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LIST OF ABBRIVATIONS

µm	Micrometer
ANOVA	Analysis of Variance
b.w	Body weight
BCT	Buffy coat technique
CD_{50} or CD_{80}	Curative dose that gives complete cure in 50 % or 80 % of the animals
CI	Confidence interval
DIGT	Drug Incubation *Glossina* Infectivity Test
ELISA	Enzyme Linked Immunosorbent Assay
HCT	Haematochrite centrifugation Technique
i.p	Interaperitonial
ILRAD	International Laboratory for Research on Animal Diseases
k	Kebele
kDNA	kinetoplast Deoxyribo Nucleic Acid
mAECT	minature Anion Exchange centrifugation Technique
MCD	Minimum curative dose
mg	Milligram
ml	Milliliter
mm	Millimeter
MOA	Ministry of agriculture
NARS	National Agricultural Research System
ng	Nannogram
NTTICC	National Tsetse and Trypanosomosis Investigation and Control Centre
OIE	Office International des Epizooties
PCV	Packed Cell Volume
RBCs	Red Blood cells
SNNPRS	Southern Nations and Nationalities People Regional State
spp	Species
STEP	Southern Tsetse Eradication Project

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ABESTRACT

The current study was conducted in tsetse and non tsetse controlled areas of the Southern Nation Nationalities and Peoples Regional State (SNNPRS) of Ethiopia. A cross sectional study to determine the prevalence of bovine trypanosomosis as well as a drug sensitivity tests on *T. congolense* in both naturally and experimentally infected cattle and mice, respectively were carried out. In the first study area, Humbo district, a total trypanosome prevalence of 4.8% (95% CI: 1.8–7.5) was recorded. In the second study area, Mareka district, a total prevalence of 20.4 (95% CI: 14–26.8) was recorded. There was statistically significant difference in the mean prevalence of trypanosomosis between the two areas (P<0.001). The mean PCV value for Humbo and Mareka was 26.2 (95%: 25.7-26.7) and 22.7 (95% CI: 22.1-23.3), respectively. There was statistically significant difference in the mean PCV value between the two study areas (P<0.001). To assess the prophylactic activity of isometamidium chloride a field trial was conducted in Humbo on nine parasitological positive zebu cattle. A breakthrough case of 66.7% (6/9) was recorded in less than five weeks. A qualitative mice assay was conducted on two *T. congolense* isolates obtained from the break through cases with ranges of doses of isometamidium chloride and diminazene diaceturate. Thereafter the mice were followed for relapse infection. Isometamidium at doses 0.5-4mg/kg b.w and diminazene at doses of 3.5-28mg/kg b.w failed completely to cure *T. congolense* infections in any of the mice. A quantitative mice assay was conducted on four *T. congolense* isolates obtained from Mareka. The four isolates were pooled into two pools (Pool-1 and Pool-2) for the quantitative mice assay. The pooled isolates were tested with the same trypanocidal drugs and ranges of doses as it was used for the qualitative mice assay. The minimum curative dose (MCD) of isometamidium chloride and diminazene diaceturate that cleared the trypanosome from mice infected with Pool-1 was found to be 4mg/kg b.w and 28mg/kg b.w, respectively. For mice infected with Pool-2 the MCD of isometamidium chloride and diminazene diaceturate that cleared the trypanosme was found to be 2mg/kg b.w and 14mg/kg b.w, respectively. Even though cloned populations were not used to proof the observed resistance was at the individual level or not the result indicated that there is the possibility of failure of "sanative pair" between the two drugs.

Keywords: *Trypanosoma congolense*, drug resistance, cattle, mice assay, isometamidium chloride (Veridium®), diminazene diaceturate (Diminasan®)

1. INTRODUCTION

Trypanosomosis is a complex debilitating and often fatal disease caused by infection with one or more of the pathogenic tsetse transmitted protozoan parasites of the genus *Trypanosoma*. The most important species responsible for the disease complex, commonly known as Nagana in livestock, include *T. brucei*, *T. congolense* and *T. vivax* (Molyneux, 1997). There are five economically important animal trypanosome species in Ethiopia: *T. congolense*, *T. vivax*, *T. b. brucei*, *T. evansi* (Langridge, 1976) and *T. equiperdum* (Alemu *et al*., 1997). A report by Abebe and Jobre (1996) indicated an infection rate of 58.5% for *T. congolense*, 31.27% for *T. vivax* and 3.5% for *T. brucei* in cattle in tsetse infested areas of southwest Ethiopia. They also indicated that 8.71% trypanosome prevalence rate was recorded in the highlands (tsetse free areas) of which 99% was due to *T. vivax*. In countries such as Ethiopia, where livestock is an important part of the agricultural sector, trypanosomosis contributes to the direct economic losses of crop-livestock production. Approximately 220,000 km^2 are infested with different species of tsetse flies, and 14.8 million cattle, 6.1 million sheep and goats, 1.0 million camels and 1.2 million equines are at risk (MOA, 1995). Chemotherapy and chemoprophylaxis are the most widely applied methods for combating trypanosomosis. Tsetse control, by comparison, is much more limited in scope, involving approximately 14% of the total tsetse-infested area. On an average, 1.5 million doses of trypanocidal drugs were administered annually at an average cost of USD 0.5 per dose (MOA, 1995). Trypanocidal drugs have been used for more than 40 years in Ethiopia to control trypanosomosis in different domestic animals (NTTICC, 1996). However, the emergence of drug resistance is seriously hampering this effort. Treatment and prevention of animal trypanosomosis relies essentially on three drugs, namely; homidium (Homidium chloride–Novidium® and Homidium bromide–Ethidium®) diminazene aceturate (Berenil®) and isometamidium chloride (Samorine®,Trypamidium®) (Uilenberg,1998). However, almost all of these trypanocidal drugs are gradually losing their efficacy due to drug resistance (Williamson, 1970). Documented accounts of trypanocidal drug resistance have been reported in at least 13 countries in sub-Saharan Africa (Geerts and Holmes, 1999). Earlier studies have shown the presence of drug resistant trypanosomes in cattle herds of Ethiopia. Scott and Pegram (1974) described the occurrence of homidium resistant populations of *T. congolense* in Didessa and Angar valleys in Wollega province. Long term occurrence of multiple drug resistance in *T. congolense* in cattle at Ghibe, southwest Ethiopia was demonstrated in laboratory studies of field isolates (Codjia *et al*., 1993; Mulugeta *et al*., 1997). Afewerk (2000) reported that *T.*

congolense field isolates from the Metekel region, expressed resistance to both isometamidium chloride and diminazene aceturate in mice. Aseffa and Abebe (2001) also described the presence of drug resistant trypanosome in naturally infected donkeys in north Omo Zone. The trypanosomes are usually not resistant to both diminazene and isometamidium at the same time. Thus, these compounds have been termed as a sanative pair for the control of bovine trypanosomosis (Whiteside, 1960). Recently, Afewerk *et al*. (2000) confirmed multiple drug resistance in cloned *T. congolense* isolated from Metekel region, northwest Ethiopia. The effectiveness of trypanocidal drugs is being eroded by the emergence of resistance trypanosomes and there is little prospect for the development of new trypanocidal drugs in the near future. Understanding the epidemiology of drug resistance in Ethiopia is increasingly important to design appropriate control measures.

Therefore the objectives of the present studies were:

- To compare the prevalence of bovine trypanosomosis in tsetse and non tsetse controlled areas of the SNNPRS of Ethiopia.

- To determine the prophylactic activity of isometamidium chloride in naturally infected cattle.

- To asses the trypanocidal activity of diminazene diaceturate and isometamidium chloride on *T. congolense* field isolates in experimentally infected mice.

2. LITERATURE REVIEW

2.1. African trypanosomosis

African trypanosomes are protozoan parasites causing trypanosomoses in animals and man. They are predominantly parasites of the blood but may exist in other sites of the body such as skin, lymph nodes or the central nervous system (CNS), where they can give rise to the distinctive sequel of trypanosome infections (Taylor and Authiė, 2004). Trypanosomes are classified in the phylum Sarcomastigophora, the order Kinetoplastida, the family Trypanosomatidae, the genera Trypanosoma (Molyneux and Ashford, 1983). Based on their difference in the course of development in their vector this genus is divided into Stercoraria and Salivaria. The pathogenic African trypanosomes belong to the later group, Salivaria (Stevens and Brisse, 2004).

2.1.1. Morphology

***Trypanosoma (Nannomonas) congolense* Broden, 1904**

The trypanosomes of this subgenus have a range in total length of 8-24 μm. There is no free flagellum at any stage in the life cycle. The posterior end of the body is usually rounded but can be slightly pointed in longer parasites. The medium sized kinetoplast is usually in a marginal and subterminal position. *T. congolense* is one of the smallest trypanosomes with a mean length of 12-17 μm (Molyneux and Ashford, 1983).The use of the term monomorphic is somewhat misleading in this species in that there is variation in size and shape between strains. Generally two variants are to be seen, a short form (9-18 μm), the typical congolense type and a longer form (up to 25μm), with individuals intermediate between the two. Recent studies have now resulted in a subdivision of the species in several types, which can be distinguished by isoenzymatic difference and molecular techniques. Only one type has received a separate name, *T. godfreyi*, as it also differ in its patogenicity for various hosts, while the others are designated as *T. congolense* savannha type, *T. congolense* Tsavo type, *T. congolense* forest type, *T. congolense* Kilifi type (Uilenberg, 1998). *T. simiae*, the porcine trypanosome, is more pleomorphic in its characteristic and the mean length is 15-19μm, slightly longer than *T. congolense*. Nannomonas trypanosomes are very active in fresh blood films but do not tend to move far across the microscope field. They also demonstrate agglutinating properties by tending to adhere to each other as well as to host tissue *in vivo* (Molyneux and Ashford, 1983).

***Trypanosoma (Duttonella) vivax* Ziemann, 1905**

The typical bloodstream form has a somewhat expanded or club shaped posterior end, which tapers towards the anterior. Movement in wet blood film is rapid and distinctive. The large, generally terminally placed kinetoplast is distinctive and is often sufficient to separate *Duttonela* from other Salivarian trypanosomes. *T. vivax* has a mean length of 20-26 µm, a long free flagellum and a large terminally placed kinetoplast, distinguishing it from the other pathogenic salivarian trypanosomes. *T. vivax* is a very mobile and lively parasite (Molyneux and Ashford, 1983).

***Trypanosoma (Trypanozoon) brucei* Plimmer and Bradford, 1899**

Blood stream forms of *T. brucei* (and *Trypanozoon*) measure from 11-42 µm in length. They are typically polymorphic, being represented by three forms: (i) long slender forms (mean lengths 23-30 µm), which possess a long free flagellum, a well developed undulating membrane, subterminal kinetoplast and narrow drawn out posterior end (ii) short stumpy trypanosomes (mean length 17-22 µm), which are stout, usually lacking a free flagellum, possess a well developed undulating membrane and have a kinetoplast near the broadly rounded posterior and, (iii) intermediate forms (mean length 20-25 µm) in which the flagellum is shorter and the posterior end blunter than in the slender form (Stevens and Briss, 2004).

***Trypanosoma (Pycnomonas) suis* Ochmann, 1905**

Trypanosoma suis is a short, stout monomorphic trypanosome with a free flagellum and a small subterminal kinetoplast. It develops in *Glossina* spp in the mid gut and salivary glands. However, salivary gland forms are reputedly not infective, fully maturation taking place in the hypopharynx of the tsetse fly. Pigs are the only domestic mammalian hosts (Stevens and Brisse, 2004). The total length of *T. suis* has a range from 13-19 µm (mean length 16 µm) (Mulligan, 1970).

