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STUDY ON THE OCCURRENCE OF DRUG RESISTANT TRYPANOSOMES IN CATTLE IN THE FARMING IN TSETSE CONTROL AREAS (FITCA) PROJECT IN WESTERN ETHIOPIA

by

Nega Tewelde Tikue

December, 2001

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A thesis submitted in partial fulfillment for the degree of Master of Science in Tropical Veterinary Epidemiology at the Freie Universität Berlin and Addis Ababa University

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bw	Body weight
CD	Curative Dose
CI	Confidence Interval
DNA	Deoxy-ribo nucleic acid
DVM	Doctor of Veterinary Medicine
ED	Effective Dose
ELISA	Enzyme-linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FHI	Food for the Hungry International
FITCA	Farming in Tsetse Control Areas
FLDP	Fourth Livestock Development Project
GIS	Geographic Information System
ICIPE	International Centre for Insect Physiology and Ecology
ILRI	International Livestock Research Institute
i.p.	Intra-peritoneal
ISCTRC	International Scientific Council for Trypanosomosis Research and Control
kg	Kilogram
km^2	Square kilometer
m	Meter
masl	Meters above sea level
mg	Milligram
ml	Milliliter
mm	Millimeter
MOA	Ministry of Agriculture
MSc	Master of Science
NARS	National Agricultural Research Systems
ng	Nano gram
NGO	Non-governmental Organization
NLDP	National Livestock Development Programme
No.	Number
NTTICC	National Tsetse and Trypanosomosis Investigation and Control Centre
OAU/IBAR	Organization of African Unity/Inter-African Bureau for Animal Resources
ORA	Oromo Relief Association
PACE	Pan African Control of Epizootics
PCV	Packed Cell Volume
SD	Standard deviation
SIM	Society of International Missionary
SIT	Sterile Insect Technique
spp	Species
ULV	Ultra Low Volume
US	United States
US$	United States Dollar
USA	United States of America

1 US$ = 8.39 Birr

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ABSTRACT

The main objectives of the study were: (1) to determine the prevalence of bovine trypanosome infection in selected areas of the FITCA project and (2) to assess the trypanocidal activity of diminazene aceturate and isometamidium chloride and the impact of isometamidium prophylaxis relative to no prophylaxis using cattle under natural challenge in the field.

The study was conducted in seven areas (Kone settlement, Loko, Shobe, Village I settlement, Ego Kofele settlement, Sore settlement and Darimu), which are located along three river valleys in western Ethiopia (upper Didessa, Birbir and Sore-Geba). The study was carried out from February to end of June 2001 and comprised of questionnaire survey, cross sectional study and isometamidium block treatment study.

Results of the questionnaire survey revealed that 95.5% of the interviewees replied that trypanosomosis was a serious problem in their areas. Apart from this, under dosing of trypanocidal drugs appeared a common practice in the areas surveyed.

Cross sectional study was carried out over 904 randomly selected animals. Cattle were examined for trypanosome infection using the buffy coat technique (BCT). Results showed that the prevalence of trypanosome infection was the highest in Kone (21.3%) and Village I (15%) settlement areas, and *T. congolense* accounted for 75% and 93% of the infections, respectively. A significant difference ($P<0.05$) was noticed between the mean PCV values in the parasitaemic (95% CI = 22.0, 23.0) and aparasitaemic (95% CI = 24.2, 25.4) cattle. Conversely, results were compared with other available background information and they indicated that the prevalence of trypanosome infection appeared to decline in many of the study areas.

Kone settlement area, which comprises of Cheleleki, Kolu and Burka villages, was selected for the isometamidium block treatment study. The study was conducted using a total of 300 cattle, 100 from each of Cheleleki, Kolu and Burka. At day -14 of isometamidium block treatment study, all animals in the three villages were treated with diminazene aceturate (7mg/kg bw). Cattle at each village were then randomized into 50 control and 50 isometamidium treated groups and they were ear-tagged to allow easy identification during each field visit. Fourteen days later (day 0), only the treatment groups of cattle were treated with isometamidium chloride (1mg/kg bw). Both groups of cattle were examined for trypanosome parasites using the BCT every 14 days until day 84 of the study. Regardless of the group, animals found infected with trypanosomes during day 0-84 were treated with diminazene aceturate (7mg/kg bw). At day-14 of isometamidium block treatment study, results showed that trypanosome prevalence was 27%, 21% and 29% at Cheleleki, Kolu and Burka, respectively. Fourteen days after diminazene aceturate treatment (day 0), none of the animals were found infected with trypanosomes. In the control groups of cattle, the mean

prevalence of trypanosome parasitaemia during the 8-week period at Cheleleki, Kolu and Burka villages was 12.8%, 9.6% and 11.0%, respectively. In the isometamidium treated groups, it was 4.5%, 5.7% and 10.1% for Cheleleki, Kolu and Burka, respectively.

Survival analysis was used to assess the impact of isometamidium prophylaxis relative to no prophylaxis. The data were analyzed and interpreted in accordance with earlier reports. The 25% survival time of the control groups of cattle was 42 days at Cheleleki and Kolu. It was only 28 days for this group of cattle at Burka. These findings indicate that the tsetse challenge was sufficiently high at all the three villages and a prophylactic regimen is more indicated if trypanosomosis is to be controlled efficiently. On the other hand, 22.9%, 20.0% and 36.0% of the isometamidium treated groups of cattle at Cheleleki, Kolu and Burka, respectively, were found infected with trypanosomes by day 56 of the isometamidium block treatment study. This again indicates that the occurrence of resistance to isometamidium chloride was insignificant and isometamidium chloride could still be used to protect cattle against trypanosomosis at Cheleleki and Kolu. However, there was strong suspicion of the occurrence of drug resistant *T. congolense* against isometamidium chloride at Burka. The ratio of the mean hazard rates of the control to the isometamidium treated groups at Cheleleki and Kolu over weeks 1- 8 were 2.75 and 2.25, respectively. As for Burka village, the ratio was 1.25. Since the ratio of the mean hazard rate for Burka was lower than two (a threshold which was proposed earlier), it may be of no value to continue using isometamidium chloride at the village.

The results of the assessment of the efficacy of diminazene aceturate treatment at day 0 showed that all animals at the three villages were not parasitaemic within 14 days of treatment with the drug. From day 0 onwards, there was no recurrence of trypanosome infection in cattle at Cheleleki 14 days after treatment with diminazene aceturate (7mg/kg bw). There were 2 recurrent infections at Kolu and 3 at Burka. However, there was no significant difference between the trypanosome incidence rate and trypanosome infection recurrence rate at each of Kolu and Burka villages. These findings indicate that they were all new cases and diminazene treatment was still effective at all the study villages.

It was concluded that where there are indications for drug resistance against isometamidium, the use of trypanocidal drugs should be supervised, the principle of sanative pairs has to be applied and chemotherapy needs to be integrated with other methods like vector (tsetse fly) control.

Key words: cattle, trypanosomosis, isometamidium chloride, diminazene aceturate, drug resistance, survival analysis.

1. INTRODUCTION AND OBJECTIVES

1.1. Tsetse and trypanosomosis in Africa

Trypanosomosis is a protozoan disease transmitted by the bite of a tsetse fly (*Glossina* spp.). The disease affects both human and domestic animals. The disease in human is known as sleeping sickness and nagana in cattle.

Tsetse transmitted bovine trypanosomosis is one of the most economically important diseases of domestic livestock in sub Saharan Africa (Gu *et al.*, 1999) and is distributed in about 40 African countries (Murray *et al.*, 1991).

Currently, tsetse flies infest approximately 11 million km^2 of Africa, about 37% of the land area of the continent; an area larger than the USA which is 9.8 million km^2 (Trail *et al.*, 1985).

A recent study has estimated the direct annual cost of trypanosomosis to be about 1.34 billion dollars (Kristjanson *et al.*, 1999). According to Budd (1999), African farmers spend 35 million US dollars per year on trypanocidal drugs to protect and cure their cattle. The disease in cattle has more impact on rural poverty than can be estimated in billions of dollars per annum (Hursey and Slingenbergh, 1995). The situation with regard to sheep, goats, pigs, donkeys, horses and camels is probably as serious but is less documented (Gu *et al.*, 1999).

It is estimated that 7 million km^2 of tsetse infested Africa would otherwise be suitable for livestock and mixed agriculture if trypanosomosis could be controlled (MacLennan, 1980).

The cost of human African trypanosomosis is extremely difficult to quantify; however, it has been estimated that at least 50 million people are at risk of contracting sleeping sickness (Kuzoe, 1991).

1.2. Tsetse and trypanosomosis in Ethiopia

Before 1960's, trypanosomosis had relatively little impact on the economy of Ethiopia. After 1960's, the magnitude of the problem has increased enormously and still it is increasing (NLDP, 1997). This is due to a number of factors which include mainly, overpopulation and overstocking of the highlands which forces people to use the tsetse infested lowlands, the advance of tsetse flies in to previously un-infested areas and the development of a widespread drug resistance by trypanosome parasites over the different types of trypanocidal drugs which are in use in Ethiopia (NLDP, 1997).

Until 1976, a total of 98,000 km^2 area of the country was infested by five species of tsetse flies (Langridge, 1976). In more recent years, tsetse flies have progressively invaded productive agricultural areas in the west, south and southwest parts of Ethiopia. Consequently, it is estimated that a total area of 220,000 km^2 is currently believed to be infested with different species of tsetse flies in which case, livestock below 2000 m contour are exposed to various levels of trypanosomosis risk (NTTICC, 1996). As a result, a total of 14.8 million cattle, 6.12 million sheep and goats, one million camels and 1.23 million equines are at risk of contracting trypanosomosis in Ethiopia (MOA, 1995). The overall economic loss due to the disease is estimated to be between US$ 1,408 and 1,540 million annually (NTTICC, 1996). None tsetse-borne trypanosomosis also affects a considerable number of animal populations in tsetse free zones of the country (MOA, 1995).

There are five economically important animal trypanosome species in Ethiopia. These are *Trypanosoma congolense, T. vivax, T. brucei brucei, T. evansi* (Langridge, 1976) and *T. equiperdum* (Dagnachew and Shafo, 1981). However, sleeping sickness is of negligible public health importance in the country.

As far as the vector is concerned, there are as well five species of tsetse flies distributed along the lowlands of western, southern and southwestern parts of the country. *Glossina morsitans submorsitans, G. pallidipes, G. fuscipes fuscipes* and *G. tachinoides* are the most important tsetse flies whilst *G. longipennis* is of minor economic importance (Langridge, 1976).

The tsetse infested lowlands of the country are highly fertile and very suitable for agriculture but considerable parts of these areas are devoid of people and livestock.

In tsetse infested regions of Ethiopia, the problem of trypanosomosis is the main cause for the decline in the number of cattle and particularly draft oxen (Abebe and Jobre, 1996; NLDP, 1997). In Ethiopia, the provision of animal draught power is basically crucial as draught oxen are more widely used in Ethiopia than in any other Sub-Sahara African country (FITCA, 1998). The loss of draft oxen, generally, causes a dramatic decline in farm size and crop production. As a result, farmers shift from cultivating higher valued teff to maize, as the latter requires less plowing (Berhanu, 1998).

Tsetse control activities against, mainly, *G. m. submorsitans* were undertaken in about 1000 km^2 of the upper Didessa valley (NTTICC, 1996). Subsequently, in the tsetse controlled areas, the apparent density of tsetse flies and the prevalence of trypanosome infection have declined to a minimum level. As a result, the total cultivated land and cultivated land per farmer has increased by 338.4% and 100%, respectively (Berhanu, 1998). In some areas, farmers were able to diversify their crops and increased teff production, which is known to be labour intensive (Berhanu, 1998).

Livestock in general and cattle, sheep, goats and equines in particular increased in number after the control programme in the area. The growth registered here was 145.7% for total livestock, 144.8% for cattle, 123.7% for sheep, 160.1% for goats and 226.8% for equines (Berhanu, 1998). The average oxen and cattle per household have also improved by 120.0% and 108.9%, respectively (Berhanu, 1998).

