, $5x10^2$ and $5x10^3$, for *T. congolense*, *T. vivax* and *T. brucei*, respectively. On the other hand, HCT is the most sensitive microscopic technique to detect *T. brucei* in bovine blood.

Animal inoculation has evolved as an alternative parasitological technique which involves the transfer of trypanosomes from a suspected case to another vertebrate or invertebrate host. This method has the advantage to conserve (stabilate) isolated trypanosomes for further investigations (Eisler *et al*., 2004). Laboratory rodents are inoculated with 0.2-0.5 ml (depending on size of rodent) of freshly collected trypanosome-positive blood. On the other hand, only 50% of *T. congolense* can be conserved in mice. *Trypanosoma vivax* rarely establishes in mice, and, if it does, the resultant parasitaemia is quite transient (Luckins, 2010).

Other less commonly employed parasite detection techniques are xenodiagnosis, 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) and the use of *in vitro* culture method for the detection of trypanosomes through inoculation of culture medium with blood but with low success rate (Zweygarth and Kaminsky, 1990).

2.3.2. Molecular technique (Polymerase chain reaction (PCR)

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 (Desquesnes, 1997; Clausen *et al*., 1998; Masake *et al*., 1997, 2002; Bengaly *et al*., 2002; Gall *et al*., 2004). In actual fact, two periods of the trypanosome infection process must be distinguished; early in an infection, parasitological and PCR techniques show a very similar sensitivity (80%), but during the chronic phase of infection, parasitological examination exhibits a very low sensitivity and PCR is much more sensitive than the parasitological methods in such situation (Desquesnes, 1997). In the Sideradougou area of Burkina Faso, an epidemiological survey carried out in 1000 herds of cattle indicated a parasitological prevalence of 5.3%; a representative sub-sampling of 260 samples tested with PCR indicated a prevalence of 11.5% (Desquesnes and Davila, 2002). In another study, carried out in 76 goats in The Gambia, the parasitological prevalence was 8% against 24% with PCR (Perreira De Almeida *et al*., 1998). In such studies, the rate of positive samples by PCR is generally two to three times higher than that by the buffy coat method (Desquesnes and Davila, 2002; Gall *et al*., 2004). The detection and identification of trypanosomes by molecular means should as a principle always be based upon stable, parasite-specific genetic characteristics (Eisler *et al*., 2004). Currently a more sensitive and species specific molecular techniques such as ITS1 PCR and PCR-RFLP are available to aid the accurate diagnosis of trypanosomosis and undertake drug efficacy studies in animals (Geysen *et al*., 2003).

2.4. Major management methods of bovine trypanosomosis

2.4.1. Vector control

Controlling the vector remains theoretically the most desirable way of containing trypanosomosis (Leak, 1999). It is a strategy that has worked well in many areas where multiple drug resistance has been reported before (Bauer *et al*., 1995). Vector control methods that are available includes the use of: Sequential aerosol technique, (SAT), Stationary attractive devices (traps and targets), live bait technique and the sterile insect technique (SIT) (Schofield and Maudlin, 2001).

The SAT involves the ultra-low volume spraying of non-residual insecticides 10-15 meters above tree canopy by fixed wing aircraft or helicopter (in more difficult terrain) in 5-6 subsequent spraying cycles, separated by 16-18 days depending on temperature (Allsopp and Hursey, 2004). The goal is to kill all adult tsetse flies in the first spraying cycle and then kill all emerging flies in the subsequent cycles before they start reproducing. Insecticide application occurs during periods of temperature inversion, i.e. at night. It remains a perfect method if done under global positioning system navigation (GPS), especially for effective area-wide tsetse suppression. The disadvantage with the method is that insecticides sprayed may also kill non-target insects.

The stationary attractive devices (SADs) attract and either kill the flies through tarsal contact with insecticides embedded in the fabric or the flies are guided and trapped in a non-return cage (Reichard, 2002). Olfactory and vision sensations are the two most important behavioral expressions in tsetse that have recognized importance in the design of odor- baited targets and efficient traps and the method exerts an additional daily mortality of 2-3% to the female segment of the fly population (Leak, 1999). The major disadvantage of SADs is that the active ingredient gets washed off by rain water, hence compromising its efficacy (Torr *et al*., 1992). Increasing the concentration of insecticide to 0.6-0.8% allows the SADs to remain deployed even during the wet season, retaining tsetse mortality rates of > 90% for about 300 days (Torr *et al.*, 1992).

The live bait technique involves the application of insecticide onto cattle as pour-ons, sprays or dips so that tsetse flies attempting to feed on the treated cattle get killed on picking up a lethal deposit of the insecticide through their tarsi and pre-tarsi. Live baits were used in Zimbabwe against *Glossina pallidipes* Austen (Hagrove *et al.*, 2000), in Burkina Faso against *G. morsitans submorsitans Newstead and G. palpalis gambiensis* Vanderplank (Bauer *et al*., 1999), against *G. fuscipes fuscipes* Newstead *and G. pallidipes* in Ethiopia (Leak *et al*., 1995) and against *G. m. morsitans* in Zambia (Van den Bosche *et al*., 2004). Unlike SADs, the live bait technique is less prone to theft and does not suffer from maintenance problems. Because of its added advantage of also controlling ticks, the use of live baits is appreciated as a private good and can easily be adopted by the rural farming community (Bauer *et al*., 1995; Torr *et al*., 2002). Disadvantages

arising from this technique are the high treatment frequency, the high cost of the insecticides, insecticide residues in cattle dung, motivation and participation of farmers and the potential development of resistance to the insecticides in ticks and insects with a high reproductive rate (Wardhaugh *et al*., 1998; Vale and Grant, 2002).

The sterile insect technique (SIT) is mainly used if the objective is tsetse eradication. As was the case in the island of Zanzibar, the introduction of the SIT helped eradicate the fly from this island in 1996 in a campaign that had been commenced two years earlier (Reichard, 2002). As a prerequisite, tsetse density has to be suppressed through the widespread application of insecticide treated SADs, live baits or fly trapping to a point where the SIT is considered feasible. In Zanzibar, a sterile insect plant producing 70,000 irradiated pupae weekly was constructed that made the release of over 7.8 million sterile male flies possible. Dispersal of the irradiated males over time was done to achieve an estimated ratio of 50 sterile males for every 1 wild male in order to overwhelm the residual wild tsetse population (Reichard, 2002). The released sterile males in the target area do out-compete the wild male population for wild females (Vreysen, 2005). Mating of the sterile males with virgin, native females results in no offspring. With each generation, the ratio of sterile to wild insects will increase, making this technique more and more efficient with lower wild female population densities. However, the SIT necessitates huge investment for rearing and releasing sterile male tsetse fly, and efficient release and monitoring methods, which have to be applied on an area-wide basis (Vreysen, 2005).

2.4.2. Trypanocidal drugs

In the 37 African countries where animal trypanosomosis is endemic, trypanocides are used for the control of the disease (Geerts and Holmes, 1998). Drugs have proven sustainably and sufficiently attractive to the livestock keepers. Three compounds - isometamidium chloride (ISM), homidium salts (homidium bromide (Ethidium®) and homidium chloride (Novidium®) and diminazene aceturate (DIM) have been and are still in use ever since their release into the market in 1950s (Holmes *et al*., 2004).

Prophylactic treatments using Isometamidium chloride (ISM) target all animals in a herd or a particular group of valuable or 'at- risk' animals (Holmes *et al*., 2004). Isometamidium administered intramuscularly (i.m.) at a dose rate of 0.5-1mg/kg b.w provides up to 3 months' (range of between 2-22 weeks) protection against pathogenic trypanosomes of cattle and against *T. vivax* especially in small stock, *T. brucei* in equidae and *T. evansi* in camels (Peregrine, 1994; Geerts and Holmes, 1998; Geerts *et al.*, 2001). Isometamidium is given either as routine block treatment (pre-determined intervals) or as strategic block treatment (when challenge reaches a predetermined threshold). It is recommended that once a year, additional to ISM, the animals are separately treated with diminazene diaceturate (DA) in order to delay the development of resistance, following the concept of 'sanative pair' as described by Whiteside (1962).

Diminazene aceturate/diaceturate and Homidium salts are the main therapeutic drugs used in the management of clinical trypanosomosis in animals (Holmes *et al.*, 2004). Diminazene aceturate is administered i.m. at a dose rate of 3.5-7mg/kg body weight; the lowest dose being effective against *T. congolense* and *T. vivax* and the highest dose against *T. brucei* (Peregrine, 1994; Geerts and Holmes, 1998). 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). Homidium on the other hand is administered at a dose rate of 1mg/kg body weight in cattle. In low tsetse challenge areas, a prophylactic effect of HOM salts has also been observed (Peregrine, 1994). However, these very important drugs are progressively exposed to resistance by the parasites due to various reasons.

2.5.Trypanocidal drug resistance

Drug resistance is the heritable loss of sensitivity of a micro-organism to a drug to which it was sensitive to before. Trypanocidal drug resistance is caused by the exposure of trypanosomes to sub-therapeutic drug concentrations, resulting from under-dosing and the irrational use of drugs and the lack of proper diagnosis (Geerts and holmes, 1998). The prolonged and frequent use of trypanocides in high tsetse challenge areas, even when used at the right doses, is also likely to cause resistance (Clausen *et al*., 1992).

Two types of resistance against trypanocidal drugs are recognized: single drug resistance and multiple drug resistance. In single drug resistance, trypanosomosis control still could be achieved by using one of the drug pairs in which resistance has not developed through the application of the sanative pair principle (Geerts and Holmes, 1998). However, the second drug should be used with caution in order to avoid resistance development against it as well. Multiple drug resistance is resistance concurrently to two or more drugs, making sanative drug pairs ineffective. Multiple drug resistance can only be counteracted by intervening at the level of the vector (Geerts and Holmes, 1998).

2.5.1. Status of trypanocidal resistance in Africa

The first case of drug resistance in trypanosomes was reported in Northern Nigeria (Naisa, 1967). Recent reports show that there are twenty-one African countries in which trypanocidal drug resistance has been reported (Delespaux *et al*., 2008; Chitanga *et al*., 2011). Examples are reports from Zimbabwe (Joshua *et al*., 1995), Kenya (Mdachi, 1999), Uganda and Tanzania (Eisler *et al*., 2000), Nigeria (Geerts *et al*, 2001), Burkina Faso (McDermott *et al*, 2003), Zambia (Shinyangwe *et al*., 2004), Mozambique (Jamal *et al*., 2005), Mali and Guinea (Diall *et al*, 2003; Grace, 2005), Cameroon (Mamoudou *et al*., 2008), Ghana and Benin (Allegye-Cudjoe, 2009) and Ethiopia (Afework *et al*., 2000, Tewelde *et al*., 2004, Moti *et al*., 2012, Shimelis *et al*, 2015) demonstrating area-wide resistance. Confirmed reports about resistance in the cotton zone of West Africa were first made particularly in Burkina Faso in the early 1980s (Pinder and Authie, 1984; Authie, 1984). These authors described stocks of *T. congolense* isolated from cattle in the Samorogouan area in 1982 which were resistant to isometamidium. Later, tests in mice showed that certain *T. congolense* strains from the same area were also resistant to diminazene aceturate indicating existence of multiple drug resistant *T. congolense* (Authie, 1984). The different species of trypanosomes and detection of resistance/multiple resistance against trypanocidal drugs are summarized in table 1.