2.1.2. Epidemiology

The epidemiology of African animal trypanosomosis is highly dependent of the parasite, vector and host factors. *Trypanosoma* species occur in a remarkable variety of genotypes with differing strains of virulence, immunogenicity and response to chemotherapeutic agents. The

severity of the disease also depends on the species and strain of trypanosomes involved. For instance, *T. vivax* and *T. congolense* are known to have high virulence in cattle. The fact that the parasite infects not only cattle but also wild animals, which constitute the reservoirs of the disease, makes the epidemiology of animal trypanosomosis extremely complicated. The animal hosts differ in their response to trypanosome infection depending on the species, breed and individual animals. The level of animal husbandry practices, nutritional status, workload and physiological states (exhaustion, lactation and parturition) also play a role in the severity of the disease (Eisler *et al*., 2004). The degree of risk to which domestic animals are exposed depends on the species and density of the tsetse present, infection rate in the tsetse, species and strains of trypanosomes, sources of these infections (wildlife or domestic animals) and feeding preferences of the flies. The extent to the flies transmitted the disease depends on the species of livestock which is there source of infection (MacLennan, 1970).

Transmission and distribution

Most tsetse transmission is cyclical, and begins when blood from a trypanosome infected animal is ingested by the tsetse fly. The trypanosome loses its surface coat, multiplies in the fly, then reacquires a surface coat and becomes infective. *T. brucei* species migrate from the gut to the proventriculus to the pharynx and eventually to the salivary glands; the cycle for *T. congolense* stops at the hypopharynx, and the salivary glands are not invaded; the entire cycle for *T. vivax* occurs in the proboscis. The animal-infective form in the tsetse salivary gland is referred to as the metacyclic form. The life cycle in the tsetse may be as short as one week with *T. vivax* or extend to a few weeks for *T. brucei* species (Itarde, 1981).

The distribution of tsetse transmitted African trypanosomes is governed by that of their vector. Apart from cyclical transmission through *Glossina* species, *T. vivax* has the capacity to be transmitted mechanically by other blood sucking diptera such as horse flies (Tabanidae) and stable flies (*Stomoxys* spp.). Mechanical transmission explains the occurrence of *T. vivax* infections outside Africa. *T. evansi*, which is pathogenic to camels and horses, is mechanically transmitted most likely by *Tabanide* and possibly also by stable fly (Molyneux and Ashford, 1983).

Pathogenesis

Distinct pathological changes may be caused by the different livestock infective trypanosome species. Pathology in tissue is associated with the relative ability of the trypanosomes to invade extravascular space and organs (Taylor and Authiė, 2004). *T. congolense* is mainly confined to the blood, while *T. vivax* and *T. brucei* also invade the tissue. There is remarkable intraspecies variation in the pathogenicity of different parasite stock, especially stock isolated from distinct geographical regions (Taylor and Authiė, 2004).

Initial replication of trypanosomes is at the site of inoculation in the skin; this causes a swelling and a sore (chancre). Trypanosomes then spread to the lymph nodes and blood and continue to replicate. Antibody developed to the glycoprotein coat of the trypanosome kills the trypanosome and results in the development of immune complexes. Antibody, however, does not clear the infection, for the trypanosome has genes that can code for many different surface coat glycoproteins and change its surface glycoprotein to evade the antibody. The variant surface glycoproteins (VSGs) are the basis of the sophisticated system of antigenic variation that allows a trypanosome infection to persist for month or years (Vanhamme *et al*., 2001). Immunologic lesions are significant in trypanosomosis. The most significant and complicating factor in the pathogenesis of trypanosomiasis is the profound immunosuppression that occurs following infection by these parasites. This marked immunosuppression lowers the host's resistance to other infections and thus results in secondary disease, which greatly complicates both the clinical and pathological features of trypanosomiasis (Uilenberg, 1998).

The mechanisms that induce, mediate and sustain anemia are not clearly defined but it is widely agreed that different mechanisms are involved in the control of anemia during the acute and chronic phase of the disease. While erythrocyte destruction is associated with both the acute and chronic phase, ineffective erythropoiesis may play a more important role in the chronic phase (Taylor and Authiė, 2004).

The endocrine system plays a central role in the regulation of important body functions such as growth, differentiation, reproduction, maintenance of the internal environment and adaptation to changes in the external environments. Trypanosome infection resulted in the dysfunction of the endocrine system. And this is may be due to the effect of trypanosomes along the hypothalamic-pituitary axis or as a result of direct injury to the organ. It was shown

that in Boran cattle infected with *T. congolense* trypanosomes were found in the microvasculature of the adenohypophysis and neurohypophysis in all experimentally infected animals. Focal degenerative changes were seen in the adenohypophyseal section of glands from the infected animals euthanatized 56 days post infection. These degenerative structural changes were confined to the somatotrophic cells. Presences of *T. congolense* in the microvasculature play a role in inducing pituitary damage and dysfunction (Abebe *et al*., 1993).

2.2. Diagnostic methods

2.2.1. Clinical diagnosis

The disease shows a variety of clinical manifestations, which are also common to other disease. The fact that the disease may run an acute, chronic, or sub-clinical course further complicate the diagnosis of trypanosome infections on the basis of clinical signs (Blood *et al*., 1989). Clinical sign of acute bovine trypanosomosis include anemia, weight loss, roughness of the hair coat, enlargement of peripheral lymph nodes, pyrexia, abortion, reduced milk yield and, in the absence of treatment, death. Case progress in to a more chronic disease state may be characterized by anemia, cachexia, poor productivity and infertility. The clinical picture depends to some extent on the species of infecting trypanosomes and the geographical location. However, these signs are all non specific and a number of other disease states that occur in the endemic area may result in the same clinical picture. Hence ideally diagnosis should be confirmed by the use of more specific diagnostic tests (Eisler *et al*., 2004).

2.2.2. Parasitological diagnosis by direct examination

Wet blood film

These are made by placing a drop of blood on a microscope slide and covering with a cover slip. The blood is examined microscopically using x40 objective lens. Approximately 50-100 fields are examined. Trypanosomes can be recognized by their movement among the red blood cells. The method is simple, inexpensive and gives immediate results. Depending on the trypanosome size and movements a presumptive diagnosis can be made of the trypanosome species. Final confirmation of the species is made by the examination of the stained preparation. The diagnostic sensitivity of the method is generally low but depends on the examiner's experience and the level of parasitaemia (Uilenberg, 1998). Sensitivity can be

improved significantly by lysing the RBCs before examination using a hemolytic agent such as sodium dodecyl sulfate (Ndao *et al*., 1995).

Thick blood film

Thick blood film is prepared by placing a drop of blood (5-10µl) on a clean microscope slide and then it is spread over an area of approximately 2cm in diameter, using the corner of another slide. The film is dried thoroughly by rapidly waving in the air without fixation. It is then dehaemoglobinised by immersion in distilled water for a few seconds and dried before staining. It is stained for 30 minutes with 4% diluted Giemsa stain in phosphate buffered water, pH 7.2. Staining time and stain dilution may vary with stain and individual technique. The stained smear is then washed with buffered water and examined with a x50 or x100 oil-immersion objective lens. Trypanosomes are easily recognized by their general morphology (OIE, 2000).

Thin blood smear

Thick smears contain more blood than thin smears and hence, have a higher diagnostic sensitivity. Thin smears on the other hand allow trypanosome species identification. Trypanosome species can be identified by their morphological characteristics (OIE, 2000):

Salient characters:

Trypanosoma vivax: 20-27 µm long, undulating membrane is not obvious, free flagellum present at the anterior end, posterior end rounded, kinetoplast large and terminal.

Trypanosoma brucei (long slender form): 17-30 µm long and about 2.8 µm wide, undulating membrane is conspicuous, free flagellum present at the anterior end, pointed posterior end, kinetoplast small and subterminal.

Trypanosoma brucei (short stumpy form): 17-22 µm long and about 3.5 µm wide, undulating membrane is conspicuous, free flagellum absent, pointed posterior end, kinetoplast small and subterminal.

Trypanosoma congolense: 8-25 μm (small species), undulating membrane not obvious, free flagellum absent, posterior end rounded, kinetoplast is medium sized and terminal, often laterally positioned. Although *T. congolense* is considered to be monomorphus, a degree of morphological variation is sometimes observed.

Trypanosoma theileri: 60-70 μm (large species), undulating membrane is conspicuous, long free flagellum present, posterior end pointed; kinetoplast is large and positioned near the nucleus. *Trypanosoma theileri* is normally non-pathogenic, but its presence can confuse the parasitological diagnosis.

Parasite concentration technique

The probability of detecting trypanosomes in a sample from an infected animal depends largely on the amount of blood examined and the level of parasitaemia. The amount of blood examined with direct examination techniques is low and parasites are often very scanty in the blood of an infected animal. Both of these factors contribute to the low sensitivity of direct examination techniques. Sensitivity can be improved by increasing the volume of blood to be examined and by concentrating the trypanosomes (OIE, 2000).

A practical method using centrifugation of microhaematocrite capillary tubes containing the blood sample and examination of the buffy coat/plasma junction under the microscope was described by Woo (1970). This method, known as the haematocrite centrifugation technique (HCT), was subsequently improved in the buffy coat technique (BCT). The improvement was done by cutting the capillary tube, expressing the buffy coat/plasma interface on a microscope slide and using dark-ground or phase contrast illumination (Murray *et al*., 1977). The advantages of these two techniques are that diagnostic sensitivity is increased, due to concentration of parasites following centrifugation, and that at the same time the packed cell volume (PCV) can be determined as a measure of anemia. The analytical sensitivity of the BCT technique depends on the species of trypanosomes, with the smallest numbers of detectable per milliliter of blood being 2.5×10^2, 5×10^2 and 5×10^3, for *T. congolense*, *T. vivax* and *T. brucei*, respectively (Paris *et al*., 1982).

As the specific gravity of *T. congolense* is similar to that of RBCs, parasites are often found below the buffy coat in the RBC layer. To improve the separation of RBCs and parasites, and increase the sensitivity for *T. congolense*, the specific gravity of RBCs can be increased by the addition of glycerol (Walker *et al*., 1972).

Xenodiagosis

Xenodiagnosis is the feeding of clean susceptible vector species on a suspected case of trypanosomosis, after which it is either dissected and examined for the presence of infection, or allowed to feed on a clean animal which is itself examined for the development of infection. In Africa, this method of diagnosis is rarely attempted for tsetse transmitted bovine trypanosomosis because of the sacristy of laboratory reared *Glossina* species. However, it is an extremely sensitive method for the diagnosis of animal trypanosomiasis. The differential susceptibility of different species of *Glossina* should be taken into account if this technique is used; for example, *Glossina palpalis* is unlikely to be infected with *T. congolense* (Stephen, 1986; Clausen *et al*., 1999).

Animal subinoculation

Sub-inoculation of blood from suspected case into another species (especially in to laboratory rodents) has been widely used, though not all trypanosomes are infectious for these species. Immunosuppression of laboratory rodents either by irradiation or using chemical immunosuppressant such as cyclophosphamide may increase the proportion of trypanosomes, resulting in infection (Eisler *et al*., 2001). Inoculation of susceptible rodents may be more effective for some trypanosome species, particularly *Trypanozoon* species, than other species. Mouse sub-inoculation failed to pick up any *T. vivax* infection. For this species, sub-inoculation of domestic ruminants (usually sheep and goats on the ground of expenses) rather than rodents may be recommended. For *T. congolense* it is shown that some of them may not be able to grow in mice (Eisler *et al*., 2001).