As an extension of the previous activities, an Eastern Africa Regional Programme "Farming in Tsetse Control Areas (FITCA)" project is operating over a total area of 4,500 km^2 of the upper Didessa valley to control tsetse flies and rehabilitate mixed farming practices (FITCA, 1998). Apart from this, operation is underway to eradicate tsetse flies from an area of 25,000 km^2 in the southern rift valley of Ethiopia using the Sterile Insect Technique (SIT).

Since vector control operation is very limited in scope, which is about 14% of the total tsetse infested area of the country, trypanosomosis control by the use of imported trypanocidal drugs is the most widely applied technique of trypanosomosis control in Ethiopia. Trypanocidal drugs were used in Ethiopia, for more than 40 years, to control the disease in different domestic animals (NTTICC, 1996). The use of these drugs was significantly increased over the past 20-30 years due to the increase in the magnitude of the problem of tsetse and trypanosomosis from year to year. Although the demand is much higher than this amount, one to two million

doses of the drugs are administered at the cost of some US $ 0.5 - 1 million per annum; excluding illegal and NGO drug imports (MOA, 1995).

In spite of all the efforts exerted so far, the emergence of drug resistance has seriously hampered the control of animal trypanosomosis in Ethiopia. Multiple trypanocidal drug resistance have been reported in the Abay/Didessa tsetse belt in Metekel district, North-west Ethiopia (Afewerk, 1998; Afewerk *et al.*, 2000) and in the Ghibe/Omo tsetse belt which is adjacent to the upper Didessa river valley (Codjia *et al.*, 1993; Rowlands *et al.*, 1993; Leak *et al.*, 1993; Mulugeta *et al.*, 1997; Ademe and Abebe, in press). Assefa and Abebe (2001) have also reported multiple drug resistant *T. congolense* in naturally infected donkeys in North Omo Zone, southern Ethiopia.

The main objectives of the research project were:

- to determine the prevalence of bovine trypanosome infection in selected areas of the FITCA project,
- to assess the trypanocidal activity of diminazene aceturate and isometamidium chloride and the impact of isometamidium chloride prophylaxis relative to no prophylaxis using cattle populations under natural challenge in the field and
- to investigate the presence of trypanosome populations resistant to diminazene aceturate and isometamidium chloride in experimentally infected mice.

2.1. Morphology of trypanosomes

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

The trypanosomes of this subgenus have a range in total length of 8-24μm. There is no free flagellum at any stage in the life cycle, which is an unusual characteristic. The flagellum, thus, terminates at the anterior end of the parasite. 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 sub-terminal position. *T. congolense* is one of the smallest trypanosomes with a mean length of 12-17 μm. *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*.

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

T. (D.) vivax Ziemann, 1905, 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. It crosses the field of a microscope rapidly, which makes it difficult to follow its movements.

2.1.3. *Trypanosoma (Trypanozoon) brucei* Plimmer & Bradford, 1899

The blood forms of *T. brucei* measure from 11-39 μm in total length. They are typically polymorphic, being represented by three forms: (a) slender forms (mean lengths 14-39 μm) possessing a long free flagellum and a well-developed undulating membrane, elongated nucleus, subterminal kinetoplast and narrow posterior end drawn out to a blunt point or sometimes truncated; (b) stumpy trypanosomes (mean lengths 16.6-20 μm) which are stout and usually without a free flagellum, undulating membrane well developed, nucleus rounded (displaced to the posterior end in posterionuclear forms), kinetoplast near broadly rounded or

obtusely pointed posterior end; and (c) intermediate forms (mean lengths 14-39 µm) in which the flagellum is shorter, the posterior end blunter and the kinetoplast nearer to this extremity than in the slender forms. The kinetoplast in *Trypanozoon* is smaller than in any of the other salivarian trypanosomes. Animal and human infective *T. brucei* are morphologically indistinguishable.

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

The total length of *T. suis* has a range from 13-19 µm (mean 16 µm) with a normal distribution, indicating that this species is monomorphic. A free flagellum is typically present. Its body is very broad and short; the posterior end usually terminates in a short point, but sometimes it is rounded. The small kinetoplast is usually situated near the posterior end and in the majority of cases occupies a marginal position, while the voluminous nucleus lies in the anterior part of the body and the undulating membrane is conspicuous.

2.2. Epidemiology of trypanosomosis

The epidemiology of African animal trypanosomosis is highly dependent on the parasite (*Trypanosoma* spp.), vector (*Glossina* spp.) and many domestic and wild mammalian hosts (Jordan, 1986).

2.2.1. Parasite

When a tsetse fly ingests trypanosome parasites together with blood meal from a mammalian host, the trypanosomes undergo a cycle of development within the insect vector. Transmission of trypanosomes after they have undergone a cycle of development which culminates in the production of infective metacyclic trypanosomes is referred to as cyclical transmission (Jordan, 1986). However, trypanosomes can also be transmitted from one mammalian host to another through the contamination of the mouthparts of various species of biting flies, and probably, including tsetse flies (Jordan, 1986). Such trypanosomes can only survive outside the mammalian hosts for a short time and thus successive hosts have to be fed upon in quick succession for the transmission to be effected. This process is known as mechanical transmission (Jordan, 1986). *T. vivax* infection has been seen at some distance from the edges of tsetse belts in Ethiopia. A similar situation has also been reported in Ethiopia, where *T. vivax* is commonly found in highlands too cold for tsetse flies to survive (Uilenberg, 1997).

Seasonal outbreaks of *T. congolense* infection have been reported outside tsetse areas in southern Sudan, associated with large numbers of tabanids, but normally this trypanosome species is confined to tsetse belts (Uilenberg, 1997). In other words, *T. congolense* has not managed to really escape from its biological vector and this is also true for *T. simiae*. Although the infection is not propagated outside tsetse areas, it was also thought that mechanical transmission by stable flies may be important for *T. simiae* once the infection has been introduced by tsetse flies (Uilenberg, 1997).

The distribution of *T. brucei* seems to be closely associated with that of its *Glossina* vectors, but *T. evansi*, and also *T. equiperdum*, which appeared to have been derived from *T. brucei* have adapted to mechanical and venereal transmission, respectively (Uilenberg, 1997).

2.2.2. Vector

There are many experimental evidences which show that host preferences and vector capacity differ greatly between groups and species of *Glossina* (Jordan, 1986).

Generally, savannah species of tsetse flies are better vectors of the pathogenic trypanosomes of livestock. Althogh the distribution of savannah (*morsitans* group) of tsetse is highly localized in the dry season, the risk of contracting the disease is widespread in areas where these species of tsetse flies are the vectors of trypanosomosis (Jordan, 1986).

As far as riverine species of tsetse is concerned, transmission of trypanosomosis occurs particularly along rivers with dense vegetation like in many parts of West and Central Africa.

Many of the forest species (*fusca* group) are confined to dense forest and are therefore not normally in contact with livestock, but sometimes may also occur on the forest edge and may locally play a significant role as vectors of African animal trypanosomosis.

Numerous attempts have been made to define the degree of trypanosome risk or challenge to which domestic livestock are exposed when they come into contact with tsetse flies. Tsetse challenge has often been simply assessed as light, medium and heavy based on the species of tsetse involved, their feeding preferences, the trypanosome infection rate and the density of the tsetse populations with which the livestock come into contact. Accordingly, the heaviest challenge to cattle and other species of domestic livestock is, undoubtedly, presented by dense

populations of the subspecies of *G. morsitans* and other species of the *morsitans* group.

The best example of light challenge is that provided by dispersed, low density populations of the *palpalis* group of tsetse.

Between these two extremes of heavy challenge from dense populations of the *morsitans* group of tsetse and light challenge from sparse populations of *palpalis* group of tsetse, there is a wide range of intermediate situations. These vary from less dense populations of *morsitans* group tsetse, to mixed infestations of *morsitans* and *palpalis* group.

2.2.3. Host

Host species and breed susceptibility are of great importance which contribute to the epidemiology of trypanosomosis. In tsetse areas, trypanosomosis is a very serious problem in susceptible animals while it may remain, practically, inapparent as far as trypanotolerant breeds of livestock are concerned. Herd management is also important when daily activity of the tsetse flies and the grazing patterns of the herds are considered. If the herds graze on infested sites at the time of the day that the flies are most active, transmission will occur more frequently.

Populations of savannah species feed mainly on mammalian hosts, particularly bovids (antelopes, buffalo, cattle, sheep, goats) and suids (warthog and bushpig), while riverine tsetse have a very wide range of preferred hosts, including reptiles and man (Weitz, 1963; Clausen *et al.*, 1998a). Zebra, certain antelopes and also carnivores have little attraction for tsetse flies. The proportion of a tsetse population found infected with pathogenic trypanosomes, therefore, depends not only on its vector capability, but also on the hosts on which it mainly feeds. For instance, reptiles do not carry pathogenic trypanosomes, and there are also major differences between suids and bovids, as the former will infect the flies particularly with *T. simiae* and *T. godfreyi*, while bovids are mainly the source of *T. vivax* and *T. congolense*.

2.3. Clinical signs of trypanosomosis

The various disease manifestations of trypanosomosis are, generally, not possible to distinguish clinically from other conditions, particularly, from those of malnutrition.

As regards to cattle trypanosomosis, *T. congolense* and *T. vivax* are the most important parasites and despite acute hemorrhagic infections due to *T. vivax*, most infections are clinically indistinguishable. The clinical signs of the disease in cattle include acute phase with elevated body temperature and death within few weeks. The chronic stage, which is more typical with indigenous African cattle, can persist for months or even years. At this stage, the animal becomes increasingly emaciated and anemic and in terminal stages, the animal is recumbent and some times comatose. Pregnant animals suffering from chronic trypanosomosis are liable to abort. There is a general understanding that *T. vivax* from East and West Africa differ in their pathogenecity. With the exception of haemorrhagic syndrome which accompanies some *T. vivax* infections, East African *T. vivax* tends to produce a milder disease which livestock in good condition can resist (Gardiner and Wilson, 1987). Though this geographic demarcation of virulence is generally true, there are several reports of an extremely acute form of *T. vivax* infection in East Africa causing a haemorrhagic syndrome in cattle (Wellde *et al.*, 1983). Although exotic breeds of cattle are highly susceptible to *T. b. brucei*, many African cattle do not show any clinical signs of the disease when infected with this species of parasite.

Horses, donkeys and mules are highly susceptible to infections with *T. b. brucei* and death of untreated animals usually occurs within 3 months. The clinical symptoms are similar to those of cattle disease. Infections of horses with *T. congolense* can be fatal but most infections of horses with both *T. congolense* and *T. vivax* often give rise to chronic disease with spontaneous recovery (Jordan, 1986).

Trypanosomosis is not a major problem in sheep and goat flocks kept under natural conditions since natural tsetse populations seldom feed on sheep or goats (Jordan, 1986). *T. congolense* is the commonest cause of trypanosomosis in sheep and goats and anemia is one of the most important symptoms of the disease.

T. simiae is the most dangerous and dramatic parasite of domestic pigs causing a massive parasitaemia with death after a pre-patent period of 4 to 6 days (Uilenberg, 1997). Pigs are probably completely refractory to *T. vivax*. However, both *T. b. brucei* and *T. congolense* can infect pigs but have relatively low pathogenecity. The rarely recorded *T. suis* is also a cause of chronic disease of pigs.

Camels can be infected by *T. b. brucei*, *T. vivax*, *T. congolense* and *T. simiae* and the disease is either acute, being fatal within a few months, or chronic, lasting several years. In general terms, symptoms are similar to those in other domestic animals. *T. evansi* transmitted by biting flies other than tsetse, is the causative organism of surra in the Middle East and Asia, as well as in Africa.