Table 1. Summary of trypanocidal drug resistance in various parts of sub Sharan Africa.

Country	Trypanosome species	Resistance to trypanocidal drug (s)	References
Burkina Faso	*T. congolense*	ISM	Pinder and Authié, 1984
		ISM, DA andHOM	Clausen *et al*., 1992
	T. vivax	ISM and DA	McDermott *et al*., 2003; Sow *et al*., 2012
Mali	*T. congolense*	ISM and DA	Mungube *et al*., 2012
	T. vivax	ISM	Mungube *et al*., 2012
Mozambique	*T. congolense*	ISM and DA	Jemal *et al*., 2005
Kenya	*T. congolense*	ISM	Gray *et al*., 1993
	T. congolense	ISM,DA and QUI	Peregrine *et al*., 1997
Zambia	*T. congolense*	ISM and DA	Chitanga *et al*., 2011
Zimbabwe	*T. congolense*	ISM and DA	Joshua *et al*., 1995
Kenya/Somalia	*T. vivax*	ISM	Schönefeld *et al*., 1987
		HOM	Ainanshe *et al*., 1992
Ethiopia	*T. congolense*	ISM, HOM and DA	Mulugeta *et al*., 1997
	T. congolense	ISM and DA	Afework *et al*., 2000
	T. congolense *T. brucei*	ISM	Tewolde *et al*., 2004
	T. congolense *T.vivax*	ISM and DA	Shimelis *et al*., 2008 Shimelis *et al*., 2015
	T. vivax	ISM and DA	Desalegn *et al*., 2010
	T. congolense	ISM and DA	Moti *et al*., 2012

2.5.2. Mechanisms involved in resistance to ISM and DA

Isometamidium is known to accumulate in two compartments of trypanosomes, the cytoplasm and the kinetoplast (Wilkes *et al*., 1997). Decreased levels of ISM accumulation have been observed in drug-resistant populations of *T. congolense* (Sutherland *et al*., 1991) and later work found indirect evidence of an increased efflux of this drug from resistant trypanosomes (Sutherland and Holmes, 1993). Mulugeta *et al*. (1997) showed that maximal uptake rates (Vmax) of ISM in resistant *T. congolense* were significantly lower than in sensitive populations. Changes in the mitochondrial electrical potential have been demonstrated in isometamidium-resistant trypanosomes (Wilkes *et al*., 1997). isometamidium resistance can be caused by a mutation in an important mitochondrial protein, the γ subunit of the F_1 ATPase, and that this mutation alone is sufficient for high levels of resistance, cross-resistance to various drugs, and a strongly reduced mitochondrial membrane potential (Eze *et al*. 2016).

The accumulation of DA has been shown to be markedly reduced in arsenical-resistant *T. brucei, T. evansi* and *T. equiperdum* due to alterations in the P2-type purine transport system (de Konig and Jarvis, 1999). In addition to this resistance mechanism, a novel gene, TeDR40, might be a factor contributing to high DA-resistance in *T. evansi* (Witola *et al*., 2004). It is suspected that DA resistance is multi-factorial and that the TeDR40 gene might be a contributing factor to resistance linked to the alteration of the gene coding for the P2-type purine transporters. A putative P2-type purine transporter TcoAT1 in *T. congolense* was identified by reciprocal blasting of the TbAT1 gene of *T. brucei* and a conserved Val306 to ile306 permutation in this gene was observed in *T. congolense* strains that show resistance to DA (Delespaux *et al*., 2006).

2.5.3. Common trypanocidal drug resistance detection methods

Field methods

Eisler *et al*. (2000) proposed a method for the assessment of prevalence of resistance to isometamidium chloride by monitoring cattle populations under natural challenge in the field. Briefly, two groups consisting of 30 to 80 cattle each are used. One group is treated with 1 mg/kg body weight ISM and the other is used as untreated control. The two groups then are exposed to natural challenge and tested for trypanosomes using the phase contrast buffy coat technique (Murray *et al*., 1977) every two weeks for two to three months. A comparison through survival analysis curves is made on the data of new trypanosome infections between the group of cattle treated with ISM and the untreated control group (Eisler *et al*., 2000; Tewelde *et al*., 2004). If >25% of the ISM treated cattle become infected within 8 weeks of exposure, drug resistance is strongly suspected (Mdachi, 1999; Eisler *et al*., 2000; Tewelde *et al*., 2004). In this regards, several epidemiological studies to map field trypanocidal drug resistance, based on the protocol by Eisler *et al*. (2000), have been documented. McDermott *et al*. (2003) working in the Kénédougou Province of Burkina Faso, Shinyangwe *et al*. (2004) working in Eastern Zambia, Tewelde *et al*. (2004) in Ethiopia, Grace (2005) in Guinea and south-eastern Mali and Allegye-Cudjoe (2009) in Ghana and in Benin are some examples.

An abbreviated version of the original 8-12 week-protocol by Eisler and colleagues was validated in the cotton zone of West Africa and found effective and reliable (Diall *et al*., 2005) for use not by researchers but by the national veterinary services. This involves a 4-week long follow-up (Diall, 2005) period in order to reduce costs and still generate data within a very short time. The abbreviated protocol is effective in areas where trypanomosis risk is high (prevalence is >10%) as has been demonstrated in the cotton zone of West Africa (Diall *et al*., 2003; Grace 2005).

Experimental method in natural hosts

Neither the single-dose nor the multiple-dose tests in mice are able to predict accurately the curative doses of trypanocidal drugs needed to clear trypanosome populations from infected cattle (Eisler *et al*., 2001). A test in ruminants should hence be used to determine whether or not drugs are principally efficacious at recommended curative doses in cattle infected with a particular trypanosome population. The test in calves may further be used for investigations on drug resistance in *T. vivax*, which is usually not infective for mice. A group of cattle or small ruminants, preferably of a breed native to the area and without prior exposure to tsetse or trypanosomosis are used (Eisler *et al*, 2001). Specific detailed protocols on this are as contained in Eisler *et al* (2001). Due to individual variation in the response to trypanocidal drug treatment among ruminants inoculated with the same *T. congolense* isolate (Ndoutamia *et al*., 1993; Kone, 1999), it is advisable to use a minimum of three and preferably six animals.

The experimental animals must be kept in a fly-proof stable or in a non-tsetse infested area to eliminate the risk of reinfection during the study. A breakthrough infection, indicative that one of the inoculated trypanosome populations was drug-resistant can be inoculated into a group of calves to determine the level of drug resistance. A variation of this method also exists whereby blood from a group of infected cattle is pooled and inoculated into a single recipient calf which is monitored and later, if parasitaemic, treated with trypanocide at the recommended dose. This technique is appropriate where laboratory facilities are limited but only allows for a qualitative assessment of resistance. Further constraints of the technique are that not all trypanosome populations might grow equally well and that sensitive isolates might overgrow resistant ones when inoculated together (Sones *et al*., 1989). A useful indication of the level of resistance can be obtained from studies in ruminants by recording the length of time between treatment and detection of breakthrough populations of trypanosomes. The shorter the period, the greater the level of resistance. If relapse occurs in more than 20% or more of the cattle tested (i.e. for a total of between one and four cattle, at least 1 relapse; for a total of 5 or 6 cattle at least 2 relapses), the isolate may be said to exhibit resistance to the dose of drug used (Eisler *et al*, 2001).

Experimental study in mice

On the other hand, mice can be used to undertake drug resistance study for trypanosome species that can grow in this species of experimental animals. 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 drug. Tail blood wet smears are checked 2-3 times per week for parasites for a period of up to 60 days. The ED50 and ED95 (effective dose that gives temporary clearance of the parasite in 50% or 95% of the animals, respectively) can be calculated as can the CD50 and CD95 (curative dose that gives complete cure in 50 and 95% of the animals, respectively). Sones *et al*. (1988) used a group of five mice, which allowed an easy calculation of ED80 and CD80 values (one out of five mice not cleared or cured). Knoppe *et al*. (2006), using the standard mouse test (SMT), screened a number of *T. congolense* isolates collected in the Kénédougou Province of Burkina Faso against isometamidium chloride at a dose of 0, 0.25, 1.0, 5.0, 10, 15 or 20 mg/kg body weight and found the method very sensitive but labour intensive.

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 difference in metabolic size (Sones *et al*., 1988). Thus, the curative dose for ruminants cannot be extrapolated from the assay results in mice. Thirdly, a danger further exists of selecting against particular trypanosome species, particularly in mixed infections. Fourthly, precise assessment of resistance requires a large number of mice per isolate.

Molecular techniques (PCR)

Because of the problems associated with the low sensitivity of the parasitological techniques (Paris *et al*., 1982) and the long follow-up time of study animals (Eisler *et al*., 2001), PCR with high sensitivity and specificity is a good solution to these problems. Gall *et al*. (2004) used this

method in Burkina Faso and found it four times more sensitive compared to the field parasitological techniques.

Molecular methods for the diagnosis of ISM resistance has been developed (Delespaux *et al.,* 2005, Afework *et al.,* 2006). The first method enables discrimination between ISM-sensitive and ISM-resistant strains of *T. congolense* by *MboII*-PCR-RFLP (Delespaux *et al*., 2005). 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. 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).

2.6. Trypanosomosis in Ethiopia

Ethiopia has the largest livestock population in Africa with 53.99 million cattle, 25.49 million sheep, 24.09 million of goats, 1.91 million horses, 0.35 million mules, 6.75 million donkeys and 0.92 million camels (CSA, 2013). Livestock is a significant contributor to economic and social development in Ethiopia at the household and national level. Livestock accounts for 15-17% of total GDP and 35-49% of agriculture GDP (CSA, 2013). Unfortunately, the development and intensification of livestock productivity in Ethiopia is hampered among others by cross-border epizootic diseases such as African animal trypanosomosis (Abebe,2005).

The most important trypanosomes, in terms of economic loss in domestic livestock include: *T.congolense, T.vivax, T. brucei, T.equiperdum* and *T. evansi* (Abebe, 2005) as indicated in (Figure 4).

