EPIDEMIOLOGY OF BOVINE TRYPANOSOMOSIS IN SELECTED DISTRICTS OF BENSHANGUL GUMUZ REGION WITH EMPHASIS ON TRYPANOSOMA CONGOLENSE TRYPANOCIDAL DRUG RESISTANCE AND ALTERNATIVE THERAPY IN MICE

MSc THESIS

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Bishoftu, Ethiopia

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A thesis submitted to the college of veterinary medicine and agriculture Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in veterinary epidemiology

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As members of the examining board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by **Abebe Bulcha Hirpa** entitled ***Epidemiology Of Bovine Trypanosomosis In Selected Districts Of Benshangul Gumuz Region With Emphasis On Trypanosoma Congolense Trypanocidal Drug Resistance And Alternative Therapy In Mice*** and recommend it to be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Veterinary Epidemiology and clinical medicine.

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STATEMENT OF AUTHOR

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LIST OF ABBREVIATIONS

AAT	Africa Animal Trypanosomosis
BSF	Bloodstream Forms
CNS	Central Nervous System
CSA	Center of Statics Agency
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
EMF	Epimastigote Forms
GDP	Gross Domestic Product
Hgb	Hemoglobin Concentration
IgM	Immunoglobulin M
ISMM	Isometamidium
ITSs	Internal Transcribed Spacers
ITT	Insecticide-Treated Traps and Targets
KDNA	Kinetoplast DNA
KM	Kilometer
MCF	Metacyclic Forms
MCHC	Mean Corpuscular Hemoglobin Concentration
P2	nucleoside transporter system
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PF	Procyclic Forms
RBC	Red Blood Cell
SIT	sterile insect technique
T. brucei	*trypanosome brucei*
T. godfreyi	*Trypanosome godfreyi*
T.congolense	trypanosome congolense
TbAT1	Trypanosoma brucei Adenosine Transporter 1
TcoAT1	Trypanosoma congolense Adenosine Transporter 1

TDR	Trypanosomosis drug resistance
USD	United States Dollar
Vmax	Maximum Volume
VSG	Variant surface glycoproteins
WBC	White blood cell

ABSTRACT

For the present study questionnaire survey, cross-sectional and experimental trials were carried out from January - May 2018: to assess the problem of bovine trypanosomosis, to determine the prevalence of bovine trypanosomosis; to assess trypanocidal drug resistant and to evaluate the effectiveness of herbal remedies extracted used for the treatment of bovine trypanosomosis. Stata version 12 was used to analysis data collected from the fled and laboratory. For the questionnaire survey 80 individuals were interviewed, focused on the constraints of trypanosomosis in the area. For parasitological survey, blood samples of 430 cattle were examined using buffy coat technique and wet blood smear. The packed cell volume (PCV), of animal sampled on field and in laboratory animals were recorded using hematocrit reader. The level of parasitaemia, body weight, packed cell volume and mean survival period of experimental animals were monitored. The overall prevalence of trypanosomosis in the study area was found to be 7.44% ($P = 0.023$). The mean PCV value (%) of parasitaemic and aparasitaemic animals during the study period were 23.12% and 26.55% respectively, which is statistically significant ($p<0.05$). *H.villosa* chloroform extracted given at a dose of 600 mg/kg bw, reduced parasitaemia 24.6 ± 8.45 ($p < 0.05$). The in vivo drug resistance tests indicated the presence of resistant parasites with the normal dose against DA isolate and suspected resistance problems were detected against ISM and DA for and isolates respectively. It was concluded that the present work evidenced that trypanosomosis has continued to pose a considerable threat to cattle production in the study area warranting an integrated control and prevention to safeguard cattle production and productivity. Therefore, trypanosomosis is a major animal health problem in study area and drug resistance is a threat in the control of trypanosomosis in both study areas. The search for alternative compounds against African trypanosomosis is justified by various limitations of existing chemotherapeutic agents. Efforts should also be directed at evaluating the possible benefits of natural products in trypanosomosis treatment.

Key words: *H.villosa, prevalence, trypanosomosis*

1. INTRODUCTION

Trypanosomosis is one of the major protozoan and neglected tropical disease that impediments to agriculture and livestock production in Africa (Shaw *et al.*, 2013). Even though the rapid human population increase and urbanization in sub-Saharan Africa are believed to increase the demand for livestock products (Thornton, 2010), this disease negatively affects the overall development in agriculture in general and to the food self-reliance efforts of the nation in particular,(Andrews *et al.*, 2008; Geerts and Holmes, 1998).

The continent has about 300 million heads of cattle, 630 million sheep and goats and 140 million camels that play an important role in the life of rural and urban communities (FAO *et al.*, 2015). The livestock sector contributes to about 30 – 50% of the total agricultural Gross Domestic Product (GDP) in some African (Hassane, 2013). Animal by-products such as wool, hides and skins add more economic value of the sector, which is valued to 14 billion United states Dollar (USD) per year of which, 9 billion USD is in the form of meat, milk and leather while 5 billion USD is in the form of organic fertilizer and draft power (AU-IBAR, 2010).

Similarly, livestock sector plays a significant role and has a great potential to assist the economic development and serve as a source of food, income and foreign exchange to the Ethiopian economy and contributes to 15 - 17% of GDP and 35 to 49% of agricultural GDP and 37 to 87% of the household incomes (Sintayew *et al.*, 2010). Ethiopia is home to Africa's largest livestock population and it is the continent's top livestock producer (MacDonald and Simon, 2011). The recent livestock population of Ethiopia estimated to be about 53.9 million cattle, 24.6 million goats, 25.5 million sheep 6.8 million donkeys and 1.9 million horses (Berihu *et al.*, 2014; CSA, 2013; MOA, 2013).

In Ethiopia, trypanosomosis is one of the most important diseases that limit livestock productivity and agricultural development due to its high prevalence in the most arable and fertile land of southwest and northwest part of the country; following the greater river

basins of Abay, Omo, Ghibe, and Baro (Abebe and Jobre, 1996)**.** Approximately 15 % of all arable land is under tsetse and trypanosomosis challenge in Ethiopia. Extensive research as well as trypanosomosis control programs has been carried out in different parts of the country and in the Ghibe valley in particular (Tadesse and Tsegaye, 2010).

Tsetse flies and a large biting flies that inhabit 37 countries in Sub-Saharan African Country (SSA) and they inhabit an estimated 8.7 million km^2 (Rogers and Robinson, 2004). Currently the distribution of tsetse in Ethiopia covers up to 200,000 km^2 in the southwest and north west parts of the country (Abebe, 2005; Sciarretta *et al.*, 2005). Four species, *G. morsitans submorsitans*, *G. pallidipes*, *G. fuscipes fuscipes* and *G. tachinoides* are responsible for the transmission of animal trypanosomosis in Ethiopia (Dinka & Abebe, 2005).

The menace of Africa Animal Trypanosomosis (AAT), still constitutes a major obstacle to food security in spite of previous attempts towards chemotherapy and tsetse control (Nakayima, 2016). Parasite control currently relies on a small group of trypanocidal compounds, and new compounds are unlikely to become available in the near future (Barrett *et al.*, 2004).

In trypanosome endemic areas, trypanocidal drugs; both prophylactic (Isometamidium chloride) (Young and Godfrey, 1983) and curative (Diminazene aceturate) (Berihu *et.al.*, 2014), are the most widely used methods of animal trypanosomosis control (Clausen *et al.*, 2010). Trypanosomosis is controlled also either by vector or parasite control, or a combination of both. various efforts to control the disease and the associated economic losses have been directed mainly against the parasite through trypanocidal drugs and against the vector through odour- baited, insecticide- impregnated targets/traps/ and insecticide-treated cattle (Shaw *et al.*, 2015; Meyer *et al.*, 2016).

According to (Faria *et al.*, 2014), now a days toxicity and resistance to the commonly used trypanocidal drugs haven been emerged in sub-Saharan Africa and interferes with effective veterinary management of trypanosomosis. The problem of drug resistance in trypanosomes chemotherapy appears to be widely spreading geographically to different regions, in which trypanosomosis occurs. At present, there are twenty-one African

countries in which trypanocidal drug resistance has been reported (Delespaux *et al.*, 2008; Chitanga *et al.*, 2011).

In addition, the occurrence of multiple (one or more of the commonly used trypanocidal drugs), trypanosomes parasites resistance has been reported in eleven Sub-Saharan African (SSA), countries: (Burkina Faso, Chad, Côte d'Ivoire, Ethiopia, Kenya, Nigeria, Somalia, Sudan, Tanzania, Uganda, and Zimbabwe) (Delespaux *et.al.*, 2008; Melaku & Birasa, 2013; Afewerk *et al.*, 2014).

Even if, the amount of trypanocidal used in Africa is very high the pharmaceutical industries interest to developing new products remains low and leaving farmers to rely on the existing drugs for many decades. As reported by (Delespaux *et.al.*, 2008; Chitanga *et.al.*, 2011) privatization of veterinary services in most parts of Africa easily access to farmers and this has resulted in rampant misuse and under-dosage of the medications.

Thus, drug resistance in trypanosomes poses a serious problem to livestock productivity unless checked and brought under control (Barrett *et.al.*, 2004). Some of the research conducted reveals that limited success had been achieved, despite enormous efforts by several workers in the field of chemotherapy and allied disciplines to discover or develop an 'ideal' trypanocide (Jennines *et al.*, 1993). In Ethiopia, the appearance of drug-resistant trypanosomes has been reported by several authors (Tewelde *et al.*, 2004; Shimelis *et al.*, 2008; Moti *et al.*, 2012; Dagnachew *et al.*, 2014).

In every effort to discover new drugs for infectious diseases, plant materials are the main focus of the researchers: which contain diverse chemical substances with biological and physiological properties (Maikai, 2010). Different research studies have shown that plants are used in traditional medicine of Africa to treat trypanosomes in humans and animals (Nibret and Wink, 2011).

It is the major component of traditional medicine, including 40,000-70,000 medicinal plants, out of which 20% of them are higher-plant species (Verpoorte *et al.*, 2006). Thus, the search for medicinal plants with trypanocidal activities continues to generate a lot of research interest (Hoet *et al.*, 2018). Although recent reports indicate anti - trypanosomal

activity exists in some medicinal plants (Feyera *et al.*, 2014), the potentials of many other plants used in folkloric medicine in Ethiopia are yet to be investigated (Feyera *et al.*, 2011). Reports by (O'Neill and Lewis, 1993) stated that close to half the world' best-selling pharmaceuticals were either natural products or their derivatives. So it is a vital task to identify and investigation those plants (natural remedies) suitable for use as a source of new drugs, focusing on efficacy and toxicity.

1.1. Statement of the Problems

Bovine trypanosomosis is one of the most economically important and constraint to livestock production in the study area; and its control mainly relay on the use of trypanocidal drugs currently available in the market. However, in addition to its constraints in agriculture and animal rearing, trypanosomosis rapidly developed resistance to multiple drug treatments and complicating the use of the few available drugs for prevention and control of trypanosomosis and the mechanisms of this parasites resistance to trypanocidal drugs were not well known. As a result the main objective of the present study was as indicated below:

General objective

Assessment of bovine trypanosomosis status, state of trypanocidal drugs used and searching for other alternative methods used locally for the control of the disease.

Specific objectives

- To determine the magnitude of bovine trypanosomosis,
- To evaluate trypanocidal activities of selected traditionally used medicinal plant extracts in mice, and
- To assess resistance of trypanosomosis to the existing trypanocidal drugs in the study area.

Hypothesis

- The prevalence of bovine trypanosomosis in both study area is same.
- The trypanosomes parasite found in the study area are not resistance to the existing trypanocidal drugs.
- The efficacy of trypanocidal drugs and herbal remedies used locally is not different.

1.2. Justification

Trypanosomosis is neglected tropical disease that is the most important constraint to agricultural activities and animal production of the country as a whole. Among regions with trypanosomosis, Benshangul Gumuz is the most infected region by this disease (Mulatu, 2016). The economic importance of trypanosomosis is expressed by reducing fertility, decreasing young growth, affect milk yields, poor quality carcass, reducing stamina and working power of animals which ended with the death of animals usually (Lelisa, 2015). As a result of tsetse flies infest a large area of the continent including the arable and fertile land of Africa, its prevalence increases from time that supported by resistance to trypanocidal drugs (Anene, 200). Even though there are several technologies exist for the control of trypanosomosis and tsetse flies, it is very difficult to be applied; because of economic problem as these technologies are so expensive to use and usually biologically unfriend to the environment. Although the use of trypanocidal drugs is the main method for trypanosomosis control, it is threatened by increasing cases of drug resistance (Geerts, 2001). Therefore, this study seeks to investigate the presence of drug resistant strains of the parasites and finds alternative therapy locally used by farmers for the treatment of bovine trypanosomosis, if any, as alternative methods of controlling the disease.

2. LITERATURE REVIEW

1.1. Background

African Animal Trypanosomosis (AAT) or ‘‘Nagana’’ is disease caused by flagellated, extracellular protozoa parasites that live in animals blood, plasma, lymph and tissue of vertebrate hosts (Pathak, 2009). Trypanosomes parasites cause a severe, often fatal disease in domestic animals, unlikely in wild animals cause relatively mild infections. The infected animals were more weakened as the disease progress and become unfit for work, hence the name of the disease "Nagana" which is a Zulu word "powerless/useless" as reviewed by (Steverding, 2008).

1.2. Taxonomy and morphology of Trypanosome congolense

Trypanosomes belong to the family of the Trypanosomatidae, the order of the Kinetoplastida, the phylum of Sarcomastigophora, and the subkingdom of Protozoa (WHO, 2013). Trypanosomes are morphologically distinguishable from each other. The parasite is an elongated, flat, unicellular organism, with a characteristic flagellum (Itard, 1989). They are haemo-flagellated parasites characterized by one nucleus and one flagellum, either free or attached to the parasites body by means of an undulating membrane, which used for species identification (Uilenberg, 1998).

There are variation in size and shape between strains trypanosomes: certain strains present a short flagellum and a more developed undulating membrane (De Waal, 2012), and the average size of trypanosome parasite are 20 μm (WHO, 2013). Depending on Size, *T. congolense* is the smallest of the pathogenic African trypanosomes, with varying size between 12 to 22 μm (AU-IBAR, 2010). Broden’s grouping of *T. congolense* was later revised and trypanosomes belonging to this group were placed in a new subgenus called ‘’Nannomonas’’ from the Greek word ‘’nannos’’ meaning dwarf (Hoare, 1970).

1.3. Etiology of Animal trypanosomosis

The etiological agent of the disease is unicellular and blood borne flagellated protozoan parasite of a genus *Trypanosoma* dwelling in various body and tissue fluids (Baral, 2010). In cattle, the most widespread and most pathogenic to animals is *T. congolense* (Mulugeta *et al.*, 2013), *T. vivax* is the second most important trypanosome to cause *nagana* (Namangala and Odongo, 2014) and to lesser extent with *T. b. brucei* (Losos and Ikede, 1972; Clarkson, 1976; Molyneux and Ashford, 1989). *T. congolense* is accountable for more than 80% of the AAT cases in domestic animals in West, Central and Southern Africa (Simukoko *et al.*, 2007). Simultaneous infection with one or more of these species is not uncommon (Molyneux and Ashford, 1989; Eshetu and Begejo, 2015).

The classification of trypanosomes has been based solely on medical and veterinary features. The genus trypanosoma is subdivided into two sections: namely the Stercoraria and Salivaria, based on how the parasites are transmitted from the insect vector to the mammalian host (Uilenberg, 1998). Salivarian are further divided into four subgenera namely; Duttonella, Nannomonas, Trypanozoon and Pycnomonas (Stevens and Brisse, 2004). Also trypanosomes have been classified into taxonomic groups based upon criteria:(morphology, development in the tsetse fly vector and preference for certain vertebrate hosts) (Hoare, 1972).

Accordingly, Trypanosomatidae is subdivided into two genera: *Trypanosoma* and *Leishmania*, which are classified according to their morphology and range of hosts the parasite, can infect (Smith *et al.*, 2007). The trypanosome species affecting man and domestic animals have been subdivided into two groups, the hematinic group (*T. congolense and T. vivax*), which remains in the plasma and the tissue invading group found in extra and intra vascular spaces (Baltz *et al.*, 1985). Because of their presence in the blood, these invading parasites produce numerous changes in the cellular and biochemical constituents of blood (Ngure *et al.*, 2008).

Table 1: The subgenus of trypanosoma in the salivaria section, species and its pathogenicity in bovine

Trypanosome subgenus	**Trypanosome species**	**Its pathogenicity in Cattle**
Nannomonas	*T.congolense*	+++
	T.simiae	-
Duttonella	*T.vivax*	+++
	T.brueci	+
Trypanozoon	*T.evansi*	++
	T.equiperdium	-

Source:(FAO, 2004): +++ - highly pathogenic, ++ - moderately pathogenic, + - low pathogenic, -: no pathogenic.

1.4. Life cycle of animal *T.congolense*

Trypanosomes has complex life cycle involving a vertebrate host and an arthropod Vector. It involves differentiation into several development stages in both the tsetse vector and the mammalian host. Development of these trypanosomes takes place in the anterior part of the tsetse gut, and infection occurs by inoculation of infected blood from diseased animal to health animal during tsetse fly feeding (Itard, 1989).

In the Stercoraria section, the metacyclic trypanosomes develop in the hindgut and are transmitted via the faeces of the insect vector. The Salivarian parasites develop into the metacyclic stage in the anterior part of the digestive tract of the tsetse fly and they are inoculated via the saliva into the mammalian host (Stevens & Brisse, 2004). The complete life cycle of the trypanosomes contains several phases both in the intermediate invertebrate host (the tsetse fly) and in the mammalian host; four developmental stages: the bloodstream forms (BSF), procyclic forms (PF), epimastigote forms (EMF) and metacyclic forms (MCF).