2.2.3. Parsitological diagnosis by indirect methods

Antibody ELISA

This test has been used in the diagnosis of trypanosomosis and is based on the detection of anti-trypanosomal antibodies in the sera of diseased animals. Usually solubilized antigens obtained from disrupted trypanosomes are used, and the soluble antigens are coated in the wells of microtrays. The test results can be read visually, but this introduces an element of subjectivity, and a special ELISA reading instrument will quickly give optical density (OD) of each well, thus helping to speed up the processing of large numbers of sera. The ELISA lends itself to standardization and automation (Uielenberg, 1998). The detection of anti

trypanosomal antibodies in serum cannot distinguish between an active infection and a cured one (Voller, 1977). As with most serological test for disease diagnosis, a single positive serological result cannot be used to demonstrate the presence of active infection, since antibody frequently persist for longer than does the infectious agent within the host. In cattle, this has been shown to be the case for up to 6 and possibly as long as 13 months following clearance of trypanosome infection (Lukins, 1992; Vanden Bossche *et al*., 2000).

Antigen ELISA

This test has been developed for the detection of circulating trypanosomal antigen. The surface antigens of trypanosomes are variable; only one or two of the many different variants are presenting the blood at any one time and, unless one would possess mixture of antibodies to all the possible variants, their detection is not reliable. Therefore, the tests that have been developed are based on so-called monoclonal antibodies against invariable (internal) antigens. Tests based on this principle, using monoclonal antibodies supposedly specific for the various subgenera, species or types of pathogenic trypanosomes, have been widely tested and distributed to National Agricultural Research System (NARS) in Africa for African animal trypanosomosis diagnosis (Uilenberg, 1998).

2.3. Control of trypanosomosis

Prevention and control of tsetse transmitted trypanosomosis depends on minimizing contact between domestic livestock, game animals and tsetse flies. In theory there are a number of control methods directed to the parasite, vector and host. However, in practice widely uses of these methods are highly variable. The methods include reducing tsetse fly population, treating infected animals, preventing animals from the disease using prophylactic drugs and using indigenous breeds of livestock that are genetically resistant to the disease. Each of these approaches is useful but has important limitations, such as expense, environmental pollution, drug resistance and poor availability (Leak, 1999).

2.3.1. Parasite control

The major strategy used to control bovine trypanosomosis in sub-Saharan Africa is based on trypanocidal drugs (Peregrine *et al*., 1994). Drugs currently recommended for chemotherapy of animal trypanosomosis come from only three closely related groups. These are the phenathridines, isometamidium and homidium and the aromatic diamidine, diminazene. Only

isometamidium and homidium are recommended for prophylaxis. The incidence of resistance to these drugs is apparently increasing and the main means of controlling is therefore under threat (Uielenberg, 1998). Resistance to one or more of the trypanocidal drugs used in cattle has been reported in at least 13 countries of sub-Saharan Africa. The occurrence of drug resistance was found in those regions where drug use was more intensive (Geerts and Holmes, 1998).

2.3.2. Vector control

Vector control may play a role by reducing the level of tsetse challenge to livestock. The development of insecticide-impregnated, odor-baited traps and targets which attract and kill tsetse offer the prospect of cheaper alternatives with less damage to the environment (Jordan, 1988). These methods had been tried in some tsetse infested areas of Ethiopia. In an attempt to control trypanosomosis at Ghibe, southwest Ethiopia, an integrated control program, involving both tsetse fly control and chemotherapeutic agents, was implemented in April 1991. Subsequent to the initiation of the target-control methodology, the relative density of the main vector at Ghibe, *G. pallidipes*, fell from a mean of 1.9 flies/trap/day before the introduction of tsetse control to a mean of 0.4 flies/trap /day during the tsetse control. The prevalence of diminazene resistant infections decreased by approximately 75% in the first 12 months following initiation of tsetse control program (Peregrine *et al*., 1994). A drop both in the apparent density of the tsetse flies (*G. tachinoides*) and the prevalence of trypanosomosis was also observed during the two years pilot vector control program in the Metekel district of northwest Ethiopia (Tilahun *et al*., 1997).

Application of deltamethrin pour-on to cattle against tsetse flies has proved to be very efficient in controlling tsetse fly vectors in the pastoral Zone of Aamorogouan, Burkina Faso (Bauer *et al*., 1995). Clausen *et al*. (1992) stressed that efficient tsetse fly control will lead to a reduction of the use of trypanocidal drugs and this will heave their role as an efficient means to cure the disease in case of an outbreak.

A biological method of control is the sterile male release technique in which artificially sterilized males compete with wild tsetse for mating with females. However, this is considered to be very expensive and the sterile males have been found to be as capable as normal male tsetse in transmitting the disease (Leak, 1999).

2.3.3. Vaccine

So fare all attempts at developing a vaccine against trypanosomosis have failed. With the rapid advances in molecular biology the situation may change at some point in the future, but so far this approach has been stranded by the almost unlimited ability of trypanosomes in the host to change their surface antigens frequently. With new antigens appearing, antibodies elicited against previous types of antigens are no longer effective and the immune system has to start all over again to produce new antibodies, until it becomes exhausted. Also, the antigen repertoire is different between different strains, type and subspecies of the same trypanosome species. Moreover, African tsetse transmitted trypanosomosis is often a mixed infection of two or even three different species (Uilenberg, 1998).

2.3.4. Trypanotolerant cattle

Introduction and keeping of trypanotolerant cattle breeds seem to be an alternative biological method to preventing clinical trypanosomosis and the economic losses for the animal holders. Trypanotolerance is a feature of both West African longhorn and shorthorn *Bos taurus* breeds such as the N'Dama and Baoulé breeds. Trypanotolerance is manifested by the ability of the trypanotolerant animals to regulate parasite growth and to prevent or reduce the rate and degree of development of anemia (Murray, 1988). However, these breeds of animals are relatively small in number and are limited in West Africa. A study was conducted on four indigenous breeds of Ethiopia, namely Abigare, Horro, Sheko and Gurage, to natural challenge of trypanosomosis in the Tolley-Gullele area of the Ghibe valley has been undertaken from August 2000 until August 2004. In this study the Sheko breed exhibited better trypanotolerant attributes than the other three breeds (Abigare, Horro, and Gurage), as measured by lower trypanosome prevalence rate, less sever anemia after infection and fewer trypanocidal treatment per annum than the other breeds. Moreover, the Sheko breed maintained its physiological functions under the prevailing trypanosomosis challenge and compared favorably with the other breeds in its reproductive performance (Lemech *et al*., 2006). In this work the authors indicated that these result need to be substantiated with further in-depth investigation including immune response, animal behavior and environmental influences (Lemech *et al*., 2006).

2.4. Chemotherapy and chemoprophylaxis

Chemotherapy and chemoprophylaxis of animal trypanosomosis relies essentially on three drugs, namely: Homidium (Homidium chloride-Novidium ®; and Homidium bromide-Ethidium ®), diminazene aceturate (Berenil ®) and isometamidium chloride (Samorin ®, Trypamidium ®) (Holmes *et al*., 2004).

2.4.1. Homidium bromide/chloride

Homidium belongs to the phenanthridine class of compounds and is manufactured as both bromide and chloride salts, which are equally active *in vivo* (Leach and Roberts, 1981). Homidium chloride and especially homidium bromide or ethidium are still widely used as trypanocidal drugs, though they are known mutagenic compounds. Their mechanism of action is not well understood but it has been shown that the drugs interfere with glycosomal functions, trypanothione metabolism and the replication of kinetoplast. The mechanism of resistance by trypanosomes to these drugs is unknown but there are indications that it is similar to that describe for isometamidium (Holmes *et al*, 2004). Both salts are generally recommended for use as therapeutic agents at a dose of 1mg/kg b.w. for the treatment of *T. congolense* and *T. vivax*. *Trypanosoma brucei* is less susceptible and the dosage for this species should be increased to 1.5 or even 2mg/kg (Uilenberg, 1998).

2.4.2. Diminazene aceturate

Dimainazene is an aromatic diamidine and is marketed in combination with phenyldimethyl pyrazolone (antipyrine), a stabilizer that prolongs the activity of the compound in solution (Fairclough, 1963). Diminazene aceturate is less effective against trypanosomes of the subgenus Trypanozoon (such as *T. evansi* and *T. brucei*) than against *T. congolense* and *T. vivax* (Uilenberg, 1998). Diaminazene is now probably the most commonly use therapeutic agent for trypanosomosis in livestock in sub-Saharan Africa (Geerts and Holmes, 1999). This is due to a number of factors: activity against trypanosomes that are resistant to most other trypanocides and a higher therapeutic index, in most animal species, than other trypanocides (Williamson, 1970). Diminazene probably exerts its action at the level of the kDNA but other targets cannot be excluded (Holmes *et al*, 2004).

2.4.3. Isometamidium chloride

Isometamidium is a phenanthridinium compound and is marketed as both a therapeutic and prophylactic agent. In the dose range recommended for prophylactic purposes (0.5-1mg/kg of b.w), the compound has been used successfully to maintain the productivity of zebu cattle exposed to tsetse challenge in both village and ranch management systems in East Africa (Moloo *et al*., 1987). However, considerable variation in prophylactic activity has been observed in that a dose of 1mg/kg b.w has been shown to confer prophylaxis to cattle for 2-22 weeks. Variation in drug susceptibility between different trypanosome populations appears to be the major factor determining the duration of prophylaxis (Pergrine *et al*., 1991). The trypanosome kinetoplast is the primary site of isometamidium accumulation. The main mode of action of the drug is the cleavage of kDNA-topoisomerase complexes. The mechanism of resistance to isometamidium is less clear. Several workers have shown that accumulation of isometamidium is significantly lower in resistant populations than in sensitive ones (Holmes *et al*., 2004).

2.5. Strategies for trypanocidal drug usage

Routine block treatments: These are generally carried out using prophylactic drugs, notably isometamidium chloride, at predetermined intervals based on the perceived duration of prophylaxis (0.5 to 1mg/kg b.w). All animals in a herd may be treated or treatment may be targeted at a particular group of valuable or at risk animals. When routine block treatment with isometamidium is practiced, it is recommended that once a year, the animals are separately treated with diminazene in order to delay the development of drug resistance following the concept of 'sanative pairs' (Whiteside, 1962).

Strategic block treatments: These are generally carried out using prophylactic drugs, though curative drugs may also be used. All animals in a herd, or particularly valuable or 'at risk' stock, are treated when challenge (as measured by the number of animals succumbing to infection) reaches a predetermined threshold (Holmes *et al*., 2004).

Monitoring and treatment of individual infected animals: Cattle are monitored using standard parasitological methods, e.g. wet blood film, haematocrite centrifugation technique or buffy coat technique. Treatment of infected animals is generally conducted using a therapeutic drug, usually diminazene aceturate (3.5 to 7mg/kg b.w) (Holmes *et al*., 2004)

Monitoring and treatment of clinical cases: This is similar to monitoring and treatment of individual infected animals but not all infected animals are treated. Cattle are treated usually with a curative trypanocide, only if the PCV falls below a predetermined threshold or if clinical signs of trypanosomosis are detected. The rationale for this strategy is the belief that cattle may acquire resistance to locally circulating strains of trypanosomes, and that treatment of animals with subclinical infections is unnecessary and may interfere with the process (Holmes *et al*., 2004).