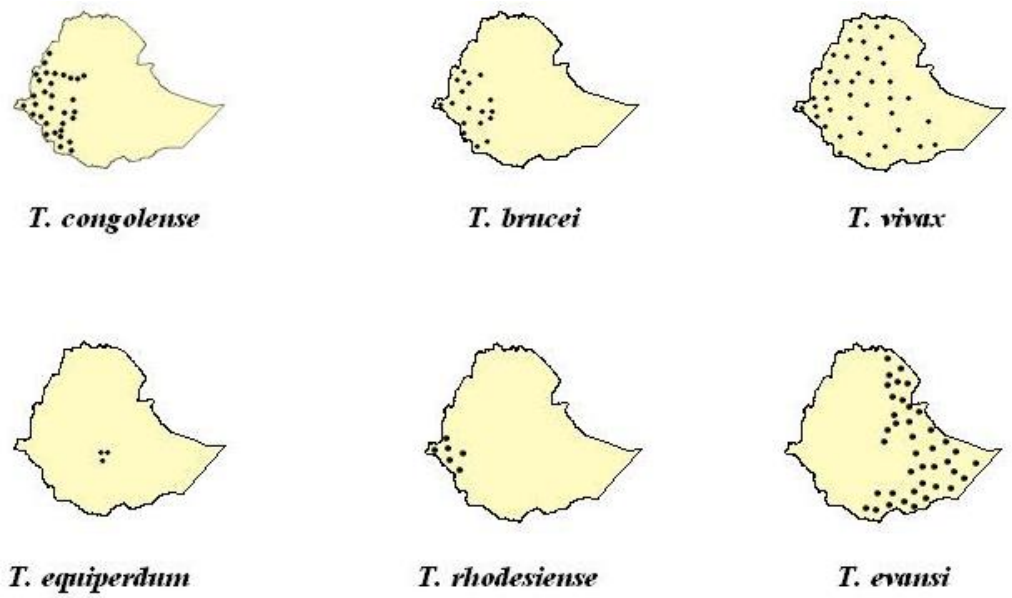

Figure 4. Distribution of pathogenic trypanosomes in Ethiopia (Source: Abebe, 2005).

Most of the studies conducted in Ethiopia on trypanosomosis focused on tsetse transmitted trypanosomosis to determine the prevalence and impact of the disease (Codija *et al*., 1993; Afework *et al*., 2000; Abebe, 2005; Dagnachew *et al*., 2005; Cherenet *et al*., 2006). In South and South-western region both tsetse and non tsetse transmitted trypanosomosis (Abebe and Jobre, 1996) are prevalent. In tsetse infested areas of Ethiopia *T.congolense* and *T.vivax* are the dominant species. Abebe and Jobre (1996) reported an infection rate of 58.5% for *T.congolense* 31.2% for *T.vivax* and 3.5% for *T.brucei* in south west Ethiopia. In the same report 8.7% prevalence rate was recorded in the highlands (tsetse free areas) of which 99% is due to *T.vivax.* Apart from the cyclical transmission of trypanosomosis by the *Glossina* species, it is highly considered that mechanical transmission through the bite of insects (Stomoxis, Tabanus, Chriosops) is also a potential threat to livestock productivity in Ethiopia (Shimelis *et al*., 2015; Shimelis et al. 2017).

Various efforts of control of the disease and losses have been directed mainly against the parasite through trypanocidal drugs and the vector through odour baited and insecticide impregnated targets/traps and insecticide-treated cattle (Slingenberg, 1992; Leak *et al*., 1996). Recently vector control operations have been implemented mainly through specifically designed joint projects

that offered some promising local results (Lemecha *et al*., 2006). However, the vector control operation is limited in areas of approximately 14% of the total tsetse infested area (MOA, 1995). Moreover, recent situation of tsetse fly advances into previously unoccupied areas and the development of trypanocidal drug resistance are thought to hamper the envisaged results of these efforts.

In Ethiopia currently the most widely used trypanocidal drugs for *T.congolense* and *T.vivax* infection are ISM and DA. The occurrence of drug resistant trypanosome across Ethiopia is not well known. Trypanocidal drug resistance particularly against *T.congolense* infection is reported in the Ghibe Valley since 1993 (Codija *et al*., 1993; Mulugeta *et al*., 1997). Since then, drug resistance has become a common report especially for *T. congolense* in several areas (Afework *et al*., 2000; Tewolde *et al.*, 2004; Shimelis *et al*., 2008; Moti *et al*., 2012) and more recently for *T. vivax* (Shimelis *et al*., 2015).

3. **MATERIALS AND METHODS**

3.1. Study Areas

The present study was conducted in three districts of the Gurage zone (Abeshege, Cheha and Enemor and Ener) and another district of South Western Shoa zone (Ameya) in southwestern part of Ethiopia some 200 km away from the capital Addis Ababa (Figure 5). These areas are collectively located between 37.52431^0 and 37.74235^0 East and between 7.96385^0 and 8.53347^0 North with altitude range from 1107-1923 meters above sea level (m.a.s.l.). The climate alternates with long summer rain fall (June-September) and a winter dry season (October-May). The area receives a mean annual rainfall of 900 mm and the mean monthly maximum temperature ranging between 29.8^0C and 44.0^0C (CSA, 2013). The study sites are composed of cultivated land, grazing land, forest shrub, bush and wood land, water bodies and rural and urban settlement areas. These areas have the same altitude, same tsetse challenge and are adjacent to Ghibe river. The livestock population includes cattle, sheep, goats and equines which are an integral part of the livelihood. Mixed crop-livestock farming is the main source of livelihoods where Maize, Teff and hot-pepper are the main cash crops. Ameya district of Southwestern Shoa zone and whole Gurage zone lie south west of the capital and are generally within the tsetse belt with relatively medium to high fly challenge (Rowlands *et al.*, 2001).

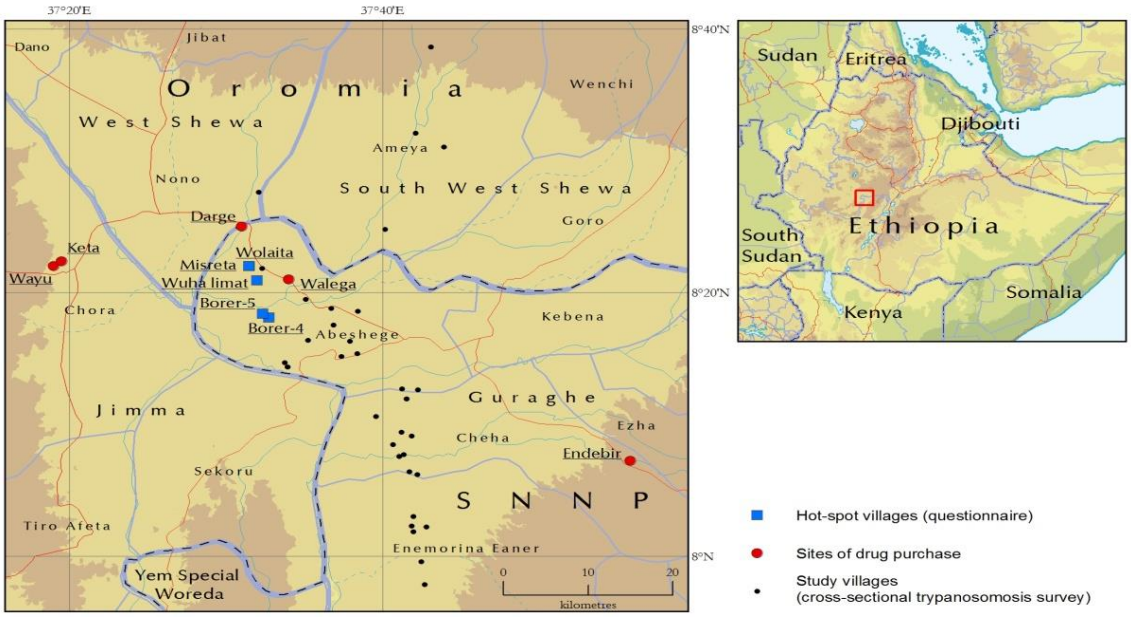

Figure 5. Map of study districts showing the study sites

3.2. Study Animals and Management

Gurage breed (Lemecha *et al.*, 2006) of cattle that occupy the major part of the study areas are considered as study population (figure 6). They are East African Zebu type breed, and also known as the Abyssinian short horned Zebu, found in the south western Ethiopia, (DAGRIS, 2004). These animals have been reported to be susceptible to trypanosomosis and trypanocidal resistance has already been reported in neighboring districts (Mulugeta *et al.*, 1997; Rowlands *et al.*, 1993).

Natural pastures from communal grazing lands were the principal feed resources for cattle and other livestock during rainy season and crop residues were the major supplements available after harvest time. Rare supplies of mineral salts (NaCl) were also available for selected animals in the herd. All age groups are allowed to graze together at the fringes of crop fields and fallow lands. They shared the same watering point during day time and housed at night on their respective farms. Animals obtain water in the rainy season from seasonal rivers while in the dry season from perennial rivers flowing in their locality.

Figure 6. Picture demonstrating conditions of cattle in the study area

3.3. Study Methodology

3.3.1. Cross sectional survey on bovine trypanosomosis

Study design and study animals

Cross sectional studies were conducted from December, 2012 to February, 2013 to determine the prevalence of bovine trypanosomosis using microscopic/parasitological and molecular (PCR-RFLP). Accordingly, each animals age was determined based on dentition according to Pace and Wake (2003) and information obtained from owners. Animals were then categorized into three age groups, cattle less than one year of age as calf; between one and three years age as young and animals above three years of age as adults. And cattle in each group were also identified based on their body condition scores according to the description of Blackwood (2013) and were categorized in to three groups as good, lean and cachectic

Sampling method and sample size determination

This study was deigned based on a cross-sectional type of investigation and was conducted from December/2013 to February, 2014. Three districts were purposively selected from the Gurage zone of the Southern Nation and Nationalities people regional State (SNNPR) and one from West Showa zone of Oromia Regional State based on the history of trypanosomsis prevalence. Then a systematic random sampling technique was employed to select 19 peasant kebeles (PK): Abeshege 6 PK, Cheha 4PK, Enemor and Ener 8 PK and Ameya 1 PK) from the list of PKs in the study woderas. To determine the nmber of animals to be sampled from each study woreda (sample size), an expected prevalence of 50%,absolute desired precision of 5% and confidence level of 95% was used according to the formula given below (Thrusfield, 2007).

$$n = \frac{z^2 pq}{d^2}$$

Where n is the calculated sample size, z is the alpha value of 95% confidence interval, p is the expected prevalence, q is 1-p and d is the desired precision.