As regards to human trypanosomosis, *T. b. gambiense* and *T. b. rhodesiense* are the causative agents of two forms of sleeping sickness. Both forms are diseases of the central nervous system and other tissues. There is an initial period of infection of the blood, which is followed by invasion of the cerebrospinal fluid and the brain. Typical features of the disease are enlargement of, particularly, the posterior cervical lymph glands and oedema of the face. *T. b. gambiense* causes a chronic disease with emaciation and sleeping symptoms from which the disease derives its name. Death of untreated victims may not occur for several years. *T. b. rhodesiense*, on the other hand, causes an acute disease with severe toxaemia and untreated patients can die within a few weeks or months of infection.

T. b. gambiense occurs in western and central Africa where as *T. brucei rhodesiense* occurs in eastern and southern Africa (Uilenberg, 1997).

2.4. Diagnosis of trypanosomosis

Diagnosis of trypanosomosis is made in the field using either clinical or laboratory findings. As regards to the former, clinical signs of trypanosomosis are not pathognomonic to the disease and diagnosis is solely attained by other methods like detecting the trypanosome parasites using thin and thick blood films. In view of the fact that trypanosomosis is generally a chronic disease, these parasitological techniques used to diagnose trypanosomosis are not fairly sensitive in detecting most of the infections. This is because, trypanosomes are often scanty in the peripheral blood at this stage of the disease. While *T. congolense* is mainly confined to the blood, *T. vivax* and *T. brucei* occur also in body tissues such as the lymph nodes, the chamber of the eye as in case of *T. vivax* and the central nervous sytem as in case of *T. brucei* (Uilenberg, 1997).

The dark ground phase contrast buffy coat technique (Murray *et al.*, 1977), which can be used under field conditions, is the most sensitive parasitological method of detecting *T. congolense* and *T. vivax* infection (Paris *et al.*, 1982).

Serological methods which may have advantage over direct methods and applied to detect anti-trypanosome antibodies, are very sensitive but often show too many false positive cases as a result of the persistence of anti-trypanosome antibodies after treatment. Tests based on detecting circulating antigens 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 Systems (NARS) in Africa for the diagnosis of trypanosomosis. It has, however, become apparent recently that the sensitivity of this type of test is not as high as it was claimed, and even positive results are not reliable (Eisler *et al.*, 1998).

There are also trypanosomosis diagnostic tests based on the principle of molecular tests which demonstrate the occurrence of sequences of nucleotides specific for a trypanosome subgenus, species or even type or strain. These tests are not only suitable for detecting parasites in the mammalian host, but also in the insect vector. However, they can only be carried out reliably in well-equipped laboratories by specifically trained staff, and are still mainly research tools (Geerts and Holmes, 1999).

2.5. Methods of trypanosomosis control

Methods used to control trypanosomosis include, parasite control, vector control and the exploitation of trypanotolerant livestock (Uilenberg, 1997).

2.5.1. Parasite control

While tsetse control has been successful in several African countries (Jordan, 1986) and use of trypanotolerant livestock is the basis for livestock development in many countries of West and Central Africa (d'Ieteren *et al.*, 1998), the major strategy used to control bovine trypanosomosis in sub-Saharan Africa is based on trypanocidal drugs (Peregrine, 1994). In other words, trypanosomosis control using trypanocidal drugs is the only approach possible for technical, logistic, financial or ecological reasons in most African countries (Jordan, 1986). It can also be argued that cattle only occur in many parts of Africa because of the availability of trypanocidal drugs (Jordan, 1986).

If trypanocidal drugs are properly used, they can provide a cost-effective and sustainable approach to trypanosomosis control (Trail *et al.*, 1985).

Drugs can be highly effective provided they are continuously available and treatments are given regularly and at appropriate dose rates. More over, drugs can offer the possibility of reducing the disease to a level where infested land can be exploited most economically with minimum risk of contracting trypanosomosis. However, if sufficient intensity of land use does not result following treatment of animals with trypanocidal drugs and tsetse habitats remain, then the presence of cattle can even cause an increase in the number of flies. This is because treated animals will serve as readily available sources of blood meal to tsetse flies. Therefore, the use of drugs to protect cattle owned by peasant farmers could be most efficacious in such circumstances when it increases the amount of land in effective cultivation, which in turn decreases the amount of suitable tsetse habitat. The Angar Gutin Settlement Scheme in Ethiopia (Boun and Scott, 1978) is an example of what can be accomplished in maintaining cattle under *G. m. submorsitans* infested areas when drugs and effective management are available. In this area of the country, *G. m. submorsitans* was abundant and the trypanosomosis challenge was high but the disease was kept under control by the strategic use of drugs and area development (Jordan, 1986). The effect of man on populations of the *morsitans* group is dramatic and is profoundly altering the distribution and abundance of these groups of tsetse flies (Jordan, 1986). For instance, *G. morsitans* occurs in areas with human population densities ranging from 0-15 people/km^2. Occasional flies of this species are found in areas 15-39 people/km^2 but never when the population exceeds 39 people/km^2 (Jordan, 1986).

Chemotherapy of trypanosomosis in domestic livestock is at present dependent upon the salts of a relatively small number of synthetic compounds; namely, homidium, isometamidium, diminazene and quinapyramine (Guelmbaye *et al.*, 1993). All four compounds have been on the market for the last 30 years and there are reports of drug resistance in *T. congolense* and *T. vivax* in many parts of Africa. Further more, because of the close chemical relationships between the compounds, the development of resistance to individual trypanosomes often appears to be associated with cross resistance to others (Whitelaw *et al.*, 1986).

2.5.2. Vector control

At the moment, there are five proven and effective tsetse control/eradication methods. These techniques are aerial spraying, ground spraying, insecticide impregnated odour baited targets, insecticide treated cattle and SIT.

Residual insecticide formulations have been applied from helicopters (Baldry *et al.*, 1981) to control/eradicate tsetse flies. Sequential aerial spraying has been used to treat several thousand km^2 in Botswana, Kenya, Nigeria, Somalia, Uganda, Zambia and Zimbabwe with varying successes (Allsopp, 1991). Aerial spraying can be used to treat large areas rapidly and is particularly appropriate in epidemic situations. It is also suitable where ground access is difficult, dangerous or undesirable (Allsopp, 1991). However, aerial spraying using ultra-low volume (ULV) and non-persisteht insecticide is expensive and cannot be implemented in areas with ragged topography. In other words, deep escarpments along river valleys do not allow for aerial spraying just above the tree canopy at nighttime.

Ground spraying has been used with great success in many parts of Africa (Allsopp, 1991). However, spraying using persistent and long acting insecticide is environmentally polluting and ground spraying is banned throughout the continent. Apart from this, the technique is too laborious and costly.

Despite successful field trials, livestock farmers and national governments in Africa have been slow to embrace traps and targets as a means of tsetse control (Baylis and Stevenson, 1998). The reasons for this were difficulties associated with deployment and maintenance of traps and targets over large and often inaccessible areas and the cost of odour attractants and insecticides. Apart from these, the scientifically rigorous approach to the design of the control programme often requires a level of infrastructure and organization rarely available to the people whose animals are at risk of trypanosomosis.

Insecticide treated cattle offer numerous advantages over odour-baited traps and targets. Cattle are used as moving targets and hence no cost on odor baits, besides cattle can be moved to spray races or dips rather than staff travelling to widely dispersed traps/targets. Moreover, it can be based on existing infrastructure like dips and spray races and can bring about significant savings in operational costs. However, fly-cattle contact is necessary if tsetse flies are to be controlled. This requires alternative means of protection of cattle using prophylactic trypanocidal drugs when they are first introduced into tsetse infested areas; and consequently, accepting a low level of disease incidence. This problem may also be recurrent if cattle are moved frequently in search of better grazing. In addition to this, tsetse numbers may need to be monitored using odour baited traps to assess the effectiveness of the control programme which re-introduces many of the short comings of traps and targets as mentioned above. Further more, blood meal analysis should be undertaken to determine the percentage of flies

feeding on cattle. Apart from this, the number of cattle population should be significantly high for the technique to be effective. The technique was field tested and found successful in a number of African countries such as Ethiopia (Keno and Mengistu, 1995), Zimbabwe, Tanzania, Zanzibar, Kenya and Burkina Faso (FAO, 1992).

The value of eradicating tsetse from the vast tsetse infested areas using the SIT lies to a large extent on the economic justification and on its sustainability. The benefits of tsetse eradication depend mainly on the rate and extent at which cleared areas are put to productive use and the sustainability of the operation which in turn is dependent on whether or not re-invasion of the cleared areas does not occur. The only sure way of maintaining eradicated area from re-invasion is to eradicate an entire fly belt. However, tsetse infested areas are, mostly, very large and eradication of the entire belt may not be economically justifiable and would take an ample amount of time. Apart from this, the SIT should succeed other tsetse suppression techniques, like traps and targets, which leads again to many of the disadvantages of trap and target techniques. SIT was applied in large scale tsetse eradication programs in Burkina Faso and Northern Nigeria (Jordan, 1986). Perhaps the most notable example of the success of the SIT, after tsetse population suppression with targets and pour-ons, is the case in Zanzibar where *G. austeni* has been eradicated from the Island.

2.5.3. Use of trypanotolerant livestock

It is well-known that genetically determined innate resistance to many diseases occurs in animal populations which have been subject to natural selection by exposure to disease pressure over many generations. This is also true for trypanosomosis. Taurine (humpless) breeds of cattle were the first to be introduced into Africa. They populated what is now the Sahara, but were pushed back further south when this area became a desert thousands of years ago. At present, they persist in the subhumid and humid northern parts of subSaharan Africa where they live and produce in tsetse areas (Uilenberg, 1997). Such taurine breeds are now mainly confined to West Africa, from Senegal to Nigeria, but they used to occur as far to the east as the central Sudan (Nuba Mountains) and even western Ethiopia (NLDP, 1997). N'Dama cattle (which originate from Guinea) have rather long horns, while breeds with short horns comprise for example the Baoulé (Burkina Faso and northern Ivory Coast) and the Muturu (Nigeria). They are "dwarf" cattle (although a N'Dama cow can weigh as much as 200 kg, similar to the size of many of the smaller zebu breeds).

Apart from cattle, breeds of sheep and goats living in tsetse areas are also relatively trypanotolerant. This is particularly true of the Djallonke sheep and dwarf goats in West Africa (Uilenberg, 1997).

There have been attempts to introduce West African trypanotolerant cattle to other areas but with limited success. Livestock owners who are used to larger cattle, are not readily attracted to the smaller trypanotolerant breeds. There are also limits to their trypanotolerance and when challenge is high even such animals may show clinical trypanosomosis. Their resistance is particularly effective in the face of riverine species of tsetse, which usually occur in lesser numbers and have a lower infection rate with pathogenic trypanosomes than the savannah species. Apart from this, they are small in mumber, poor in productivity and highly susceptible to other diseases compared with other breeds of cattle (Jordan, 1986).

2.6. Trypanocidal drug resistance

Drug resistance is defined as a loss of sensitivity by a strain of an organism to a compound to which it had previously been susceptible (Uilenberg, 1997).

Because of misuse of trypanocidal drugs and lack of essential information dissemination at all levels, the effectiveness of trypanocidal drugs is often limited (Connor, 1989), and this is mainly due to the development of drug resistance (Ndoutamia *et al.*, 1993). In general, drug resistance has been closely associated with easy availability and improper use of trypanocidal drugs (Connor, 1992).

Resistance to trypanocidal drugs has been reported from many African countries (Jones-Davies, 1967; Williamson, 1970; Mwambu and Mayende, 1971; Lewis and Thomson, 1974; Pinder and Authié, 1984; Schönefeld *et al.*, 1987; Moloo and Kutuza, 1990; Clausen *et al.*, 1992; Mohammed-Ahmed *et al.*, 1992).