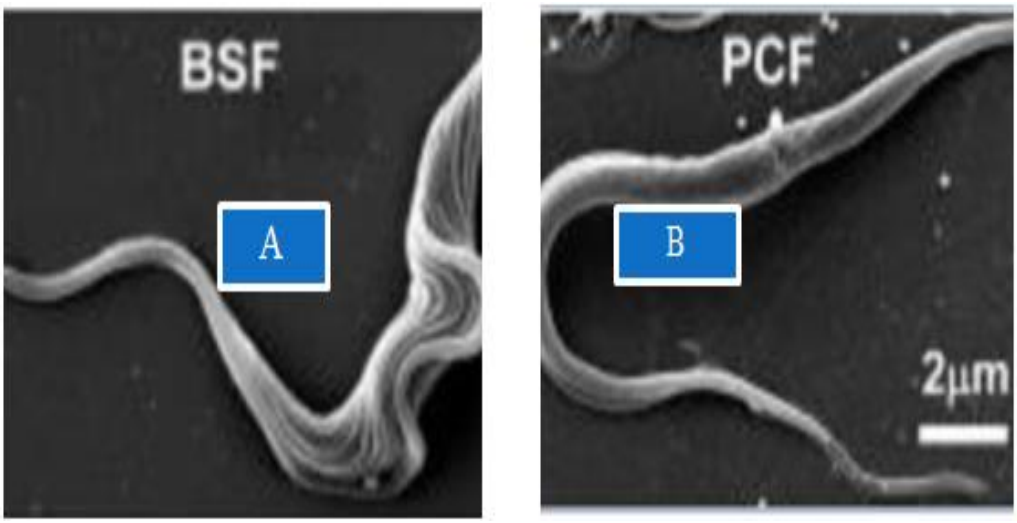


Figure 1: Scanning electro micrographs of the A). BSF versus B). procyclic form (PCF) of African trypanosomes(*Sourse:* Maclean *et.al.* 2013):

When BSF trypanosomes are consumed by a tsetse during its blood meal, they enter the insect mid gut and differentiate to procyclic forms (PF) that are adapted to life in the midgut (Brett *et al.*, 2011). Once established in the fly, a small subset of the PF migrate from the mid gut and begin to differentiate, becoming non-motile epimastigote forms (EMF) that adhere to the fly's salivary glands (Moser *et al.*, 1989) or to the fly's proboscis and mouth parts (Jamal *et al.*, 2005).

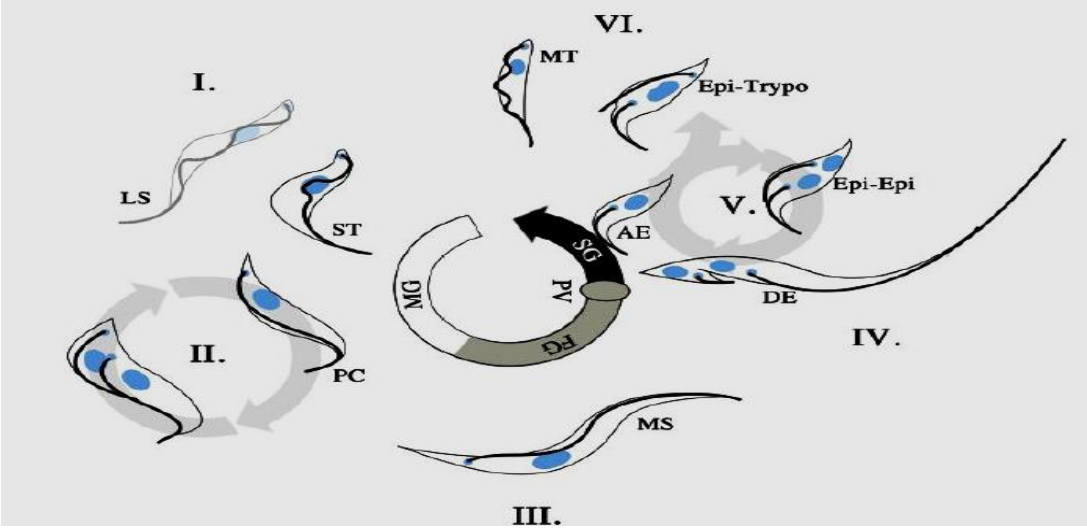


Figure 2: The main phases in the life cycle of the trypanosome, both in the intermediate host (Tsetse fly) and in the mammalian host. (Source: *http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3826061/*):

Life cycle of trypanosomes in tsetse fly: (1), the fly ingests the long slender (LS) and short stumpy (ST), Trypomastigotes forms which are then present in its midgut. The ST forms differentiate into the procyclic trypomastigote forms which are proliferative in nature (Portugal, 1994). After infection is established, the PC trypomastigotes migrate toward the anterior portion of the midgut, elongated into mesocyclic trypomastigotes (MS), which are found in the foregut (Connor, 1989 335 /id), in the process (iii). Once the MS forms arrive at the proventriculus (PV), they differentiate into epimastigote which then divided (DE) asymmetrically into long and short epimastigote (IV). Long and short epimastigotes travel to the salivary glands then undergo normal cell division (Epi-Epi) in order to establish infection in the SG or asymmetrical division (Epi-Trop) to produce infective metacyclic trypomastigotes (MT) (VI) (Hoare, 1972).

Finally, the dividing, adherent EMF differentiate into non-dividing, non-adherent, VSG expressing and infectious metacyclic forms (MCF) and are injected into the host bloodstream when tsetse take a blood meal. The parasites multiply at the site of the fly bite for 5–9 days after which they invade the bloodstream and lymphatic system, becoming BSF and thus completing their life cycle (Brett *et.al.*, 2011).

T. congolense is reproducing by binary fission and according to (Roditi, I. and Lehane, M.J. 2008), for reproduction of trypanosome the exchange and recombination of genetic materials may take place in the tsetse fly between two trypanosomes, but it is unknown how frequently this occurs. The kinetoplast divides first. Then the second para-basal body develops, from which a second flagellum develops. The nucleus divides next, followed by the rest of the trypanosome body duplicating all the structures present in the cytoplasm. The body then divides into two daughter cells, beginning at the anterior end. The process is rapid and may result in a vast population in the host within a short period of time (Roditi and Lehane, 2008). Unlike other trypanosomes, *T. vivax* does not multiply

in the tsetse midgut, but remains confined to the insect proboscis, where it completes its short life cycle (Gardiner, 1989).

1.5. Epidemiology and economic importance of *T. congolense*

The epidemiology of vector-borne diseases is complex due to variability in the ecology of the different actors involved, *i.e*. parasites, vectors and hosts. Due to environmental changes (land use, demographic changes and deforestation) the epidemiology of animal trypanosomosis is changing (Van den Bossche and Delespaux, 2011). Tsetse-borne trypanosomosis is a widespread protozoal disease-complex affecting wildlife, livestock and people in sub- Saharan Africa, with a range of pathologies, from chronic and long lasting to acute and rapidly fatal, depending on circumstances (Bourn *et al*., 2001).

The epidemiology of Africa Animal Trypanosomosis (AAT) in tsetse infected areas of Africa is determined by four biological factors, namely: trypanosomes, tsetse flies, reservoir hosts and livestock. However, cattle are the domestic species in which the disease is most frequently diagnosed and treated. When dealing with the tsetse-transmitted trypanosomosis, much depends on the distribution and the vectorial capacity of *Glossina species* responsible for transmission. Of the three groups of Glossina, the savannah and riverine are the most important since they inhabit areas suitable for grazing and watering (Urquhart *et al.*, 1987).

These factors are modulated by local environmental changes that can substantially affect their dynamics and consequently influence the disease transmission patterns. Anthropogenic environmental changes, such as the increased human pressure and the simultaneous demand for arable lands, result in deforestation and loss of suitable habitats for tsetse flies and their hosts. Considering the cyclical transmission of the parasite, the diversity of tsetse fly habitats considerably affects the interactions among vectors, hosts (livestock and wildlife) and parasites (levels of virulence) (Van den Bossche *et al.*, 2010). For the savannah species, the disease is widespread due to the large dispersion of these flies (Dale and Maudlin, 1999; Dale and Maudlin, 1999).

For the riverine species (*G. palpalis gambiensis, G. tachinoides*), the areas of contacts with the hosts are limited. In this case, the risk of infection does not only depend on the density of the flies, but also on the intensity of space-time interfaces between tsetse and livestock (Dicko, 2016). Tsetse flies are living for millions of years in proximity of wildlife constituting the sylvatic trypanosomosis transmission cycle. Nowadays, the importance of livestock as a source of food for tsetse flies is increasing as a consequence of human encroachment (deforestation for cultivation) and the reduction in wildlife species (Van den Bossche *et.al.,* 2010).

These drastic changes in tsetse flies habitats and hosts availability have resulted either in their elimination or in many cases in their adaptations to those new conditions (Bourn *et.al.,* 2001). The use of specific microclimatic niches (Terblanche *et al.*, 2008; Van den Bossche *et.al.,* 2010) and their opportunistic feeding behavior regarding the choice of the host made this adaptation possible (Krafsur, 2009). Parasite virulence is also an important factor influencing the epidemiology of AAT. For example, West Africa's *T. vivax* strains are rapidly fatal when compared to those of East and Central Africa. Similarly, *T. congolense* induces a more chronic disease in West Africa compared to East and Central Africa, except in the vicinity of protected game reserves (Leak, 1999; Van den Bossche *et al.*, 2011).

Moreover, within the *T. congolense* group, the three subgroups i.e. Savannah, Kilifi and Forest (Hide and Tait, 2004) show important differences in virulence, with the Savannah subgroup strains being the most virulent (Bengaly *et al.*, 2002). Even within *T. congolense* Savannah subgroup, substantial differences in virulence are observed between strains (Masumu *et al.*, 2006; Masumu *et.al.,* 2006) and between transmission cycles, with significantly higher virulent strains in the sylvatic transmission cycle (Chitanga *et.al.,* 2011).

Tsetse flies inhabit 8.7 million km2 of sub-Saharan Africa known as the "tsetse belt". This area represents approximately a third of the continent. In the areas where tsetse is prevalent, agricultural output is suboptimal because of the risk of African animal trypanosomosis (James, 2007). *Trypanosoma congolense* Forest is distributed mainly in

Western African forest biotopes. The distribution of *T. congolense Forest* seems to be linked with that of the tsetse fly vector *Glossina palpalis*, Perhaps explaining its distribution across the continent (Moti *et.al.,* 2012). *T. congolense* Kilifi is largely restricted to East and Southern Africa. The detection of *T. congolense Forest* and *Kilifi* in the geographical 'outliers' described above has mostly been undertaken in tsetse flies (but also in pigs and wildlife (Hamill *et al.*, 2013).

1.6. Transmission methods of trypanosomes parasites

The *Glossina* is responsible for tsetse-transmitted trypanosomosis due to *T. congolense, T. vivax* and *T. brucei* in 10 million square kilometers of Africa (Hoare, 1972). This wasting disease is spread by the bite of the infamous tsetse Salivaria (subgenera *Duttonella, Nannomonas, Trypanozoon*): transmission occurs by inoculation when the vector injects its saliva at the time of the bite, which precedes the blood meal (Mulugeta *et.al.,* 2013).

The disease is widespread in sub-Saharan Africa, where it is cyclically transmitted by the tsetse fly (Matovu *et al.*, 2003; Bauer *et al.*, 2012). In animals, tsetse flies can also transmit trypanosomes mechanically when they begin a blood meal on an infected host and end it on another one, provided that the time between the two meals is short enough to ensure survival of parasites in the insect mouthparts (Moloo *et al.*, 2000).

Non-tsetse transmitted *T. vivax* infection in cattle is also recognized in parts of Africa, for example in regions of Ethiopia, Chad and Sudan (Ahmed *et al.*, 2016). Mechanical transmission of *T. congolense* has been shown under experimental conditions (Desquesnes and Dia, 2003) and can therefore not be excluded from contributing to its spread in Africa (Desquesnes *et al.*, 2009).

1.7. Host range

Trypanosomosis is a widely spread protozoan disease complex which affects cattle and other wide range of hosts in sub-Saharan Africa (Bourn *et.al.*, 2001). The host

preferences of each trypanosome species may differ, but *T congolense, T. vivax* and *T. brucei* have a wide host range among domesticated animals (FSPH, 2009). A wide range of domestic animals such as, horses, camels, donkeys, mules, water buffalo, pigs, goats and dogs are victim to trypanosome infection (Stevens and Brisse, 2004). Mice are also susceptible to *T. congolense* infections (Shi *et al.*, 2007a).

The host range is wide (Uilenberg, 1998). *T.congolense* is considered the most pathogenic trypanosome in cattle (followed by *T. vivax*), but it also causes infections in horses, sheep, goats, pigs and dogs. Apart from bovines, *T. vivax* can affect sheep, goats, horses and camels (Osorio *et al.*, 2008). *T.b. brucei* is found in various domestic ungulates, but it is particularly virulent in dogs, camels and horses, the latter often succumbing to infection within a few months in the absence of treatment.

In areas where more than one trypanosome species is present, mixed infections in domestic animals are often encountered (Kihurani *et al.*, 1994; Auty *et al.*, 2008; Biryomumaisho *et al.*, 2013; Takeet *et al.*, 2013; Moti *et al.*, 2015) and modern molecular techniques (Desquesnes and Davila, 2002), facilitate speciation. Many wild animal species in Africa also host one or more trypanosome species and can serve as reservoirs for both human and domestic animal infective trypanosomes (Mulla and Rickman, 1988; Auty *et al.*, 2012). Similarly, wild South American fauna can harbor *T. vivax* and act as reservoir of infection (Osorio *et.al.*, 2008).

1.8. Mechanisms for evading immune responses

African Animal Trypanosomosis are protozoan parasites living in the blood stream of the mammalian host. Different species of African trypanosomes cause different patterns of disease (Mulligan and Potts, 2006), but they all share the characteristic of being covered by a dense layer of variant surface glycoproteins (VSG) (Cross *et al.*, 1990; Gerald *et al.*, 1996; Adam *et al.*, 2012).

The relevant genes and their products are unknown. Control of parasitaemia is due to antibodies specific for VSG (Jackson *et al.*, 1978; Brun *et al.*, 2017; Bussler *et al.*, 2017).

Most of the initial antibodies specific for the VSG of *T. congolense* or *T. brucei* are of the immunoglobulin M (IgM) class and are produced in a *T cell* independent fashion (Shi *et al.*, 2007; Campbell *et al.*, 2013).

1.9. Pathogenesis and clinical signs

The pathogenicity of trypanosomal infections varies considerably depending on several factors, including parasite-related aspects (species and virulence), host (species, breed, age, immunological status, nutritional status, presence of co-infection and physical condition), vector (species, density, infection rate and host preference), epidemiological situation (endemic or epidemic) and the environment (***e.g.*** the availability of food and water and the season) (Leach & Roberts, 1981; Van den Bossche and Delespaux, 2011).

The course of the disease may run from a chronic long lasting to an acute and rapidly fatal depending on the vector-parasite-host interactions. The disease is mainly characterized by intermittent fever, progressive anaemia, and loss of condition of susceptible hosts which if untreated leads to heavy mortalities (Bourn *et.al.,* 2001).

Initial replication of metacyclic trypanosomes begins at the site of inoculation, inducing lymphadenopathy, anaemia, and tissue damages dominate the pathology of trypanosomosis and swelling and a sore called '*chancre*'. The chancre reaches a maximum diameter, of some 100 mm, in 10 - 14 days after an infective tsetse fly feed, its development preceding invasion of the by bloodstream trypanosomes, and is accompanied by enlargement of the draining lymph nodes which is known as lymphadenopathy. This 'chancre' disappears after a few days (3 to 15 days) when the trypanosomes spread to the lymph nodes and blood, and continue to replicate (De Waal, 2012).

In cattle, the pathogenesis is dominated by three features: anaemia, tissue lesions and immunosuppression. Other symptoms include pyrexia, splenomegaly, ataxia, lethargy, weight loss, oedema, abortion and decrease in milk production. Anaemia is a cardinal sign of trypanosomosis in many domestic animals, and the etiology is probably similar in

all species (Taylor and Authié, 2004), and in conjunction with other systemic lesions, can contribute to death through eventual congestive heart failure.

The cause of anaemia is complex and involves a variety of mechanisms. The anaemia caused by animal trypanosomosis could be associated with decrease in PCV, hemoglobin and RBCs counts in different animal species trypanosomosis infected: cattle, goats, sheep, dogs and rabbits as reported by (Silva *et al.*, 1999), which may result from massive erythrophagocytosis by an expanded and active mononuclear phagocytic system of the host (Njiru *et al.*, 2005).

This anaemia could also be due to the haemolysins such as proteases, phospholipases and neuraminidases induced by the trypanosomes, (Soulsby, 1982). Although haemolysins are released by trypanosomes, intravascular haemo lysis is not a prominent feature, and anaemia is rather attributed to erythrophagocytosis by cells of the mononuclear phagocytic system in the spleen, bone marrow, lungs and lymph nodes; these cells are stimulated by the formation of complexes between immunoglobulin specific for trypanosomes and antigen or complement attached to red cells. The immunosuppression caused by trypanosomes can affect animal health by interfering with vaccination against other diseases (Singla *et al.*, 2010), or by increasing susceptibility of the host to other infections.

Pathology in tissues is associated with the ability of the parasites to invade extravascular spaces, organs and species of trypanosomes parasites. *T. congolense* remains confined to the vascular system and induce changes in the endothelium of capillaries, and a generalized dilatation of capillary beds, which alters the hemodynamics, is observed (Connor and Van den Bossche, 2005).

Damage to endothelial cells by parasite products, immune complexes, vasoactive amines and cytokines increases vascular permeability. *T. b. brucei* is distributed in both the circulation and in the tissues and *T. vivax* although primarily a vascular parasite, has also been found in extravascular locations (Taylor and Authié, 2004).

1.10. Vector of trypanosomes

Several species of hematophagous tsetse flies of the genus *Glossina* are the biological and/or mechanical the vectors of African trypanosomosis and are responsible for cyclical transmission of the parasitic protozoan between numerous vertebrate hosts. The vector is distributed over wide range of habitats covering about 10 million square kilometers of potential grazing lands in 37 countries which are rendered unsuitable for livestock breeding and farming across the African content (Kuzoe, 1993). As a result agriculture revolution which is a key element in the fight against poverty and the improvement of food security in developing countries failed in tsetse infested areas of sub-Saharan Africa (IAEA, 2002).

Tsetse flies are vector of trypanosomes, constitute a potent and constant threat to humans and livestock in sub-Saharan Africa, (Gooding and Krafsur, 2005).There are currently 31recognized species and subspecies of tsetse flies, and they are normally divided into three different subgroups; the savannah (subgenus *Morsitans*),forest (subgenus *Fusca*) and riverine type (subgenus *Palpalis*) (Solano *et al.*, 2010).

Tsetse feed exclusively on blood; they are holometaboulos insects with females giving birth to full-grown larvae which rapidly pupate in the soil (Hargrove, 2004).The tsetse fly is very sensitive to environmental conditions - it will not survive in areas that are too hot, too dry, or too high (Pagabeleguem *et al.*, 2016). When the tsetse flies suck blood, development of trypanosomes in them depends on the species of Trypanosoma. *T. vivax* only colonizes the proboscis, *T. congolense* and *T. simiae* the midgut and the proboscis, whereas *T. b. gambiensis*, *T. b. rhodesiense and T. b. brucei* develop in different regions of the intestine (Gibson and Bailey, 2003).

1.11. Distribution of tsetse flies in Ethiopia

Ethiopia, located in the horn of Africa between latitude from 3^0 N to 15^0 N of the equator and longitude from 33^0 E to 48^0E, is an agrarian country (Abdi, 2016). The coverage of

tsetse distribution in Ethiopia ranges between 66,000 km^2- 200,000 km^2 in the South and West parts of the country (Sciarretta *et.al.*, 2005; Cecchi *et al.*, 2008).