Therefore, for each districts, 384 animals were required with over all sample size of 1536 animals for the four woredas. However, to increase the statistical efficiency at kebele level 2000 animals were sampled (815 female and 1185 male) from the study areas. Through a systematic technique, 50 animals were sampled per village to achieve the 2000 sample size. Then 40 villages were randomly selected from within the 19 PKs by a lottery method.

Parasitological examination and PCV determination

Blood sample was collected in ethylene diamine tetra-acetate (EDTA) coated 5ml vacutainer tube from the jugular vein of sampled animals. The vacutainer tubes were labelled and stored in an ice-packed cool box awaiting examination within 2 hours of collection. Micro haematocrite capillary tubes were filled with the blood sample and sealed at one end using cristaseal. The capillary tubes were centrifuged at 9000 revolution per minute (rpm) for 5 minutes to concentrate the trypanosomes in the buffy coat layer (Woo, 1970). First, the packed cell volume (PCV) was measured soon after centrifugation using the Hawksley microhaematocrit reader (Hawksley, Lancing, United Kingdom). Then the capillary tube was placed in a Woo viewing chamber with a cover slip of 24 mm × 24 mm and the buffy-coat plasma junction was examined under the microscope for the presence of trypanosomes. Trypanosome species were identified based on their movement and morphology using x 25 objective lens. For the positive ones: 2 Eppendorf tubes of 2ml were filled with 0.75ml of blood mixed with 0.75 ml of Guanidine Protecting Buffer (GEB) and two negative samples for each positive sample were also similarly prepared and kept in the refrigerator for DNA extraction and molecular analysis.

DNA extraction and Molecular analysis

For the molecular analysis, 75 of the 90 Woo positive samples (excluding those detected for *T. theileri* and another five samples with low DNA quantity) and 151 parasitologically negative samples (two negative for every one positive) were analysed using PCR-RFLP for confirmation and species identification. Briefly, 750µl of whole blood was mixed with equal volume of Guanidine Protecting Buffer (GEB). These samples were kept in refrigerator until DNA extraction. The DNA extraction was conducted using the QIAamp® DNA Mini Kit (Qiagen,

Germany) according to the manufacturer's instructions and DNA was eluted in 100 µl of elution buffer. Extracted DNA samples were stored at -20 °C until the molecular analysis.

The molecular analysis was conducted using Polymerase Chain Reaction-Restricted Fragment Length Polymorphism (PCR- RFLP). First the DNA in the samples was amplified in a reaction volume of 25 µl containing 12 µl of 2X Phusion Flash High-Fidelity PCR Master Mix (Thermo Scientific, LTC Tech, South Africa), 0.5µl of forward primer 18ST nF2 (5'-CAACGATGACACCCATGAATTGGGGA-3') and 0.5µl of reverse primer 18ST nR2 (5'-GTGTCTTGTTCTCACTGACATTGTAGTG-3') nuclease-free water 7µl and 5µl of DNA template. The cycling condition of the reaction was initial denaturation at 98 °C for 10seconds and then 35 cycles each of denaturation at 98 °C for 1 sec, annealing at 58 °C for 5 sec and elongation at 72 °C for 15 seconds, and a final extension step at 72 °C for 1min. The amplification products were examined for the presence of trypanosome DNA on a 2% agarose gel. A 100 bp DNA ladder (Thermo Scientific, LTC Tech, South Africa) was included in every gel for fragment size determination. The samples were run for 35 min at 120 V in 1x Tris/Acetic acid/EDTA (TAE) buffer, stained with Ethidium bromide and photographed under UV illumination using the ChemiDoc™ XRS system (Bio-Rad Laboratories, South Africa). Detection of trypanosomal DNA was regarded as a positive infection (amplicons between 600 and 700 bp were obtained (Geysen *et al* 2003). A negative control consisted the PCR mixture without DNA template and the positive control of a known *T. congolense Savannah* DNA sample was included during each PCR.

A double-digest reaction was performed on the positive PCR amplicons in 15 µl reactions using the restriction enzymes *Msp*1 (New England BioLabs, InqabaBiotec, South Africa) and *AcuI* (New England BioLabs, InqabaBiotec, South Africa) in the CutSmart® Buffer according to the manufacturer's instructions. Four microlitres of the restricted PCR product was mixed and 2 µl of loading dye buffer on to a 10% Tris/Boric acid/EDTA (TBE) polyacrylamide gel (PAGE) and the fragments were thereafter separated by gel-electrophoresis in 1x TBE buffer at 100 V for 95 min. A 100 bp DNA ladder (Thermo Scientific, LTC Tech, South Africa) for fragment size determination was also included in each TBE-PAGE gel. The gel was then stained in 1x TBE buffer with SYBR Green I gel stain (Roche, South Africa) for 30 min and photographed using

the ChemiDoc™ XRS system (BioRad, South Africa). The species' of trypanosomes were identified based of characteristic banding pattern as described in Gysen *et al*., 2003.

3.3.2. Trypanocidal drug utilization practices in the study area

Questionnaire survey

A structured questionnaire was used to collect data on the awareness of trypanosomosis and the practice of trypanocidal drug usage among farmers in five selected study villages already identified in the cross-sectional survey. Based on the formula given by Arsham (2002) for questionnaire surveys, a total of 100 randomly selected farmers (20 farmers in each village) were interviewed.

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

$$= 0.25/0.05^2 = 100$$

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%.

The respondents were selected from farmers who were voluntarily presenting their cattle for trypanosomosis screening during the study period. The type of drug used to treat trypanosomosis, the frequency of usage, practice of trypanocidal administration, etc. were the subjects of the interview.

3.3.3. Field trypanocidal drug sensitivity/resistance study

Study villages

The trypanocidal drug sensitivity study was carried out in Abeshege District, Gurage zone south west Ethiopia. Five hot-spot villages, purposively selected based on their higher trypanosomosis prevalence and corresponding to the questionnaire survey sites, were considered in this study as already depicted in figure 3. Two of the selected villages are from Borer peasant association (PA) whereas the remaining three are in Hudad-4 PA.

Study design and protocol

An abbreviated 28 day field protocol was used to estimate resistance to 3.5 mg/kg/body weight diminazine aceturate and to 0.5 mg/kg/body weight isometamidium chloride in trypanosome-positive cattle as described by Diall *et al*. (2005). Cattle were screened for trypanosomes using the woo technique (Woo, 1970) until 16 positive animals were identified positive in each selected village. In each of the five villages, the 16 positive animals were randomly allocated to Diminazene (group DA) and Isometamidium (group ISM) treatment groups (8 animals/treatment). Therefore, the total number of animals treated with Diminazene was 40 and another 40 for isometamidium group. The animals were ear-tagged and their weights estimated using a weighing band as described by Mekonnen and Biruk, (2004) to determine the dose of the drugs to be administered. All animals in group DA received 3.5 mg/kg body weight of 7% solution Diminazine aceturate (Veriben®, Ceva Animal Health Inc., France) whereas group ISM was treated with 0.5mg/kg body weight of 2% solution Isometamidium chloride (ISM) (TRYPAMIDIUM-SAMORIN®, Merial, France). Deep intramuscular injections were made in gluteal muscle. The drug treatment day was considered as day 0. Treated cattle were monitored for trypanosomes and PCV on days 14 and 28 post-treatment using parasitological technique.

3.3.4. Trypanocidal drug quality assessement

Collection of drug samples

Fifty samples of trypanocidal drugs (diminazene aceturate and isometamedium chloride) were collected from different vendors (authorized and unauthorized) and wholesalers. The sampling points were purposely selected for consistency with the drug supply facilities mentioned by respondent farmers during the questionnaire survey (Figure 7). Sampling points included authorized markets (Veterinary Clinics, pharmacies and wholesalers) and unauthorized sources or open markets (Table 2). Drug samples, sealed in plastic bags and identified by a unique ID number, were sent to LACOMEV in Senegal; one of the reference laboratories of the World Animal Health Organisation (OIE). Information like: trade name, manufacturer, origin, date of manufacture and expiry, place of purchase (source) were recorded in excel spread sheet. To ensure sufficient quantity, each sample contains at least 5-10 sachets depending up on the quantity within each sachet. For the 2,36g sachet of granules/powder containing diminazene diaceturate, 10 sachets per sample were needed and for the 23,6g sachet of granules/powder containing diminazene diaceturate, five sachets per sample were collected. Similarly, for the 125 mg sachet of granules/powder containing isometamidium, 10 sachets per sample were taken and for the 1g sachet of granules/powder containing isometamidium, a minimum of five sachets per sample were collected.

Table 2. Sources of trypanocidal drugs used for quality analysis

	Whole seller[a]	**Govt.clinic**[a]	**Private Vet. Pharmacy**[a]	**Open market**	**Total**
ISM	5	3	2	7	17
DA	5	2	8	18	33
Total	**10**	**5**	**10**	**25**	**50**

Drug quality testing

The drug quality was assessed by (i) galenic tests (physicochemical nature), (ii) identification and (iii) measurement of concentration of the respective active ingredient according to standard operating procedures prepared by GALVmed, FAO and IFAH in collaboration with Manchester Metropolitan University and IAEA (Sutcliffe *et al*., 2014). The galenic testing included pH measurement, solubility/limpidity of ready-made solutions as well as solutions prepared from DA granules or ISM powder according to the manufacturers' recommendations. The pH was measured using a Metler MP 230 pH meter with a pH between 4 and 7 considered as compliant. The limpidity of solutions was assessed visually with the naked eye for the absence of visible solid particles . Identification and concentration of the active ingredient were assessed using high performance liquid chromatography (HPLC) (Sutcliffe *et al*., 2014). Each sample was simultaneously measured with a reference standard. The standards for diminazene and isometamidium were manufactured by VETOQUINOL (Paris, France) and CEVA (Libourne, France), respectively (Sutcliffe *et al*., 2014) and were provided to LACOMEV by the consortium GALVmed/FAO/AIEA/IFAH. They were stored in a refrigerator at 4° +/- 1°C until use.