Drug resistant *T. congolense* strains have also been reported in Ethiopia (Scott and Pegram, 1974; Codjia *et al.*, 1993; Rowlands, *et al.*, 1993; Leak *et al.*, 1993; Mulugeta *et al.*, 1997; Afewerk, 1998; Afewerk *et al.*, 2000). Assefa and Abebe (2001) have also reported multiple drug resistance of *T. congolense* against diminazene aceturate and isometamidium chloride in naturally infected donkeys in southern Ethiopia.

Resistance to one or more of the three trypanocidal drugs used in cattle (isometamidium, diminazene and homidium) has been reported in at least 13 countries in sub-Saharan Africa. These include, Burkina Faso, Central African Republic, Chad, Cote d'Ivoire, Ethiopia, Kenya, Nigeria, Somalia, Sudan, Tanzania, Uganda, Zambia and Zimbabwe. In 8 out of the 13 countries, multiple drug resistance has been reported (Geerts and Holmes, 1999).

Diminazene aceturate and isometamidium chloride have been termed as sanative pairs since one drug will eliminate parasites which have developed resistance to another (Whiteside, 1960). However, later reports on multiple drug resistance (Clausen *et al.*, 1992; Codjia *et al.*, 1993; Mulugeta *et al.*, 1997) suggest that the concept of sanative pairs might no longer always be valid (Afewerk *et al.*, 2000).

2.7. Detection of drug resistance

Currently, three types of techniques are commonly used to identify drug resistance: tests in ruminants, the mouse test and *in vitro* assays (Geerts and Holmes, 1999; Geerts *et al.*, 2000).

2.7.1. Test in ruminants

This test commonly consists of infecting a group of cattle or small ruminants with the isolate under investigation and later on when they are parasitaemic, treating them with various doses of trypanocidal drugs. The animals are then regularly monitored over a prolonged period (up to 100 days) to determine the Effective Dose (ED): the dose of the drug able to clear temporarily the parasites from the circulation and Curative Dose (CD): the dose of the drug able to provide a permanent cure (Geerts and Holmes, 1999). For these studies, the animals must be kept in fly-proof accommodation or in a none-tsetse area in order to eliminate the risk of re-infection during the study.

The advantages of studies in ruminants are all trypanosome isolates for cattle are able to grow in these hosts and the data obtained are directly applicable to the field. The disadvantages are a long duration of follow-up of 100 days is necessary to detect relapse infections and the cost of purchase and maintenance of animals is expensive. Further more, if only one isolate per animal is used, it is usually too impractical and expensive to examine a large number of isolates.

To overcome these constraints a method, for the assessment of trypanosomosis risk as well as the level and prevalence of resistance to isometamidium, has been developed which utilizes cattle populations under natural tsetse challenge in the field (Eisler *et al.*, 2000). This is assessed in the field using survival analysis of time (Geerts *et al.*, 1999; Eisler *et al.*, 2000): the time to first detection of trypanosome infection in cattle following a prophylactic isometamidium chloride treatment. The survival analysis of time provides a rapid and accurate assessment of isometamidium chloride resistance in the field and the impact of drug use relative to no treatment (Eisler *et al.*, 2000). In general, this type of approach is inexpensive and less time consuming (Eisler *et al.*, 2000). The approach has been applied to assess drug resistance at field situations in Burkina Faso (McDermott *et al.*, 1999), Kenya (Mdachi, 1999; Ndungu *et al.*, 1999), Tanzania and Zambia (Ndungu *et al.*, 1999). Trypanosome isolates can also be obtained from cattle and tested using a single discriminatory or multi-dose of isometamidium chloride and diminazene aceturate in mice (Geerts *et al.*, 2000; Eisler *et al.*, 2001). Although there was reasonable correlation between drug sensitivity data in mice and cattle (Codjia *et al.*, 1993), the exact curative dose in cattle can not be directly extrapolated from the results obtained in mice (Geerts *et al.*, 2000). Therefore, test in calves should be used to determine whether or not drugs are efficacious at recommended curative doses in cattle infected with a particular trypanosome isolate. The test in calves can also be used to investigate drug resistance in *T. vivax*. The test, which was standardized by Eisler *et al.* (2001), uses 3.5mg/kg bw for diminazene aceturate, 0.5mg/kg bw for isometamidium chloride and 1mg/kg bw for homidium bromide/chloride. Calves, which are 3-6 month-old and previously not exposed to tsetse or trypanosomosis, are used for this test.

2.7.2. Test in mice

Using a multi-dose test in mice (Eisler *et al.*, 2001), the degree of resistance of individual *T. congolense* and *T. brucei* can be determined precisely. This test may be used if detailed comparison of the level of drug resistance of different trypanosome isolates is required. In general, it can be used to maximize the information obtained where only a limited number of stabilates can be obtained from an area of interest.

After an expansion of an isolate in a donor mouse, groups of six mice (five treatments and one control) are inoculated with $1x10^5$ trypanosomes. Twenty-four hours later or at the first pick of parasitaemia, each group except the control group is treated with different drug doses. The mice should be monitored three times a week for 60 days. The test, which was also

standardized by Eisler *et al.* (2001), uses 1-60mg/kg for diminazene aceturate and 0.01-20mg/kg for both isometamidium chloride and homidium chloride/bromide.

The ED50 or 95, Effective Dose which gives temporary clearance of the parasites in 50% or 95% of the animals as well as the CD50 or 95, Curative Dose which gives complete cure in 50% or 95% of the mice can be calculated (Geerts and Holmes, 1999).

The advantage of the mouse assay is that it is cheaper than the test in cattle. However, there are several disadvantages. Most *T. vivax* and also some *T. congolense* isolates do not grow in mice (Geerts and Holmes, 1999). Apart from this, higher doses of drug must be used in mice in order to obtain comparable results to those obtained in cattle because of the vast difference in metabolism size. Normally, mice should receive doses ten times higher than those used in cattle (Geerts and Holmes, 1999). Further more, assessment of the degree of resistance needs a large number of mice per isolate. This makes it rather labor-intensive test.

However, the identification of a discriminatory dose, the dose above which an isolate should be considered resistant (Geerts *et al.*, 2000), could drastically reduce the number of mice and the amount of work to be carried out. The test was similarly standardized (Eisler *et al.*, 2001) and uses 20mg/kg for diminazene aceturate and 1mg/kg for isometamidium chloride. The difference between the single- and multi-dose tests is that in the former, comparison is possible between geographic areas and the later provides detail information on individual isolates. But both take 60 days to evaluate the drug sensitivity of an isolate (Geerts *et al.*, 2000).

In general, the test in mice can only be used as a screening test; where as, the test in ruminants should be used as a confirmation test (Geerts *et al.*, 2000).

2.7.3. *In vitro* assays

In vitro techniques have been examined for their usefulness in assessing drug susceptibilities (Elrayah and Kaminsky, 1991).

One of these assays, the Long-term *In Vitro* Viability Assay, is based on the cultivation of trypanosomes on feeder layer cells (Kaminsky *et al.*, 1990). This assay can be performed in laboratories with tissue culture facilities. However, it is necessary to adapt trypanosomes to

culture conditions before their drug susceptibility can be assessed. This adaptation is possible for most *T. brucei* field isolates. *T. congolense* and *T. vivax* isolates in contrast, are difficult to grow continuously under culture conditions (Kaminsky *et al.*, 1990).

Another test, which does not require adaptation to culture conditions and which therefore can be applied also for *T. congolense* is the ^{3}H-hypoxanthine Incorporation Assay (Brun and Kunz, 1989). The incorporation of ^{3}H-hypoxanthine by bloodstream trypomastigotes in short-term culture systems without feeder layer cells is examined in the presence of varying concentrations of drugs.

Further progress has been made in the field of *in vitro* assays to determine drug sensitivity of trypanosomes using metacyclic or blood steam forms instead of procyclic forms (Geerts and Holmes, 1999). The advantage of this technique is that large numbers of isolates can be examined and tests with metacyclic trypanosomes correlate well with field observations. However, the assays take up to 40 to 50 days for an *in vitro* incubation to generate metacyclic trypanosomes (Geerts and Holmes, 1999). Apart from this, *in vitro* cultivation of blood stream forms is only possible using laboratory pre-adapted lines and not using field isolates directly from naturally infected animals.

In general, *in vitro* assays are expensive to perform and require good laboratory facilities and well-trained staff (Geerts and Holmes, 1999). All *in vitro* tests mentioned have been carried out for a limited number of well-defined laboratory trypanosome stocks. In addition, they still have to be evaluated for all methodologies whether consistent results can be achieved always when uncloned field isolates are used. Moreover, results obtained from *in vitro* tests can not be extrapolated to apply to ruminants. Therefore, it is also necessary to carry out experimental work using ruminants before making any conclusions on the aspect of chemoresistance. For this purpose it is necessary to evaluate the potential of the available diagnostic techniques in assessing the effectiveness of therapy in cattle.

The Drug Incubation Infectivity Test (DIIT) is a combination of *in vitro* and *in vivo* techniques. In DIIT, trypanosomes are incubated for a defined time in the presence of a trypanocidal drug. Thereafter, the trypanosome suspension is inoculated into mice, which then are screened for 20-30 days for the appearance of parasitaemia. The test is very sensitive in assessing isometamedium susceptibility and is also useful for distinguishing between trypanosome susceptible and resistant to diminazene (Kaminsky *et al.*, 1990). It can even be

established in only moderately equipped cell culture laboratories.

Drug Incubation *Glossina* Infectivity Test (DIGIT) (Clausen *et al.*, 1999a) is a modification of the DIIT. Blood stream trypanosomes are incubated for a defined time in the presence of a trypanocidal drug *in vitro*. The trypanosomes are then assessed for their infectivity to tsetse (*Glossina*). Results of the work conducted by Clausen *et al.* (1999a) has clearly showed that diminazene- or isometamidium resistant *T. congolense* populations can be distinguished from drug-sensitive populations using the DIGIT. The advantage of this test is that it allows direct assessment of large numbers of field isolates of all trypanosome species without using experimental animals but the availability of tsetse is the main limiting factor of the technique (Clausen *et al.*, 1999a).

2.7.4. Other techniques

As an alternative to the above mentioned tests, the use of trypanocidal drug-ELISA in combination with parasite detection tests has given promising results for the detection of drug resistant trypanosomes (Geerts and Holmes, 1999). A competitive ELISA which allowed the detection of small amounts of isometamidium in serum of cattle was further improved by Eisler *et al.* (1993) and has been validated in cattle under experimental and field conditions (Eisler *et al.*, 1994; Eisler *et al.*, 1997). The test is both sensitive (> 0.5 ng/ml) and specific (Geerts and Holmes, 1999). It allows the monitoring of drug levels over extended periods and the evaluation of factors influencing drug disappearance rates from the plasma.

The advantage of isometamidium ELISA is that large numbers of sera can be tested within 12 to 24 hours. The disadvantage is that further studies are required to confirm the correlation of the parasitological results with the isometamidium concentration in the serum and it is not yet possible to draw firm conclusions on the sensitivity or resistance of the trypanosome population at the level of the individual animal. It might, however, give some indication of the resistance situation at the level of the herd.

Rowlands *et al.* (1993) has also showed that the application of a computer model to parasitological data, collected over a long period on a monthly basis (longitudinal parasitological study), allowed to distinguish the incidence of new infections from recurrent infections. The advantage of this kind of data is that they are directly applicable to the field. However, the disadvantages are the true prevalence of drug-resistant infections seems to be

underestimated. Besides to this, results are retrospective by at least 6 months and this technique is quite expensive if longitudinal study is not carried out for other purposes (Geerts and Holmes, 1999).

Diagnostic methods which are based on the amplification of trypanosomal DNA using the Polymerase Chain Reaction (PCR) and DNA probe hybridization techniques, can also be used to monitor the efficacy of trypanocidal drugs in the control of animal trypanosomosis (Clausen *et al.*, 1998b). The techniques were successfully used to monitor the efficacy of diminazene aceturate in cattle experimentally infected with *T. brucei* (Clausen *et al.*, 1999b) and sheep similarly infected with *T. congolense* and *T. vivax* (Bengaly *et al.*, 2001). DNA-based results and parasitological findings together with results from *in vivo* and *in vitro* drug sensitivity studies have also shown a high level of isometamidium sensitivity among the trypanosome populations present in Mukuno County in Uganda (Clausen *et al.*, 2001). Gall *et al.* (2001) has also demonstrated the superiority of the PCR over the buffy coat technique (BCT).