Five species of tsetse are found in Ethiopia: *Glossina longipennis*, *G. m. submorsitans*, *G. pallidipes*, *G. fuscipes fuscipes*, and *G. tachinoides*, and are confined to Southern and Southwestern regions of the country (Langridge, 1976). Except *Glossina longipennis* the rest of these tsetse fly species are responsible for most AAT transmission in Ethiopia (Dinka and Abebe, 2005).

G. m. submorsitans is usually found in deciduous woodland and wooded grassland, often interspersed with evergreen vegetation. *G. pallidipes* is almost invariably associated with extensive and fragmented thickets, including evergreen species. *G. longipennis* is found in dry acacia, thorn-bush and is very active after sunset and before nightfall. *G. fuscipes fuscipes* and *G. tachinoides* inhabit gallery forest, thickets and fringing vegetation on streams, rivers and lake shores (Langridge, 1976).

1.12. Reservoir of African Animal Trypanosomosis (AAT)

Numerous wild animal species also seem to be naturally infected with *T. vivax*, *T. congolense* and *T. b. brucei* (Mulla and Rickman, 1988). Certain species, like gazelle, dik-dik, jackal, bat-eared fox and aardvark, seem to usually die as a result of the infection, whereas other species, like eland, hyena, bushbuck and impala are susceptible to infection and remain parasitaemic for quite a while (Anderson *et al.*, 2011). Warthogs, bush pigs and porcupines only seem to show transient infection (Ashcroft *et al.*, 1959) as cited by, (Mulla and Rickman, 1988; Njiokou *et al.*, 2004).

The level of parasitaemia and anaemia in wildlife was much lower than in domestic animals. It was suggested that the level of infection in wild animals can be controlled by specific host anti-bodies, efficient phagocytosis, non-immunological responses and innate trypanolytic factors. This phenomenon is called ‘trypano-tolerance’ and is an important aspect of their role as a wildlife reservoir for both human and animal trypanosomosis (Mulla and Rickman, 1988).

1.13. Control of vector and reservoir of trypanosomes

The control of animal trypanosomosis relies primarily on the use of insecticides or traps to control the vector (especially in the case of tsetse-transmitted trypanosomosis), and on the use of trypanocides to control the parasite (Holmes, 2013). As a vector control can be expensive when used on a large scale and is not always sustainable or effective, administration of trypanocidal drugs represents the main intervention tool in most poor rural endemic areas, ensuring maximum effects at relatively little cost (Grace *et al.*, 2009).

Vector control, which aims at reducing the population of tsetse flies, is a widely used method to control the transmission trypanosomosis. If the fly can be controlled, so can trypanosomosis. Among others some of the methods tried and used are fly traps, the sterile insect technique, insecticide spraying and pour-on of insecticide on livestock (Allsopp, 2001).According to the (WHO, 2013), direct control of trypanosomosis in wildlife is not an option. Many wild animals are protected species and mass screenings would be unethical and too expensive. Game elimination, as a way to control the wildlife reservoir, was done in the past, but these methods are no longer used because of their negative influence on biodiversity (Clarke, 1964).

However, even though trypanotolerant animals are able to survive and reproduce in tsetse and trypanosomosis infested areas, they may still be affected by the level of the challenge (ILCA, 1979).Thus, production and reproduction of tolerant animals could be increased by strategic control methods during high challenge periods (Murray *et al.*, 2004).

1.14. Diagnosis of trypanosomosis

The diagnosis of trypanosoma infection is based on clinical signs and on the demonstration of the parasites by direct or indirect methods. The clinical signs of the AAT are indicative, but are not sufficiently pathognomonic and diagnosis must be confirmed by laboratory methods. The classical direct parasitological methods for the

diagnosis of trypanosomosis, namely microscopic examination of blood or lymph node material, are not highly sensitive, but a number of techniques, including enrichment of the sample, rodent inoculation and molecular methods may increase the sensitivity (Murray and Mcintyre, 1977; Murray *et al.*, 1982; Solano *et al.*, 2002)

Indirect methods rely on serological tests by detecting specific antibodies developed by the host against the infection or, inversely, to demonstrate the occurrence of circulating parasitic antigens in the blood by the use of characterized specific antibodies. The detection of antibodies indicates that there has been infection, but as antibodies persist for some time (weeks, sometimes months) after all trypanosomes have disappeared from the organism (either by drug treatment or self-cure) a positive result is no proof of active infection. On the other hand, circulating trypanosomal antigens are eliminated quickly after the disappearance of the trypanosomes, and their presence therefore shows almost always that live trypanosomes are present in the animal (FAO, 2004).

Compared to standard parasitological techniques and serological methods, molecular diagnostic tools in particular the polymerase chain reaction (PCR); allow the detection of trypanosome infections with much lower parasite numbers, both in the vertebrate and in the insect host (Desquesnes *et.al.,* 2009; Fernández *et al.*, 2009; Thumbi *et al.*, 2010).

1.15. Treatment strategies and challenges

Treatment and prophylaxis of pathogenic trypanosome infections in animals relies on (Diminazene aceturate, Homidium bromide (chloride) and Isometamidium chloride, most dating back to the first half of the 20^{th} century (Leach and Roberts, 1981). Most trypanocides have therapeutic rather than prophylactic activity, but Isometamidium chloride is mostly used for its prophylactic effects (Stevenson *et al.*, 1995).

It has been estimated that about 35 million doses of trypanocides are administered each year. Consequently treatment given by livestock owners is not without serious drawbacks, because most farmers do not have adequate knowledge on diagnosis and appropriate use of drug used in areas of high prevalence of trypanosomosis. Trypanocides

are frequently used in the absence of diagnosis or used to treat conditions for which they are not effective (Barrett *et.al.,* 2004).

Improperly used, veterinary drugs waste scarce resources, occasion avoidable sickness and death, mask poor production and promote drug resistance leading to exacerbated disease in animals and humans (Grace *et.al.,* 2009). Although there is a continuous demand for trypanocides by livestock keepers, the African market of trypanocides estimated at about US$ 30 million (Grace, 2003), is not considered sufficient to justify investment by large pharmaceutical companies in the development and licensing of new animal trypanocides; the cost of which is estimated from 200 to 800 million dollars (DiMasi, 2014).

As new compounds are not likely to become available in the near future, prudent use of those already on the market is paramount, though in field settings drug usage is often difficult to monitor and regulate. In hyper-endemic African countries, trypanocides are usually administered directly by farmers, who can easily obtain them at local markets for a relatively affordable price (Grace *et.al.,* 2009). In unregulated market, poor quality or counterfeit trypanocides are widespread in some areas, especially in Africa, where documented product specifications are scarce (Sutcliffe *et al.,* 2014; Tchamdja *et al.,* 2016).

Besides correct dosage administration, various other options to extend the life of current trypanocides exist *i.e*. alternative formulations have been considered in order to improve therapeutic efficacy (Kroubi *et al.,* 2011; Unciti-Broceta *et al.,* 2015).

1.16. Drugs currently in use against animal trypanosomosis

Diminazene aceturate: was introduced for the treatment of African trypanosomosis and Babesiosis in livestock in 1955. This day it is the most commonly used trypanocide in different species of animals, due to its activity against both *T. congolense* and *T. vivax (*Peregrine and Mamman, 1993). After rapid absorption (the peak blood level is reached within 1 hr of dosing. elimination half-life values following intramuscular administration varied from 11–19 hr in sheep and goats, to 74 to >200 hr in cattle (Mamman *et al.,* 1993;

Mdachi *et al.*, 1995; El Banna *et al.*, 1999). Diminazene residues may persist for several weeks in the edible tissues of cattle and other food-producing animals, especially in the liver and kidney, whereas the drug levels in milk peak at 6 hr and fall to below detection limits after 48 hr (El Banna *et.al.*,1999).

The mode of action of Diminazene aceturate has not been completely elucidated. The compound binds the minor groove of the DNA at AT-rich sites (Wilson *et al.*, 2008). In trypanosomes, the kDNA is a known target of the drug, and kDNA binding can cause inhibition of replication and kDNA loss (Shapiro and Englund, 1990), possibly exacerbated by an inhibitory effect on mitochondrial type II topoisomerase (Portugal, 1994). It had long been believed that loss of the kinetoplast might not be sufficient to kill trypanosomes, as viable dyskinetoplastic strains do occur naturally and also can be produced artificially in the laboratory (Schnaufer *et al.*, 2002).

It has been suggested that Diminazene can also modulate the host immune response by dampening pro-inflammatory cytokines and excessive immune activation, which might also influence the in vivo effects of the drug (Kuriakose *et al.*, 2012). Due to its charged nature, Diminazene can only cross membranes via specific carriers and this has three important consequences: (a) the drug is not active on CNS infections; as it cannot cross the blood–brain barrier; (b) the compound is selectively toxic to trypanosomes, as they express transporters that specifically accumulate Diminazene; and (c) trypanosomes may become resistant to the drug by losing these transporters or their activity. Diminazene uptake in *T. brucei* mainly occurs via an amino-purine transporter called P2 or *Tb*AT1 (Carter *et al.*, 1995; de Koning, 2008).

Loss of P2/TbAT1 activity was shown to cause Diminazene resistance in *T. b. brucei* (Matovu *et.al.*, 2003), *T. b. equiperdum* (Barrett *et al.*, 1995; Stewart *et al.*, 2010) and *T. b. evansi (Witola et al., 2004)*. Loss of P2/*Tb*AT1 alone is sufficient to give high level of resistance to this latter drug (Matovu *et.al.*, 2003). A putative P2/ *Tb*AT1-type transporter, *Tco*AT1, was identified in *T. congolense* and a particular allele proposed to be associated with Diminazene resistance. This conclusion was curious, given that the so-called resistance allele was not always associated with resistant form parasites isolated in

one region (Delespaux *et.al.,* 2006) and was also abundant in areas where Diminazene had not been used (Chitanga *et.al.,* 2011).

Isometamidium chloride: is a hybrid phenanthridines with amphiphilic and cationic properties. It has both curative and prophylactic properties and, since its launch in the 1960s, it has remained the only drug available for chemoprophylaxis of AAT, after Quinapyramine was discontinued due to problems linked to toxicity and the induction of multi-drug resistance (Peregrine, 1994; Geerts and Holmes, 1998).Isometamidium is used primarily to treat and prevent *T. congolense* and *T. vivax* infections in livestock in Africa. The drug is administered to cattle at single doses of 0·25–1·0 mg kg^{-1} for cure, and at doses of 0·5–1 mg kg^{-1} for prophylaxis (Leach and Roberts, 1981).

Multiple intramuscular administrations this drug can cause severe fibrous lesions, hence damaging the carcass and meat quality from livestock. Intravenous administration has been successfully used to abrogate muscular damage, but it has been suggested that this could result in compromised prophylactic activity, due to the lack of a drug depot at the injection site (Dowler *et al.*, 1989; Munstermann *et al.*, 1992). Isometamidium plasma concentrations reach their peak within 1hr after administration and then fall relatively quickly during the first week post-treatment and thereafter more gradually (Kinabo, 1993; Eisler *et al.*, 1994).

Three months after cattle had been injected, the circulating drug concentration was measured at 0·75 ng mL^{-1} (Eisler *et.al.*,1994). In sheep and goats isometamidium appears to be eliminated more rapidly than in cattle (Wesongah *et al.*, 2004). The drug accumulates in the liver, kidneys and spleen as well as at the injection site, and from here it is slowly released to the plasma exerting its prophylactic activity (Kinabo and Bogan, 1988). Persistence of isometamidium residues is much longer than for diminazene.Excretion occurs mainly via bile and levels in cattle milk are generally very low (Kinabo, 1993).

Isometamidium may be used as part of a sanative pair with diminazene, the two drugs being used sequentially to minimize the risk of resistancedevelopment (Leach and Roberts, 1981; Peregrine, 1994b). Despite this recommendation, there are multiple

reports of field isolates, from many African countries, indicating isometamidium resistance; particularly in *T. congolense,* but also in *T. brucei* species and *T. vivax*, sometimes detailing cross-resistance with Diminazene (Afewerk *et al.*, 2000; Sinyangwe *et al.*, 2004; Mamoudou *et al.*, 2008).

By taking advantage ofIsomethamidium's intrinsic fluorescence accumulation in the kinetoplast was observed (Wilkes *et al.*, 1995; Boibessot *et al.*, 2002). Although closely related to the intercalating phenanthridine homidium, isometamidium is not known to be carcinogenic, and was reported to bind kDNA with an unconventional 'sideways' geometry (Dougherty and Waring, 1982). The drug would still accumulate preferentially in the mitochondrion, as the mitochondrial membrane potential is unaffected by the loss of the kinetoplast in cells carrying a compensatory mutation in the γ-subunit of the F1F0-ATP synthase, providing a driving force for cations (Dean *et al.*, 2013).

A mutation in this ATP synthase subunit is sufficient to cause a substantial level of isometamidium and homidium resistance, although further drug pressure was shown to increase this even further. Accordingly, very high level of resistance is indeed associated with a loss of mitochondrial membrane potential, preventing further isometamidium accumulation in this organelle (Eze *et al.*, 2016).Resistance to isometamidium is encountered in the field. In *T. congolense* amechanism behind resistance was proposed to relate to diminished mitochondrial membrane potential (Wilkes *et al.*, 1997).

1.17. **Herbal Treatment**

In many countries in Africa, herbal treatment of various diseases is a common practice (Freiburghaus *et al.*, 1996; Nibret and Wink, 2011). Because of the drug resistance, relapse and side-effects, phytotherapy is therefore being restored to, in developing countries, owing to the low price and easy accessibility. It would thus be a valuable effort if some attention is paid to the therapeutic remedies which have been used in treating trypanosomosis as is practiced by herdsmen village elders and others who keep animals. It has also been observed that natural products derived from plants offer novel possibilities to obtain new drugs that are active against trypanosomes.

Many investigators targeted finding new anti-trypanosomal agents to combat the trypanosomosis by screening extracts of medicinal plants (Mann and Ogbadoyi, 2012). However, there are some herbs that are used for veterinary purposes, and some diseases peculiar to animals had been treated with such herbs (Nwude and Ibrahim, 1980). Among all Plants have always been the most common sources of medicaments, either processed as traditional preparations, or used to prepare pure active principles. As reported by (Feierman, 1981), traditional herbal treatments of human and animal diseases in Africa still holds a strong position in medical care.

Many natural products of plant origin, with a wide range of different chemical structure, have been reported to have activities against different species of protozoan parasites including *Plasmodium*, *Trypanosoma*, *Leishmania* and *Entamoeba.* A major problem in discovering new antiprotozoal drugs is that protozoa, unlike pathogenic bacteria, share many common biochemical pathways with the animal host. (Barbara, 2018), indicate that the preparation of antiprotozoal activity without sufficient selectivity to kill the parasite only and not damaging animal host is of limited value to drug development.

As a general will working with plant materials there are some common problems should have to be kept in mind is that, while working with anti-parasitic plant compounds: ***a)*** highly active plant substances are very often are toxic, ***b)*** active compounds *in vitro* do not necessarily display activity in rodent models and ***c)*** crude extracts are more active than purified compounds indicating synergetic activity of different compounds (Kirby, 1996). The other problem is that plant materials extract measurement techniques do not exist in African cultures and that the plants do not contain highly active or toxic constituents (Kirby, 1996).

There are many of authors evaluate medicinal plants for their *in vitro* and/or *in vivo* trypanocidal activity in different time and areas as try shown on the following chart.

Table 2: Ethiopian plants authenticated for their antitrypanosomal activity

Plant species	treated spp	Parts used	Author's name
Dovyalis abyssinica (Flacourtiaceae)			(Nibret and Wink, 2011)
Albizia schimperiana			(Tadesse *et al.*, 2015)
Ocimum urticifolium (Lamiaceae)			(Tesfaye *et al.*, 2015)
Acokanthera schimperi (Apocynaceae)	*T. b. brucei*		
Chenopodium ambrosioides (Chenopodiaceae**),**			
Solanecio angulatus	*T. brucei*	Flowers	(Nibret *et al.*, 2009)
*Crotalaria phillipsiae(*twigs) and	*brucei*,	leaves	
Solanecio manni			
Artemisia absinthium	*T. brucei*	leaves and	(Nibret and Wink, 2010)
Artemisia abyssinica	*brucei*	aerial parts	
Artemisia afra, and			
Artemisia annua)			
Hagenia abyssinica (Rosaceae)	*T. brucei*	seeds	
Leonotiso cymifolia (Lamiaceae),	*brucei*		(Nibret and Wink, 2010)
Moringastenopetala(Moringaceae)			
Artemisia abyssinica	*T.congolense*	aerial parts	(Feyera *et.al.*, 2014)
Dovyalis abyssinica (Flacourtiaceae)	*T. congolense*		(Tadesse *et.al.*, 2015)

1.18. Trypanosomosis drug resistance

It is the loss of sensitivity by a strain of an organism to a drug to which it had previously been susceptible and implies failure of treatment and prevention of a disease (Uilenberg, 2017), or when the use of trypanocidal does not produce the expected outcome (cure or protection), there is a tendency to assume that drug resistant has arisen (Leach and Roberts, 2015).

From an analysis of properly kept records of routine monitoring, it is possible to determine whether administration of a therapeutic dose of a trypanocidal cures or not an infected animal. There may be different reason for the failure of drug treatment. There is little control on drug sale and an evident lack of information on the correct condition of trypanocides storage and use. All of these factors, associated to the change of farming systems (extensive to more intensive farming in peri - urban zone with increased treatments) and the absence of new products favor the development of Trypanosomosis drug resistance (TDR) (Anonymous, 2004).

Resistance seems to develop in a step-wise manner with trypanosomes resistant to a low dose of a trypanocides being removed by a higher dose of the same compound. The problem is that, because of the narrow therapeutic indices of the trypanocides, there is only limited scope to overcome resistance by increasing the dosage (Connor, 1989).

Table 3: Status of animal trypanosomosis drugs resistance in Ethiopia

study Site	Drugs type	Species tested	researchers
Gibe valley	ISM & DA	*T.congolense*	(Chaka and Abebe, 2003; Moti, *et al.*, 2012 ; Mulugeta *et al.*, 2014)
Upper Didessa	ISM	*T.congolense*, *T.vivax &T.brucei*	(Tewelde *et.al.*, 2004)
Bedelle	ISM &DA	*T.congolense*	(Chaka and Abebe, 2003)
Metekel	ISM &DA	*T.congolense*	(Afewerk *et.al.*, 2014),
Sodo	DA	*T.congolense*	(Chaka and Abebe, 2003)
Arba-minch	DA	*T.congolense*	(Chaka and Abebe, 2003)
Omo valley	ISM &DA	*T.congolense*	(Ademe, 1998)
Tselemti	ISM &DA	*T.vivax*	(Kebede and Abebe, 2010)

1.19. Mechanisms of trypanocidal drug resistance

The discovery of trypanocidal drugs with preventive action raised high hopes that their use would make it possible to run subtropical African into flourishing livestock production area. Although, these drugs do provide protection, all of them frequently give rise to the formation of drugs resistant trypanosome strains. This drugs resistance occurs, when the trypanosomes are in contact with a trypanocidal administered in a sub curative dose insufficient to ensure the destruction of the parasites (Das *et al.*, 2004).