2.6. Chemoresistance

Resistance to one or more of the trypanocidal drugs used in cattle has been reported in at least 13 countries of sub-Saharan Africa (Geerts and Holmes, 1998). In East Africa, drug resistant *T. congolense* strains have been described in Ethiopia (Scoot and Pegram, 1974; Codjia *et al*., 1993; Rowlands *et al*., 1993; Leak *et al*., 1993) and in Kenya (Gitatha, 1979). Isometamidium and diminazene are usually prescribed as a "sanative pair" in the control of bovine trypanosomosis (Whiteside, 1960). However development of multiple drug resistant strains of *T. congolense* isolated in the Bobo-Dioulasso region of Burkina Faso (Clausen *et al*., 1992) and Ethiopia (Codjia *et al*., 1993; Mulugeta *et al*., 1997; Afewerk *et al*., 2000; Aseffa and Abebe, 2001) suggested that the concept of sanative pairs might no longer be valid in certain regions.

An understanding of the mechanisms of drug resistance by trypanosomes is important as it can lead to the identification of potential and novel drug targets and provide direction to how new chemotherapeutic strategies can be used to reduce development of resistance. Trypanocidal drug resistance could be innate, such as in resistant individuals without previous exposure to the particular drug, or acquired (induced) as a result of drug exposure/pressure, cross-resistance or sometimes by mutagenesis (ILRAD, 1990). The biochemical basis of trypanosome resistance to trypanocides has not been fully characterized. However, drug resistance can arise either as a consequence of changes in drug concentration at the target site or alteration in the target, or both. There are experimental evidences that drug resistant trypanosome clones accumulate less amount drugs than their sensitive counterparts. Fluorescence microscopic and flow cytometric studies showed a reduction in uptake and accumulation of isometamidium chloride by resistant clones of *T. congolense* (Zilberstein *et al*., 1993). Sutherland *et al*. (1992) showed that the uptake of isometamidium chloride by both

sensitive and resistant clones of *T. congolense* was through an energy-dependent, specific, receptor-mediated transport system on the parasite surface. Resistance appeared multifactorial, including an alteration in a specific receptor on the cell surface of the resistant trypanosomes and a putative enhanced efflux mechanism (Zilberstein *et al*., 1993).

Carter and Fairlamb (1993) evidenced that drug sensitive bloodstream forms of *T. brucei* expressed at least two different nucleoside transporters (P1and P2) through which they salvage adenosine from their mammalian hosts. Several studies have shown that purine nucleoside transporters are involved in the uptake of trypanocidal drugs and that resistance is associated with changes in them (Barrett and Fairlamb, 1999). For instance, loss or alteration of the P2-type transporter was shown to account for the resistance of *T. brucei* to melaminophenyl arsenicals (Carter and Fairlamb, 1993) and diamidines (Carter *et al*., 1995). Studies of isometamidium uptake in the presence or absence of the metabolic inhibitor salicylhydroxamic acid (SHAM) and glycerol, and a range of calcium flux-modulating compounds (such as verapamil and prazosin), provide indirect evidence that energy-dependent drug-efflux mechanisms may also play a role in reduced trypanocidal drug accumulation in and resistance by trypanosomes to these drugs (Sutherland *et al*., 1992).

To demonstrate that drug resistant trypanosomes are present it is necessary first to discount other problems such as inadequate drug preparation, handling and administration, and secondly to demonstrate the response of the trypanosomes to treatment under carefully controlled conditions (Eisler *et al*., 2001). Ideally this should be carried out in the host species of interest, i.e. *Bos indicus*, for strains isolated from East African Zebu cattle. Rowlands *et al*. (1993) showed that the application of a computer model to parasitological data collected over a long period on a monthly basis allowed the incidence of new infections to be distinguished from recurrent infections.

The reduction of selection pressure by the drugs, i.e. decreasing the number of treatments, is considered as the most efficient way to delay the development of drug resistance. This is important particularly in high tsetse challenge areas, which are commonly associated with frequent treatments in a short period (Whiteside, 1960). Drug resistance often emerges in such situations as a result of increased frequency of drug usage. Additionally, systematic mass treatment could hasten the development of resistance. Therefore, in well monitored situations limiting treatments to individual clinical cases may be important. In such situations drug

resistance problems can be minimized and acquired immunity encouraged (Scott and Pegram, 1974).

2.7. Assessment of chemoresistance

2.7.1. *In vivo* techniques

Tests in mice: Test in mice can be used as single-dose test or as multi-dose test. In the latter case the objective is to obtain more detailed information by determining the CD_{50} or CD_{80} values (curative dose that gives complete cure in 50 % or 80 % of the animals) for a given trypanocidal drug. In the case of single-dose test, a large number of trypanosome isolate is tested at a single discriminatory dosage (1mg/kg b.w for isometamidium and 20mg/kg b.w for diminazene). This single-dose test is conducted with the objective of characterizing the geographical area of origin of the isolates in terms of the extent of drug resistance, rather than in-depth characterization of individual isolate (Eisler *et al*., 2001). The advantage of the mouse assay is that it is cheaper than the tests in cattle. There are several disadvantages, however. Firstly, most *T. vivax* isolates, and also some *T. congolense* isolates do not grow in mice. Secondly, although there is a reasonable correlation between drug sensitivity data in mice and in cattle, higher doses of drug must be used in mice in order to obtain results comparable to those from cattle because of the vast difference in metabolic size. The results in mice cannot be directly extrapolated to calculate the curative dose to be used in cattle. Thirdly, precise assessment of the degree of resistance requires a large number of mice per isolate. This makes it rather labor-intensive test. Finally, it takes as long as 60 days to evaluate the drug sensitivity of an isolate (Eisler *et al*., 2001).

Tests in ruminants: These tests provide direct information from studies in ruminants using recommended dose of trypanocides. The tests commonly consist of infecting a group of cattle or small ruminants with the isolate under investigation and latter, when they are parasitaemic, treating them with various dosages of trypanocides. It is preferable to use at least three animals in each group, because it has been shown that results obtained after inoculation and treatment of one animal are not always reliable. The animals are regularly monitored over a period of 100 days to determine the efficacy of standard drug doses in terms of their ability to provide a permanent cure. For these studies the cattle or small ruminants must be kept in fly-proof accommodation or in a non-tsetse area in order to eliminate the risk of reinfection during the study. A variation on this technique is to inoculate blood from several different

infected cattle into a single recipient calf. This technique is useful in situations where laboratory facilities are very limited but it only allows a qualitative assessment and does not indicate how many of the isolates inoculated into a single calf were resistant. Further constraints to this technique are not all populations grow equally well and that sensitive isolates might overgrow resistant ones when inoculated (Eisler *et al*., 2001).

2.7.2. *In vitro* techniques

For the *in vitro* evaluation of drug sensitivity procyclic, metacyclic or bloodstream forms of trypanosomes can be used. The advantage of *in vitro* assay is that large numbers of isolates can be examined. However, there are several disadvantages. The use of metacyclic and bloodstream forms is considered more reliable than the use of procyclic forms. Test with metacyclic trypanosomes correlates well with field observations, but it may take up to 40 to 50 days of *in vitro* incubation to generate metacyclic trypanosomes. *In vitro* cultivation of bloodstream forms is only possible using preadopted lines and not using isolates directly from naturally infected animals. A simplified axenice culture system has been developed, but further research is still necessary to study the correlation with field data. *In vitro* assay is expensive to perform and require good laboratory facilities and well-trained staff (Holmes *et al*., 2004). An interesting alternative is the drug incubation *Glossina* infectivity test (DIGT), in which the trypanosomes are exposed to the drug *in vitro* for a short time and thereafter fed to tsetse flies to check whether or not they develop into metacyclic forms. This technique distinguishes resistant from sensitive isolates and does not require experimental animals, but it does require a ready supply of teneral tsetse flies from an artificially reared colony (Clausen *et al*., 1999).

2.8. Assessment of the therapeutic effectiveness

When trypanosome infected animals are treated successfully, circulating drug levels are by definition adequate to protect against trypanosome challenge and therefore breakthrough infections may only occur where drug levels are inadequate, possibly as a result of inadequate dosage regime. All relapses are not due to drug resistance. Trypanosome species of the Trypanozoon group could reach tissues (CNS, eye etc.) where trypanocidal drugs could not reach (Whitelaw *et al*., 1985). Alternatively, infections may occur where the challenge population of trypanosomes expresses drug resistance (Holmes and Torr, 1988). The use of trypanocidal-ELISAs in combination with parasite detection tests has given promising results

for the detection of resistant trypanosomes. A competitive ELISA, which allowed the detection of small amounts of isometamidium in serum of cattle, has been validated in cattle under experimental and field conditions (Eisler *et al*., 1994). The test is both sensitive and specific and it allows the monitoring of drug levels over extended periods and the evaluation of factors influencing drug disappearance rates from the plasma. The drug disappeared more rapidly in animals infected with drug resistant trypanosome isolates than in those infected with sensitive trypanosomes. Preliminary observations showed that the presence of trypanosomes in animals with an isometamidium concentration of >5ng/ml strongly suggests resistance (Eisler *et al*., 1994).

3. MATERIALS AND METHODS

3.1. Study area

The present study was conducted in two different areas of the Southern Nations and Nationalities People Regional State (SNNPRS) of Ethiopia. The first study area, Humbo, is one of the *districts* of Wolyta Zone. It is located near to Arbaminch where there is active tsetse suppression program conducted by Southern Tsetse Eradication Project which comprise the sterile insect technique as its final component to eradicate tsetse fly from the region (STEP, 2004). The second study area, Mareka, is located in the Dawero Zones of Southern Nations and Nationalities People Regional State (SNNPRS). In Dawero Zones there is no tsetse control programme. The experimental work was conducted at the Faculty of Veterinary Medicine, Debre Zeit.

Humbo: Humbo is one of the *districts* of Wolyta Zone and it is located in Southern Nation and Nationalist People Regional State (SNNPRS) about 420 km south of Addis Ababa. It is located at 6.4^0-46^0 N and 37^0-45^0 E. The rainfall pattern is bimodal and maximum rainfall is received during the long rain season (mid-June to mid-September). The short rain season is in March and April. The annual rainfall in the surrounding area ranges from 1200 mm to 1300 mm. The average monthly maximum and minimum temperature ranges from 20^0C-25^0C and 10^0C-15^0C, respectively. The area coverage is about 86,646ha and the cattle population in the area is 53,631 (Wolyta Zone Finance and Economics Department, 2005).

Mareka: Mareka is one of the five *districts* of the Dawuro Zone. It is located in the Southern Nations and Nationalities People Regional State (SNNPRS), southwest Ethiopia. It is located about 500 km from Addis Ababa. The area coverage is about 466,082ha and bounded at southeast by Wolita Zones. The mean daily temperature is 16.9-29.4^{o}C and has annual rainfall of 1409mm. The area is situated at 1300-1750 above sea level. The cattle population in the area is 53,631(Dawro Zone Office of Agriculture, 2003).