3. MATERIALS AND METHODS

3.1. Study areas

Before commencing the study, areas located within the FITCA project were identified and listed down based on the magnitude of tsetse and trypanosomosis problem (NTTICC, 1996). Study areas were then selected from the list by convenience sampling method such as prior information on trypanosomosis challenge/risk and the problem of drug resistance, farmers willingness to cooperate during the study, accessibility of the study areas and based on the existing back ground information of the area (NTTICC, 1998).

The study was conducted in seven areas located along three river valleys in western Ethiopia, namely, upper Didessa, Birbir and Sore-Geba (Table 1).

Table 1
Study areas and their locations

Study area	Location
Kone settlement area	Valley floor of upper Didessa valley
Loko	"
Shobe	Escarpment of the upper Didessa valley
Village I	Valley floor of Birbir river
Ego Kofele	"
Sore	Escarpment of Sore-Geba river
Darimu	"

Among the seven areas, Kone settlement and Loko are located in the valley floor and Shobe is located along the escarpment of the upper Didessa river valley (Fig. 1). On the other hand, Village I and Ego Kofele are located in the valley floor of the Birbir river. Apart from this, Sore and Darimu are located along the escarpments of Sore-Geba river valley.

In general, the river valleys belong to two main tsetse belts, namely, Abay/Didessa and Baro/Akobo. Birbir and Sore-Geba rivers belong to the later fly belt. Geba river takes Sore

from the left to form Sore-Geba and empties further down in to the Birbir river.

The altitude of the three river valleys range from 1300 at the floor to 2000 masl along the escarpment of the valleys. The annual mean minimum and maximum temperatures of the river valleys is about 12^0C and 26^0C, respectively (Berhanu, 1998). The annual rainfall is above 1100 mm (NMSA, 1996).

The upper Didessa river valley is adjacent to the Ghibe/Omo tsetse belt where multiple drug resistance of *T. congolense* was reported (Codjia *et al.*, 1993; Rowlands *et al.*, 1993; Leak *et al.*, 1993; Mulugeta *et al.*, 1997). The area is also the upper most limit where Afewerk *et al.* (2000) has reported similar findings. On the other hand, there was a report of trypanocidal drug failure at Keto Settlement area (NTTICC, 1996).

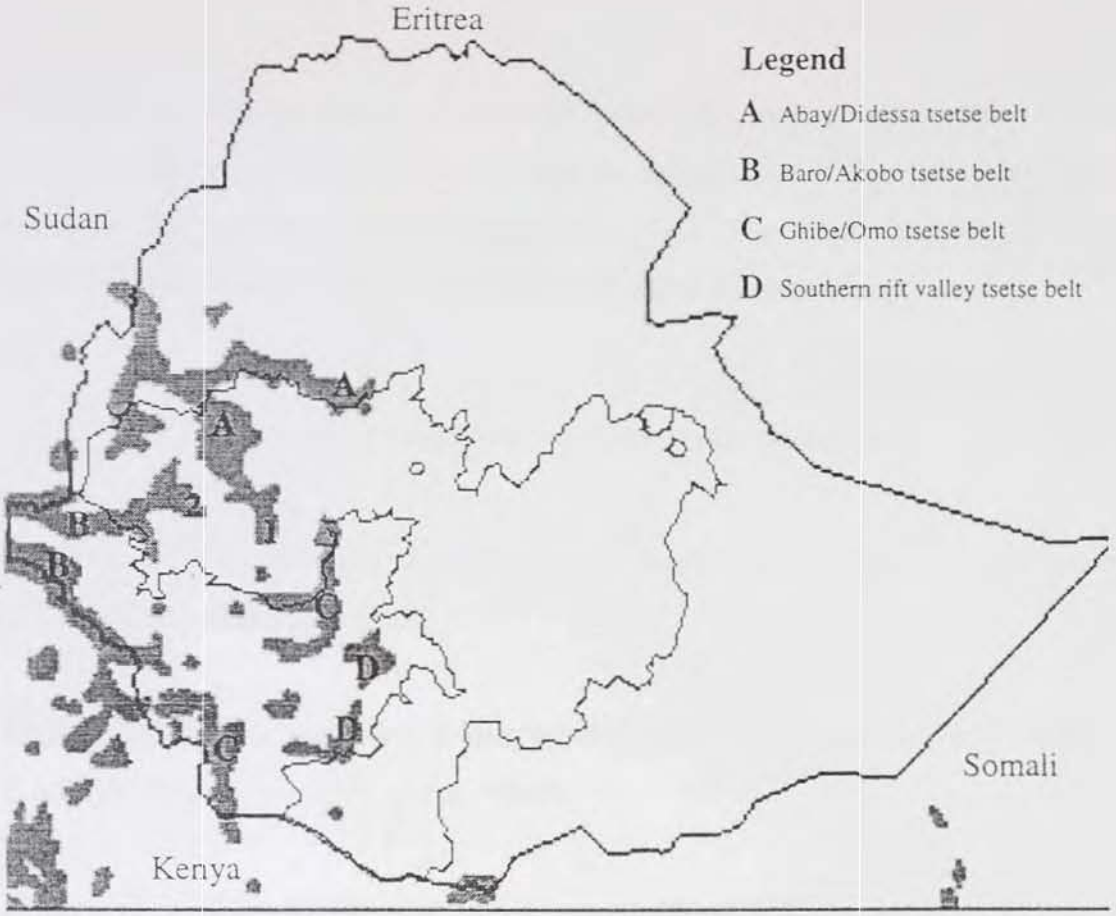

Figure 1. Map of Ethiopia showing tsetse belts (shaded in dark gray) and study areas in the upper Didessa river valley (1) and Birbir and Sore-Geba river valleys (2).

3.1.1. Vegetation

The habitat of several species of tsetse in western Ethiopia is associated with high grass low tree savannah and bamboo zones. Among the tall grasses, *Hyperrhenia* spp. *Hyperthelia* spp and *Exotheca* spp are common (Langridge, 1976). The main channels for the extension of tsetse to highlands are the river valley and associated vegetation.

3.1.2. Tsetse and trypanosomosis distribution

Two species of tsetse occurred in the upper Didessa valley. These are *G. m. submorsitans* and *G. tachinoides* (Erkelens, 2000).

As regards to trypanosomosis, *T. congolense*, is the most dominant followed by *T. vivax* and *T. brucei* (Nega *et al.*, 1997; Nega, 1999).

Birbir river valley, on the other hand, is infested with three species of tsetse flies and *G. pallidipes* is the most important species followed by *G. fuscipes fuscipes*. The valley also has a very low density of *G. m. submorsitans* (NTTICC, 1996). In many areas which are close to settlements, the forest and woodland next to Keto river, a tributary of the Birbir river, has disappeared and only scattered trees remain in an agricultural landscape (Krafsur, 2000). Further down the valley, Sore-Geba river is infested with only *G. f. fuscipes* (NTTICC, 1998).

3.1.3. Human and livestock population

Kone settlement, Village I, Ego Kofele and Sore settlement areas are inhabited by settlers coming from the drought stricken northern part of the country. The rest of the areas (Loko, Shobe and Darimu) are occupied by local inhabitants. Like in many other areas, animal draught power is widely applied during crop production in western part of the country and the availability of drought power is essential for plowing of heavy vertisol (Slingenbergh, 1992).

A total of six million cattle are estimated to be at risk of contracting animal trypanosomosis along the escarpments of the southwestern part of the country (NTTICC, 1996).

3.1.4. Crop production

Western Ethiopia is suitable for agriculture except for some waterlogged areas. In the lowlands, maize and sorghum are the main crops while the highland is predominantly coffee producing area. The predominant farming system in the highlands is characterized by mixed livestock and crop production.

3.2. Study design

3.2.1. Questionnaire survey

A total of seven areas, namely, Kone settlement, Loko, Shobe, Village I settlement, Ego Kofele settlement, Sore settlement and Darimu were selected for a questionnaire survey. In February/March 2001, about 67 farmers were interviewed using a questionnaire to collect preliminary information on herd composition, major animal health problems, livestock management, socio-economic activities, sources and usage of trypanocidal drugs and suspected drug failures (see Annex I).

3.2.2. Cross sectional study

The main aim of the study was to determine the prevalence of trypanosome infection and select areas for isometamidium block treatment study. Cross sectional study was carried out over 904 randomly selected animals found in Kone settlement, Loko, Shobe, Village I settlement, Ego Kofele settlement, Sore settlement and Darimu between end of February and mid March 2001. To determine the sample size required to conduct the cross sectional study, the following formula was used (Thrusfield, 1995).

$$n = \left(\frac{t_x \sqrt{P_x(1 - P)}}{L} \right)^2$$

Where:

n = sample size; t= Student's t-value (e.g. 1.96 at 95% confidence level); P= estimated prevalence of trypanosome infection; L= accepted absolute error or precision (e.g. 5%).

Blood samples were collected from the ear vein of each animal into heparinized capillary tubes. After capillary tubes were sealed at one end using a placticin, they were centrifuged at 12,000 rpm for 5 minutes. Once the PCV was recorded, the contents of the capillary tubes were expulsed onto microscope slides by breaking the tubes, using a diamond pen, slightly below the buffy-coat layer. The slides were then examined for trypanosome parasites using a phase contrast buffy coat technique (Murray *et al.*, 1977). In case of parasitaemic animals, species of trypanosomes was identified using Giemsa stained thin blood smears.

3.2.3. Isometamidium block treatment study

Based on results of the cross sectional study, Kone settlement area was selected for the isometamidium block treatment study using a purposive sampling method because it had a trypanosome prevalence above 20%. Although Village I had high prevalence of trypanosome infection, it was excluded from the isometamidium block treatment study for logistical reasons since it was far away from the NTTICC. Isometamidium block treatment study was conducted from early April to end of July 2001.

Kone settlement area has three separate villages, namely, Cheleleki, Kolu and Burka. Although all the three villages are surrounded by tsetse infested bush, they are located in such a way that Cheleleki is located in between the two villages, namely, Burka and Kolu. During the study, it appeared that there was a variation in the degree of exposure to tsetse flies among herds between the villages and herds within the same village. The reason for this observation was that during the time when the study was conducted, there was a scarcity of grazing area since more grazing land was changing to cultivated land. Therefore, the situation was pushing the grazing area into tsetse infested bush. Apart from this, it was not possible for farmers to

manage large herds and as a result, collective herds were often split into smaller groups and graze in less cultivated areas.

All the three villages found in Kone settlement area, namely, Cheleleki, Kolu and Burka were included in the study and a total of 300 animals (100 from each village) were selected using a simple random sampling method. Two weeks after the cross sectional study (day-14), all animals were blanket-treated with diminazene aceturate (Diminazene™, Lot No. 5677, Exp. 05-2003, Farvet Laboratories B.V., Bladel-Holland) at a dose rate of 7 mg/kg bw to eliminate existing trypanosome infections. Cattle which are found in each village were then randomized into 50 isometamidium treatment and 50 control groups (Rowlands, 2000; Eisler *et al.*, 2000). Animals were grouped after owners were allowed to draw a random number. Animals in each group were ear-tagged using yellow plastic tags, which allow easy identification of animals during each field visit.

After two weeks (day 0), one group was treated with isometamidium chloride (Veridium™, Lot No. 31 A1, Exp. 06-2005, Sanofi Sante Nutrition Animale, Liborne, France) at a dose rate of 1mg/kg and the other group was left as untreated control. Diminazene™ and Veridium™ are among the most widely used trypanocidal drugs in Ethiopia. Veridium™ was as effective as Samorin™ in Kenya (Stevenson *et al.*, 2000).