This situation may be due to one or more of the following factors: the application of insufficient doses, underestimating the weight of animals, the formation of abscesses followed by partial rejection of the drugs, the formation of cyst-forming reaction which prevents the diffusion of the drugs, preventive treatment at too long or irregular time intervals, halting the application of trypano-prophylactics while the animals are still exposed to the risk of infection and may be due to the use of preventive drugs in curative treatments (Rowlands *et al.*, 1994).

It is important to understand the mechanisms of trypanosomes drugs resistance, to identify the potential and novel drugs targets and provides direction to how new chemotherapeutic strategies can be used to reduce development of trypanocidal drugs resistance (Barrett and Fairlamp, 1990).

Trypanocidal drugs resistance could be innate (resistant individual)without previous exposure to a particular drug or acquired (induced) as a result of drug exposure, cross-resistance or sometimes by mutagenesis (ILRAD, 1990). Reduction in drugs accumulation by the target cell or organism and reduce drugs activities in immunosuppressed animals can contribute to the emergence of drugs resistance (Frommel and Balber, 1987). Some trypanocidal drugs are well-known mutagenic compounds and might induce mutations (Hayes and Wolf, 1990).

Trypanosome kinetoplast is the primary site of Isomethamidium (ISM) accumulation and decreased levels of drug accumulation have been observed in drug resistant populations of *T. congolense* (Sutherland *et al.*, 2016) and (Sutherland and Holmes, 2004), found

indirect evidence of an increased efflux of drug from resistant trypanosomes. (Mulugeta *et.al.,* 2014), showed that the maximal uptake rates (V_{max}) of ISM in resistant *T. congolense* were significantly lower than sensitive populations.

It remains to be shown whether this is caused by a decreased number of protein transporters of ISM in the plasma membrane and/or by changes in the balance between influx and efflux. The role of nucleoside transporters in resistance to ISM by *T. congolense* remains to be examined, although changes in these transporters have been associated with resistance to arsenical drugs in *T. brucei* (Ross and Barns, 2009). Although DA probably exerts its action at the level of the kinetoplast DNA, this has not been proven in in vivo (Delespaux, 2004; Berger *et al.*, 2007), showed that the accumulation of Diminazene was markedly reduced in arsenical-resistant *T. brucei* owing to alterations in the nucleoside transporter system (Barrett e*t.al.*, 1995).

Pathogenicity and interaction of drug resistant trypanosomes with tsetse flies: Whether drug-resistant trypanosomes are less pathogenic than susceptible ones remains a controversial issue, have observed a loss of virulence in drug-resistant trypanosomes (Mutugi *et al.*, 1995; Berger *et al.*, 2016). Transmission by tsetse flies does not appear to affect the sensitivity of trypanosomes. Recent studies at the International Livestock Research Institute used four populations of *T. congolense*, ranging from extremely sensitive to strongly resistant to ISM and found that no differences in virulence between them; where only the most resistant one showed a reduced viability, *i.e*. it took longer to establish parasitaemia than the other three (ILRI, 1996).

1.20. Detection of trypanocidal drug resistance

Drug resistance is suspected when treatment failure occur using standard drug dosages. However, in the field, this interpretation can be erroneous (incorrect), as treatment failure can result from many factors other than the parasite's increased tolerance to drugs. The presence of parasites in treated animals could correspond (to be the same) to a new infection rather than to recrudescence, particularly in areas of high challenge (Rowlands *et al.*, 2001a).Using microsatellite DNA markers to strain type *T. congolense* from cattle

in Ethiopia following treatment with Diminazene, essentially equal occurrences of new infection (Donelson, 2003) and actual relapse (37·5%) were proposed (Moti *et.al.,* 2015).

Other causes of treatment failure not linked to true drug resistance could be related to the poor health state of the animal (e.g. malnutrition, immunosuppression, concurrent infections), or to incorrect drug use (e.g. irregular treatment or prolonged intervals between treatments), or to under-dosage. The latter can result from poor drug quality (either due to inappropriate storage or to the use of counterfeit products) (Sutcliffe *et.al.,* 2014), or from incorrect drug usage (wrong dilution, use of unsterilized water or erroneous dosage due to inaccurate estimation of the animal weight) (Grace *et.al.,* 2009; Van den Bossche *et.al.,* 2011).

So methods to assess true resistance are crucial. Several methods have been described to identify drug resistance in trypanosomes (Peregrine, 1994). The four techniques most commonly used to identify drug resistance: tests in ruminants, in mice, in vitro assays and molecular detection also possible.

Tests in vivo: A standardized protocol for the assessment of susceptibility and resistance to trypanocidal drugs in mice or in ruminants has been described by (Eisler *et al.,* 2001). Although there is a good correlation between the tests in mice and in ruminants, the curative dose that must be used in ruminants cannot be extrapolated from the results in mice directly. The advantage of mouse assay is that it is cheaper than the test in cattle.

There are several disadvantages, however: 1) most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice; 2) higher doses of drug must be used in mice (normally 10x higher) in order to obtain comparable results to those obtained in cattle, because of the vast difference in metabolic size; 3), precise assessment of the degree of resistance needs a large number of mice per isolate, which makes it a labor intensive test identification of a discriminatory dose, and 4) it takes as long time to evaluate the drug sensitivity of an isolate (Subekti *et al.,* 2015).

In vitro assays: In vitro evaluation of trypanocidal drug sensitivity test of procyclic, metacyclic or blood stream forms trypanosomes can be used. In vitro cultivation of blood stream forms is only using pre adapted lines and not using isolates directly from naturally infected animals. Even though this method has some advantages it also disadvantages expensive to perform, require good laboratory facilities, well trained stuff, and some trypanosome species are difficult to cultivate *i.e. T.congolense*. If this technique can be improved to adapt trypanosomes isolates to grow in vitro more rapidly, these may become more popular especially in those laboratories where culture facilities are already established (Coustou *et al.*, 2010).

Molecular tests: Polymerase chain reaction (PCR) has also has been used to monitor the efficacy of trypanocidal drugs treatment in cattle naturally and experimentally infected with *Trypanosoma spp*. (Tran *et al.*, 2014). Under natural challenge, PCR and DNA probe hybridization were used to confirm the effectiveness of Isometamidium chloride prophylaxis in cattle infected with *T. brucei* and *T. vivax* populations (Delespaux *et al.*, 2008b). The potential of PCR as a method to detect drug failures in cattle was also reported by (Gall *et al.*, 2004).

1.21. Diagnosis of African Animal Trypanosomosis

Clinical diagnosis: The diagnoses for AAT are classified into: clinical, microscopy, immunological and molecular diagnoses (Nakayima, 2016). The clinical manifestation of AAT is influenced by the host as well as the trypanosomes species and "strain" (Bezie *et al.*, 2014). Animals which survive often remain infected for several months or years, exhibiting a low level of fluctuating parasitaemia which serves as a reservoir for the disease (Anderson *et.al., 2*011). It is characterized by severe anaemia, loss of production, pyrexia, lacrimation, pallor of the mucus membrane, weight loss, infertility and abortion, with death occurring in some animals during the acute phase of the disease (Maigari *et al.*, 2015; Maigari *et.al.,* 2015). So, these varied clinical manifestations, diagnosis of trypanosomosis cannot be based on clinical signs alone.

Parasitological diagnosis: The demonstration of parasites based on standard trypanosome detection methods as developed by (Woo, 1969). The methods include wet mount, animal inoculation, thick/thin smears, haematocrit centrifugation techniques (HCT) (buffy coat methods (BCM). The sensitivity of direct microscopic examination was improved through concentration (buffy coat technique) of the parasites by centrifugation. Examination of the buffy coat is thus more sensitive than examination of blood films (Hou *et al.*, 2010). Thick smear is used for detection, but not speciation based on parasite morphology; whereas thin smear is used for speciation based on parasite morphology.

Similarly, Wet film is used for speciation based on parasite motility. *T. vivax* moves/swims very fast across the microscope field. *T. brucei* actively moves, but in one position. *T. congolense* attaches onto Red Blood Cells (RBCs) and just purveys in one position (Nakayima, 2016). Blood from suspect animals can also be inoculated into susceptible laboratory animals, usually mice or rats. Animal inoculation has the added advantage that trypanosome isolates can be collected for other studies in the laboratory. This technique is more sensitive than direct microscopic examination of the blood sample (Nakayima, 2016).

This, however, is not a practical technique because diagnosis is not immediate (Yusuf *et al.*, 2015), the cost of maintaining the animals makes the method prohibitively expensive for routine diagnosis, especially in the field (Nakayima, 2016). and some trypanosome isolates, notably East African *T. vivax* and *T. simiae* do not infect laboratory rodents (Yusuf *et al.*, 2015).

Antibody Detection Tests: Antibody detection techniques include complement fixation test (CFT) that has been used in the diagnosis of *T. equiperdum*, which causes dourine in equines, and indirect fluorescent antibody test (IFAT) which was used in herd diagnosis of trypanosomes, (Hussain *et al.*, 2014). The IFAT has proven to be both sensitive and specific in detecting trypanosomal antibodies, although there is cross-reactivity between the trypanosome species, in addition to its being expensive its subjectivity can make

comparison of results quite difficult, requiring sophisticated microscopy (Nakayima, 2016).

Card agglutination test for trypanosomosis (CATT), is the simplest test used for *T. evansi* (Ezeani *et al.,* 2008). CATT uses the formalin fixed variable antigen types of *T. evansi* that are used in the agglutination test. However, the major draw backs of this method is that, when antibodies are detected, they do not distinguish between current and past infections, in addition to cross reactions among trypanosome species (Silbermayr *et al.*, 2013).

Enzyme-linked immunosorbant assays(ELISA) (Kedir *et al.*, 2016), has particularly been used for epidemiological surveys to detect trypanosome antibodies. However, the detection techniques involve use of either whole parasite or crude parasite lysate as the antigen, which are not often standardized. Tests using crude solicited trypanosomal extracts showed that the ELISA had a sensitivity and specificity similar to the IFAT (Ezeani *et.al.,* 2008).

Thus, the introduction of ELISA was a major breakthrough in the field of immunodiagnostic (Fleming *et al.,* 2014). This is because ELISA requires simple equipment, the technique is straight-forward and sensitive, and can be used for large scale screening of samples. However, a major limitation of ELISA in its application as a routine diagnostic test is the nature of the antigens used in the assay. The antigen is usually a crude trypanosome lysate, the quality of which is ill-defined. This makes the test difficult to standardize with regard to specificity and sensitivity (Nakayima, 2016).

Antigen Detection Tests: To detect trypanosome-specific antigens in the blood of infected animals have been advocated as an alternative approach to antibody detection (Birhanu *et al.*, 2015). Hence, the demonstration of trypanosome antigens is an indicator of active infection, if animal has not been recently treated for the disease and thus, is equivalent to parasitological diagnosis. Many studies have shown that, Antigen ELISA technique may give false negative results even in parasitologically proven cases (Nguyen *et al.*, 2015). The monoclonal antibody used in antigen ELISA is directed at an internal or

somatic unsecreted antigen that is only released after trypanosome lysis (Birhanu *et.al.*, 2015).

Thus, before the first parasitaemic peak, the test can give negative results due to absence or low levels of antigens in blood (Nguyen *et.al.,* 2015). It is, therefore, important to combine antigen detection ELISA with the parasitological techniques for effective diagnosis of trypanosomosis. Currently, there is a promising use of recombinant antigens to improve on the available trypanosome cell lysate to detect antibodies (Nakayima, 2016a). A tube-ELISA, as opposed to a microtiter plate-ELISA, has since been developed. This test gave similar results, thus providing, for the first time, a potentially suitable test for immunodiagnostic of individual animals in the field (Nguyen *et.al.*, 2015).

Molecular Diagnostic Methods: Molecular detection techniques have been developed for the diagnosis of infections with African trypanosomes in humans, animals and tsetse flies (Büscher, 2014) and first performed in 1983 now has various primer sets available that can amplify different trypanosome subgenus, species and types (Yusuf *et al.,* 2015), DNA-based identification methods tap into the intrinsic genetic identity of the organism provides an unchanged genetic signature is particularly useful for parasites where individual life cycle stages may show little resemblance to each other (Gibson, 2009).

Therefore, DNA based methods have a wider range of applicability and efficacy in epidemiological studies than morphological examination alone, and are now indispensable (crucial0 tools for the study of trypanosomosis, because of the improved sensitivity and specificity. Additionally, species-specific probes are now available to identify trypanosome to sub species level (Gibson, 2009). PCR can detect infection as early as 5 days following an infective tsetse bite (Thumbi *et al.*, 2008).

Moreover, using the quantitative PCR confers an additional advantage of identification as well as establishing the parasite burden (Zarlenga & Higgins, 2001). Size variation among trypanosome species in the internal transcribed spacer of the ribosomal RNA (rRNA) locus provided the necessary discriminatory power and this methodology has

been widely applied for identification of both tsetse midgut and livestock blood samples (Cox and Balik, 1994; Adams *et al*., 2006).

In the ITS-1 generic PCR test, the PCR primers flank the ITS-1 spacer, which is variable in both length and sequence; the primers are chosen in the 18S and 58S rRNA genes such that they match all trypanosome species of choice. The size of the single PCR fragment amplified from a trypanosome sample is then measured by gel electrophoresis against a DNA size marker and compared with known values from reference species (Adams *et al.*, 2006; Gibson, 2009).

1.22. Control of African animal trypanosomosis

(Allsopp, 2001), suggests that the vector control operations were covering approximately 128,000 km^2 of Africa in 2001, a mere 1.3% of the tsetse infested area; although extensive trypanosomosis and tsetse control operations have been running since the beginning of the 20^{th} century. Tsetse infestation in sub-Saharan Africa has hardly receded; even if, geographic variation in trypanosomosis, tsetse species distribution and eco-epidemiology of the disease, as well as disparities in resource distribution, infrastructure and political stability within and between sub-Saharan Africa countries raises questions as to the plausibility of long term sustainable AAT control at sub-continent level (Allsopp, 2001).

Before the 1950s, Tsetse & trypanosomes control mostly involved methods with negative environmental impacts: (such as bush clearing, ground spraying with dichlorodiphenyltrichloroethane (DDT) and wildlife culling). From the 1980s, more ecologically and politically acceptable methods were developed such as selective bush clearing, sequential aerial spraying (Sutcliffe *et.al.,* 2014), insecticide-treated traps and targets (Hamilton *et al.,* 2008), insecticide-treated cattle used as live baits and eventually the sterile insect technique (SIT) (Vreysen *et al.*, 2013). Recently, several studies showed that restricted applications of insecticides on cattle (spray on lower body parts, footbaths) were an effective cheaper control option (Bouyer *et al.*, 2009; Muhanguzi *et al.*, 2015).

Each of these approaches is useful, but has important limitations, such as expense, environmental pollution and drug resistance. In the absence of vaccines and effective and affordable drugs, African trypanosomosis control relies heavily on vector control with eventual impacts ranging from reduction of fly populations to total eradication. Targets and traps have been effective in controlling populations locally and have been used extensively in agricultural settings and considerable success has been achieved by directly applying insecticides on animals (pour-on) (Rowlands *et al.*, 2001).

Since vector control can be expensive when used on a large scale and it is not always sustainable or effective. Discriminative spraying of just the resting sites of tsetse would reduce costs, cause less environmental pollution and would be easier to carry out as only a small percentage of the total tsetse habitat would be sprayed. However, the technique is labor intensive, demands high level of supervision and has effects on non-target organisms (Schofield and Kabayo, 2008). An alternative approach that is area-wide in nature is the Sterile Insect Technique (SIT), which was applied in large scale tsetse eradication programs in some parts of Africa including: Burkina Faso, Tanzania, and Zanzibar (Vreysen *et al.*, 2000).

Africa Animal Trypanosomosis (James, 2007), control measures are mainly targeted towards elimination of the parasite from host blood and prevention of tsetse bites through vector control. Parasite elimination is mainly achieved through use of trypanocides which can be either prophylactic or curative (Young and Godfrey, 1983; Berihu *et.al.,* 2014), are commonly used to achieve the respective goals. In trypanosome endemic areas, trypanocidal drugs are the most widely used methods of trypanosomosis control (Clausen *et.al.,* 2010). Control of parasites with chemotherapeutic and chemo-prophylactic agents has the double effect of limiting the losses caused by the infection and eliminating the transmissible trypanosome reservoir (Welburn *et al.*, 2015).

Some indigenous African livestock breeds (N'Dama, Muturu and Dahomey) are more resistant to trypanosome infection than imported breeds. This phenomenon is called '*trypano - tolerance*' and is defined as the 'capacity to survive and remain productive after trypanosome infection' (Murray *et.al.,* 1982). The use of Trypanotolerant breeds

has helped livestock productivity in various endemic regions in Africa and elsewhere, and it is often advocated as an important control strategy. A major factor enabling these animals to cope with trypanosome infections is a better capacity to limit both anaemia and parasitaemia (Naessens, 2006).

3. MATERIALS AND METHODS

3.1. Study areas

The study was conducted in Benshangul Gumuz Region from January 2018 to May 2018 in two selected districts namely; Assosa and Bambasi districts of Assosa zone, which is located about 676 and 633 km respectively to the North-West direction of Addis Ababa, Ethiopia. Assosa district is bordered in the north-east by the district of Bambasi and in the north-west by the by northern and southern Sudan, in the north direction by Amhara region and Oromia region in south west direction. And this district composed of seventy administrative peasants and four Assosa town kebeles, which is located at 9.600 and 10.45^0 N and 34.200 and 34.58^0E longitude.

The altitude of Assosa zone ranges from 580 to 1544 m.a.s.l. Its total area is 2317 Km^2 of which is characterized by low land plane. According to National Meteorology Service Agency the average annual rainfall of 1316 mm, with uni-modal type of rain fall that occurs between May and October. Its mean annual temperature ranges between 16.75 ^{0}c and 37.9 ^{0}c, (IRI, 2011).

The total human population of the district is estimated to be 104,147, According to the information collected from the district's office of Agriculture and rural development in 2017-2018 the populations of livestock in this district in species level were: 36,916 cattle, 35,500 goats, 14,325 sheep, 5890 donkeys and 35,125 poultry, (CSA, 2016).

On the other hand Bambasi district is located in Benshangul Gumuz Region, in the Southwest direction of Assosa town and 616 Km in the West of Addis Ababa at 9.45 – 9.75^0 N and 34.35 – 34.88^0 E, with a minimum and maximum altitude of 1350 and 1770 m.a.s.l. The district is composed of 42 administrative peasant associations and two Bambasi town kebeles. The total area of the district is 2100 Km^2; of which the average minimum and maximum annual rainfall are 900 mm and 1200 mm; while the average of minimum and maximum temperature is 23 ^{0}c and maximum 32 ^{0}c. The total human population of the district is 62,693. Bambasi district has the livestock population of

38,964 cattle, 11,990 goats, 3452 sheep, 1995 donkeys and 38442 poultry according to the information obtained from the office of Agriculture and rural development of the district in 2017.