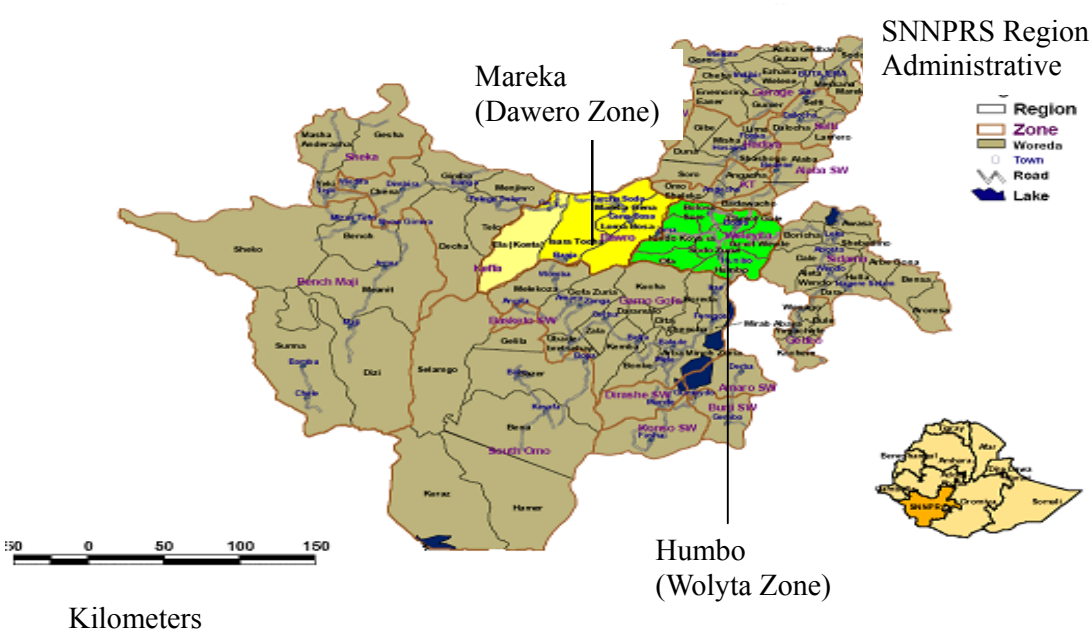

Figure1. Map of study areas

3.2. Study animal

The cattle in the two study areas were indigenous East African zebu breeds. They were kept under traditional extensive husbandry systems with communal herding. During the day time, cattle were grazed together and looked after by herdsmen. Male cattle over 3 years of age were used as ploughing oxen. Animals work usually in the morning, particularly during the wet season, and graze the rest of the day. They were watered in small and big rivers. Cattle return to their owner's homestead in the evening.

3.3. Study design

3.3.1. Questionnaire survey

During the first 2 weeks of November 2006, questionnaire survey was carried out with the major objective of identifying cattle herds highly suspected to be infected with drug resistant trypanosome populations. A total of 60 farmers were randomly interviewed from three different villages with the aid of a questionnaire about herd composition, diseases and usage of trypanocidal drugs (Annex 1). The questionnaire was pretested in the field before its administration to the study population for time and relevance of type of questions included. The questionnaire was administered to individual farmers.

3.3.2. Cross sectional study

In order to determine the prevalence of trypanosome infections in the study population a cross sectional study was carried out in December 2006. A total of six different villages (three villages from each study area) were selected. The villages were selected by purposive sampling on the basis of prior information on the problem, farmer's cooperation, accessibility and economical reasons. The sample size for the cross sectional study was determined using the formula given by Thrusfield (1995). A precision level of 5% and a 95% confidence interval with an estimated trypanosome prevalence of 10% (Awoke, 2000) was used to calculate the sample size. Representative numbers of animals were sampled from each village. The sampling method used in each village was simple random sampling and parameters like age, sex, breed and previous history of treatment were recorded.

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

Where, n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

Humbo: The selected three villages for the cross sectional studies were *Humbo Kebele*, *Abela* and *Tebela*. These villages were coded from I-III, respectively. A total of 189 animals were sampled during the cross sectional study period. Humbo is the area where tsetse control is in progress.

Mareka: The three villages selected from Mareka for the cross sectional studies were *Odoro*, *Wara keble* 1 and *Wara kebele* 2. These villages were coded from IV-VI, respectively. A total of 152 animals were sampled during the cross sectional study period. Mareka is the area where there is no tsetse control program.

Dark ground/ phase contrast buffy coat method (Murray *et al*., 1977) was employed to study the prevalence of trypanosome infections. Two heparinised microhaematocrit capillary tubes were used for each blood sample collected from the ear vein. Blood from the ear vein was added into the capillary tubes by capillary attraction until the tubes were filled ¾ ways. Each capillary tube was sealed at one end using plasticine. After centrifugation at 10, 000 revolutions per minute for 10 minutes, the capillary tubes were cut 1 mm bellow the buffy

coat to include the uppermost layer of the red blood cells. The contents, including the first few mm above the buffy coat, was then gently expressed on to a clean slide, so that some plasma was included with the buffy coat. The expressed contents were carefully mixed and covered with coverslip (22x22 mm). The preparation was then examined using a microscope with x40 objective to identify trypanosomes by their motility. Thin blood smear stained with Giemsa was used to confirm the *Trypanosoma* spp. The PCV of each sample was estimated using a Hawksley microhaematocrit reader (Hawksley and Sons, Lancing, UK) before the buffy coat was examined for the presence of trypanosomes.

3.3. 3. Longitudinal study

The interest of this study was to evaluate the prophylactic effect of isometamidium chloride to natural trypanosome infections. From the two study areas Humbo was selected purposely based on accessibility, financial feasibility and willingness of the farmers. In this study all positive cattle during the cross sectional studies were selected. Each of parasitologically positive animals was identified using a plastic ear tag. Numbering of cattle for the longitudinal study was started from positive cattle found at village-I and ends on positive cattle found at village-III. For calculating the treatment dose, the body weight of each animal was estimated before treatment using measurements of heart girth and (MOA, 1989). The animals were treated intramuscularly with isometamidium chloride (Veridium®, Lot No.92A1; Libourne, France) at a prophylactic dose of 1mg/kg of b.w.

Monitoring infection

Blood samples were collected from the ear vein and examined to determine PCV and parasitemia using the phase contrast buffy coat method (Murray *et al*., 1977). Cattle were bled and examined every week for 9 weeks (60 days) post-isometamidium treatment to detect development of parasitemia. The PCV was determined to evaluate the relationship between treatment and recovery of PCV values. Blood from the relapse/breakthrough cases was inoculated via the intraperitoneal (i.p) route into mice in the field. Thereafter animals, with relapse cases, were treated with 7mg/kg b.w of diminazene diaceturate (Diminasan®) and released. The mice were then transferred to the Faculty of Veterinary Medicine, Debre Zeit, for mice assay study. The mice were kept in a fly proof cage during the study period.

3.3.4. Drug sensitivity studies of trypanosomes in mice

Experimental mice

Swiss white mice, 8-10 weeks of age and weighing 25–30 gram, were obtained from the breeding colony of the Ethiopian Health and Nutrition Research Institute and maintained on a commercial pelleted ration and water adlibitum. They were housed in a fly-proof room at the Faculty of Veterinary Medicine, Debre Zeit.

Experimental design

***Trypanosoma congolense* isolates**: For the drug sensitivity test a total of six *T. congolense* isolates were used. Two isolates were obtained from Humbo and four isolates were obtained from Mareka. Isolates were transported to the Faculty of Veterinary Medicine, Debre Zeit, for experimental studies after inoculating 0.2 ml of blood intraperitonially into mice from representative animals.

(a) Qualitative mice assay: For this assay the two isolates brought from Humbo were used. These two isolates were obtained from two animals which showed a breakthrough cases during the longitudinal study. These isolates were used to infect the experimental mice after two passages.

(b) Quantitative mice assay: For this assay the four isolates brought from Mareka were used. These isolates were obtained from animals which were positive for *T. congolense* in village-IV. For this mice assay village-IV was selected purposely based on complain of the farmers about the efficacy of trypanocidal drugs in the area and accessibility of these areas for transportation. The four isolates were pooled into two Pools by using modification of the standard drug sensitivity test set by Eisler *et al*. (2001). The isolates were pooled after one passage on mice and the pooled isolates used to infect the experimental mice after one more passages. For the sake of convenience, the pooled isolates were designated as Pool-1 and Pool-2, where each pool contained two isolates.

Infection of mice

The field isolates of *T. congolense* was expanded in mice and used for infection of experimental mice. Thin smear was made to confirm that the trypanosome under investigation

was *T. congolense* before infection of mice. Blood was collected aseptically from ether anaesthetized donor mouse by cardiac puncture for infection of experimental mice. One milliliter of blood with estimated parasitemia of $5x10^6$ trypanosomes/ml (Paris *et al*., 1982) was collected and diluted in 4ml of sterilized buffered saline water so that each mouse receive approximately $2x10^5$ trypanosomes in an inoculum of 0.2 ml (Eisler *et al*., 2001).

For each drug sensitivity test 30 mice were divided randomly into five experimental groups of six mice each (I–V). The first four groups (group I–IV) formed the infected groups treated with different doses of trypanocidal drug. The fifth group (group V) served as untreated infected controls.

Treatment and monitoring

Isometamidium chloride (Veridium®, Lot No. 127A1FAB; Libourne, France) and diminazene diaceturate (Diminasan®, Batch No. 05L12.I; JA Woerden, Holland) were purchased and used for treatment of infected mice in the treatment group. (Table 1 and 2) Each mouse in group I–IV was weighed on a flat pan balance prior to administration of trypanocidal drugs for calculation of dosages. Mice in the treatment group were treated 24 hrs post infection. Isometamidium chloride (Veridium®) was administered i.p at doses of 0.5, 1, 2 and 4.0mg/kg b.w (Table 1) and diminazene diaceturate (Diminasan®) was given i.p at doses of 3.5, 7, 14 and 28mg/kg b.w (Table 2). The control groups received the same amount of sterile distill water without drug by the i.p route. Injection solution was prepared by dissolving the required quantity of each compound in sterile distilled water before use according to respective manufactured instruction using clean container and new syringes (Annex II and III). The required dose of drug was administered in 0.2 ml of solution for all treatment groups (Eisler *et al*., 2001).

Table 1. Isometamidium chloride (Veridium®) treatment group.

Group	No. Mice	Isometamidium chloride (mg/kg b.w)
I	6	0.5
II	6	1
III	6	2
IV	6	4
V (control)	6	-

Table 2. Diminazene diaceturate (Diminasan®) treatment group.

Group	No. Mice	Diminazene diacetutate (mg/kg b.w)
I	6	3.5
II	6	7
III	6	14
IV	6	28
V (control)	6	-

Mice were monitored every other day for 60 days for the presence of trypanosomes by microscopic examination of wet smears of blood obtained from tail. Presence of trypanosomes in the blood smear was considered as indication of drug resistance. When mice were found positive they were euthanized with ether.

3.5. Statistical analysis

The Chi-square test was used to compare the prevalence of trypanosomosis between villages of their respective area and between the two study areas. One way ANOVA was used to compare the mean PCV values between villages of their respective area. The Student's *t*-test was used to compare the mean PCV value between the two study areas. SPSS® (Version 13, 2004) for Microsoft windows was used for the statistical analysis.

4. RESULTS

4.1. Questionnaire survey

Animal management: Free grazing covered the largest proportion (93.3%) of the livestock feed. Most of the cattle in the area grazed in small herds. Livestock feed was better available during the rainy season (July-September) than the dry season (November-April). The majority (86.7%) of the farmers used to move their cattle for as long as 3 km for grazing during the dry season. The grazing areas were in most cases very close to livestock watering points. Majority of treatment for trypanosomosis was found to be given during the dry season.

Livestock diseases: The questionnaire survey indicated that among diseases affecting livestock in the area, trypanosomosis, bacterial (blackleg and anthrax) and gastrointestinal helminthosis were the most important. During the survey majority (68.3%) of the interviewed farmers ranked trypanosomosis as moderate, while 18.3% as major and 13.3% as least important. The survey indicated that trypanosomosis affect cattle both during the rainy and dry season, although their cattle look in better condition during the rainy season. The farmers claimed trypanosomosis to cause reduced appetite, rough hair coat, diarrhea, emaciation, weakness and reduction in the working power of their ploughing oxen.