Trypanocide drug samples were dissolved in ultrapure water for DIM and 25% acetonitrile in ultrapure water for ISM to obtain a solution of 0.1mg/ml of active ingredient. The solution was poured into vials and introduced into the chromatograph that was programmed to automatically conduct the process of identification and concentration measurement. The mobile phase for the analysis of DA used a mix of 10% methanol, 10% acetonitrile and 80% ammonium formate buffer (20mM, pH 4.0). Similarly, the mobile phase for ISM used 25% acetonitrile and 75% ammonium formate buffer (50mM, pH 2.8). A Water Kromasil C18® HPLC column (150 x 4.6 mm, 5µm - AkzoNobel, Separation Products, Bohus, Sweden) was used to run the test. The procedure was performed twice with a maximum acceptable divergence between analyses being 2%. In case of a higher divergence, the procedure was repeated until it fell within the 2% range. For DA, a measured concentration within ±10% variation from the manufacturers' label claim was considered as compliant (Tettey *et al*, 2002). For ISM, the following criteria were used to declare compliance: (i) presence of the four isomers (I, II, III, IV), (ii) a proportion of isomer I (principal component) equal to or greater than 55%, (iii) a proportion of isomers II, III and IV equal to or less than 40% and (iv) a proportion of the four isomers falling between 95 and 102%.

All along the quality assessment process, names of pharmaceutical companies producing the drugs were kept anonymous.

3.4. Ethical consideration

All animals involved in this study were handled according to standard guidelines for the use and handling of animals in research. Questionnaire surveys were administered after obtaining full informed consent from each respondent. Ethical approval was obtained from the Animal Research Ethics review committee (**Certificate Ref. No: VM/ERC/05/07/07/2015) of the college of Veterinary Medicine and Agriculture, Addis Ababa University.**

3.5. Data Analysis

All data was recorded during sample collection and stored in the Microsoft Excel spread sheets to create a data base and imported to STATA® version 11 for analysis. Descriptive statistics (percentage) were used to measure prevalence and responses obtained from respondents to summarize results in tables. In the event that a category contained fewer observations, the fisher exact test was used to see the effect of origin of the two drugs separately. Linear regression was employed to compare the mean PCV of the parasitaemic and aparasitaemic animals.For the drug quality compliance study, logistic regression for binary compliance was employed to compare the difference in non-compliance with regards to trypanocidal drug origin (Asia/Europe), the two groups of trypanocides (DA/ISM) and the marketing channel (official/unofficial). Pearson chi^2 was used to compare treatment failures between villages. Through out the analysis, *p*-value < 0.05 was considered to have statistically significant difference.

4. RESULTS

4.1.Cross sectional survey on bovine trypanosomosis

4.1.1. Parasitological findings

Out of the total of 2000 animals examined using parasitological technique, the overall prevalence of trypanosomosis in the study population was 4.5% (95% CI= 3.4-5.4%). In the four districts the prevalence of trypanosome varied from 1.5%- 7.12% and the difference was significant between districts/woredas (P=0.000) being higher in Abeshege and Cheha (Table 3). At a zonal level, there was significant difference ($\chi 2$ = 9.79, P = 0.00) in prevalence of trypanosome infection between the study zones where higher overall prevalence was encountered in Gurage zone (4.8%) compared to the 1.5% overall prevalence in South-West Shoa. Among the trypanosome species identified in the study area, significantly higher prevalence was detected for *T.congolense* (61.1%) compared to *T.vivax* (17.8%) and Mixed infection of *T.congolense* and *T. vivax* (10%) as well as *T. theilerie* (11.1%) as described in table 4.

Table 3. Number of animals sampled and prevalence of bovine trypanosomosis in four districts of Southwestern Ethiopia

Parasite	**Districts**				
detection	Abeshege	Enemor and Ener	Cheha	Ameya	**Total**
Parasitaemic	57 (7.12%)	11 (2%)	19 (4.22%)	3 (1.5%)	90 (4.5%)
Aparasitaemic	744	538	431	197	1910
Total	801	549	450	200	2000

Pearson chi square ($\chi 2$) = 24.9867 P = 0.000

Table 4. Microscopic prevalence of different species of trypanosome infection in selected districts of South Western Ethiopia

Study areas		N	*T.c*	*T.v*	*T.v/T.c*	*T.th*	Number of negative animals
Zone	Gurage	1800	55 (3.1)	13 (0.7)	9 (0.5)	10 (0.6)	1713
	South-West Shoa	200	0 (0)	3 (1.5)	0 (0)	0 (0)	197
District	Abeshage	801	36 (4.5)	12 (1.5)	9 (1.1)	0 (0)	744
	Enemor-Ener	549	11 (2.0)	0 (0)	0 (0)	0 (0)	538
	Cheha	450	8 (1.8)	1 (0.2)	0 (0)	10 (2.2)	431
	Ameya	200	0 (0)	3 (1.5)	0 (0)	0 (0)	197
Total		2000	55 (2.8)	16 (0.8)	9 (0.4)	10 (0.5)	1910

T.v= Trypanosoma vivax, T.c.= Trypanosoma congolense, Tth= Trypanosoma theileri, T.v/T.c (mixed*)=Trypanosoma vivax, Trypanosoma congolense*

Prevalence of trypanosomosis did not significantly differ between calves, young and adult animals (p=0.708) (Table 5). Similarly, body condition (P = 0.584) (Table 6) and sex (P=value = 0.165) had no significant influence on the prevalence of the disease when parasitological technique was used to detect parasites in blood (Table 5).

Table 5. Prevalence of bovine trypanosomosis in different age, sex and body condition of animals examined in four districts south western Ethiopia.

Parameters		Parasitaemic	Aparasitaemic	X^2 value	P-value
Age	Calf	5(4.4%)	108	0.6904	0.708
	Young	17(3.8)	431		
	Adult	68(4.7%)	1371		
Sex	Male	43(5.2%)	772	1.9278	0.165
	Female	47(4%)	1138		
Body condition	Good	55(4.8)	1100	1.0746	0.584
	Lean	5(3%)	162		
	Cachectic	30(4.4%)	648		

4.1.2. *Haematological findings* (PCV)

The packed cell volume ranged between 5% and 31% for parasitologically positive animals and 15-42% for aparasitemic animals at the time of sampling. Generally, the overall mean PCV was significantly reduced in parasitaemic animals compared to aparasitaemic animals (P=0.000) (Table 6).

Table 6. Mean PCV of parsitaemic and aparasitaemic cattle in the four study districts

Infection status	**No. of animals**	**Mean PCV%**	**95% CI**	**Reg. coef.**	**P-value**
parasitaemic	90	22.6	21.46-23.73	3.00	0.000
aparasitemic	1910	25.6	25.41-25.78		
Total	2000				

Species based comparison has also proven that except *T. theilerie*, both *T. congolense* and *T. vivax* have resulted in significant anaemia compared to parasitologically negative cattle (Table 7).

Table 7. Mean PCV of animals infected with different species of trypanosomes and non-infected animals.

Infection type	**No of animals**	**% PCV**		**P-value**
		Mean	S D	
Negative	1910	25.60	4.04	Ref
T.congolense	55	22.25	5.515	0.011
T.vivax	16	20.00	4.872	0.001
T.vivax/T.congolense	9	24.56	4.927	0.330
T.theilerie	10	26.90	3.929	0.845
Total	90			

4.1.3. *Detection of trypanosomes by PCR-RFLP.*

Among the 75 samples detected positive by Woo technique and verified by PCR-RFLP, 57 (76%) were found to be positive by the latter. On the other hand, out of the 151 samples considered negative by the parasitological technique, 29 (19.2%) were found positive by PCR-RFLP. Comparison of the two diagnostic tests also indicated that the detection efficiency of these diagnostic tools varies from species to species of the trypanosomes (Table 8). By Woo technique, it was easier to identify *T. congolense* much better than *T. vivix* or mixed infection of the two species. *T. theileri* was often confused with *T. vivix* under Woo technique whereas PCR seems to miss some positive cases. In this regards, out of the 55 samples that were taken as positive for *T congolense* by Woo technique, only 74.5%were found positive by PCR-RFLP of which only 78% were *T.congolense.*

Table 8. Detection and confirmation of trypanosomes by PCR-RFLP technique.

Woo test	**PCR-RFLP**						
	Negative	Positive	*T.congo.*	*T.vivax*	*T.v/T.c*	*T.theileri*e	Unknown
Negative	122	29	22	3	0	4	0
	(80.8%)	(19.2%)	(75.9%)	(10.3%)		(13.8%)	
Positive	18	57	36	10	5	5	1
N=75	(24%)	(76%)	(63.2%)	(17.5%)	(8.8%)	(8.8%)	(1.8%)
T.congo	14	41	32	3	3	2	1
N=55	(25.5%)	(74.5%)	(78%)	(7.3%)	(7.3%)	(4.9%)	(2.4%)
T.vivax	3	8	2	3	0	3	0
N=11	(27.3%)	(72.7%)	(25.0%)	(37.5%)		(37.5%)	
T.v/T.c	1	8	2	4	2	0	0
N=9	(11.1%)	(88.9%)	(25%)	(50%)	(25%)		

T.v= *T. vivax*, T.c. *T. congolense*

4.2. **Trypanocidal Drug Utilization Practices**

The questionnaire survey revealed that for all respondent farmers communal or free grazing was the major livestock management practice in the study villages and trypanosomosis was ranked as biggest animal health constraint among other livestock diseases prevailing in the area. For the control of trypanosomosis, all respondents ascertained to depend mainly on the two trypanocidal drugs diminazene aceturate and isometamidium chloride rather than other methods of control. The majority of respondents of all villages (56%; 95% CI= 46.3-65.7%) get trypanocidal drugs from unauthorized markets (Figure 7).

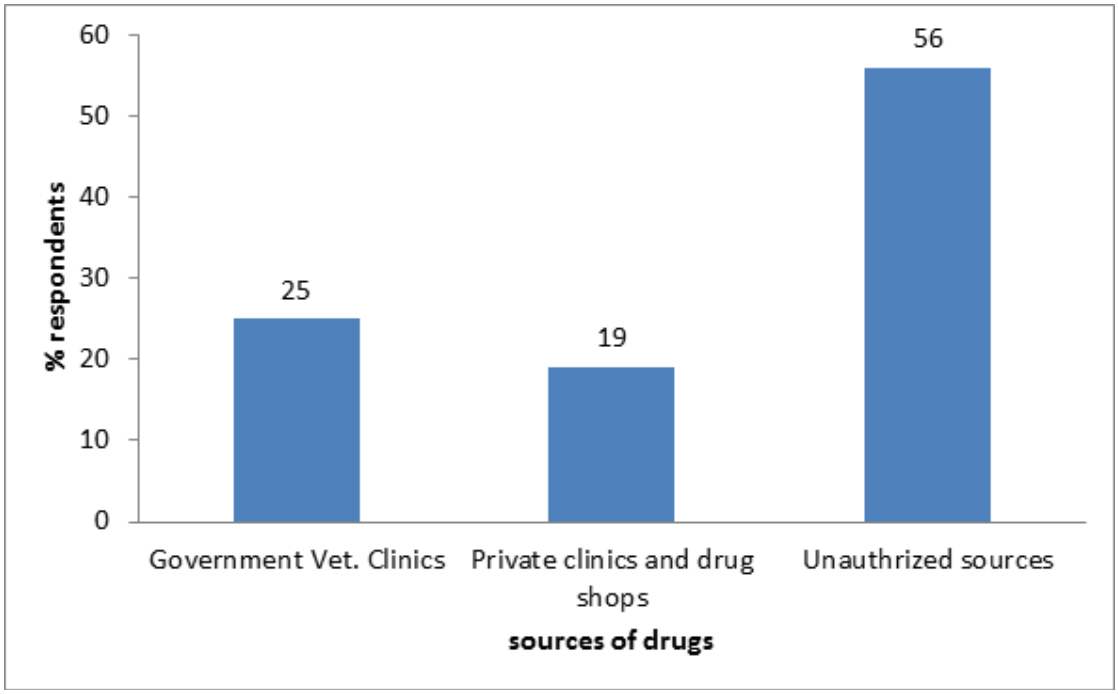

Figure 7. Questionnaire response on the sources of trypanocidal drugs from hot spot areas.