To represent the study area four kebeles from Assosa and four kebeles from the Bambasi were selected purposively, on the bases of tsetse flies distribution in the districts and depending on the earlier history of trypanosomosis occurrence in the area. The study area of both districts was similar, which is low land and plane agro-ecology. The map of both study areas are shown as below on the map of Ethiopia (*Fig. 3*).

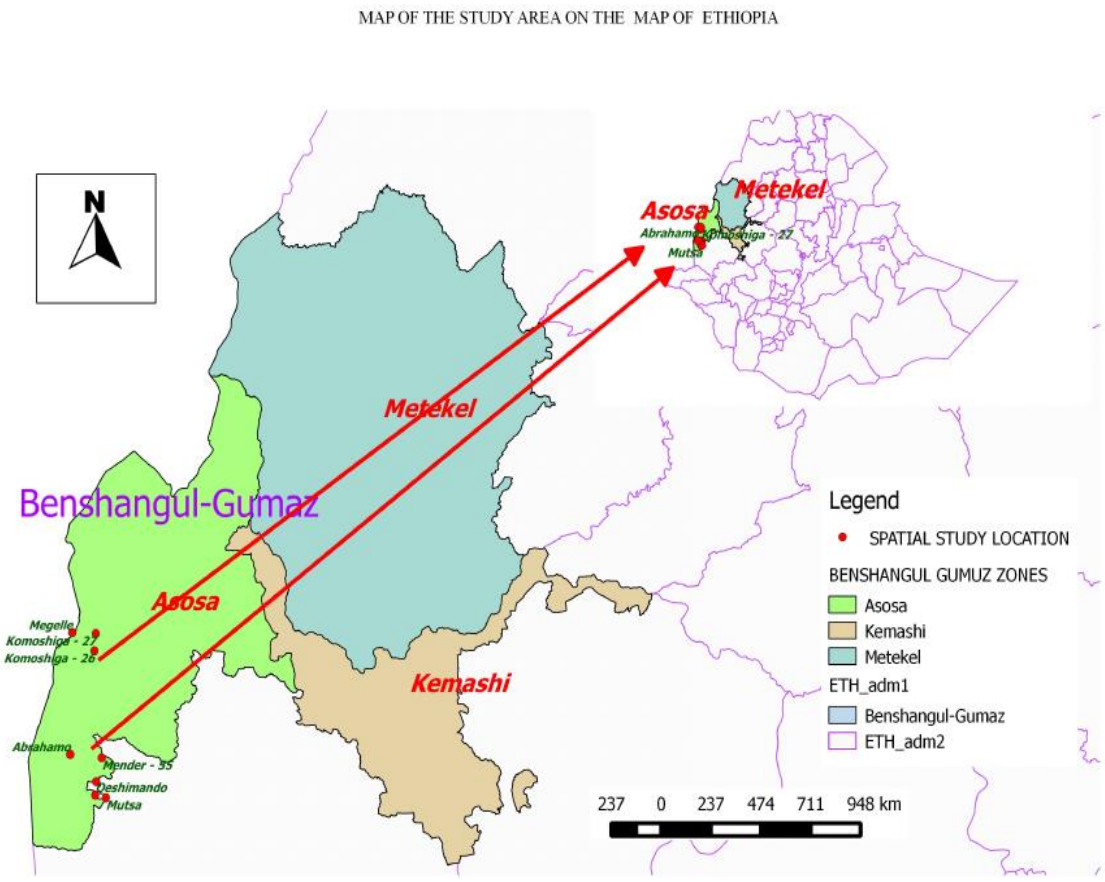


Figure 3: The study location on the map of Ethiopia. *[Source: Geographical information system]*

3.2. Study Methodology

3.2.1. Study design, Sampling methods and Sample size determination

Epidemiological cross-sectional study was used with systematic sampling in Assosa and Bambasi districts of Benshangul Gumuz region, which are lowlands with similar agro-ecological in one season of the year, from January - May 2018: for the isolation of *trypanosome congolense* for the drug resistance testing, determination of the magnitude of bovine trypanosomosis as well as measurement of packed cell volume (PCV) between parasitaemic and aparasitaemic animals in the study areas. Experimental study on trypanocidal drug resistance with emphasis on *trypanosome congolense* and alternative screening tests for herbal remedies traditionally used by animal owners to treat bovine trypanosomosis in mice model.

Sampling frame were list of villages in the study district; with multistage sampling one sampling technique were implemented (Village >Herds). Four villages were purposely selected from each district based on the information collected from Animal health professional and technician who gives veterinary services to the community at both districts regard. From the herds found in each village those chosen for sampling were selected based on the willingness of the owners and a temporary code assigned to each herd. In this case herd were a group of animals those grazing together which make up of a number of household herds.

During field work all parameters including: sex, body condition, body weight and age were recorded for the sampled animals. The sample size calculation was performed using 95% confidence interval with the absolute error of 5% and the expected prevalence of 25%, ((Tesfaye and Ibrahim, 2017) , and 21% (Tikuye and Fantahun, 2017), for Assosa and Bambasi district respectively (Smith, 2000 , because of the reason of having similar ecological and prevalence of the trypanosomosis in the area.

The desired sampling size was calculated according to the formula given by (Thrusfieled, 2018):

$$N = \frac{1.96^2 * P_{exp}(1 - P_{exp})}{d^2}$$

Where: n required sample size

Pexp expected prevalence

d desired absolute precision

1.96^2 the value of z at 95% confidence interval

Pexp expected prevalence

$$n = \frac{1.96^2 * PexpX\ (1\text{-}Pexp)}{d^2}$$

$$\frac{3.84 * 0.25\ (1\text{-}0.25)}{(0.05)^2} = 288$$ this is sample size for Assosa district

$$n = \frac{1.96^2 * 0.21X\ (1\text{-}0.21)}{(0.05)^2} = 254$$ this is sample size for Bambasi district

***Criteria for inclusion*:** Cattle be sampled in this study animals were those age of above six months. However, those animals that were treated for trypanosomosis cases before a week were not included in the study, to minimize the false negative result.

3.2.2. Questionnaire survey

A questionnaire survey was conducted to gather data on the problems of trypanosomosis, practices of trypanocidal drugs usage and the presence of other alternatives used to combat bovine trypanosomosis. The aims of the survey were explained to the respondents before starting the interview and the information were collected in ‘*’Amharic language*’’ using translator. Structure questionnaire format were prepared for the herders and/or

animals owners. In each village 10 animal owners those had cattle and voluntary to be interviewed were included and interviewed regarding to the history and impacts of bovine trypanosomosis and treatment frequency. Other information about livestock management including animal health and extension services (type and frequency of treatment) regarding trypanosomosis and grazing system were collected.

3.2.3. Study animals

The animals used for this study were indigenous zebu cattle (Mamman *et.al.,* 1993), which are usually kept under an extensive husbandry system, depends on natural grazing and crop residues, and kept in a traditional communal management system at both study districts. The study animals were categorized into three depending on their age group (<2 years, 2–5 years, and greater than six years) based on dentition and the body condition score was grouped into poor, thin, medium and good body conditioned animals based on the appearance of ribs and dorsal spines applied for zebu cattle (Nicholson and Butterworth, 1986).

3.2.4. Parasitological examination and PCV determination

Cattle were bled, and paired blood samples were collected from the peripheral ear vein after cleaning and disinfecting the area using micro hematocrit capillary tubes, that were filled 3/4 of the height and sealed with crystal seal. The capillary tubes were centrifuged at 12,000 rpm for 5 minutes in a micro-hematocrit centrifuge to measure the PCV as described in (Murray *et al.,* 1982; Young and Godfrey, 1983). After centrifugation capillary tubes were placed in a hematocrit reader and the length of the red cells column was expressed as a percentage of the total volume of blood. Animals with PCV less than 24% were considered to be anemic.

For parasitological examination: the capillary tubes were cut with a diamond pointed pen 1mm below the buffy coat to include the upper most layer of RBC, the contents of the capillary tube will be expressed on a clean microscope slide, mixed and covered with a cover slip (22X22nm) (Paris *et al.,* 1982) and examine the slide under a microscope

using X40 objectives, the species of the trypanosomes were determined based on the movement of parasite under the microscopic field (Murray and Mcintyre, 1977).

3.2.5. Isolation of *T. congolense* parasite

Depending on parasitological examination blood sample was collected, isolation of *T. congolense* parasites were carried out during sampling period from March – May 2018; for the isolates were collected in cryomedium form from naturally mono-infected zebu cattle with *T. congolense* using a parasitological technique (Paris *et.al.,* 1982; Uilenberg, 1998), in the field and stored in liquid nitrogen (-196 ^{o}C). The cryomedium used for the preparation of stabilates was 14% glycerol. The stabilates were prepared by mixing equal volume (1:1) of infected blood with cryomedium. These stabilates were passage into mice to diagnosis trypanocidal drug resistance, and tested with PCR (Masiga *et al.*, 1992), to confirm pure *T. congolense.*

Trypanosome count: The rapid matching wet-count technique Herbert and Lumsden reviewed by (Clarkson, 1976) was used. This entailed examining a drop of mouse blood under the x 40 magnification of a microscope and counting the number of trypanosomes in each field. Each count per field was matched with the log figures obtained from the reference table (Herbert and Lumsden, 1976). The log figures were converted to absolute number of trypanosomes per ml of blood.

Ethical Consideration

All Ethical clearance protocol, guidelines and principles of the Animal Care and Use followed and obtained for this study from Addis Ababa University College of veterinary Medicine and Agriculture Minutes of Animals Research Ethics and Review Committee. A seven page request for explanation of the purpose of carrying out the studies and all possible care planned to reduce animal reduced from suffering due to sampling was given to the committee. After the committee evaluated the significance of this research, approval was given (*Minutes No. and date of review:* VM/ERC/08/05/10/2018). Accordingly, before sampling animals, consent was asked from the owners and only animals from volunteer owners were sampled.

The experimental study was undertaken from March – May 2018 in laboratory animal units house found in the Veterinary Teaching Hospital (VTH) premises of the College of Veterinary Medicine and Agriculture Addis Ababa University at Bishoftu, Geographically 9°6'N and 37°15'E with in altitude of 1920 m.a.s.l. about 47 km east of Addis Ababa, Ethiopia. The experimental was conducted on albino Swiss white mice brought from house of the Ethiopian Health and Nutrition Research Institute and School of Pharmacy, Addis Ababa University; for the comparison effectiveness of traditional remedies and conventional trypanocidal drugs on the treatment of bovine trypanosomosis emphasis on *trypanosoma congolense*.

3.3. Experimental design

The efficacy of trypanocidal drugs [Diminazene aceturate (DA) and Isomethamidium chloride (ISM)] were tested against *T. congolense* isolates in experimentally infected mice based on previous established protocols (Eisler *et.al.,* 2001; Geerts *et al.,* 2001) and assessing the effectiveness of locally used traditional herbal remedies collected from the study area used for the treatment of bovine trypanosomosis. The experimental mice were randomly assigned into eight groups of five mice per group for assessment of trypanocidal drugs resistance and herbal remedies screening test.

3.3.1. Experimental mice

Swiss white albino mice weighing 25 –35 gm and age of 8–12 weeks; were bought from the laboratory of animal house; Ethiopian Health and Nutrition Research Institute and School of Pharmacy, Addis Ababa University. Animals were housed in polypropylene cages: 5 mice per cage kept and allowed to free access to feed (pellet) diet and clean water ad libitum. They were classified either as donors or experimental (ILAR, 2000).

3.3.2. Donor and experimental mice parasite inoculation

Those donor mice infected with *T. congolense* isolated from the study area followed up to peak parasitaemia; after then the mice were kept in chloroform reagent and immediately blood collected by cardiac puncture and blood was diluted in PSG to increase the blood volume and number of animals injected with the infected blood collected from the donor mice. Each mouse was injected with 0.2 ml of diluted blood which contains~10^5 trypanosomes (Eisler *et.al.,* 2001; Yayeh *et al.*, 2018).

Accordingly, healthy Swiss albino mice were divided into four groups: four for conventional drug and another four groups for traditional herbal remedies screening test, given intraperitonealy and orally respectively. A total of 70 (seventy) mice were used for both trypanocidal drugs resistance testing, screening test on traditional herbal and toxicity testing of the herbal remedies. The treatment with the herbal remedies extracts and trypanocidal drugs began on day when peak trypanosome parasites first detected post-infection (Clarkson, 1976).

Reagents and drugs used for the experiment: The reagents used were Methanol^{RS} (CARLO ERBA Reagents S.A.S, Batch No.: V6I635186L, EXP: 2019/10, France), Chloroform^{RS} (CARLO ERBA Reagents S.A.S, Batch No.: D6N002076N, EXP.2020/12, France) and10X Phosphate Buffered Saline (10x PBS, PH 7.4), Batch No.: X.7382819, Mfg. Date: 03/2014, Sisco Research Laboratories Pvt. Ltd. 26 Navketan Ind., M.C.Rd., Andheri (E), Mumbai 93, India. The amount of extraction reagents for 50gram of each plant extract was 250ml.

The trypanocidal drugs used in this experiment were Diminazene aceturate (Veridium 125mg®, LOT: No.254A2, FAB: 03/2016, Exp. 06/2021, Ceva Sante Animale, Libourne-France and Diminal®, Batch: No.17111703, DOM: 2017/04/20, Exp: 2020/04/19, Made in Korea). For the treatment of infected mice with *T. congolense* the two commonly used (Isometamidium and Diminazene aceturate) trypanocidal drugs were injected intraperitonealy on the basis of accurate body weight measurement taken

immediately before treatment using digital weighing machine (METLER TOLEDO, TDNR: 0.1.7.201.81.0, SNR; 1125092703, Switzerland), dose rate determined *(Annex:4),* by dissolving in 12 ml and 15 ml sterile water respectively at the onset of parasitaemia; after examining the collected blood sample from the experimental mice under microscope by direct wet film technique (Murray *et al.*, 1977), and the experimental animals were monitored until the end of experiment.

3.4. Plant materials and its preparation

The two plants collected for this study were selected based on information collected from local traditional healers on their curative effect in the treatment of animal trypanosomosis and other internal parasites of animal and human in Benishangul Gumuz region from one of the study districts, Bambasi. The fresh plant materials collected from the study area were wrapped by plastic sheets and transported to Addis Ababa University College of veterinary medicine, Bishoftu, Ethiopia; to pharmacology laboratory for extraction preparation. Taxonomic identification was done, and a voucher specimen was deposited (Collection EM/001 and 002) at the National Herbarium identification, College of Natural sciences, Addis Ababa University, Ethiopia. These plants were identified as *Eriosema montanum* and *Hypoxis villosa.*

Eriosema montanum: belongs to the Family: FABACEAE *Erect, rarely prostrate or climbing,* herbs or subshrubs**:** it is known by its vernacular name as; Qumbu, inhabiting in lowlands. The chemical composition of this plant was not identified.

Hypoxis villosa: - *Hypoxis villosa* of the Family: Hypoxidaceae. The picture of *Hypoxis villosa* (*Annex: 5*). It is known locally by its vernacular name: - ‘’Africa potato’’, inhabiting lowland area, distribution- rare and its chemical composition were not identified.

The whole plant sample of each plant after collection and transportation were shade dried and then later pulverized (ground) to powder using a mortar and pestle. The powder was collected in polythene bags and stored at room temperature until it was passed through

the extraction procedure. The plants were extracted for primary *in vivo* evaluation screening by extracting 50gm of each dried roots of *Hypoxis villosa* and *Eriosema montanum*.

The powder of each plant sample was extracted with chloroform and methanol by Soxhlet extraction method. A fivefold (250 ml) quantity of solvent in relation to the plant material was used for the extraction (*Fig: 4).* All extracts were concentrated on a rotatory evaporator (BüCHI Rotavapor R-200, Switzerland) at 65^0C and 62^0c for Methanol and Chloroform extraction respectively, at low pressure coupled to a thermoregulatory device in order to obtain dry extract as see bellow *(Fig: 5).*

Figure 4*:* Soxhlet machine for the extraction of plant materials

Figure 5*:* Rotatory evaporator apparatus used during preparation of the powder from the extracted plant materials

For each solvent extraction was performed depending their boiling point and the time taken for one extraction range from 3-4 hr. The solvent-free extracts (solid extract obtained): were removed, weighed 1.2gm., 2.2gm., 2.5gm.and and 2.6 gm. were collected and stored at 4^{o}C until use. The chlorophormic extract was dissolved in Dimethyl Sulfoxide (DMSO) propylene glycol while the methanol extract was dissolved in Phosphate buffer distilled water.

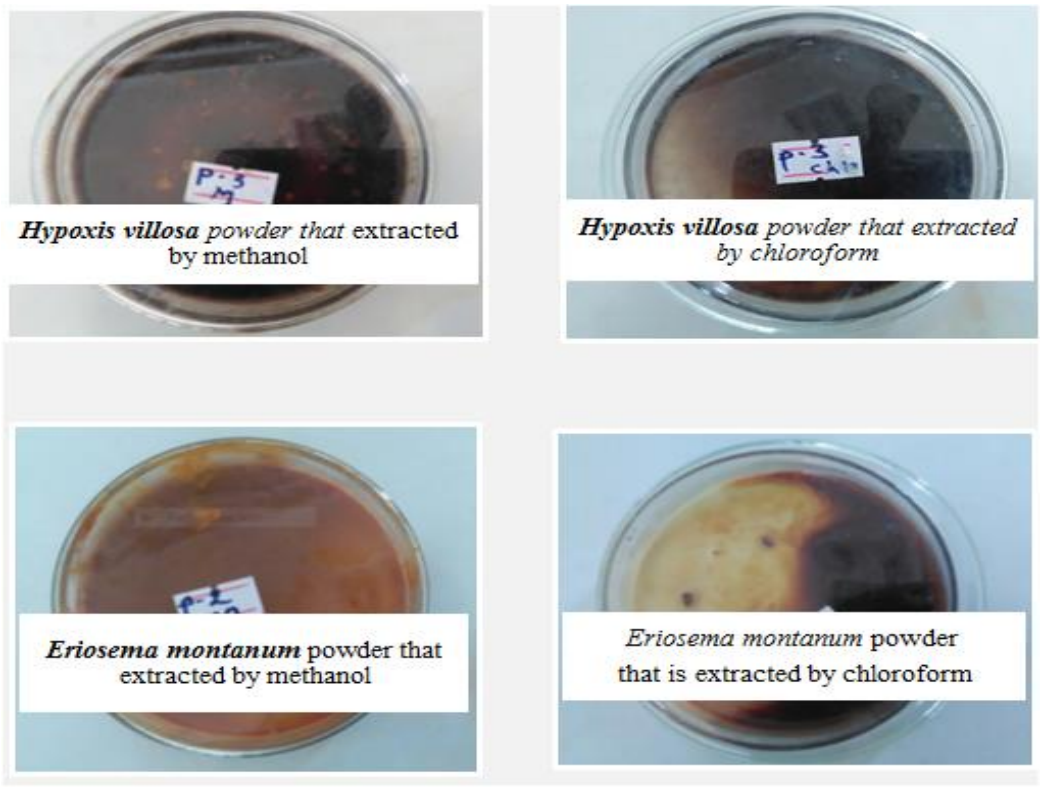


Figure 6: The powder obtained after evaporation of extracts of *E. montanum* and *H.villosa*

3.4.1. Experimental design for plant

Table 4: The parasite species, extraction used and dosage

Trypanosome inoculated	**Herbal remedies type**	**Extraction used**	**Groups**	**No. of mice**	**Dosage**
T. congolense	*Hypoxis villosa*	Methanol extract	G1	5	600mg/kg
		Chloroform extract	G2	5	600mg/kg
	Eriosema montanum	Methanol extract	G3	5	600mg/kg
		Chloroform extract	G4	5	600mg/kg

SPP: Species of the parasites, *T. congolense*

3.4.2. Administration of the plant extracts

The above plant materials powder (*Fig: 7*) prepared in the peterdish; distilled water used for those extracted by methanol and the other extracted by chloroform reagent powder were dissolved in the Dimethyl Sulfoxide (DMSO)and administered orally using nasogastric tube to the experimental mice. The dose of the prepared extract given to each experimental mouse was calculated on the body weight (mg/kg), administered orally for five days consecutively at a dose rate of 600mg/kg bw for each group mouse of different extract of plant material powder and monitored until the disappearance of trypanosomes or end of the experiment and Animals were euthanized immediately the end of the experiment.