Body weight of each animal was estimated using heart girth measuring tape (Arora *et al.*, 1981). The two groups of cattle were herded together to ensure similar levels of disease challenge. Examination of animals for trypanosome infection was conducted every two weeks, starting from the day of blanket treatment (day-14) up to 12 weeks following isometamidium block treatment which was day 0, 14, 28, 42, 56, 70 and 84. Cattle were examined for trypanosome infection using the methods described in the cross sectional study. Apart from this, sex, age, date of treatment with trypanocidal drugs and dosage used was recorded.

In each group, cattle which were found to be infected with trypanosomes were treated with diminazene aceturate at a dose rate of 7 mg/kg bw. The efficacy of diminazene aceturate treatment was, therefore, ascertained on the basis of whether parasitaemia followed each diminazene aceturate treatment of any cattle on day 0, 14, 28, 42, 56, 70 and 84.

3.2.4. Trypanocidal drug resistance testing in mice

Blood from nine parasitaemic cattle at Cheleleki (tag No. 53 and 87), Kolu (tag No. 131, 154 and 194) and Burka (tag No. 237, 261, 277 and 284) of the isometamidium treated groups were subinoculated i.p. into mice. Out of the nine infected blood samples inoculated, three (33.3%) were able to grow in mice. The isolates were coded according to WHO (1986) as MBOI/ET/01/BURKA 261, MBOI/ET/01/BURKA 237 and MBOI/ET/01/KOLU 154. After the isolates were sub-passaged three times in mice, one isolate from Burka (MBOI/ET/01/BURKA 261) was used for drug sensitivity testing in mice using a single discriminatory dose of isometamidium chloride (1mg/kg bw) and diminazene aceturate (20mg/kg bw) (Eisler *et al.*, 2001).

Groups of 6 mice each for diminazene aceturate and isometamidium chloride and one control group of 6 mice were inoculated i.p. with the trypanosome isolate to be tested (Eisler *et al.*, 2001). Twenty-four hours after inoculation of the trypanosomes, trypanocidal drug treatment was administered to the treatment groups by the i.p. route. Following drug treatment, parasitological examination of a wet blood smear, obtained from the tails of the mice, was conducted twice a week using phase-contrast microscopy of magnification x 250. When parasitaemias were observed in the control mice, the number of days to parasitaemia were recorded and the mice euthanased. The treated groups were followed until a relapse occurs.

Results were interpreted with the following conditions (Eisler *et al.*, 2001). If at least 5 out of the 6 control mice are not parasitaemic, the test is repeated. Likewise, if more than one control mouse dies prior to detection of parasitaemia, the test is repeated. If one control mouse dies prior to detection of parasitaemia, the test is valid provided that parasitaemia is detected in all of the remaining 5 mice.

A trypanosome isolate was considered as drug-sensitive if at least 5 out of the 6 treated mice are cured, i.e. they remain aparasitaemic until the end of the 60-day observation period. If fewer than 5 mice are cured, the isolate may be considered to express resistance in mice to the dosage used. If one test mouse dies prior to detection of parasitaemia, the isolate is considered as drug-sensitive if at least 4 out of the remaining 5 treated mice are cured; if fewer than 4 of the remaining 5 mice are cured, the isolate may be considered to express resistance in mice to the dosage used.

The three trypanosome isolates were then stabilized in liquid nitrogen in case if the study requires further investigation.

3.3. Data analysis

Data on questionnaire survey were presented using frequency distribution and percentage.

In cross sectional study, the prevalence of trypanosome infections was calculated as the number of parasitologically positive animals as examined by dark ground phase contrast buffy coat technique (Murray *et al.*, 1977) over the total number of animals examined at a particular point in time multiplied by 100. 95% CI was used when presenting trypanosome prevalence of the seven study areas. Haematological (PCV) values of animals in the seven study areas were presented using percentage and 95% CI. Student's t-test and frequency distribution were used when comparing the pooled data of PCV values of aparasitaemic and parasitaemic animals of all the seven study areas.

Data from isometamidium block treatment studies were presented as trypanosome prevalence, trypanosome incidence rate and cumulative incidence rate. Trypanosome prevalence was calculated in the same way as in the cross sectional study. Mean trypanosome prevalence was calculated to compare study villages during 8 weeks period as the average of the two-weekly trypanosome prevalence during day 0-56. The incidence rate of trypanosome infection at the eighth week of isometamidium block treatment study was calculated as the ratio between the number of new cases of trypanosome infections and the sum of cattle-days at risk during the eight weeks period. Trypanosome incidence rate difference and incidence rate ratio between the isometamidium chloride treated and control (untreated) groups of cattle was analyzed using a 95% CI. Since all animals at each village were not presented at regular intervals, the cumulative incidence rate was calculated as the number of animals which contract trypanosome infection at day t and before divided by the number of animals at risk at the start of the study minus half the number withdrawals. When calculating the cumulative incidence, it was assumed that sanative dose of diminazene aceturate clears all the infections diagnosed and as a result, repeated infections of an animal were treated as new cases. Mean slope of PCV values and 95% CI were used to assess for any change in PCV value of the isometamidium treated and control groups of cattle over 8 weeks period.

Drug resistance of trypanosomes against isometamidium chloride, in village cattle in the field, was assessed using survival analysis of time: the time to first detection of trypanosomes 8 weeks post treatment with isometamidium chloride at 1mg/kg bw (Eisler *et al.*, 2000). According to Eisler (2000), survival analysis of time provides a rapid and accurate assessment of isometamidium chloride resistance in the field and the impact of isometamidium prophylaxis relative to no prophylaxis. Moreover, it is applicable not only to those trypanosomes that are infective to mice but also to all species and strains, which are pathogenic to cattle.

Based on Eisler (2000), survival analysis was used to address the following three practical questions which cattle keepers are facing:

Was challenge sufficient to warrant prophylaxis in the study villages?

Was there evidence of isometamidium resistance in each study village?

If resistance was suspected, had the drug nevertheless sufficient effect to make its use worthwhile?

Accordingly, three measures were proposed to resolve these que-tions (Eisler *et al.*, 2000):

The time after prophylaxis by which 25% of the herd becomes infected,

The proportion of cattle becoming infected by 8 weeks following prophylaxis and,

The ratio of the mean hazard rate for the sentinel and prophylaxis herds over weeks 1-8.

Finally, survival data was interpreted as follows (Eisler *et al.*, 2000):

If fewer than 25% of sentinel cattle became infected within 8 weeks of exposure, then challenge was insufficient to warrant isometamidium prophylaxis, which would be undesirable on grounds of cost, possible side effects and unnecessary drug pressure tending to develop drug resistance.

If more than 25% isometamidium treated cattle became infected within 8 weeks of exposure, this was strongly suspicious of the occurrence of drug resistant trypanosomes, provided there was good evidence that the drug was administered correctly, which was proved using close supervision when the drug was administered.

Where there was evidence of drug resistance on the grounds of the number of isometamidium treated cattle becoming infected within 8 weeks of exposure, it may nevertheless be worth continuing prophylaxis in situations where the ratio of the mean hazard rate for the sentinel and prophylaxis herds over weeks 1-8 was greater than 2.

Accordingly, the 25% survival time: the time by which 25% of the animals were parasitaemic as a result of trypanosome infection, after the start of isometamidium block treatment study, was determined using a software (Stata Corporation 2000) as follows (Klein and Moeschberger, 1997).

$$p\text{th percentile} = \frac{p\sqrt{g}}{\sqrt{S(tp)}f(tp)}$$

where g is the Greenwood pointwise standard error estimate for $S(tp)$, and $f(tp)$ is the estimated density function at the pth percentile. The upper confidence limit for pth percentile was defined as the first time at which the upper confidence limit for $S(t)$ (based on a Ln{-Ln $S(t)$} transformation) is less than or equal to p, and similarly, the lower confidence limit was defined as the first time at which the lower confidence limit of $S(t)$ is less than or equal to p.

95% CI was used to analyze the difference between the 25% survival times of the control and isometamidium treated cattle.

The proportion of cattle becoming infected by 8 weeks after isometamidium treatment was calculated as the number of cattle infected (failure) during 8 weeks after the start of isometamidium block treatment study divided by the total number of cattle presented at day 14 when the first case(s) diagnosed.

Mean hazard rate and hazard ratio of the control and isometamidium treated groups of cattle over 8 weeks period were calculated as follows (Frankena and Graat, 1997).

$$\text{Mean hazard rate (hi)} = \frac{\text{no. became infected (failures)}}{\sum ti \text{(total time at risk)}}$$

$$\text{Hazard ratio} = \frac{\text{hi (control)}}{\text{hi (treatment)}}$$

Since all survival times were not exactly known, Kaplan-Meier survival curves were plotted, for the control and isometamidium treated groups of animals found in the three villages, to estimate probability of surviving up to 8 weeks post isometamidium block treatment study (Frankena and Graat, 1997). Log-rank and Wilcoxon (Breslow) tests were used for statistical evaluation of the equality of the survivor functions of the control and isometamidium treated groups of cattle (Stata Corporation, 2000).

On the other hand, the efficacy of diminazene aceturate treatment was assessed on the basis of whether parasitaemia followed two weeks after treatment of cattle with the drug at a dose rate of 7mg/kg bw. To analyze data on diminazene aceturate, trypanosome incidence rate and trypanosome infection recurrence rate (Rowlands, 2000) at each village were compared using Fisher's exact test (Stata Corporation, 2000). The test was used because some cells in chi-square table had expected values of less than 5. Trypanosome infection recurrence rate was defined as the proportion of animals (cattle) which were found infected with the same species of trypanosome among the total number of animals which were treated with diminazene aceturate at a dose rate of 7mg/kg bw before two weeks.

Mean relapse intervals (days±SD) were used to analyze drug sensitivity test results in mice using the single discriminatory dose of diminazene aceturate (20mg/kg bw) and isometamidium chloride (1mg/kg bw).

4.1. Questionnaire survey

A total of 67 farmers who live in seven areas located along three river valleys, namely, Didessa, Sore-Geba and Birbir were interviewed. They were mainly questioned on herd composition, major animal health problems, livestock management, socio-economic activities, sources and usage of trypanocidal drugs and their efficacies. The questionnaire was administered in February/March 2001. The response rate was 98% and there was no apparent difference between the responses of the seven areas.

About 73% of the informants who participated in the questionnaire survey came from the tsetse and trypanosomosis affected areas of the upper Didessa valley.

4.1.1. Herd composition and major health problems

All the individuals interviewed had livestock and cattle accounted for 63% of the animals. The farmers reported major livestock diseases such as trypanosomosis (Gendi), anthrax, blackleg, pasteurellosis, and internal and external parasites (Fig. 2). Among the few animal diseases named by farmers, trypanosomosis (Gendi) was the most important animal health problem. The interviewees characterized the disease, among many other signs, by emaciation, rough hair coat, and geophagia (Fig. 3). In 40% of the cases, trypanosomosis was reported to occur in the dry, 25% at the start of the rain season and 32% during both seasons. At least 76% of the farmers reported trypanosomosis to be transmitted by biting flies. Apart from this, none of the farmers were able to differentiate tsetse from other biting flies. Although trypanosomosis occurred in the area a long time ago (NTTICC, 1989; Slingenbergh, 1992), farmers believed that the magnitude of the disease was getting worse in more recent years.