Diminazene aceturate was the most preferred drug because of the cheap price and availability of the drug at a single dose in all sources (79%) over ISM (21%). On the other hand, significant numbers of farmers in the study villages 59% (95%; CI= 49.4-68.6%) administer trypanocidal drugs by themselves or through family members because of the distance of the veterinary service station from their village (Figure 8) and about 85% of them (95% CI= 78-92%) confirmed to treat their animals more than six times per year (Figure 9). All respondents revealed that perceived treatment failures are common. In this respect, 58% of the interviewed farmers believe

that treatments were more likely to be successful when the drugs are sourced from government veterinary clinics and authorized private sources than from unauthorized open markets.

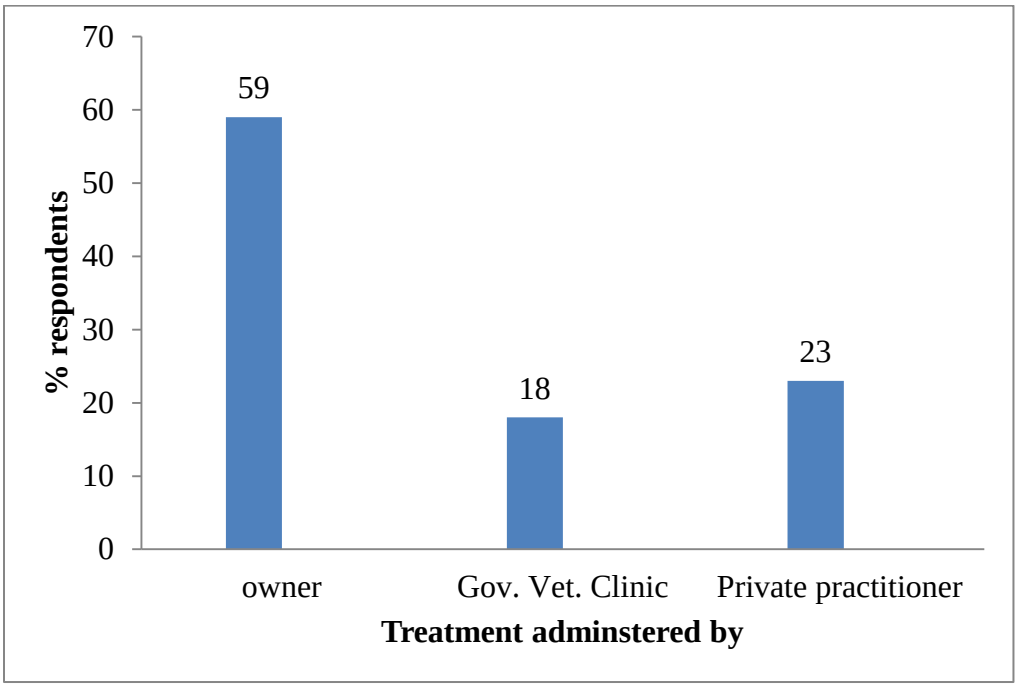

Figure 8. Questionnaire response on administration of trypanocidal drugs on cattle

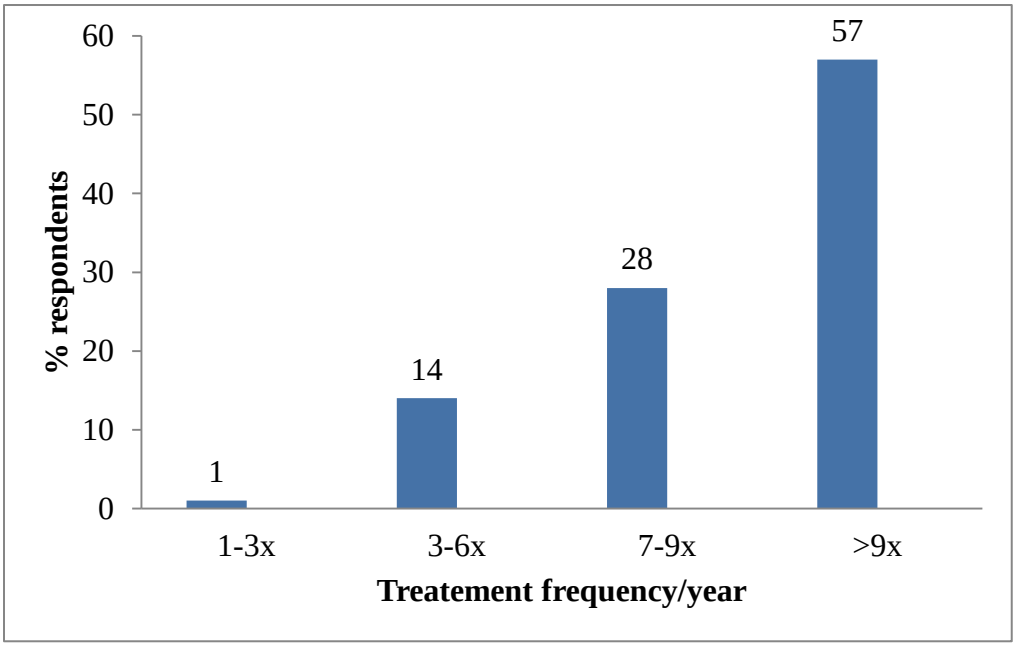

Figure 9. Questionnaire response on trypanocidal drug treatment frequencies/animal/year in the hot spot areas

4.3. Trypanocidal Drug Quality Assessment

From the 50 trypanocidal drug samples collected, 26 were from European companies and 24 from Asian companies. The 50 samples represent 19 different trade names (9 for ISM and 10 for DIM). Although galenic tests show all drugs were upto the standard because they had good solubility, the overall result showed that 28% (14/50) of the drugs were non-compliant due to insufficient active ingredient detected by HPLC. The difference in non-compliance between the two drugs was not significant (P>0.05), being 27.3 % for DA and 29.4% for ISM. Similarly, clients of authorized and non-authorized markets are at similar risk of purchasing non-compliant trypanocides (Table 9).

Table 9. Proportions of non-compliant samples according to the marketing channel

Source	**Drugs**		**Total**	**Non-compliance (in %)**	**P-value**
	ISM	DIM			
Authorized	10	15	25	24	
Unauthorized	7	18	25	32	
Total	17	33	50	28	P>0.05

The logistic regression analysis revealed differences in drug compliance depending on the origin of pharmaceutical companies (P<0.05) (Figure 10). Accordingly, trypanocides from Asia are much less compliant than those from Europe (P< 0.05). This was especially the case for ISM where 55.6% of the tested products from Asia did not comply with standards. However, the interaction between marketing channel (official and unofficial) and trypanocidal origin (Asia and Europe) shows no significant association.

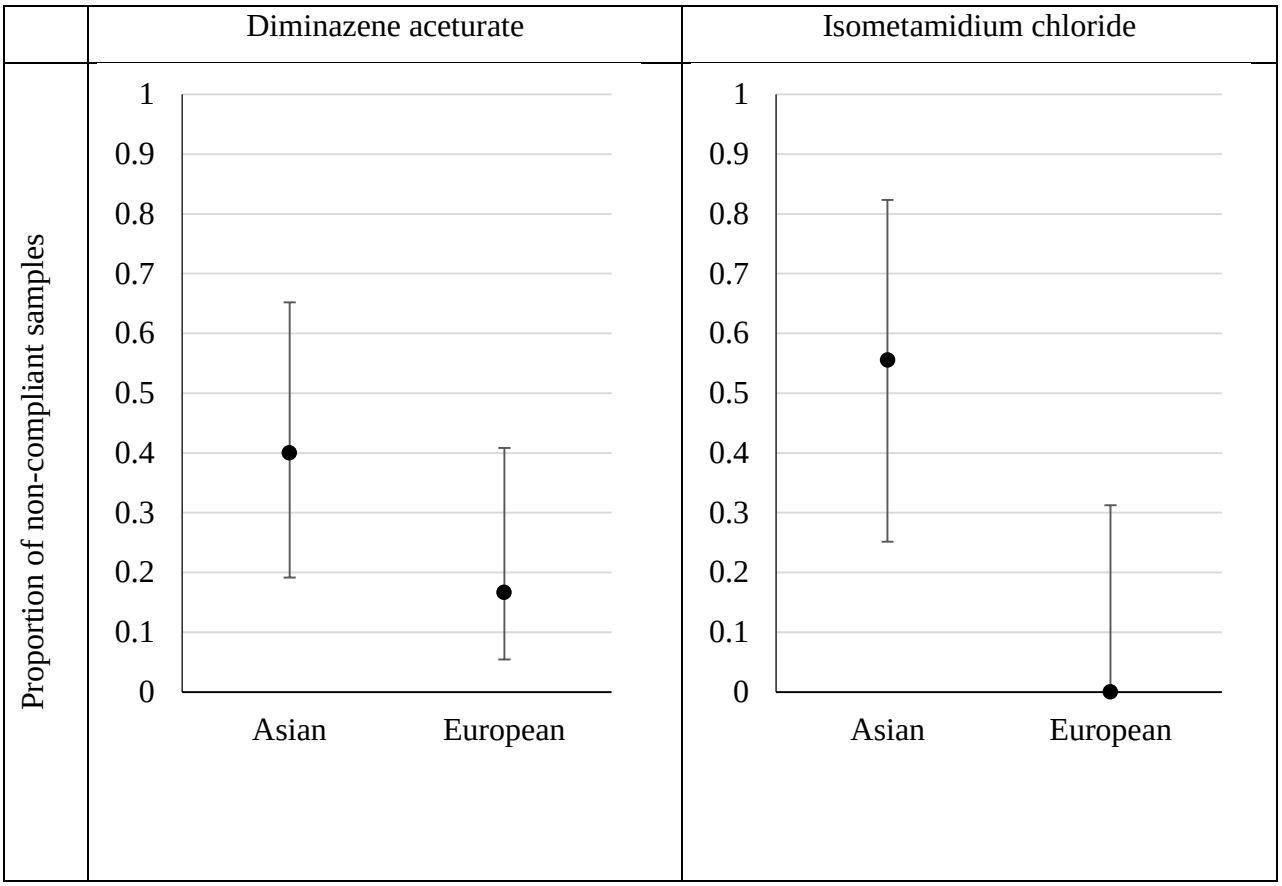

Figure 10. Proportion of trypanocidal drugs compliance by the origin of the country.