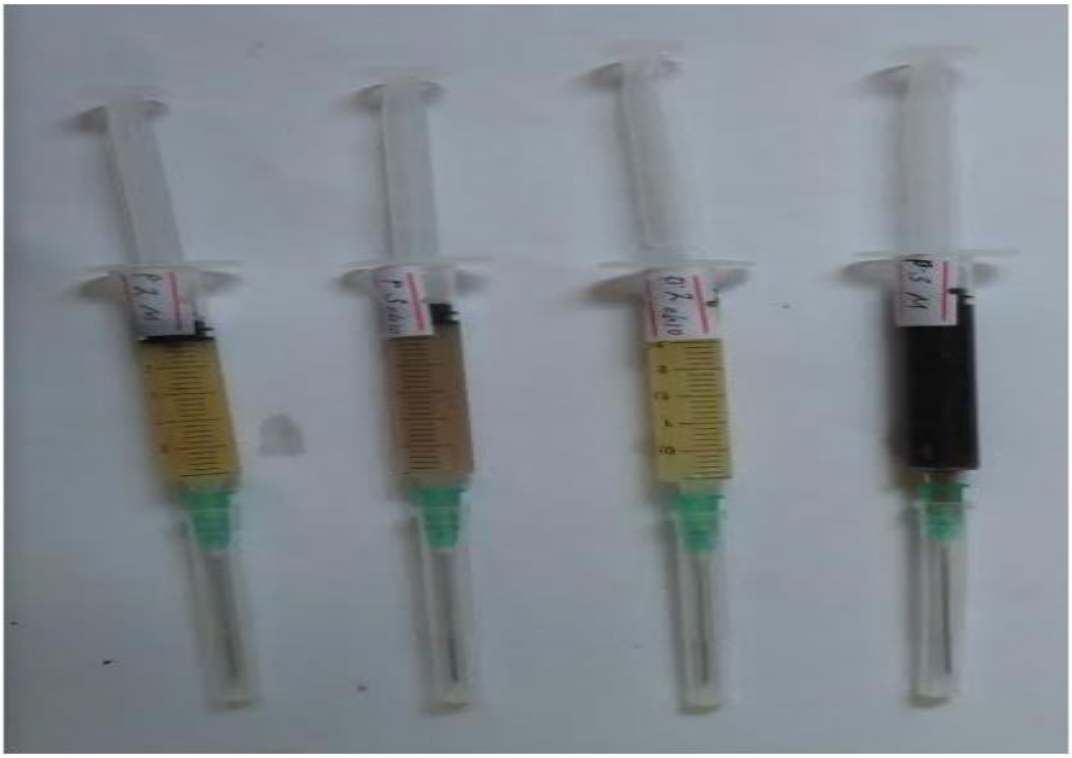


Figure 7: The last result of plant materials prepared for oral feed of experimental mice. (Source: from powder of *E. montanum* and *H.villosa* extracted powder).

3.4.3. Determination of acute toxicity

The study was conducted for each extract in female and male Swiss albino mice after adaptation of new environment; acute toxicity study was investigated in accordance with the (Lorke, 1983) method. To investigate whether the plant is toxic or not, groups of eight mice each group contain 3 mice for *Eriosema montanum* methanol 300mg/kg and 600mg/kg bw and chloroform extract were given at a dose of 300mg/kg and 600mg/kg. Similarly, for that of *Hypoxis villosa* methanol 300mg/kg and 600mg/kg and chloroform extract also given at dose of 300mg/kg and 600mg/kg orally using a nasogastric tube.

All animals were kept under strict observation for behavioral, neurological, autonomic, or physical changes such as alertness, motor activity, restlessness, convulsions, coma, diarrhea, and lacrimation. These observations continued for further 14 days for any signs of overt toxicity. The mice were observed and the mortality was recorded for a period of three weeks. Among the dose given the lowest dose which killed one mouse (minimum toxic dose) and the highest dose which had not killed any mouse (maximum tolerated dose) were noted.

3.4.4. Parasitological and observation of the clinical signs of the experimental

Starting from the day of experimental study animals were controlled daily for clinical signs and parasitological findings (parasite load, PCV and body weight measurement) using direct microscope examination (buffy coat technique), by collecting blood from the tail of mice in to paired heparinized capillary tube (Murray *et.al.*, 1977) and digital balance every two days for two weeks. According to (Herbert and Lumsden, 1976), the level of parasitaemia was assessed using rapid matching method.

3.5. Data Analysis

All information collected from the field and laboratory results was entered to and stored in the Microsoft office excel data sheet. The data collected from the field observation and questionnaire survey was analyzed by stata 12 and presented using percentages, mean $\pm$ standard error and frequency tables. Prevalence was analyzed by determining total positive cases out of the total number of animals sampled. Infection rate on the basis of sex, age, and body condition was compared using χ^2 tests (chi-square). Mean PCV in parasitaemic and aparasitaemic animals were compared using *t*-test. Significance test was set at 5% alpha and 95 confidence interval. Statistical significances of differences between levels and combination in the survival duration for the experimental studies were analyzed using (ANOVA) one way analysis of variance. Variation in survival duration was compared using between different groups and the p values less than 0.05 were considered as statically significant difference between considered variables (Rodrigues *et al.*, 2014).

4. RESULTS

4.1. **Questionnaire survey results**

In the study area the farmers were rearing varied animals species: indigenous zebu cattle 90%, sheep 25%, goat 15% and equine 40%, which were kept under an extensive husbandry system that depends on natural grazing, private and crop residues and kept in a traditional communal management system in herd, 25% or in small group and free grazing 75% in the two study districts. In the area cattle was primarily reared for draught purpose and income generation. However, these animals were threatened by several types of animal diseases. The respondents complained that there are number of animals' diseases that affect livestock production and husbandry practices. Among these, trypanosomosis is one of the most important constraints to agriculture and animal health problem in the study area.

All respondents agreed that the recurrence of the disease increases during rainy season due to high infestation fly population in the summer, but the opposite is true during the dry season of the year. According to the respondents' suggestions trypanosomosis is getting worse in the study area due to failure to cure the diseases while using commercially available trypanocidal drugs (i.e., the development of trypanocidal drugs resistance by trypanosomes species). The questionnaire survey results also showed that there was reduction in the trypanosomosis burden and tsetse fly population during the implementation of traps and application of insecticides (deltamethrin spray or pour on).

Because of trypanocidal drugs failure for the treatment of trypanosomosis (locally named, ''Ghendi''), 10% of those individuals interviewed in both districts had acquainted with preparation and using the home herbal remedies and indigenous knowledge for the treatment of bovine trypanosomosis; when the animals showing signs of this disease (*Annex: 5 and 6).* The traditional healers indicated a tea cup powder of the above mentioned herbal remedies mixed with common salt (NaCl), were used together and drench to adult cattle showing the signs of trypanosomosis. In addition to using the

mentioned medicinal plants for the treatment of bovine trypanosomosis, they also used these herbal remedies for internal parasites of humans and animals for a long period of time and they were very confident that these herbal remedies are effective to treat the mentioned above diseases.

Assessment on practices of farmers about trypanocidal drugs usage indicated that Isomethamidium chloride and Diminazene aceturate are the two most commonly used trypanocidal drugs in the study area. The result also showed that animal owners in both study districts started to inject concurrently two sachets of Diminazene aceturate and Isomethamidium chloride at time to one animal; because when animals treated with either of one of this drugs alone, they relieved from the disease only for one or two weeks. It also stated that Isomethamidium chloride is the better one and relieved their animals at least for two to three weeks in the both districts. Out of the total respondents that used trypanocidal drugs, 18% of them showed that they inject their animals by their own whereas 82% send their ill animals to animal health post and veterinary clinic.

All 100% of respondents in both study districts believed in that the trypanocidal drugs currently used to treat trypanosomosis in the study area are not effective; and believed that those drugs from the private veterinary shops were better than those drugs provided by Minister Agriculture and Rural Development (Mo ARM). In these study areas 90% of the interviewed individuals knew how trypanosomes transmitted and some of the clinical signs of the disease (decrease appetite, depressed, the infected animal is stand alone, weakness and rough hair coats, mentioned by respondents).

As the information collected from the respondent the prevalence of trypanosomosis were high in Assosa as compared Bambasi district (*Fig 8).* All interviewed individual from both study districts confirmed that among livestock reared by the farmers, cattle are the most affected animal (100%), equine 40%, shoat 4% and those animals sick of trypanosomosis showed different sign, such as weakness, loss of body condition, grinding teeth, diarrhea, rough hair coat, lacrimation and decrease feed intake.

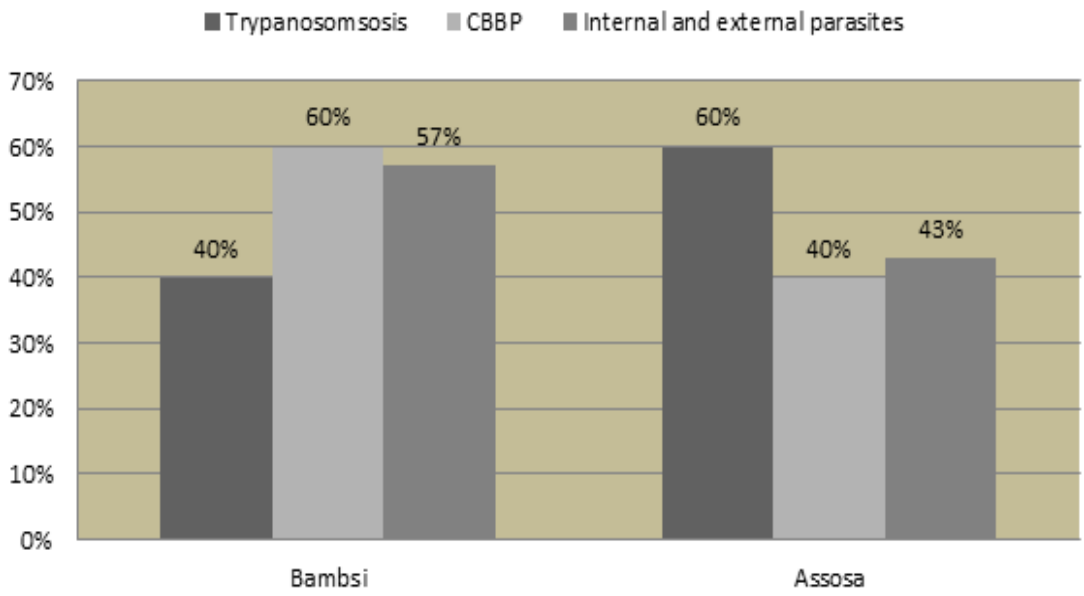


Figure 8*:* Prevalence of bovine trypanosomosis in the two districts of study area.

According to the respondents, the production loss, reduced working power, loss of body condition and mortality were the major impacts of bovine trypanosomosis. The burden of trypanosomosis as the information collected from the community in the study area revealed that production loss is the highest significance of trypanosomosis which was followed by reduction in animals body weight as shown in *(Fig: 9).*

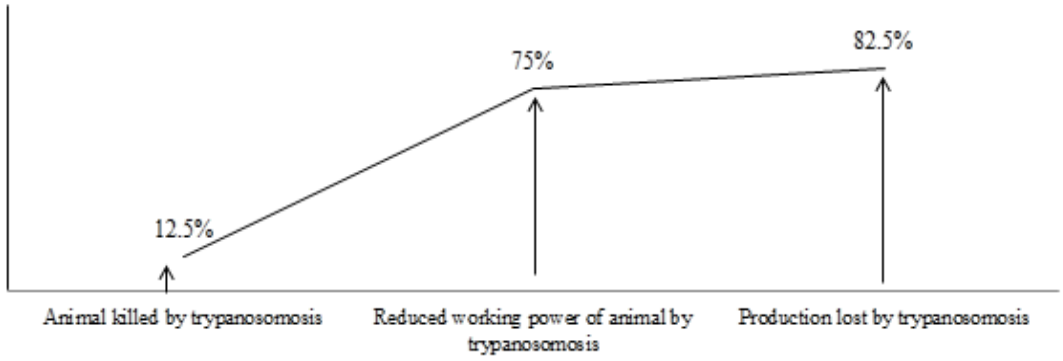


Figure 9: Impact trypanosomosis in animal production

4.2. Parasitological findings during field work

From the overall 542 animals examined, 33(6.08%) were found to be infected with trypanosomes. During the primary parasitological screening, the three species of trypanosomosis, namely *T.congolense, T.vivax, T.brucei* and mixed infection were detected. The point prevalence obtained from the cattle screened for isolation was 10.5% in Assosa district and 4.78% in Bambasi district as in (*Table: 10*).

Table 5: Results of parasitological trypanosomosis survey conducted in each district

Districts	**Cattle sampled**	**Total positives**	**Prevalence in each area**	**Species of parasites detected**			
				T.c	T.v	T.b	mixed
Assosa	288	21	7.3%	17	1	-	4
Bambasi	254	11	4.33%	6	1	1	3
Total	542	33	11.6%	23	2	1	7

T.c *Trypanosome congolense*, T.v; *Trypanosome vivax*, T.b: *Trypanosome brucei*

The prevalence and the proportion of the trypanosomes species according to the results obtained from parasitological examination were in the *(Figure: 10)*.

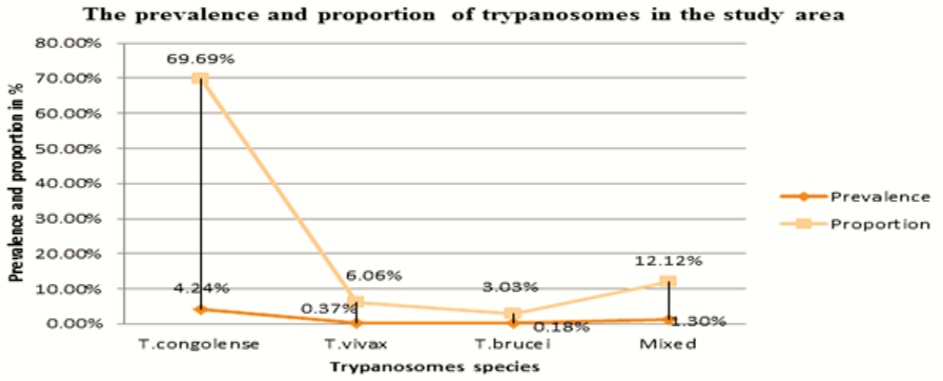


Figure 10: Prevalence and Proportion of trypanosomes species in the study area

4.3. The prevalence of bovine trypanosomosis depends on age, sex and body condition

The prevalence of trypanosomosis was higher in females (5.12%) as compared to male animals (33.33%). However, the difference was not statistically significant ($P > 0.05$). The highest prevalence was observed in animals those thin and the variation in prevalence between the different body condition groups was also not statistically significant ($P > 0.05$). The prevalence of trypanosomosis between body condition scores was 0.23% in good, 2.79% in medium, 3.72% and 0.69%in good body conditioned animals and it was statistically not significant ($P > 0.05$).

Table 6: Prevalence of trypanosomosis according to Age, Sex, and Body Condition at both districts

Hosts at risk	**Animal examined**	**Animal infected**	**prevalence**	**P value**	**Chi^2**
Female	340	23	6.76%	0.469	0.534
Male	203	10	4.92%		
Total	542	33	6.08%		

Table 7: The variation of trypanosomosis prevalence depending on different age categories

Age	**Animal examined**	**Not infected**	**infected**	**prevalence**	**P value**	**Chi^2**
<3	180	168	10	5.6%		
3-6	282	260	18	6.4%	0.876	0.2657
>6	80	75	5	6.25%		
total	542	503	33	6.08%		

Table 8: The influence of body condition on the prevalence of trypanosomosis

Body condition	Animal examined	Not infected	Infected	Prevalence	P value	Chi^2
Good	21	20	1	4.76%		
Medium	237	222	12	5.06%	0.833	0.868
Thin	247	227	17	6.88%		
Emaciated	37	33	3	8.10%		
Total	542	502	33	6.08%		

The prevalence of bovine trypanosomosis was studied separately in sex, body condition, and age groups of cattle and significant variation was not observed ($P > 0.05$).

4.4. Condition of sampled animals PCV during the field

The frequency distribution of PCV of the overall studied 430 cattle was recorded. The mean PCV value of 26.30% was registered during the study period. The most frequently recorded PCV value of infected animals was 24% which was recorded in four animals and the minimum PCV of infected animal was 10% which recorded only in one animal from the overall studied animals in the two districts. The maximum PCV recorded in positive animals were 41% and the minimum PCV value of negative animals examined to be 11%. The mean PCV values of the studied animals were significant influenced by trypanosomes infection ($P = 0.015$).

Table 9: Mean PCV comparison between infected and non-infected animals

Group of animals	examined	Mean	Std. Dev.	t-value	p value	95% Conf. Interval
Non-infected	509	26.55528	5.742651			25.98937 - 27.12118
Infected	33	23.1875	6.620776	3.1542	0.0009	20.80046 - 25.57454
Total	542	26.30465	0.2831252			25.74817 26.86114

4.5. Experimental trial results

All mice inoculated with fresh blood containing *T. congolense* isolates taken from the field were parasitological positives. Parasitaemia was proven after 15 days of inoculation in donor mice and peak parasitaemia was detected 20% after inoculation. Then after transferred to experimental mice, parasitaemia detected 10 days of post infection in all groups and blood samples collected 5 days after treatment was given to check for the clearance of parasitaemia. In this studies bleeding and wound in the abdominal areas of three mice, one from Isomethamidium treated group with 1mg/kg bw, one from Diminazene treated group at a dose 7mg/kg bw and the other one from *H. villosa* chloroform extract product at a dose 600mg/kg bw on the fourth days of treatment commenced by the extract. During experimental period three mice died from Isomethamidium 1mg/kg bw on second day of treatment, Diminazene 7mg/kg bw on the fourth day of treatment and *H. villosa* methanol extract on the seven days of treatment, which accounts 20% of each of the treated groups by the products.