Trypanocidal drug usage: The majority of the cattle owners (85%) had used trypanocidal drugs a year before the interviewed time (Table 3). Of the farmers used either isometamidium chloride or diminazene aceturate, 17.6% of them used both drugs on different time of the year.

Table 3. Farmers used trypanocidal drugs a year before the questionnaire survey.

No. farmers interviewed	Trypanocidals users (%)	Trypanocidal drug users (%)		Used both drug at different time
		Diminazene aceturate	Isometamidium chloride	
60	85 % (51/60)	31.4 % (16/51)	68.6 % (35/51)	17.6% (9/51)

Drug purchase and administration: Majority of the farmers (62.7%) bought trypanocidal drugs from veterinary pharmacy, 21.6% from veterinary clinics and the remaining 15.7% got from black market. The proportion of personnel who administered trypanocidal drugs are summarized in Figure 2.

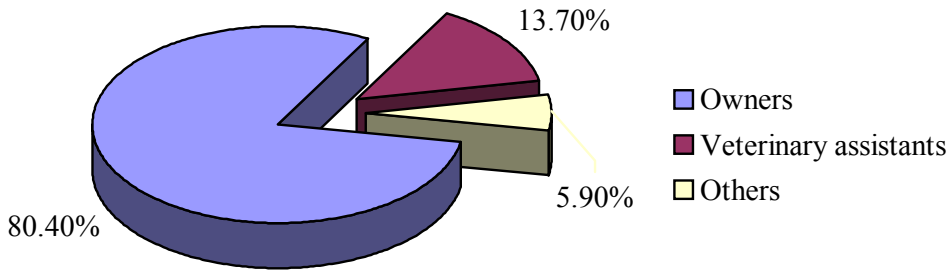

Figure 2. Administration of trypanocidal drugs by different personnel a year before the questionnaire survey.

Interview made about the fate of the left over drugs indicated that 64.7% of users kept residual isometamidium chloride powder for later use while 35.3% sold the residual drugs.

The questionnaire survey also indicated that a relatively higher portion (54.3%) of farmers administered isometamidium chloride underdose and a relatively correct dose was given using diminazene aceturate (Figure 3).

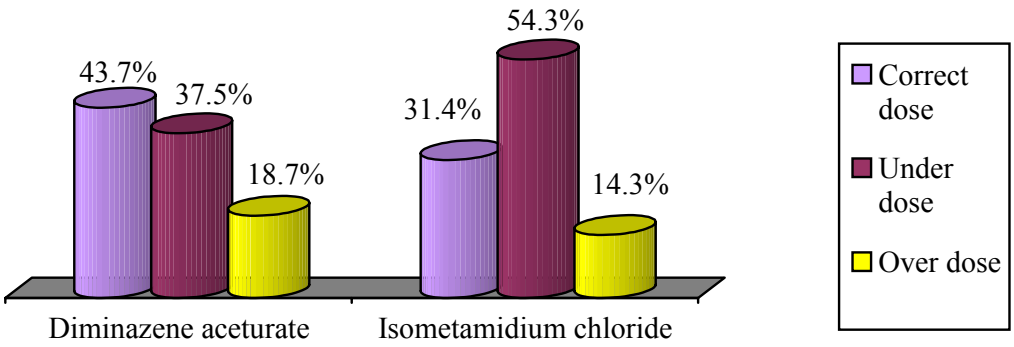

Figure 3. Dosage of trypanocidal drugs used by the farmers of Humbo.

Reason for trypanocidal drug usage: The questionnaire survey indicated that 37.5% of the diminazene used and 45.7% of isometamidium used were given for suspected cases of having trypanosomal infection (Table 4). Trypanocidals given due to trypanosomal diagnosis was 18.7% and 8.6% for diminazene aceturate and isometamidium chloride, respectively.

Table 4. Reasons for the use of diminazene aceturate and isometamidium chloride for the treatment of cattle in Humbo district.

Reasons for use	Diminazene aceturate (%)	Isometamidium chloride (%)
Trypanosomosis diagnosed	3 (18.7)	3 (8.6)
Trypanosomosis suspected	6 (37.5)	16 (45.7)
To prevent trypanosomosis	3 (18.7)	7 (20)
Animal sick (unknown reason)	4 (25)	9 (25.7)

4.2. Cross sectional study

4.2.1. Parasitological findings

The trypanosome prevalence for each of the six villages of the two study areas during the cross sectional study period is presented in Table 5 and 6.

In the present study revealed an overall trypanosome prevalence of 4.8 % (95% CI: 1.8–7.5) in Humbo (Table 5). During the study period the most prevalent trypanosome species was *T. congolense* and mixed infection was also recorded in village-II. The chi-square test indicated that there was no statistically significant difference (P > 0.05) in the mean prevalence of trypanosomes between the three villages.

Table 5. The prevalence of trypanosomosis in the three villages of Humbo district.

Villages	No. of cattle examined	Positive animals (%)	Trypanosoma spp. (%)				95% CI:
			T.b	T.c	T.v	Mixed	
I	61	4 (6.6)	1 (25)	3 (75)	0	0	
II	64	4 (6.3)	0	2 (50)	1 (25)	1 (25)	
III	64	1 (1.6)	0	1 (100)	0	0	
Total	189	9 (4.8)	1 (11)	6 (66.7)	1 (11)	1 (11)	1.8-7.8

T.b: *Trypanosoma brucei*, T.c: *T. congolense* T.v: *T. vivax*.
Mixed: *T. congolense* and *T. vivax*
Numbers in parenthesis refer to prevalence.

The present study also reveled an overall trypanosome prevalence of 20.4% (95% CI: 14–26.8) in Mareka district (Table 6). The most prevalent trypanosome species was *T. congolense*. The chi-square test indicated that there was no statistically significant difference (P>0.05) in the mean prevalence of trypanosomes between the villages.

Table 6. The prevalence of trypanosomosis in the three villages of Mareka district.

Villages	No. of cattle examined	Positive animals (%)	Trypanosoma spp. (%)				95% CI:
			T.b	T.c	T.v	Mixed	
IV	51	9 (17.6)	1 (11)	6 (66.7)	0	2 (22.2)	
V	47	10 (21.3)	0	8 (80)	1 (10)	1 (10)	
VI	54	12 (22.2)	1 (8.3)	9 (75)	1 (8.3)	1 (8.3)	
Total	152	31 (20.4)	2 (6.4)	23 (74.2)	2 (6.4)	4(13)	14-26.8

T.b: *Trypanosoma brucei*; T.c: *T. congolense*; T.v: *T. vivax*.
Mixed: *T. congolense* and *T. vivax*
Numbers in parenthesis refer to prevalence.

The statistical test competed for the two study areas (Humbo and Mareka) indicated that there was significant difference (*t*-test, $P<0.001$) in the mean prevalence of trypanosomes being higher in Mareka.

4.2.2. Hematological findings

The mean PCV value obtained during the cross sectional study for each village with their respective study area is indicated in Table 7 and 8. The mean PCV (%) value of the total number of cattle tested in Humbo and Mareka was 26.2% (95% CI: 25.7-26.7) and 22.7% (95% CI: 22.1-23.3), respectively.

Table 7. Mean PCV value (%) of the three villages of Humbo district.

Study areas	Mean PCV value (%)			
	Village-I	Village-II	Village-III	Total
Humbo	26.1	25.9	26.6	26.2
95% CI	25.3-27	25.9-26.7	25.8-27.4	25.7-26.7

Table 8. Mean PCV value (%) of the three villages of Mareka district.

Study areas	Mean PCV value (%)			
	Village-IV	Village-V	Village-VI	Total
Mareka	22.5	22.7	22.9	22.7
95% CI	21.3-23.6	21.6-23.9	22-23.8	22.1-23.3

The one way ANOVA computed for the mean PCV value between villages for each study area indicated that there was no significant difference in the mean PCV values between villages of the respective study areas. However, there was significant difference in the mean PCV value between Humbo and Mareka ($P<0.001$, independent Student t-test) with a higher value found in Humbo.

During the cross sectional study 48.4% and 82.2% of the cattle sampled from Humbo and Mareka districts had PCV values less than 27%, respectively. The PCV distribution of the two study areas is shown in Figure 4. Majority of the animals which had a PCV value less than 27% were aparaisitemic while only few animals were parasitemic with PCV value greater than 27%.

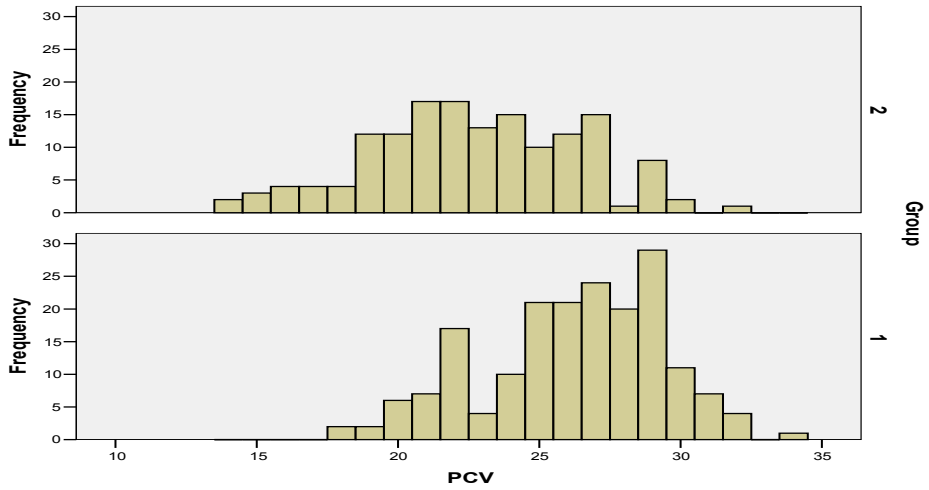

Figure 4. Histograph showing the PCV (%) distribution for the two study areas.

Group 1: Humbo, with tsetse control program

Group 2: Mareka, with no tsetse control program

4.3. Longitudinal study

The result obtained during the longitudinal study in Humbo is summarized in Table 9. A total of nine cattle were found to have infection with trypanosome during the cross sectional study in Humbo and these animals were used for the longitudinal study. A break through case of 66.7% (6/9) was recorded from cattle treated with isometamidium chloride at 1mg/kg b.w. The relapse of infection was recorded from village-I and village-II during the follow up period. All the relapse/breakthrough cases were recorded from *T. congolense* infection. The PCV (%) value of all treated animals during the follow up period showed an improvement of 2% in average.

Table 9. Relapes/breakthrough cases after treatment with isometamidium at a prophylactic dose of 1mg/kg b.w in Humbo district.

Villages	I	II							III
Animal ID	102	103	104	105*	106	107*	108	109	110
Trypanosome species	T.b	T.c	T.c	T.c	T.c	T.c	T.v	Mixed	T.c
Weeks of relapsed	-	3rd	2nd	2nd	3rd	3rd	-	4th	-

T.b: *Trypanosoma brucei*, T.c: *T. congolense*, T.v: *T. vivax*

Mixed: *T. congolense* and *T. vivax*

* : The two *T. congolense* isolates used in the qualitative mice assay were obtained from these animals.