4.4. Trypanocidal drug sensitivity study

4.4.1. Parasitological responses to drug treatments

Trypanocidal treatment trial was undertaken using an abbreviated 28 days field protocol to verify the presence of trypanocidal drug resistance using 80 *Trypanosoma congolense* positive cattle from Abshege district. The overall findings revealed multiple drug resistance to recommended doses of Diminazine aceturate and Isometamidium chloride (Table 10). Accordingly, out of 40 trypanosome-positive cattle treated with DA at a dose of 3.5 mg/kg body weight, 47.5% had persistent infections at day 14 post treatment 1. Similarly, of the 40 trypanosome-positive cattle

treated with isometamidium chloride at a curative dose of 0.5 mg/kg body weight, 27.5% had persistent infections at day 14 post treatment. There was no significant difference between the five villages in this regards. All cattle that showed treatment failure against curative dose of ISM were subjected to 3.5 mg/kg body weight DA whereas those that have resisted recommended dose of DA were again treated with 0.5mg/kg body weight ISM. After another 14 days (post treatment 2), five animals from each group had still relapsing parasitaemia suggesting multiple resistance.

Table 10. Proportion of trypanocidal drug resistance following treatment with diminazine aceturate and curative dose of isometamidium chloride as recommended by the manufacturer

Treatment schedule	**Drug**	**No. of animals**	**Relapsing cases**	**% relapse**
Treatment 1 (Day 0)	DA	40	19	47.5
	ISM	40	11	27.5
Treatment 2 (D28 After Relapse)	DA	11	6	54.5
	ISM	19	5	26.3

4.4.2. Packed cell volume of treated animals

The mean PCV of parasitaemic (relapsing) animals 14 days post treatment with Diminazine aceturate at 3.5 mg/kg body weight was significantly lower than those animals rendered parasitologically negative following the same treatment ($P<0.01$). The same scenario was observed including day 28 following treatment with Isometamidium chloride ($P<0.05$) at curative doses of 0.5 mg/kg body weight (Table 11).

Table 11. Mean PCV of parasitaemic cattle treated with Diminazine aceturate at 3.5 mg/kg body weight and Isometamidium chloride at 0.5 mg/kg body weight.

Parameters	Groups	Treatment response	No of animals	Mean PCV (%)	95% CI		SD	Reg coeff	P Value
					LL	UL			
	DA	Positive	19	21.8	20.36	23.33	3.2		0.004
Day 14	N=40	Negative	21	25.1	23.55	26.64	3.5	-3.253	
PT1	ISM	Positive	11	22.3	21.32	23.22	1.6	-2.279	
	N=40	Negative	29	24.6	23.4	25.7	3.2		0.028
	ISM	Positive	5	18	15.9	20.1	2.45		0.000
Day 28 (Day 14 PT2)	N=19	Negative	14	25.0	23.83	26.16	2.0	-7	
	DA	Positive	6	20.8	18.64	23.02	2.40		
	N=11	Negative	5	24.0	18.82	29.18	5.20	-3.167	0.213

5. DISCUSSION

Trypanosomosis is a major constraint to the utilization of large land resources as it severely affects livestock, particularly cattle which have a major role in the agricultural economy of Sub-Saharan Africa (Jemal and Hugh-Johns, 1995). The field control of animal trypanosomosis has, over the years, relied on two broad strategies; using chemotherapeutic agents on infected animals and the cyclical vector control. However, despite many efforts and often huge investment, the problem is still rampant probably as a result of inadequate and/or inappropriate implementation of control strategies and the nature of the parasite and the vectors themselves. It is therefore, necessary to undertake a thorough periodic investigation into prevalence of the disease, and problems contributing to the persistence of the parasite in endemic areas.

5.1. Current situation of bovine trypanosomosis in the study areas

The present study reports a mean prevalence of bovine trypanosomosis ranging between 1.5% and 7.15% at the time of sampling. This is in concordance with previous reports (Afework *et al*., 2000; Tewelde, *et al*., 2004; Dagnachew, *et al*., 2005; Chernet, *et al*., 2006; Shimelis, *et al*., 2011). *Trypanosoma congolense* was the dominant species over *T.vivax*. Similar findings were reported in tsetse infested areas of Ethiopia (Dagnachew, *et al*., 2005; Fikru *et al*., 2012). The predominance of *T. congolense* infection in cattle may be due to the high number of serodomes of *T congolense* as compared to *T.vivax* (Gardiner, 1989). The lower prevalence reported for *T.vivax* is in agreement with various workers done in tsetse infested areas (Cherenet *et al*, 2006; Sinishaw *et al*, 2006). On the other hand, trypanosome infection rate was much more significant in Abeshege district than the other three districts included in this study. This might be related to tsetse fly distribution as the area is bordering the Gibe Valley (Rowlands *et al*., 1993). This is further supported by the fact that most of the infections in Abeshege were due to *T.congolense* as compared to the other study sites suggesting the existence of the cyclical vector, tsetse flies.

Animal trypanosomoses is known to cause significant anaemia in infected animals (Cherenet *et al*., 2006; Dagnachew, *et al*., 2005; Sinishaw, *et al*., 2006; Degu, *et al* 2006). Anaemia appears

with progressing parasitaemia and there is lysis of large numbers of red blood cells resulting in a drop in PCV%, haemoglobin and RBC counts (Silva *et al*., 1999) which may result from massive erythrophagocytosis by an expanded and active mononuclear phagocytic system of the host. In support of the above fact, this study has shown that animals detected parasitaemic with parasitological technique had significantly reduced PCV as compared to those categorized as aparasitaemic by the same technique. Rowlands, *et al*. (2001) in Ghibe observed that with a decrease in PCV value, the proportion of infected animals increased and hence showing the direct relationship between trypanosome infection and development of anaemia *T. congolense* and *T. vivax* were the main species of trypanosomes responsible for the occurrence of trypanosomosis in the study area.

5.2. Perception and practices of farmers on trypanocidal drug utilization

Several control approaches, with varying successes, are available to overcome the impacts of African animal trypanosomosis and its biological vector. Treatment using trypanocidal drugs such as diminazene aceturate and isometamidium chloride is often times the only method available to farmers for containing the disease in many parts of Ethiopia. In this respect, there is a growing risk of drug resistant trypanosomes as already reported by previous studies (Shimelis *et al*., 2015; Moti *et al*., 2012 ; Desalegn *et al*., 2010; Shimelis *et al*., 2008; Tewelde *et al*., 2004; Afework *et al*., 2000). This study assessed trypanocidal drug utilization practices in selected animal trypanosomosis hot-spot areas in the south western part of Ethiopia. In agreement with previous reports from other parts of the country (Zewdu *et al*., 2013), trypanosomosis was ranked by the local farmers as biggest animal health constraint in the study areas. For the control of the disease, all respondents reported to depend mainly on the two trypanocidal drugs; diminazene aceturate and isometamidium chloride rather than other control methods. It was observed that risk factors for drug resistance such as the presence of unofficial drug sources and frequent treatments are unequivocally prevalent in the areas studied. A similar report was documented by Zewdu *et al.,* (2013) where annual treatment frequencies range between one and 12 injections/animal and where a significant number of farmers gave injections on their own without cosulting a veterinarian. Accoring to Uilenberg *et al*., (1998) and Holmes *et al*. (2004), a

high number of annual trypanocidal treatments suggest the development of drug resistance in a given area. Therefore, the high frequency of trypanocidal treatments of more than six per year reported in this study, as well as the indiscriminate access and use of the drugs obtained from unauthorized sources and the practice of treating animals by untrained personnel is likely to increase the risk of trypanocidal resistance in the study areas and neighboring localities.

5.3. Quality of trypanocidal drugs circulating in the local markets

Regular quality assessment of trypanocides is a prerequisite to ensure better management of trypanosomosis and prevention of drug resistance (Clausen *et al*.., 2010). accordingly, Although it was below the 71.4% reported in ivory coast (Assoumy *et al*., 2010), 100% reported in Cameroon (Teko-Agbo *et al*., 2009) 70% reported in Senegal (Akoda *et al*., 2008), 40% reported in Togo (Tchamdja *et al*., 2016) and 42.3% observed in Burkina Faso (Teko-Agbo *et al*., 2011), the trypanocidal drug quality test result with 28% non compliance reported in this study is a truly alarming indication of the quality of drugs being marketed in Ehtiopia. Since the drugs were supplied by big companies who are national distributers, such non con compliant products pose a serious threat to successful AAT treatments country wide. The samples were purchased from both authorized and unauthorized markets. The galenic and HPLC tests were performed to confirm the identity and estimate of the dosage of active ingredients. This enabled us to evaluatethe efficacy and safety the subjected products by applying minimal characteristics.

The non-conform veterinary medicinal products identified by this study were found in both authorized and unauthorized markets. This is different from reports from Togo and Mali (Tchamdja *et al*., 2016; Abiola *et al*., 2002) where non-conform drugs were more often found on unauthorized markets. The non-compliance may be attributed to poor storage conditions and the doubtful sources of supply in this market channel.

The 2012 Proclamation for Veterinary Drugs and Feed Administration and Control of Ethiopia capitalizes on regulating “the production, distribution and use of veterinary drugs to ensure the safety, efficacy and quality of the products”. It also focuses on “prevention and control of the

illegal production, distribution and use of veterinary drugs" (Federal Democratic Republic of Ethiopia, 2011). Therefore, the observation of such a significant frequency of non-conform trypanocides undermines the basic objective of the existing law. Although there are encouraging signs, the current situation signals the urgent need to enforce the legislative framework at all levels to reduce and prevent illegal marketing and use of veterinary drugs.