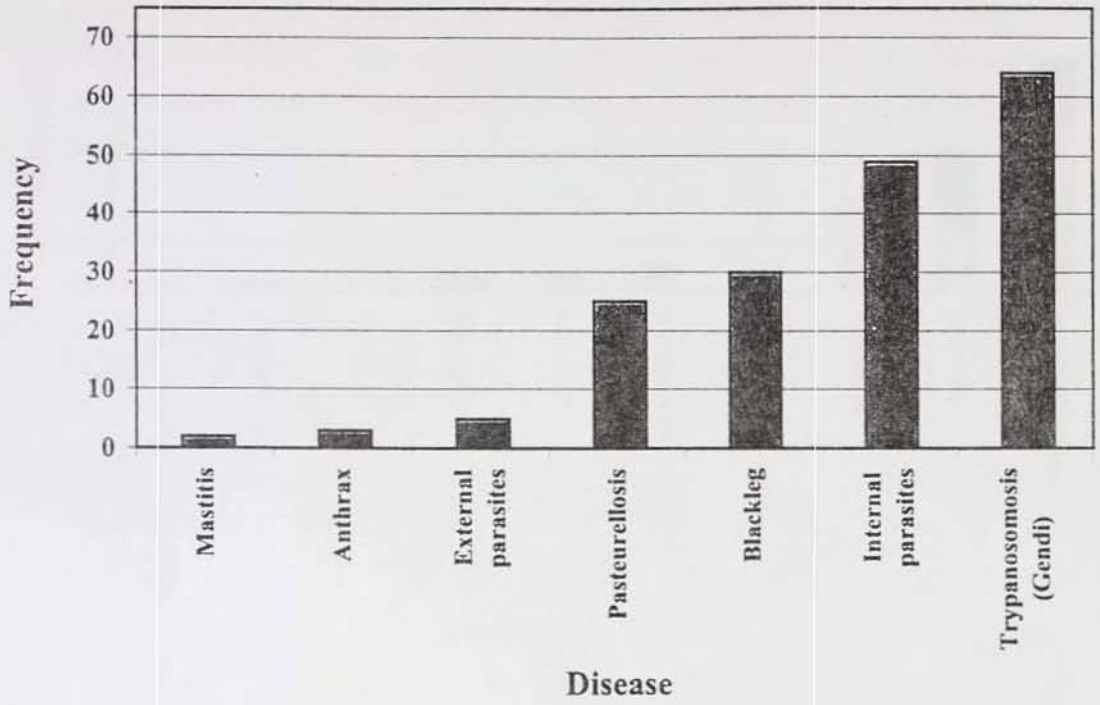

Figure 2. Major diseases affecting livestock as reported by interviewees in seven areas along three river valleys (Didessa, Sore-Geba and Birbir), in western Ethiopia, in February/March 2001.

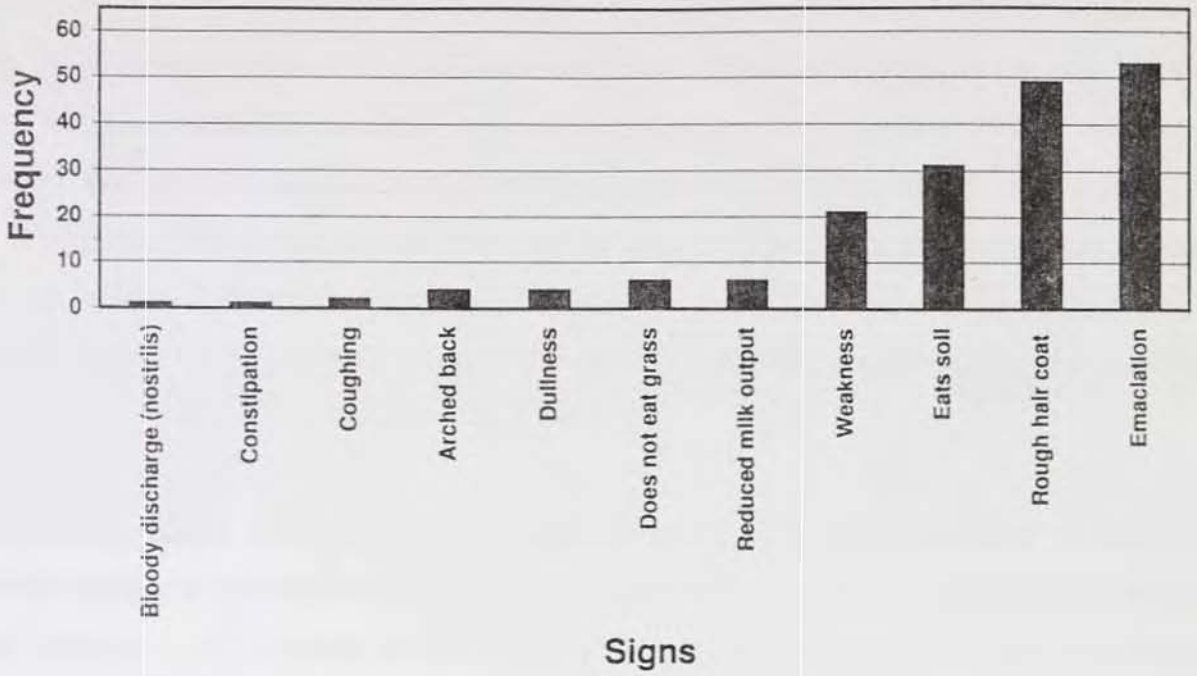

Figure 3. Major signs of trypanosomosis in cattle as reported by interviewees in seven areas along three river valleys (Didessa, Sore-Geba and Birbir), in western Ethiopia, in February/March 2001.

4.1.2. Livestock management

All the farmers reported free grazing of livestock as the only source of feed. Over 76% of the animals graze in the surrounding bush areas. In 42% of the cases, the watering points were not far away from the villages.

4.1.3. Socio-economic activities

Seventy two percent of the interviewees reported mixed crop and livestock production as the main source of income and 60% of them kept animals as a source of milk and drought power.

4.1.4. Usage of trypanocidal drugs

Fifty seven percent of the farmers interviewed used trypanocidal drugs for the last 20 years. However, they indicated increased usage over the last 10-15 years. Over 70% of the farmers reported that each animal was treated for trypanosomosis at interval of 2.5 - 3.5 months (Fig. 4). Otherwise, 79% of the animals were treated one to two months before the questionnaire was administered. However, out of the total animals treated for trypanosomosis, the farmers reported that 34% of them did not recover from the disease. On the average, each farmer spends 7 Birr (0.8 USD) to treat an animal once.

Ninety five percent of the respondents used isometamidium and diminazene to treat their animals against trypanosomosis. Eighty five percent of the farmers replied that trypanocidal drug treatments were applied to sick animals. According to the questionnaire, trypanocidal drugs were applied in 8% of the cases by farmers themselves, 43% by animal health personnel and 49% by other uncertified individuals. From the responses of the farmers, 45% and 40% of the diminazene aceturate and isometamidium chloride treatments were used below the recommended doses, respectively (Fig. 5). On the other hand, about 40% and 48% of the farmers reported that they did not have any idea on the dosages of diminazene aceturate and isometamidium chloride, respectively. None of the farmers interviewed volunteered to show any trypanocidal drugs.

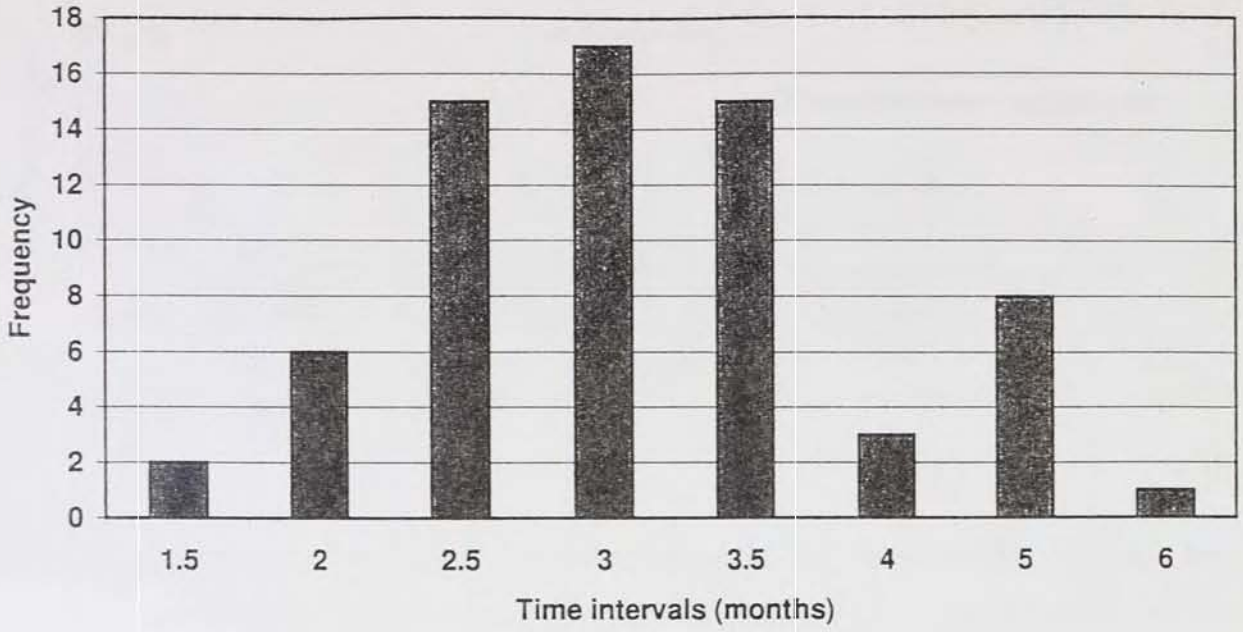

Figure 4. Time intervals between trypanocidal drug treatments reported by the interviewees in seven areas along three river valleys (Didessa, Sore-Geba and Birbir), in western Ethiopia, in February/March 2001.

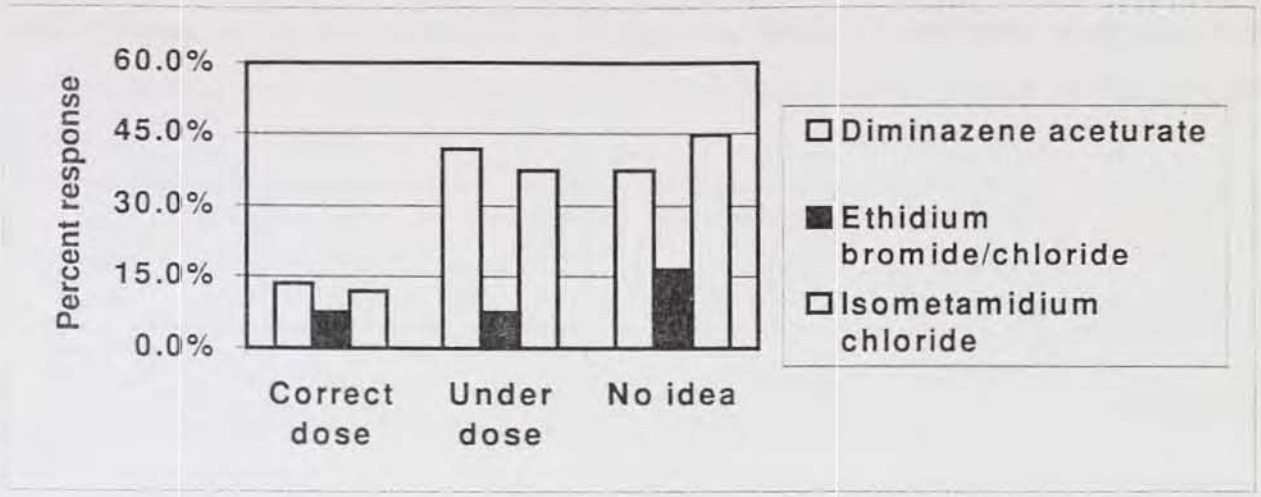

Figure 5. Dosage of trypanocidal drugs reported by interviewees during the questionnaire survey in seven areas along three river valleys (Didessa, Sore-Geba and Birbir), in western Ethiopia, in February/March 2001.

NB: Correct dose - one sachet of diminazene aceturate or one tablet of Ethidium bromide/chloride for one adult cow or one sachet of isometamidium chloride for 10 adult cows. Under dose - one sachet of diminazene aceturate or one tablet of Ethidium bromide/chloride for more than two adult cows or one sachet of isometamidium chloride for 15-20 adult cows. No idea - when farmers have no idea for the specific question.