4.6. Sensitivity status of trypanocidal and extracts of *E. montanum* and *H. villosa*

Dosages of Isomethamidium chloride and Diminazene aceturate of different doses used for the comparison of cattle doses in mice have initially cleared the parasitaemia. Fast apparent clearance observed in Diminazene aceturate used at both doses for the

experiment after two days of treatment given and three days post treatment for Isomethamidium chloride used at both doses intervals, with comparative therapeutic doses in mice.

4.7. The clinical finding and development of parasitaemia in experimental mice

The measured mean parasitaemia load of the infected mice with *T. congolense* and treated by extracts of *E. montanum* and *H. villosa* herbal remedies were significant ($P < 0.05$) as compared to each other (*Table :10).*

Table 10: The average parasitaemia load recorded during laboratory activities in mice treated with *E. montanum* and *H. villosa*

Extracted plant types	**Mean value of parasitaemia load**				
	1st day	2nd day	3rd day	4th day	5th day
E. montanum chloroform ext.	29.2± 4.17	31.2±4.96	31.6±5.15	30.4±4.9152	33.2±5.16
E. montanum methanol ext.	35.6± 7.38	38.4±7.54	40±8.62	43.2±10.36	43.2±9.26
H. villosa methanol ext.	35.2± 4.23	39.5±1.65	32.5±4.34	36.75±1.49	34±2.94
H. villosa chloroform ext.	25.2± 6.34	24±6.84	26±7.53	25.6±7.87	24.6±8.45

Ext: extracted, *E. montanum: Eriosenama montanum, H.villosa: Hypoxis villosa*

The parasite load during the experimental study was measured at different days of the experimentation and was recorded, and the result obtained were compared and computed as the logarithmic values described in by Herbert and Lumsden (1976) (*Figure: 11*).

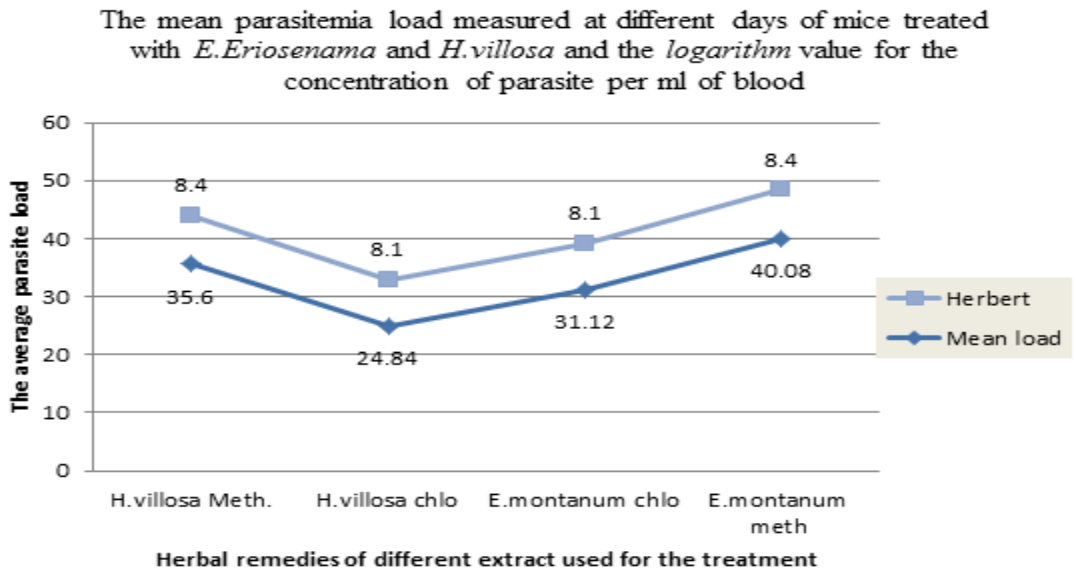


Figure 11: The effect of the extracts *E.montanum* and *H. villosa* on the parasitaemia load

As compared the mean body weight of those experimental mice treated by traditional medicinal plant extracts and convectional trypanocidal drugs were highly significant ($p < 0.05$).

4.8. The PCV of infected mice with *T. congolense* and treated with *E. montanum* and *H. villosa*

The mean PCV value of experimental mice infected with *T. congolense* and treated with extracts of *E. montanum* and *H.villosa* herbal remedies were significant ($P < 0.05$) as compared each other (*Table: 11),* because of there is decrease PCV value of the experimental mice.

Table 11: Impact of methanolic and chloroform extracts of medicinal plants on PCV of the *T. congolense* infected mice

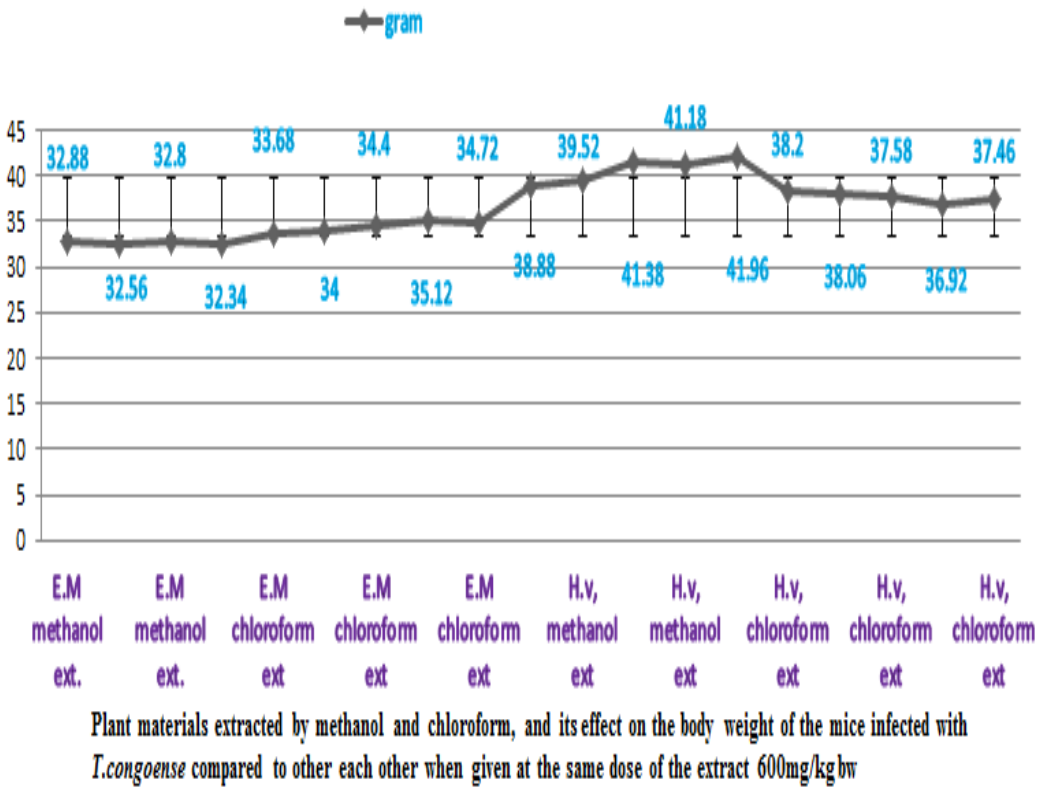
Groups	Rx	Packed cell volume in mice				
		Day 1	Day 2	Day 3	Day 4	Day 5
***H.villosa* chloroform ext.**	5	34.8±0.86	34 ±0.70	33±0.70	32.2±0.66	32+0.70
***H.villosa* methanol ext.**	5	37.6±0.5	37.2±0. 73	36±0.89	35.2±0.86	34.4+0.92
***E.montanum* chloroform ext.**	5	38.25±0.47	37.75±0.47	37.5±0.6	36.75±0.8	36+0.40
***E.montanum* methanol ext.**	5	39.4±0.5	39.2±0.37	37.6±.50	38±0.70	37.8+0.58

ext.: extract, Rx: treatment

SE: standard error, n = 5, even though the mean PCV values were slightly changed as gone through the row of the table, and the p values ($p < 0.05$) indicate that there is significant difference in the mean PCV in a group of mice infected with *T. congolense* and treated with methanol and chloroform extracts of *E. montanum* and *H. villosa.*

4.9. Effects of *E. montanum* and *H. villosa* extracts on body weight of mice infected with *T. congolense*

The extracts of *E. montanum* and *H. villosa* prepared by methanol and chloroform extract were orally administered at a maximum dose rate of 600mg/kg for each group of mice containing five animals per group, and as the time progresses the animals become getting decreased in body weight *(Figure:12).*



E.M: *Eriosenama montanum*; H.v: *Hypoxis villosa*; ext.: extract

Figure 12: The effect of *E. montanum* and *H. villosa* on the weight of experimental mice

4.10. The effect of *E. montanum* and *H. villosa* extract on the survival time of mice infected with *T. congolense*

The group of experimental mice infected with *T. congolense* and treated with similar dose of 600mg/kg of bw; methanol and chloroform extracts of *E. montanum* and *H. villosa.*

Table 12: Mean survival time of mice infected with *T. congolense* and treated by *E. montanum* and *H. villosa*.

Type of Rx	**Condition of experimental mice**					**Mean survival time (days±SE)**
	Rx	Cured	Survived	Death	relapse	
*E.M.*600mg/kg, meth. Ext.	5	Initially cleared	5	-	Not cured	30±00
*E.M.*600mg/kg, chlor. Ext.	5	Initially cleared	4	1	Not cured	28±2
H.v. 600mg/kg, meth. Ext.	5	Initially cleared	4	1	Not cured	27±3
H.v.600mg/kg, chlor. Ext.	5	Initially cleared	5	-	Not cured	30±00

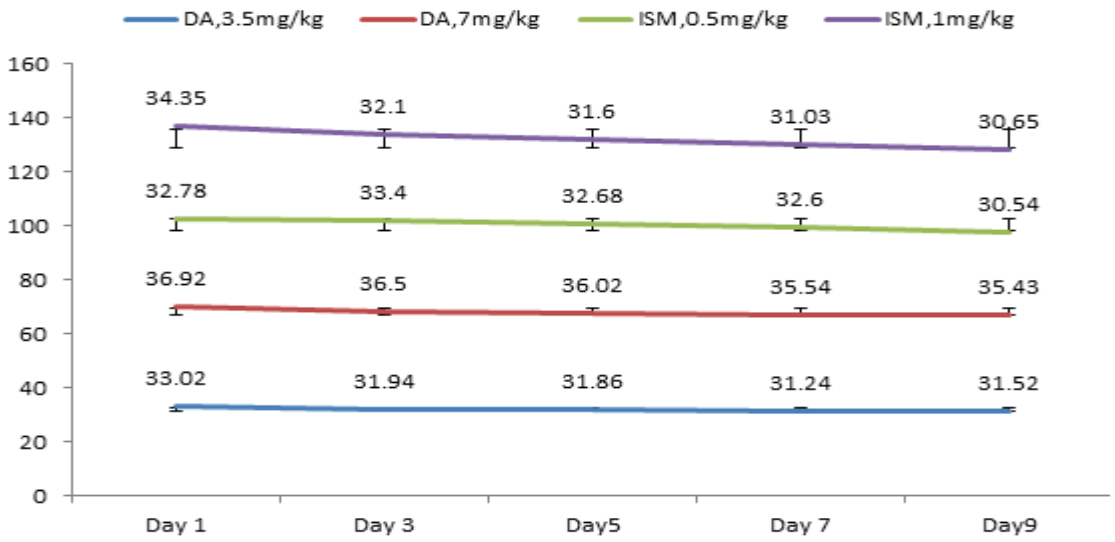
E.M.: Eriosenama Montunam, H.V.: Hypoxis villosa, chlor: chloroform, *Meth*: methanol

Table 13*:* Mean survival time of mice with *T.congolense* and treated by Diminazene and Isomethamidium

Type of Rx	Condition of experimental mice					Mean survival time (days ±SE)
	Rx	Cured	Survived	Death	relapse	
DA 3.5mg/kg bw	5	Initially cleared	3	2	3	30±00
DA 7mg/kg bw.	5	Initially cleared	3	2	2	26±2.75
ISM 0.5mg/kg bw	5	Initially cleared	5	-	2	30±00
ISM 1mg/kg bw	5	Initially cleared	4	1	2	28±2

DA: Diminazene, *ISM:* Isomethamidium, *Rx:* treated, *SE:* standard error

The body weight of those infected experimental mice and treated by Diminazene aceturate and Isomethamidium chloride as indicated in the (*Fig. 13*, there is a slight decrease from time to time in their body weight.



ISM; Isomethamidium chloride, ***DA***: Diminazene aceturate

Figure 13: The average body weight of experimental mice

5. DISCUSION

The result of questionnaire survey conducted in the two selected study d districts (Assosa and Bambasi), confirmed that trypanosomosis is most economically important disease in the area. Among the 80 individuals interviewed in both districts, 90% of them indicated that trypanosomosis is the first constraints to agricultural activities and animal production. Similar findings have already reported by different researchers (Afewerk *et al.*, 2000), from Metekel district of Northwest Ethiopia, (Tewelde *et.al.,* 2004), from Western Ethiopia and (Dagnachew *et al.*, 2005), from Northwest Ethiopia where tsetse fly are present. In these area farmers are rearing different species of animals and managed under extensive husbandry system which depends on natural grazing and communal management system in the area.

All respondent (100%) agreed that the recurrence of the disease increases during rainy season. This may be due to the favorable condition during this season for hatching of tsetse flies pupas, which buried under the ground during the dry season of the year. Ninety percent (90%) indicated that; the commonly used trypanosomosis control methods in the area, were by using the available trypanocidal drugs in the local market (Isomethamidium chloride and Diminazene aceturate), similar results indicated by (Seyoum *et al.,* 2013), who conducted research on the prevalence of bovine trypanosomosis and its impacts in tsetse fly infested districts in Baro-Akobo and Gojeb river basins, Southwestern Ethiopia and others were used the locally prepared herbal remedies for the treatment, as animal showing the clinical signs of the disease. .

In study reported by (Delespaux *et al.*, 2002), in eastern province of Zambia, all farmers bought Isomethamidium chloride from veterinary assistance or directly from veterinary office, in the same to this in the study area 80% the respondents indicated that the sources of these drugs were veterinary clinics and shops and the remaining 20% was from unknown or unauthorized veterinary drugs vender, as reported by (Shimelis *et al.*, 2008),

during his study on the prevalence of bovine trypanosomosis and assessment of trypanocidal drugs in tsetse and non- tsetse infested area of northwest Ethiopia. Furthermore, according to the respondents suggestions trypanosomosis is getting worse in the study area due to the development of trypanocidal drugs resistance by trypanosomes species, as reported by (Sinshaw *et al.*, 2006). Because of trypanocidal drugs failure for the treatment of trypanosomosis (locally, ‘’Ghendi’’), 10% of those individuals interviewed in both districts had acquainted with preparation and using the home herbal remedies and indigenous knowledge to treat of bovine trypanosomosis.

In both the study districts farmers were practicing concurrent administration of two commonly used drugs (Diminazene aceturate and Isomethamidium chloride) to one animal at a time. This was practiced because of treated animals were received from the signs and symptoms of the disease only for not more than 14 days. Besides, out of the total respondents that use trypanocidal drugs, 18% of them practice self-medicine prescription and inject their animals by their. This finding is agreed with various reports done in Ethiopia, (Afewerk *et al.*, 2004; Tewelde *et al.*, 2004; Seyoum *et al.*, 2013). Incontray to this,. 82% of the respondents said that they send their animals to animal health post and veterinary clinics, as also reported by (Shimelis *et al.*, 2008).

According to the information collected from the respondent the prevalence of trypanosomosis were high (60%) in Assosa as compared Bambasi district, that could causes considerable losses due its mortality and morbidity, treatment cost, reducing milk yield and working power animals. The overall prevalence of bovine trypanosomosis in the study area was 7.44%. On the other hand, in the present study prevalence of bovine trypanosomosis in Assosa and Bambasi districts of Benshangul Gumuz region were 4.18% and 3.25% respectively, which slightly higher than the prevalence of trypanosomosis conducted in Dale Wabera district of Kellem Wollega Zone, Western Ethiopia; which was 2.86%. The prevalence of trypanosomosis recorded in Assosa district is similar with the result reported by Shimelis *et al.*, (2005), 4.91% in the non-tsetse infested northwest of Ethiopia. But, the present result is relatively similar to results recorded by (Tafese *et al.*, 2012), in East Wollega zone using buffy coat technique and

found prevalence rate of 8.5%. . This result was also lower as compared to (Abebe & Jobre, 1996) at tsetse infested areas of Ethiopia (17.67%), Shimelis *et al.*, (2005), in Dembecha and Jabitehenan (12%) and (Mekuria & Gadissa, 2011) reported 12.41% prevalence in Metekel and Awi zones of Northwest Ethiopia. Oppositely, the present study prevalence different from studies conducted by (Cherenet *et al.*, 2006), who record 20.9% and 25.7% prevalence respectively, in the tsetse-free and the tsetse-infested areas of Amhara region Northwestern part of Ethiopia, using molecular diagnostic method.

Out of the total recorded 6.08% prevalence of trypanosomosis, *T. congolense 4.24%*, *T. vivax* 0.37%, *T. brucei* 0.18% and mixed infection accounts about 1.93% of the totally examined animals. In this the prevalence recorded for *T.vivax* was similar to the result reported by (Cherenet *et.al.,* 2006; Sinshaw *et.al.,* 2006), 2 – 9% in the tsetse infested area using buffy coat technique. Sex wise the prevalence of trypanosomosis was high in females compared with males (8.52%), which is opposite to the result reported by (Fetehanegest *et al.,* 2012). The study finding showed that proportion of trypanosomosis infected animals *T. congolense* 81.82%, *T. vivax* 3.03%, and *T. brucei* 3.03% and mixed infection 12% recorded. The higher proportion of *T. congolense* in this study was in agreement with the previous results of for tsetse infested areas of Ethiopia (58.5%) (Abebe & Jobre, 1996) and (Muturi, 1999); at Mereb Abaya, South Ethiopia (66.1%) .

Moreover, the results of (Woldeyes & Aboset, 1997), at Arba Minch Zuria districts (85.2%) and (Rowlands *et al.*, 1999), in Ghibe valley, Southwest Ethiopia (84%), had also shown higher results of *T. congolense*. The predominance of *T. congolense* infection in cattle suggests that the major cyclical vectors or *Glossina* species are more efficient transmitters of *T. congolense* than *T. vivax* in East Africa since 1976 (Langridge, 1976).