4.4. Drug sensitivity studies in mice

The results obtained during the qualitative mice assay are summarized in Tables 9 and 10. The result obtained for the qualitative assay on mice indicated that isometamidium chloride administered intraperitonially at doses of 0.5–4mg/kg b.w failed completely to cure mice infected with *T. congolense* isolate brought from Humbo (Table 10). Likewise, diminazene aceturate at doses of 3.5–28 mg/kg did not cure any of the infections of the two *T. congolense* isolates brought from Humbo (Table 11).

Table 10. Drug sensitivity of two *T. congolense* isolates (Humbo/2006/FVM/105 and Humbo/2006/FVM/107) in mice treated with isometamidium chloride.

Drug	Doses mg/kg b.w	Number of mice relapsed/treated	Mean relapse interval in days ± S.D	
			Humbo/2006/FVM/ 105	Humbo/2006/FVM/ 107
Isometamidium chloride	0.5	6/6	5.17 ± 0.983	5.33 ± 0.816
	1	6/6	7.17 ± 0.753	7.5 ± 1.049
	2	6/6	9.67 ± 1.75	9.83 ± 1.835
	4	6/6	16.5 ± 1.76	15.67 ± 0.816

Table11. Drug sensitivity of two *T. congolense* isolates (Humbo/2006/FVM/105 and Humbo/2006/FVM/107) in mice treated with diminazene diaceturate.

Drug	Doses mg/kg b.w	Number of mice relapsed/treated	Mean relapse interval in days ± S.D	
			Humbo/2006/FVM/ 105	Humbo/2006/FVM/ 107
Diminazene diaceturate	3.5	6/6	5.67 ± 1.033	5.83 ± 0.753
	7	6/6	9.5 ± 1.378	7.17 ± 1.722
	14	6/6	12.33 ± 1.366	12.83 ± 1.722
	28	6/6	13 ± 2.066	17 ± 2.00

The results obtained during the quantitative mice assay are summarized in Tables 11 and 12. *Trypanosoma congolense* isolates obtained from Mareka were sensitive for both isometamidium chloride and diminazene aceturate at higher doses. The MCD of isometamidium chloride required to clear *T. congolense* from experimentally infected mice was 4mg/kg and 2mg/kg for Pool-1 and Pool-2, respectively (Table 12). The MCD of

diminazene aceturate required to clear *T. congolense* from experimentally infected mice was 28mg/kg and 14mg/kg for Pool-1 and Pool-2, respectively (Table 13).

Table 12. Drug sensitivity of two pooled *T. congolense* isolates (Pool-1 and Pool-2) in mice treated with isometamidium chloride.

Drug used	Doses used (mg/kg b.w)	Number of mice relapsed/treated	Mean relapse interval for pooled isolates in days (±S.D.)	
			Pool-1	Pool-2
Isometamidium chloride	0.5	6/6	10.8 ± 0.7	9.8 ±1.2
	1	6/6	11.5 ± 1.5	11.7 ± 1.5
	2	6/6	14.3 ± 1.0	0
	4	6/6	0	0

Table 13. Drug sensitivity of two pooled *T. congolense* isolates (Pool-1 and Pool-2) in mice treated with diminazene diaceturate.

Drug used	Doses used (mg/kg b.w)	Number of mice relapsed/treated	Mean relapse interval for pooled isolates in days (±S.D.)	
			Pool-1	Pool-2
Diminazene diaceturate	3.5	6/6	10.3 ± 2.2	8.8 ± 1.9
	7	6/6	12.2 ± 1.3	12.2 ± 1.9
	14	6/6	15.5 ±1.97	0
	28	6/6	0	0

There was a clear relationship between the time of relapse/cure and dose of drug used. Mice treated at lower doses relapsed after a shorter time than mice treated with higher doses. All mice in the control groups (group V) showed high level of parasitemia and died between 18 and 43 days after infection.

5. DISCUSSION

The questionnaire survey indicated most of the farmers in Humbo used free grazing system for their animals. As a result of this animals had to travel long distance when there is scarcity of water and feed during the dry season. This situation might have increased the chance of cattle being infected with trypanosomes.

Majority of the farmers (68.3%) ranked the situation of trypanosomosis as moderate. This result can be attributed to the tsetse control program conducted by STEP and it indicates that tsetse control brought down the importance of the disease from major to moderate condition in the area (Abebe *et al*., 2004).

Most of the trypanocides were obtained from veterinary pharmacies. This may avoid the use of generic products which have unknown efficacy. A relatively higher proportion of trypanocides (43%) was used for trypanosomosis suspected cases by the farmers. This might have increased incidence of drug resistance. Isometamidium chloride was more preferred than diminazene aceturate. This could be due to the fact that isometamidium chloride has both curative and prophylactic activity. A relative higher proportion of correct dose was given using diminazene aceturate. This might be because the active principle is available in a sachet for single animal usage.

During the cross sectional study statistically lower trypanosome prevalence was recorded in Humbo. The lower prevalence of trypanosome infections recorded in Humbo might be due to the active vector control program performed by STEP (Abebe *et al*., 2004) and the better veterinary services provided in the area. They reported that the use of insecticide impregnated target and application of pour-on on cattle in Arbaminch has suppressed the tsetse population from 4.1 to 0.9 fly/trap/day. As a result the prevalence of bovine trypanosomosis has dropped from 27 to 6% in two years time (Abebe *et al*., 2004). The cross sectional study also indicated that the dominant trypanosome species in Humbo and Mareka was *T. congolense*. This finding is in accordance with earlier reports from other parts of Ethiopia. Rowlands *et al*. (1993) reported a prevalence of 37% in cattle for *T. congolense* in southwest Ethiopia. Abebe and Jobre (1996) reported a prevalence of 58.5% for *T. congolense* in tsetse infested areas of Ethiopia. In the cross sectional study the prevalence of *T. brucei* and *T. vivax* infection was very low in both study areas. This is may be due to the fact that *T. brucei* and *T. vivax* cause mild infection in cattle and the disease is well tolerated by the animal (Leak, 1999). The lower

ratio of *T. vivax* to *T. congolense* infections in this study may supports earlier findings (Maclennan, 1970), which suggested that cattle developed immunity to *T. vivax* than *T. congolense* more readily. Low prevalence of *T. vivax* and *T. brucei* in the study areas may also be due to more sensitivity of these trypanosomes to trypanocidal drugs than *T. congolense*. Godfrey *et al*. (1964) reported that, in Nigeria, drug treatments did not control *T. congolense* to the same degree as *T. vivax*. The epidemiology of African trypanosomosis is complex and poorly understood and requires large-scale field based investigation (Cox *et al*., 2005). Studies using other more sensitive diagnostic techniques could give a better picture about the epidemiology of the disease in the study areas.

The higher PCV (%) value of cattle in Humbo indicates that control measures taken to suppress tsetse fly has impact in decreasing the disease incidence and thereby improve PCV value of the animals. The appearance of parasitologically negative animals with PCV values of less than 27% may be due to the low sensitivity of the BCT compared to other parasitological diagnosis techniques, e.g. from mAECT (Lumseden *et al*.,1979) animal subinoculation and xenodiagnosis (Stephen, 1986; Clausen *et al*., 1999). The observed low PCV value in parasitologically negative animals might be also explained as follow. During the chronic phase of anemia, erythrocyte synthesis is not sufficient to compensate for erythrocyte destructions, which suggest impairment of bone marrow function (Taylor and Authiė, 2004). The trypanocidal effect on infected cattle depends on the stage of infection. In the early phase, treatment usually results in rapid clinical and hematological recovery. In contrast, during the longstanding chronic disease, despite clearance of parasite by the drug therapy, clinical recovery may be low (Taylor and Authiė, 2004). The appearance of a PCV value less than 27% in parasitologically negative animals may be also due to host factors such as age, nutritional status and breed (Murray and Dextere, 1988). There is growing evidence that nutrition can have a profound effect on the pathophysiological features of animal trypanosomosis (Holmes *et al*., 2000). The appearance of positive animals with PCV of greater than 27% may be explained by recent infections of the animals. The kinetics and degree of parasitaemia are highly variable, influenced by both the virulence of the parasite and the innate and acquired resistance of the host (Taylor and Authiė, 2004).

All cases of the relapsed/breakthrough infections during longitudinal study were due to *T. congolense*. The results have shown that the period of prophylaxis conferred by isometamidium against *T. congolense* was less than 5 weeks, which is in accordance with

earlier reports from different parts of Ethiopia (Scott and Pegram, 1974; Codjia *et al*., 1993; Sutherland *et al*. (1991) reported that the period of prophylaxis conferred by 1mg/kg b.w isometamidium chloride was less than 28 days in cattle challenged with a clone of *T. congolense.* Itard (1981) indicated isometamidium chloride at a dose of 1mg/kg b.w protect cattle for a period of 2-4 months. However the current study showed that the prophylactic activity of isometamidium chloride at a dose of 1mg/kg b.w is less than five weeks.

The qualitative mice assay indicated that isometamidium chloride administered intraperitoneally at doses of 0.5-4mg/kg b.w and diminazene diaceturate at doses of 3.5-28mg/kg b.w failed completely to cure mice infected with isolates brought from Humbo. Since both drugs failed to cure the infections in all doses tested, the minimum curative dose (MCD) for each of the isolates could not be determined. However, the minimum curative dose (MCD) for the two isolates appeared to be greater than 4mg/kg b.w for isometamidium and greater than 28mg/kg b.w for diminazene. This finding is in agreement with the work of Afewerk *et al*. (2000) where isometamidium and diminazene failed to clear *T. congolense* infection in mice at a dose 0.5-4 and 3.5-28mg/kg b.w, respectively. Aseffa and Abebe (2001) also reported similar result on isolate brought from naturally infected donkeys and tested for trypanocidal drug sensitivity on mice. Under normal condition mice infected with a sensitive strain of *T. congolense* could be cured with 0.5mg/kg b.w of isometamidium chloride or 3.5mg/kg b.w of diminazene aceturate (Sones *et al*., 1998; Codjia *et al*., 1993). The advantage of using mice in the present assay is that it is cheaper than the use of cattle. There are several disadvantages, however. Firstly, most *T. vivax* isolates and some *T. congolense* isolates do not grow in mice. Secondly, because of the vast difference in metabolic size, the results in mice cannot be directly extrapolated to calculate the curative dose to be used in cattle. Thirdly, precise assessment of the degree of resistance requires a large number of mice per isolate. This makes it rather labor-intensive test. Finally, it takes as long as 60 days to evaluate the drug sensitivity of an isolate (Eisler *et al*., 2001). Codjia *et al*. (1993) and Afewerk (2000) reported multiple resistance phenotype observed in their study. It is not known in this qualitative mice assay whether the drug resistance is at the individual level or not. If multiple drug resistance is expressed at the level of the individual trypanosome, chemotherapy can become increasingly ineffectual.

The quantitative mice assay conducted on pooled isolates indicated that isolates were sensitive for trypanocidal drugs at higher doses. The result obtained in this assay has

similarity with the work of Chaka and Abebe (2003). They did drug sensitivity test on four *T. congolense* isolates obtained from southwest of Ethiopia using diminazene aceturate. In their result three of the isolates were sensitive for 7mg/kg b.w of diminazene aceturate. The result of this mice assay is also similar with the work of Chitambo and Arakawa (1992), in which a single Mumbwa derived clone of *T. congolense* was sensitive to both Berenil® and Samorine® with MCD of 7mg/kg and 2mg/kg, respectively. Although some drug resistance is apparent in the current study, diminazene aceturate and isometamidium chloride can still be expected to be effective as a sanative pair. Since pooled isolates were used in this mice assay, it was not possible to identify which isolate was resistant. However, these data confirm the presence of *T. congolense* population in Mareka, which require higher doses of trypanocidal drugs as shown in the quantitative mice assay. The advantage of this mice assay is large number of isolates can be tested for the drug sensitivity tests. The result obtained in this quantitative mice assay cannot be extrapolated for cattle due to the same reason explained earlier for the qualitative mice assay (Eisler *et al*., 2001). A further constraint to this technique is that not all populations grow equally well and that sensitive isolates might overgrow resistant ones when inoculated (Eisler *et al*., 2001). The obtained result in this assay did not indicate whether it is at the individual level or not and further studies are required using large number of isolates to get clear picture about the degree of drug resistance in Dawero Zone.