Although this study did not demonstrate significant variation in non-compliance between the two drugs, coupled with the high pressure on diminazene due to its short biological half-life and hence more frequent administration (Suleman *et al*., 2016; Kaur *et al*., 2000), this loss of quality would mean a greater risk for the development of resistance under local conditions. Various brands of trypanocides originating from several countries in Europe and Asia have shown different levels of compliance to drug quality thereby casting doubt on the reputability of some of the manufacturers of veterinary products (Tchamdja *et al*., 2016). In this respect, the detection of non-compliant drugs within samples sourced from wholesalers strongly suggests that some of these drugs, might be defective right from their origin. The impact of such quality defects in veterinary trypanocidal drug products can be very significant. A lack of efficacy will cause financial harm due to loss of animal productivity and mortality, increased dosage and residues in the muscle tissue damaging for consumers when the concentration is above the prescribed limit (Eisler *et al*., 1996; Montiero, 1998). Some specialists (Tettey *et al*., 2002) quite rightly believe that the circulation of counterfeit drugs in most sub-Saharan African countries, inevitably leads to the persistence of animal trypanosomosis. This is a proof for urgent need to introduce systematic controls on the supply, distribution and utilization channels of veterinary trypanocidal products in Ethiopia.

5.4. Sensitivity of *T.congolense* against diminazine aceturate and isometamidium chloride

The present study employed an abbreviated 28 days field protocol to assess resistance to recommended doses of Diminazine aceturate (3.5 mg/kg) and Isometamidium chloride (0.5 mg/kg) in trypanosome-positive cattle. This method was choosen because of several reasons: 1) it takes only one month to generate results at a reasonably low cost, 2) the abbreviated protocol

uses cattle of known trypanosome infection status in comparison to longitudinal studies that start off with susceptible animals to follow them for eventual trypanosome infections and 3) the efficacy of both Diminazine aceturate and Isometamidium chloride was evaluated simultaneously, making it possible to estimate the resistance of trypanosomes to both drugs.

Accordingly, single and multiple drug resistant populations of *T. congolense* were detected in cattle at Abeshege district. In the absence of parasite clearance from blood, PCV of such animals was significantly reduced suggesting that treatment, when effective, have contributed for betterment of the PCV in clinically cured animals. This finding is in agreement with several other previous reports in the country (Afework *et al*., 2000, Tewelde *et al*., 2004; Moti *et al*., 2012) and elsewhere in Africa (Mc Dermott *et al*., 2003; Diall *et al*., 2003; Grace, 2005; Knoppe *et al*., 2006). It is also in line with the malpractices of farmers in the use of trypanocidal drugs (frequent injections by untrained farmers) in the study areas and the poor quality of some of the trypanocidal brands imported from Asian countries (Geerts and Holmes, 1999; Van den Bossche *et al.,* 2000; Tchamdja *et al*., 2016).

The abbreviated 28 days field protocol to assess resistance seems to have limitations linked to under estimating the resistance situation as observed by Gall *et al*. (2004) and Knoppe *et al*. (2006). This could be due to the fact that it is based on a parasitological technique that can not detect trypanosomes when parasitaemia is low (Paris *et al*., 1982) and some animals may need more time to develop detectable parasitaemia especially when it is in the chronic stage. We believe that more resistance cases could have been detected if molecular techniques such as PCR (Desquesnes and Dàvila, 2002) were used. In support of this argument, this study has already demonstrated that significant number of animals tested negative by the Woo technique were turned out to be positive by PCR during the cross-sectional survey.

In general, it is strongly believed that inappropriate trypanocidal drugs use (drug administration by untrained personnel, high treatment frequency, etc) by the farmers and supply of low quality products by traders have partly culminated into the persistence of trypanosomosis and consequently is contributing to loss of production and productivity of the livestock in the areas. The demonstration of resistance to both drugs (multiple resistance) in individual trypanosomes

suggests that the concept of use of sanative pair (Mamoudou *et al*., 2008) is no longer efficacious in at least in the areas included in this study. Similar observations were also noted from the nearby Ghibe valley of Ethiopia (Mulugeta *et al*., 1997) and coastal areas of Kenya and Tanzania (Mdachi, 1999; Eisler *et al*., 2000).

Owing to the high costs of developing and licensing new trypanocides, there is little interest for pharmaceutical companies in developing new trypanocides for use in either animals or humans. Hence, concerted efforts must be made to conserve the efficacy of existing drugs to prevent further escalation of the problem due to complete loss of efficacy. Multiple drug resistance can be contained by minimizing disease transmission, as was demonstrated in Ethiopia by Peregrine *et al*. (1994), Burkina Faso by Bauer *et al*. (1995), and in the Mkwaja ranch in Tanzania by Fox *et al*. (1993).

6. CONCLUSION

Trypanosomosis is one of the major parasitic diseases of livestock in many parts of Ethiopia for which control is still far from a reality. Despite extensive efforts to control the transmission of trypanosomes, the spread of parasite populations resistant to drugs and the lack of effective vaccines against them contribute to their persistence as major livestock health problems. The present study attempted to evaluate the situation of cattle trypanosomosis in south western part of Ethiopia and assessed major contributing factors to the development of trypanocidal resistance. The cross sectional study revealed that bovine trypanosomosis was prevalent with different magnitude within the study districts. *T.congolense* followed by *T.vivax* was the dominant species of trypanosomes responsible for the occurrence of the disease in cattle. Cognizant of the severity and prevalence of trypanosomosis and owing to the free access to trypanocidal drugs on open markets, farmers in the study area are using both diminazene aceturate and isometamidium chloride for many years at an alarming frequency/animal per year, often injecting their animals without veterinary assistance. Prompted by the above fact, the quality of trypanocidal drugs circulating on the local markets was assessed. This unequivocally revealed that counterfeit drugs (both DA and ISM) with significantly reduced active ingredient are widely distributed and legal importers are not immune from this fact especially when they import from some Asian companies. Since improper utilization and under dosage are the main factors contributing to drug resistance, the above observations have further led to trypanocidal drug efficacy/resistance study. This last section of our study has undoubtly confirmed the wider prevalence of trypanocidal resistance often with strains resistant to both trypanocides. Altogether, the findings of this study demonstrate that trypanosomosis is an important problem in the study areas and the available drugs to combat the problem are endangered due to misuse and widespread prevalence of non-conforming trypanocides.

7. RECOMMENDATIONS

Based on the above conclusion the following recommendations are forwarded

- The control of trypanosomosis in the study area necessitates intensification of vector control activities and restriction of the use of trypanocides to clinical cases coupled with rational utilization of the few available trypanocidal drugs.
- Corrective measures must therefore be taken towards effective implementation of regulatory and legislative frame works towards:
    - quality control of veterinary trypanocidal drugs
    - improving the coverage and quality of veterinary services and
    - regular monitoring of trypanocidal drug efficacy

  To conserve the efficacy of the few existing trypanocides, a veterinary extension program should educate all livestock owners on the threats of drug resistance and appropriate procurement, handling and use of the drugs.
- Efforts should be intensified for the discovery of new trypanocidal drug compounds and or effective vaccines.

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9. APPENDICES

9.1.Questionnaire survey

Questionnaire survey on the trypanocidal drug utilization assessment in the five hot-spot villages of Abeshege district, Gurarage zone, south west Ethiopia.

Respondents name_____

Region_____Zone_____Ditrict_____

Village_____

Have _____ cattle_____Sheep_____Goat_____

Q. 1. Sources of trypanocidal Drug

A. Vet clinics B. Private vet. Pharmacies C. Open markets D. other, specify----------

Which one is more effective in your opinion? _____

Q. 2. How often you treat your animals per year

A. 1-3 B. 4-6 C. 7-9 d. >9

Q.3. Who is treating your animals

A. My self B. Vet personnel C. Others

Q . 4. Which of the trypanocidal drug are you using?

A. Brown B. Yellow C. Other

Q.5. Why?

Q6. How is the condition of your animals after treatment?

A. Improved B. No change

Q7. Do you think the drugs are as effective as before? A. Yes B. No_____

Questionnaire filled by_____

Position_____+

Signature_____

9.2. Cross sectional survey format

Data collection format for the cross sectional study

Zone _____ Altitude_____

District_____ Longitude_____

Kebele_____ Latitude_____

Village_____ Breed_____

Date of collection_____ Species_____

S.N	Owners name	Age	Sex	Body weight	PCV	Infection				
						T,c	*T.v*	*T.b*	Mixed	*T.th*

9.3.Trypanocidal drug quality survey format

Zone _____ Altitude_____

District_____ Longitude_____

Kebele_____ Latitude_____

Village_____

Sample ID	Brand Name	Manufacturer name	Trypanocide	Quantity on package	Unit(gm or mg)	Batch (Lot)	Manufacturing Date	Exp.date	City of purchase	Purchase from	Made in	Date of purchasing

9.4.Block treatment data collection format

Zone _____ Altitude_____

District_____ Longitude_____

Kebele_____ Latitude_____

Village_____

Animal ID	Owners name	Sex	Age	Body condition	weight	Infection/ day zero	PCV/day zero	Type of drug	Infection day 14	PCV day 14	Infection day 28	Type of drug	PCV

9.5.Comparison between diminazene and isometamidium-based injectable preparations subjected to the limpidity test

9.6. Diminazene-based injectable preparations (poor limpidity on the left with residue present, and limpid solutions on the right)

9.7. Picture showing an illicit drug saller, with many brands of trypanocidal drugs

10. CURRICULUM VITAE

Telahun Tekle was born in Addis Ababa, Ethiopia on the 1st of June/1962. After he finished secondary school in Addis Ababa, Ethiopia and he obtained a DVM degree in 1986 in the University of Camaguey Cuba and Master's degree in Tropical Veterinary epidemiology in 2007 at Addis Ababa University. After his studies, he worked in several institutions such as district veterinary officer, regional animal health research officer and researcher at the National Animal health Diagnostic and Investigation Center, Sebeta.

Telahun started his PhD in January 2013 in a joint program under the supervision of Dr. Getachew Terefe and Dr. Hagos Ashenafi at the College of Veterinary Medicine and Agriculture of the Addis Ababa University and Dr. Thomas Cherene from the Federal Ministry of Agriculture and from the Institute of Tropical Medicine Vincent Delespaux and Prof. Jan Van Den Abelle. The topic of his PhD project is "TRYPANOSOMOSIS AND TRYPANOCIDAL DRUGS: DISEASE PREVALENCE, DRUG QUALITY, EFFICACY AND UTILIZATION PRACTICES IN CATTLE IN SELECTED HOT SPOTS OF SOUTH-WESTERN PART OF ETHIOPA". The PhD project is funded by Trypanosomosis Rational Chemotherapy (TRYRAC) which is part of the European Union-funded initiative "Global Programme on Agricultural research for Development (ARD) that supports agricultural research on global basis" and supplemented by the Addis Ababa University.

11. PUBLICATIONS

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