Rowlands *et al.*, (1995), have indicated the problems of drug resistance are higher in *T. congolense*, and *T. congolense* is mainly confirmed in the blood, while *T. vivax* and *T. brucei* also invade the tissues (Stephen, 1986). The prevalence of bovine trypanosomosis was studied in different sex, body condition, and age groups of cattle and statistically not significant value observed ($P > 0.05$). This result is in agreement with the previous researches reported by (Abebayehu *et al.*, 2011; Tafese, Melaku & Fentahun, 2012).

During the diagnosis of sampled animals at field area, to measure the PCV of animals, a number of animals had anaemia without having trypanosomosis; even though anaemia is characteristic of this disease. On the other hand there were also other factors that are anticipated to affect the PCV profile of animals (internal parasites those sucking blood, vector-borne diseases, and nutritional deficiencies can also cause reduced PCV (Van den Bossche & Rowlands, 2001). Some animals were infected by trypanosome but their PCV was normal and anaemia was not recorded in them. The other reason might be due to some infected animals being able to keep their PCV within the normal range for a certain period of time. And negative animals with PCV values of less than the threshold value set 24% may be due to inadequacy of the detection method used (Murray & Mcintyre, 1977) and other anaemia causing diseases, or delayed recovery of the anaemic situation after current treatment with trypanocidal drugs and positive animals with PCV of greater than 24% might be thought of as recent infections of the animals (Van den Bossche & Rowlands, 2001).

During the study period the most frequently recorded PCV value of infected animals were 24% which is recorded in four animals and the minimum PCV of infected animal was 10% which recorded only in one animal from the overall studied animals in the two districts. The maximum PCV recorded in positive animals were 41% and the minimum PCV value of negative animals examined to be 11%. The mean PCV values of the studied animals were significant influenced by trypanosomes infection ($P = 0.015$).

Experimental trails in mice were conducted to assess trypanosomes resistance status to trypanocidal drugs and the effectiveness of locally used herbal remedies for the treatment of bovine trypanosomosis with emphasis on *T. congolense* isolates from the study area. The experimental trails aimed at tracing trypanocidal drugs resistance for Diminazene and Isomethamidium chloride. These drugs were temporarily clear *T. congolense* isolates from experimental mice on second day's treatment by Diminazene aceturate and third days post treatment by Isomethamidium chloride of the both doses given, and relapse occurs in tenth days in two mice treated with 3.5mg/kg bw of Diminazene aceturate and fourteen days post treatment with Isomethamidium given at a dose rate of 0.5mg/kg bw.

Similar study results were reported on experimental sensitivity conducted on the *T.congolense* isolates from Ghibe, Arba-minch, Bedele and Sodo by (Chaka & Abebe, 2003), indicating the failure of trypanocidal drugs to clear the parasites from the experimental mice using bovine doses. In the research conducted by Afewerk *et al.*(2000) the *T. congolense* isolates from Benshangul Gumuz was not respond to trypanocidal drugs given at a dose a rate of 28mg/kg bw Diminazene aceturate and 4mg/kg bw Isomethamidium chloride in experimental mice. Similarly, the results of many studies in Ethiopia and in others African countries indicate the development of multidrug resistance: In Ethiopia (Codjia *et al.*, 1993; Mulugeta *et al.*, 1997) and in Burkina Faso (Clausen *et al.*, 1992) indicate resistance to sanative pair of trypanocidal drugs for *T.congolense* isolates.

However, there is an experimental trial study that reveals the effectiveness of trypanocidal drugs conducted in mice. For instances, study conducted in Zambia as reported by (Sinyangwe *et.al.*, 2004) indicates even using discriminatory doses at 0.1mg/kg bw Isomethamidium chloride and 20mg/kg bw of Diminazene aceturate, from *T.congolense* isolates 53.5% were sensitive to both drugs used in experimental trial. The clone of identity IL 3000 from Burkina Faso, also resistance to 1mg/kg bw Isomethamidium chloride was successfully treated with 5mg/kg bw dose of Isomethamidium chloride in mice (Knoppe *et al.*, 2006). As reports from Nigeria, by (Nnia Egbe-Nwiyi *et al.*, 2006) indicated, *T.congolense* isolates were cleared by Diminazene aceturate when given at a dose rate of 10.5mg/kg from the experimental rats.

In Ethiopia field observation conducted indicate that the prophylactic coverage of Isomethamidium chloride can extends up to four weeks of post treatment, in opposites to manufacturer 6 to 16 weeks prophylactic coverage of this drugs. From the study results of three villages of Kindo Koysha, southern Ethiopia (Ademe & Abebe, 2000) documented that the prophylactic efficacy of Isomethamidium chloride was less than thirty days. Similarly, in Woinma field observation on *T. congolense* isolates from the area revealed four weeks for the prophylactic activities of Isomethamidium chloride on the isolates. On the other hand, researches conducted in different parts of Africa reveals effectiveness of trypanocidal drugs in the field observations. As the reports from Kenya, by (Hagos *et al.*,

2014) Samorin and Veridium, which is Isomethamidium based products were effective in prophylactic activities for 70 days when administered at a dose rate of 0.5mg/kg bw.

At start of present experimental trial, the initial and the peak parasitaemia level were demonstrated on 15 days and 20 days of respectively, post inoculation of the parasites in the mice used for isolation and amplification. In the experimental mice parasitaemia was demonstrated 7 – 11 days of post inoculum of the parasite to the mice. A similarly observation by (Olila *et al.*, 2002) detect parasitaemia 5 – 7 days of post infection using primary isolates of *T.brucei.* The mice treated during peak parasitaemia 11^{th} days post infection. Nnia Egbe-Nwiyi *et al.*, (2006), from Nigeria, reports that the procedure for the treatment of experimental mice have to be after 14 days of infection at peak parasitaemia was followed in more recent experimental trial conducted.

The outcome of the present trypanocidal drugs resistance test in mice clearly shows the presence of trypanosome isolates have developed drugs phenotype to the currently available trypanocidal drugs. The reports by (Peregrine, 1994), indicate that the in the areas where multiple trypanocidal drugs resistance is expressed at the level of individual parasite, chemotherapy becomes increasingly ineffective and intervention at the level of the control of vector is far most important.

The used herbal remedies (*E. montanum* and *H. villosa)* or *''Africa potato''* known as for its long history of medicinal use in Africa continent (Snijman, 2000), of different extract were used for the screening test to detect their anti-trypanosomosis activities given at 600mg/kg bw. In the South Africa primary health care community currently using *Hypoxis* as immuno-stimulant for the patients with HIV/AIDS, A daily dose of 2400 mg of raw plant purported to be therapeutically effective (Albrecht *et al.*, 1996). The results of the used herbal remedies *E. montanum* and *H. villosa* indicate that one mouse was died from the group treated with *H.villosa* on the fourth days of treatment started. The mean parasitaemia load of the infected mice with *T. congolense* those treated by extracts of *E. montanum* and *H. villosa* extracts were significant ($P < 0.05$) as compared each other.

During the period of experimental activities in the laboratory the average parasitaemia level measured in each group treated with herbal remedies indicate that the average

minimum parasite load counts (24.84 average wet film field count of parasite for five days) (*Fig: 14)*, detected in those group treated with *H.villosa* extracted product using chloroform extracted and in *E.montanum* chloroform extract and *H.villosa* methanol extracts relatively similar parasite load detected during the study period. Similar to (Maikai *et al.*, 2008), who report that the preliminary screening of *X. americana* methanol and aqueous extract reduce motility of *T.congolense* in vitro, *H.villosa* chloroform extracted used in this experiment also indicate reduction in the parasite loads in mice treated orally by this herbal extract.

Similarly, researches were conducted by different researcher on herbal remedies against trypanosomosis. As reviewed by (Assefa, 2017), in 2013 *Abedo,* showed *in vitro* activity was observed on nine extracts from plant materials of *Tapinanthus globiferus* and *G. latifolium* were against *T.congolense* at various concentration. In Ethiopia, reports (Nibret & Wink, 2011), speculate that *Dovyalis abyssinia* might be promising candidate for phytotherapy of trypanosomosis. Feyera et al (2014) also reported that methanol extract of *Artemisia abyssinica* showed appreciable *in vitro* and *in vivo* antitrypanosomal activity against field isolates of *T.congolense*.

In this study the PCV value result observed in all groups of infected mice treated with *E. montanum* and *H. villosa* extract was reducing from time to time, which indicate that the parasite load was not cleared or reduced to the minimum level that the animals can cop up with. Besides, the body weights of the experimental mice assigned to groups of the three extracts: *E.montanum* methanol extract, *E.montanum* chloroform extract and *H.villosa* chloroform extract showed similar measurement; and *H.villosa* treated group showed there was no reduction in body weight measurement. The study results also revealed that the body weight of experimental mice treated with trypanocidal drugs and herbal remedies shows similar pattern of reduction in weight even though there was initially clearance of the parasite from those groups treated with trypanocidal drugs

6. CONCLUSION AND RECOMMENDATIONS

Bovine trypanosomosis caused by *T. congolense* and *T. vivax* was found to be an important disease of cattle in Assosa and Bambasi districts of Benshangul Gumuz region, North West of Ethiopia. In addition to this in chronic infection may reduce and the animals are too weak to be used for ploughing. This chronic infection often ends in the death of the animals. Totally the disease affects each household and, in the area, the socio-economic importance of the disease is a single most important constraint to improve livestock productivity in the area. Despite limited number of trypanocidal drugs, they are more widely used than means to control the disease. As a result, trypanosomosis develop resistance to the existing trypanocidal drugs and it is very unlikely that new trypanocidal drugs will be released in to the market in the near future. It is clear that trypanocides remain a vital part of the armory used by farmers across Africa to control trypanosomosis and it is in every one's interest that these drugs remain as effective as possible for as long as possible. In order to achieve this, it is important to monitor, on regular bases drugs, usage patterns by farmers, and prevalence and incidence of drugs resistance in different countries, and to put in place measures to ensure that only drugs of the highest quality find their way on to the markets in Africa. The other alternatives were to search for herbal remedies used locally by traditional healers. Different researches conducted on indigenous African medicinal plants have indicated that African medicinal plants have potential to treat and manage the devastating parasitic diseases common in Sub Saharan Africa. Therefore, need to put emplace or in place_measures for vale addition of Africa traditional medicine and its subsequent incorporation into mainstream medical care system.

7. FEATURES DIRECTIONS

Despite the continued use of medicinal plants by traditional healers to treat parasitic diseases, the scientific data generated has not been translated into polices, guidelines or products. This coupled with variations in dosage regimens amongst practicing traditional healers which predispose patients to acute toxicity, (Gericke 1996). The mode action of these medicinal plants has not been fully explored; if studies are carried out to fill in these knowledge gaps, they would inform on safe and effective dosage determination modalities, drugs presentation and value addition (Annapurna *et.al.* 2014).Advances in metabolomics, genomics, proteomics, bioinformatics, and cheminformatics should be utilized as a leeway to drug discovery and development. This calls for partnerships b/n traditional medicine practitioners and established research institutions, both private and government. Biotechnological advances should be used as a high throughput screening plat forms of the indigenous natural plant products. This is essential for it will allow for value addition process like preservation of medicinal extracts for extended shelf life, preparations of tablets, herbal tea and infusions, freeze dried products, or even be fortified into foods.

Patenting of indigenous knowledge should be considered so that all stake holders can feel safe to share information that can be lead to development of natural product proto type that can be commercialized. Apart from this, sustainable harvesting, protection and conservation of these plants need to be implemented. Polices that regulate harvesting from natural habitats like forest ought to be enforced as well as the promotions of community – nurseries to preserve the heritage and avoid depleting the precious plant resource.

Conflict of interest statement

The authors declare that they have no competing interests and have no any financial or personal relationships that could inappropriately influence or bias the content of the paper

Limitation of the paper

The present study was conducted to find solution concerned with the problem of bovine trypanocidal drugs resistance. And during this study all measurements needed to include in this paper was not included: (The chemical compositions of the herbal remedies used for this study were not identified, the length of experimental period was shortening, due to this and other reason the pathological and biochemical change may be caused by the traditional remedies treatment were not diagnosed).

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9. ANNEXES

Annex 1: Questionnaire survey format (for individual interview)

Points of Discussion

 Livestock management General Animal Health List of animal diseases in the area

 Trypanosomosis epidemiology and impacts on farmers' livelihood

 Trypanocidal drugs and therapeutic management practices commonly known in the area

II. Questionnaire for individual cattle owners

District……Kebele/Village……..Date……

1. Livestock management

1.1. Which livestock species are kept? Cattle, Sheep, goat and Equines Others

1.2. What is the grazing management of your animals? Communal and free grazing; live at the outside of the farmers' house in beret system; Private and free grazing; live at the outside of farmers' house; Tether; Stall feed

1.3. If management is based on free grazing system, are they in herd or in small groups?..

1.4. Where do animals graze? ……...……

2. General Animal Health: Major diseases of livestock

2.1. List of animal diseases in your locality in their order of importance

2.2. Perception and incidence of trypanosomosis

A. Does trypanosomosis occur in this area? (Yes, no, other) If yes, what is the rank of trypanosomosis with regard to animal losses compared to other diseases? …….

2.3. Which livestock does trypanosomosis most affect? *Cattle, Sheep, Goat Equine, Others*……..

2.3. What signs do you commonly observe when your animals get sick with trypanosomosis?.....

2.4. Does the disease problem: A) Kill the animal B) cause production loss C) cause loss of work efficiency of oxen?

2.5. In which season/month do livestock most often get the disease (trypanosomosis)?. ...

2.6. How is the disease transmitted? A) By Flies B) by Ticks C) by treatment materials D) Others specify

3. Management of trypanosomosis

3.1. Is there any trypanosomosis and fly control operation in this area? Yes…, No…

If yes, what kinds of control method(s) employed in your area? A) Fly control using insecticides B), resting animals from work C), Treatment of affected animals D), others specify…….

3.2. If using fly replant insecticides employed, where are the common insecticides sources? .....

A), Federal government B), Regional government C), NGO D), others……

3.3. Who are applying the insecticides to the animals? A), Animals owners B), Animal health personnel (professional) C), other people specify……..…….……...

3.4. Which insecticides are most commonly used in the areas (Name, types, color etc.)? Modern,specify……,Traditional, specify……...

3.5. What is the cost of insecticides/animal in the area? .....

3.6. Since when have you been using each of the insecticides you mentioned? .....

3.7. What is the situation of trypanosomosis from your experience? A) Improving B) no change C) increasing D) do not know

3.8. When was your animals lastly treated against tsetse fly?..Did you see any improvement? ……

3.9. Are the insecticides effective? Yes, No; if yes, how do you describe the effectiveness?...

A), Reduction in clinical signs B), Working efficiency C), Milk production D) Body condition E), reduced mortality F), money invested on treatment G) working efficiency of your animals H), other specify…..……

3.10. Are there traditional method of treatment and management practices for controlling and prevention of trypanosomosis……

4. Therapeutic Practices

4.1. How do you combat trypanosomosis when it exists in your herd? A), Traditional medicine locally available B), Buying and administration of veterinary drugs by their own, C), travelling to nearby veterinary clinic D), all alternatives are employed

4.2. Do you use traditional medicine to treat your animals? Yes, No If yes what type of traditional medicine you use for your bovine when affected by trypanosomosis?.....

4.3. How do you prepare and administer to your animals? ……

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

4.5. Do you use veterinary drugs to treat trypanosomosis? Yes, No If yes, which type of veterinary drugs you know and experienced as treatment for your animals? A), Diminazene/yellow powder/ ‘’Bicha’’, B), Isometamidium/coffee/Buna, C), Ethidium (red tablet), D), others (specify) ……..……

4.6. Who administer drugs to your animals? A. Yourself..... B. Experienced villager, C. Any other professional.....

4.7. How do you apply /prepare injectable solutions?

1. **Measurement:** A), Standard measurement /Gramm Other local measurement, B), locally adopted measurement/metallic probe, C). Simple guess

2. Solvent used (water, others) ……

3. Other substances added**:** A), antibiotics, B), Oil/salt C), nothings

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

A). Route of administration……, B) Site of administration ……

4.9. What amount of injectable solution do you give for each of your animals with trypanosomosis? A. for calves' B. for heifers C. for adults

4.10. How many of your animals get cured after your treatment? ¼, ½, ¾

4.11. How many times you treat your animals for trypanosomosis per year? 2X, 3X, 4X, 5-10X, >10

4.12. Where do you get trypanocidal drugs? a. Pharmacy/Vet. Or human b. Shop/Others

5. Questionnaire (for professionals engaged in veterinary service).

District……Place of Work……..…Status/DVM/AHA/AHT

5.1. Are there trypanosomosis in your area? Yes, No

5.2. What general procedures are used before treatment of trypanosomosis? .....

5.3. What type of trypanocidal drugs have you been using?.....,A). Trade mark/active ingredient…….…..B), at what dosage?

Drugs nameNormalExtra label

1…….…… …….. …….

2. …….…… …….. …….

5.4. Are there any relapses/therapeutic failures encountered? Yes / No At what time? Duration

5.5. What measures taken to overcome relapses? .....

5.6. Can you estimate relapse rates? .....

Thank You

Name of interviewer ……Date…….. Signature…….....

Annex 2: The overall summary of traditional plant remedies and the used dosages

Plant materials	*Regents used*	*Dosage*	*Number of animal treated*	*Experimental group*
E.montanum	*Methanol extract*	*600mk/kg bw*	*5*	*Group 1*
	Chloroform	*600 mg/kg bw*	*5*	*Group 2*
H.hypoxis	*Methanol extract*	*600mk/kg bw*	*5*	*Group 4*
	Chloroform	*600 mg/kg bw*	*5*	*Group 5*
	Methanol extract	*600mg/kg bw*	*5*	*Group 6*

bw – body weight, kg – kilograms, mg – milligram

Annex 3: Cryomedium preparation procedure

- Mix one tablet of PBS in 200µm of distilled water
- Add two grams of glucose .Now you have prepared PSG
- Mix the newly prepared PSG with 14% pure glycerol
- Mix equal volume of positive blood the above solution

Annex 4: Standardized protocols for testing trypanocidal drug resistance in mice

Drugs type by trade name	Group of mice	The number of parasite injected	Doses	Route of injection	Parasitological examination
Veriben®	5	10^5	3.5mg/kg	IP	Tail blood wet smear
	5		7 mg/kg		
Isometamidium chloride	5		0.5mg/kg		
	5		1 mg/kg		

IP: intraperitoneal, mg: milligram, kg: kilogram

Annex 5: Picture of guenus of *Hypoxis villosa*

Annex 6: Picture of guenus of *Eriosena montanum*

Annex 7: The powder prepared for the further extraction process

Powder of Genus:Hypoxis villosa

Powder of Genus:Eriosema montanum

Figure: the powder of the two plant species prepared for extraction

Annex 8: The extracted *Hypoxis villosa* and *Eriosema montanum* by different reagents