6. CONCLUSIONS AND RECOMMENDATIONS

Trypanosomosis is a major constraint to the livestock production in Ethiopia. Controlling trypanosomosis using either vector control or trypanocidale drugs has a profound effect in improving the PCV value of the animals. Drug therapy has been main strategy used in the past to control trypanosmosis in the study site and throughout Ethiopia. However, development of drug resistance by trypanosomes jeopardize the prophylactic and/or therapeutic activity of the available trypanocidal drugs in the study areas. The prophylactic activity of isometamidium chloride is less than five weeks in the study area. Hence, prophylaxis and/or chemotherapy of trypanosomosis using isometamidium should be conducted with carefully monitoring of the prevalence of trypanosomes and prophylactic and/or therapeutic activity of isometamidium. Development of drug resistance to both isometamidium and diminazene by *T. congolense* in the study area is evident in the current study. Even though cloned populations were not used to proof the observed resistance was at the individual level or not the result indicated there is the possibility of failure of "sanative pair" between these two drugs. Since there is little prospect for the development of new trypanocidal drugs in the near future, control of trypanosomosis in the study area and other tsetse infested part of Ethiopia needs to focus on the careful use of the available antitrypanosomal compounds.

Based on the above conclusions the following pointes are forwarded:

- Large scale field based studies using other more sensitive diagnostic techniques is required to understand the epidemiology of the disease in the study areas

- Strategies should be planned to adopt an integrated tsetse and trypanosomosis management so that trypanocides are used within the broader context of trypanosomosis control.

- Detailed experimental work in the field as well as under laboratory conditions to monitor the extent of drug resistance in pathogenic trypanosomes in the study areas should be conducted.

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8. ANNEXES

Annex 1

Annex 1 Questionnaire set to interview farmers about herd structure, diseases and usage of trypanocidal drugs in Humbo

Respondent

Name…… Date.....…

Address…… Code.....

Village.....

I. Cattle data

1. Herd structure data

Male……. Female…….

Young (1-3 years)……. Young (1-3 years)……

Adult (above 3 years)…… Adult (above 3 years)…….

2. Livestock Management

2.1. How do you manage cattle?

a) Free grazing……. c) Grazing and supplementation……

b) Indoor feed…….. d) Other (specify)……..

2.2. Where do cattle graze?

.....…

.....…

2.3. Where is the location of livestock watering point?

.....…

.....…

2.4. How long is the distance of watering point from the grazing area?

.....

.....

2.5. In which season/month of the years is livestock feed most available?

.....

.....

2.6. In which season is it least available?

.....……

.....……

2.7. Can you mention the feed types available in different seasons?

Season	Feed types
.....	.....
.....	.....
.....	.....

II. Livestock Diseases

1. What are the most common diseases affecting your livestock?

.....

.....

2. Does trypanosomosis occur in this area?
   (Yes, No, Other)
3. If yes, would you rank trypanosomosis with regard to cattle loses compared to other diseases (Major, Moderate, Least)?

.....

.....

4. Which livestock trypanosomosis most affect?

a) Cattle (Yes, No, Other) b) Small ruminants (Yes, No Other)

c) Others (specify)

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

.....

.....

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

.....

7. Is trypanosomosis getting worse, getting better or unchanged in this area in the last few years?

   a) it is getting worse b) it is getting better

   c) it is the same d) I do not know

8. State how the transmission of the disease (trypanosomosis) is affected.

III. Treatment

Diminazene aceturate (Berenil®) Use

1. Have you used diminazene aceturate (Berenil®) since this time last year?

a) Yes b) No

1.1. If yes, why did you treat your animals with diminazene aceturate (Berenil®) ?

a) Because I thought the animals were sick with trypanosomes

b) Because the animals were sick but I didn't know the reason

c) Because the veterinary assistant diagnosed trypanosomes and advised me to use Berenil®

d) Any other reason (specify)……

1.2. When you treat your cattle with diminazene aceturate (Berenil®), do you give the same dose to Young (0-1 years), young cattle (1-3 year) and mature cattle (> 3 years)?

a) Yes b) No

1.3. If no,

a) How many adult cattle (>4 years) would you normally treat with one sachet of diminazene aceturate (Berenil®)

b) How many calves (<1 years) would you normally treat with one sachet of diminazene aceturate (Berenil®)

c) How many young cattle (1-3 years) would you normally treat with one sachet of diminazene aceturate (Berenil®)

Isometamidium chloride (Veridium®)

1. Have you used isometamidium chloride (Veridium®) since this time last year?

a) Yes b) No

1.1. If yes, why did you treat your animals with isometamidium chloride (Veridium®)?

a) Because I thought the animals were sick with trypanosomes

b) Because I wanted to prevent them from getting sick with trypanosomes

c) Because the animals were sick but I didn't know the reason

d) Any other reason (specify)……

1.2. How many of your animals did you treat with isometamidium chloride (Veridium®) since this time last year?

1.3. When you treat your cattle with isometamidium chloride (Veridium®), do you give the same dose to calves (<1 years), young cattle (1-3 year) and adult cattle (> 4 years)?

a) Yes b) No

1.3. If no,

a) How many adult animals would you normally treat with one sachet of isometamidium chloride (Veridium®)

b) How many calves (<1 years) would you normally treat with one sachet of isometamidium chloride (Veridium®)

c) How many young cattle (1-3 years) would you normally treat with one sachet of isometamidium chloride (Veridium®)

1.4. If you have any isometamidium chloride (Veridium®) left over after you have treated all your cattle what do you do with it?

a) I dilute it and keep it in a bottle or container to use latter

b) I keep it as a powder to use later

c) I through away any of the diluted mixture that I don't use

d) I sell the remaining mixture or powder to another farmer

e) I use the remaining mixture or powder to treat my small stock (sheep/goat)

g) Other (specify)……

Treatment general

1. When you use diminazene aceturate (Berenil®) or isometamidium chloride (Veridium®) to treat your animals, do you dilute the powder with
    a) Water that you have boiled?
    b) Water straight from a river or dam?
    c) Water straight from a well?
    d) Other source (specify)……

2. Who treats (injection) your animals with diminazene aceturate (Berenil®) or isometamidium chloride (Veridium®)?
    a) I treat them myself
    b) A veterinarian treats them
    c) A veterinarian assistant treats them
    d) Any combination of the above
    e) Other (specify)

3. What are the sources to obtain trypanocidal drugs
    (a) Veterinary clinics
    (c) Black markets
    (b) Veterinary pharmacies
    (d) Non governmental organizations

Annex. II. Calculation of treatment doses: Diminasan® treatment group

The maximum dosage used for diminazene diaceturate treatment groups in the present study was 28mg/kg b.w

(a) Treatment group: 28mg/kg b.w

For 1kg (1000g) b.w → 28mg/kg b.w

25g mouse → $\frac{25g \times 28mg/kg\ b.w}{1000g}$ = 0.7mg diminazene diaceturate

Therefore we need 0.7mg diminazene diaceturate for a mouse weighing 25g

2.36g Berenil® contain 1.05g of diminazene diaceturate. Thus the required amount of Berenil® to treat 25g mouse is

$$\frac{0.7mg\ diminazene\ diaceturate \times 2.36g\ Berenil®}{1.05g\ diminazene\ diaceturate} = 1.57mg\ Berenil®/mouse$$

Dosage per mouse → 1.57 mg Berenil®/0.2ml of distilled water (157mg Berenil® is dissolved in 20ml of distilled water)

→Inject 0.2ml of this solution into each of the six mouse in the fourth treatment group

(b) Treatment group: 14mg/kg b.w

→To obtain 14mg/kg b.w diminazene diaceturae the preparation for 28mg/kg b.w is double diluted i.e. 10ml of the solution prepared for 28mg/kg b.w is diluted with 10ml of distilled water.

→From this preparation 0.2ml of solution is injected into each of the six mice in the third treatment group

(c) Treatment group: 7mg/kg b.w

→To obtain 7mg/kg b.w diminazene diaceturae the preparation for 14mg/kg b.w is double diluted i.e. 10ml of the solution prepared for 14mg/kg b.w is diluted with 10ml of distilled water.

→From this preparation 0.2ml of solution is injected into each of the six mice in the second treatment group

(d) Treatment group: 3.5mg/kg b.w

→To obtain 3.5mg/kg b.w diminazene diaceturae the preparation for 7mg/kg b.w is double diluted i.e. 10ml of the solution prepared for 7mg/kg b.w is diluted with 10ml of distilled water.

→From this preparation 0.2ml of solution is injected into each of the six mice in the first treatment group

Annex. III. Calculation of treatment doses: Veridium® treatment group

(a) Treatment group: 4mg/kg b.w

1kg (1000mg) → 4mg/kg b.w

25g mouse → $\frac{25g \times 4mg/kg\ b.w}{1000mg}$ = 0.1mg isometamidium chloride/mouse

→ Dosage per mouse → 0.1 mg isometamidium chloride/0.2ml of distilled water (10mg isometamidium chloride is dissolved in 20ml of distilled water)

→ From this preparation inject 0.2ml of solution into each of the six mice in the fourth treatment group

(b) Treatment group: 2mg/kg b.w

→To obtain 2mg/kg b.w isometamidium chloride the preparation for 4mg/kg b.w is double diluted, i.e. 10ml of the solution prepared for 4mg/kg b.w is diluted with 10ml of distilled water.

→From this preparation 0.2ml of solution is injected into each of the six mice in the third treatment group

(c) Treatment group: 1mg/kg b.w

→To obtain 1mg/kg b.w isometamidium chloride the preparation for 2mg/kg b.w is double diluted.

→From this preparation 0.2ml of solution is injected into each of the six mice in the second treatment group

(d) Treatment group: 0.5mg/kg b.w

→To obtain 2mg/kg b.w isometamidium chloride the preparation for 1mg/kg b.w is double diluted.

→From this preparation 0.2ml of solution is injected into each of the six mice in the first treatment group

9. CURRICULUM VITAE (CV)

I. Personal data

Name:	Miruk Assefa
Sex:	Male
Place of birth:	April 25, 1978
Date of birth:	Addis Ababa
Marital status:	Single
Language skill:	Amharic and English
Qualification:	DVM
	Computer literate

II. Educational background

Elementary school:	Adventist Mission
High School:	Kokebe Tsebeha Secondary School
University:	Addis Ababa University Faculty of Veterinary Medicine

III. Research experience

DVM thesis: Parasitic Causes of Carcass/Organ Condemination at Asella Municipality Abattoir

MSc course work seminar paper: Current Knowledge on Sterile Male Tsetse Flies Mass Rearing for the Eradication of Wild Tsetse Flies

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Signed Declaration

This thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name_____

Signature_____

Date of submission_____

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

Dr. Hagos Ashenafi (DVM, MSc, Assistant Professor) _____

Dr. A.K. Basu (DVM, MSc, PhD, Associate Professor) _____