4.2. Cross sectional study

4.2.1. Parasitological findings

In order to select areas for the isometamidium block treatment study and assess drug resistance in the field, a total of 904 cattle in seven areas along three river valleys, namely, Didessa, Sore-Geba and Birbir were screened for the presence of trypanosome infections. Parasitological results are presented in Table 2.

Among the seven areas, a trypanosome prevalence of 21.3% was recorded in Kone settlement area. However, it was not significantly different ($P>0.05$, CI = -0.0237, 0.150) from the 15% infection rate recorded at Village I in Keto settlement area. Nevertheless, a total of 52 trypanosome infections were detected in Kone settlement area. Seventy five percent of the overall infections were due to *T. congolense*, 21.2% due to *T. vivax* and infections due to *T. brucei* alone and mixed infection of *T. congolense* and *T. vivax* accounted for 1.9% each.

Table 2

Parasitological results of the cross sectional study conducted in seven areas along three river valleys (Didessa, Sore-Geba and Birbir), in western Ethiopia, between end of February and mid March 2001.

Study area	No of animals	No. of animals infected		No. of *Trypanosoma* spp. diagnosed				95% CI*
		Total	%	Tb	Tc	Tv	Mix	
Kone settlement	244	52	21.3	1	39	11	1**	0.162-0.264
Loko	100	3	3.0	1	1	1	0	0.006-0.085
Shobe	160	0	0	0	0	0	0	
Village I settlement	100	15	15.0	1	14	0	0	0.087-0.235
Ego Kofele settlement	100	0	0	0	0	0	0	
Sore settlement	100	0	0	0	0	0	0	
Darimu	100	0	0	0	0	0	0	
	904	70	7.74	3	54	12	1	0.061-0.097

NB: Tb=*Trypanosoma brucei;* Tc= *T. congolense;* Tv= *T. vivax;* Mix= Mixed infection of trypanosome species;
*No. of animals infected (%); **Tv+Tc

4.2.2. Haematological findings

Results of the haematological findings are presented in Fig. 6 and 7. Accordingly, cattle herds found in Kone settlement area had the lowest mean Packed Cell Volume (PCV) values compared with the other areas (Fig.6). There was statistically significant difference ($P<0.05$; CI = 0.286, 2.29) between the mean PCV values of cattle found at Loko and Kone settlement area.

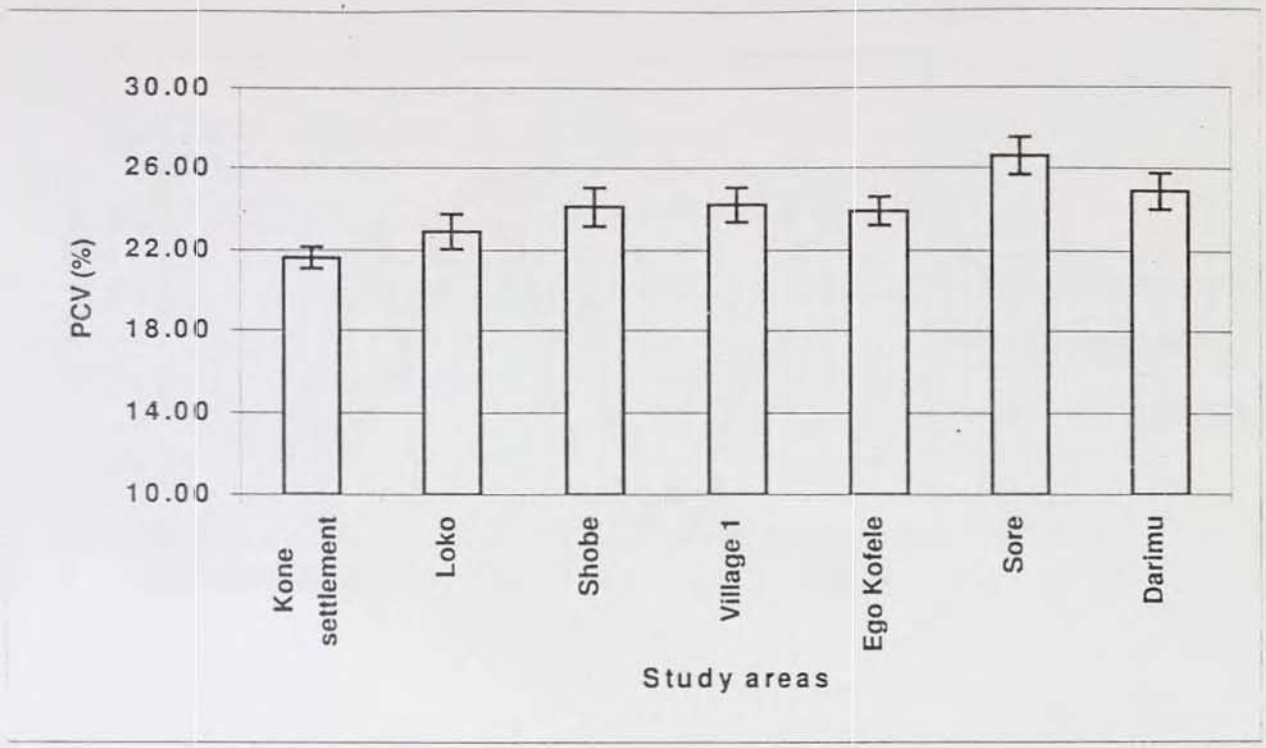

Figure 6. Mean and 95% CI of PCV (%) values of cattle during the cross sectional study conducted in seven areas along three river valleys (Didessa, Sore-Geba and Birbir), in western Ethiopia, between end of February and mid March 2001.

A significant difference ($P<0.01$) was observed between the mean PCV values in the parasitaemic (CI = 22.0, 23.0) and aparasitaemic (CI = 24.2, 25.4) animals. On the other hand, over 36% of the parasitaemic and 45% of the aparasitaemic animals had PCV values greater than or equal to 26% (Fig.7).

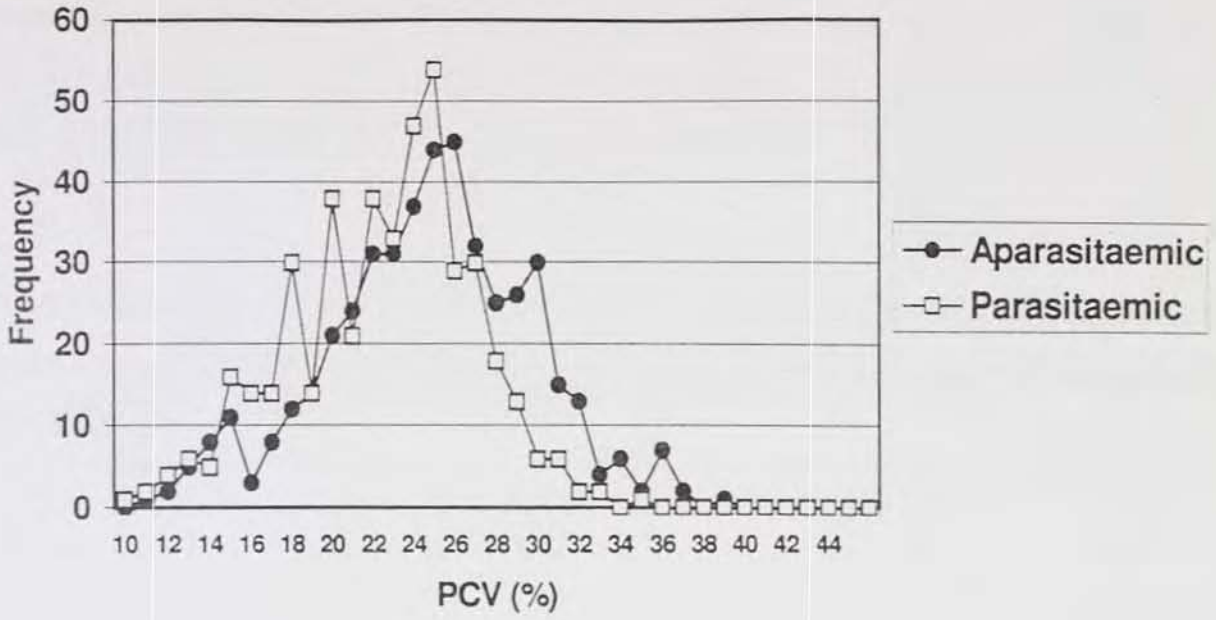

Figure 7. Frequency distribution of the PCV (%) values of aparasitaemic and parasitaemic cattle during the cross sectional study conducted in seven areas along three river valleys (Didessa, Sore-Geba and Birbir), in western Ethiopia, between end of February and mid March 2001.

4.3. Isometamidium block treatment study

4.3.1. Parasitological findings

Out of the seven study areas used for the cross sectional study, Kone settlement was selected for isometamidium block treatment study. Kone settlement area contains three villages, namely, Cheleleki, Kolu and Burka. The other six areas were excluded from the study for the reason of low trypanosome prevalence. Although Village I had high prevalence of trypanosome infection, it was excluded from the isometamidium block treatment study for logistical reasons since it is far away from the NTTICC.

4.3.1.1. Trypanosome prevalence

Fourteen days prior to the commencement of the isometamidium block treatment study, a total of 27, 21 and 29 trypanosome infections were diagnosed at Cheleleki, Kolu and Burka

villages, respectively (Table 3). Out of these, *T. congolense* was the most prevalent trypanosome parasite at Cheleleki (55.6%) and Kolu (52.4%). The prevalence of *T. vivax* was 40.7% at Cheleleki and 33.3% at Kolu. At Burka, each of *T. congolense* and *T. vivax* accounted for 44.8% of the total trypanosome infections.

Table 3

Parasitological results of cattle 14 days prior to the commencement of isometamidium block treatment study, at three villages in Kone settlement area located in the upper Didessa valley, western Ethiopia.

Study area	Village	No of animals	No. of animals infected		Mean PCV (%)	No. of *Trypanosoma* spp. diagnosed				95% CI****
			Total	%		Tb	Tc	Tv	Mix	
Kone settlement	Cheleleki	100	27	27.0	21.4	0	15	11	1*	0.186-0.368
"	Kolu	100	21	21.0	20.4	2	11	7	1**	0.135-0.303
"	Burka	100	29	29.0	21.8	0	13	13	3***	0.204-0.389
	Total	300	77	25.7	21.2	2	39	31	5	0.207-0.306

Tb=*T. brucei*; Tc=*Trypanosoma congolense*; Tv=*T. vivax*; *=Tv+Tc; **=Tv+Tc; ***= Tv+Tb (2) and Tb+Tc (1)

****No. of animals infected (%); Mix= Mixed infection of trypanosome species

Starting from day 0 up to day 84, animals were examined for trypanosome infection every two weeks and parasitological results are attached in Annexes II and III.

Fourteen days prior to isometamidium block treatment study, all animals were treated with diminazene aceturate at dose rate of 7mg/kg bw. At day 0 of the study, there was no trypanosome infection diagnosed at any of the three villages (Fig. 8, 9 and 10). Fourteen days later, a total of five trypanosome infections were detected at Cheleleki, seven at Kolu and 13 at Burka villages. Out of the total trypanosomes detected at each village, Cheleleki and Kolu had one trypanosome infection in each of the isometamidium treated groups of cattle. There were three trypanosome cases in the isometamidium treated group of cattle found at Burka. As far as the species of trypanosome is concerned, *T. vivax* and *T. congolense* accounted for the two trypanosome infections detected in the isometamidium treated groups of cattle found at Cheleleki and Kolu villages, respectively. At Burka, three of the trypanosome infections were due to *T. congolense*. The mean prevalence of trypanosome infection during the 8-week period at Cheleleki, Kolu and Burka villages was 12.8%, 9.57% and 11.01% in the control groups of cattle, respectively. In the isometamidium treated groups, it was 4.51%, 5.72% and 10.08% for Cheleleki, Kolu and Burka, respectively.

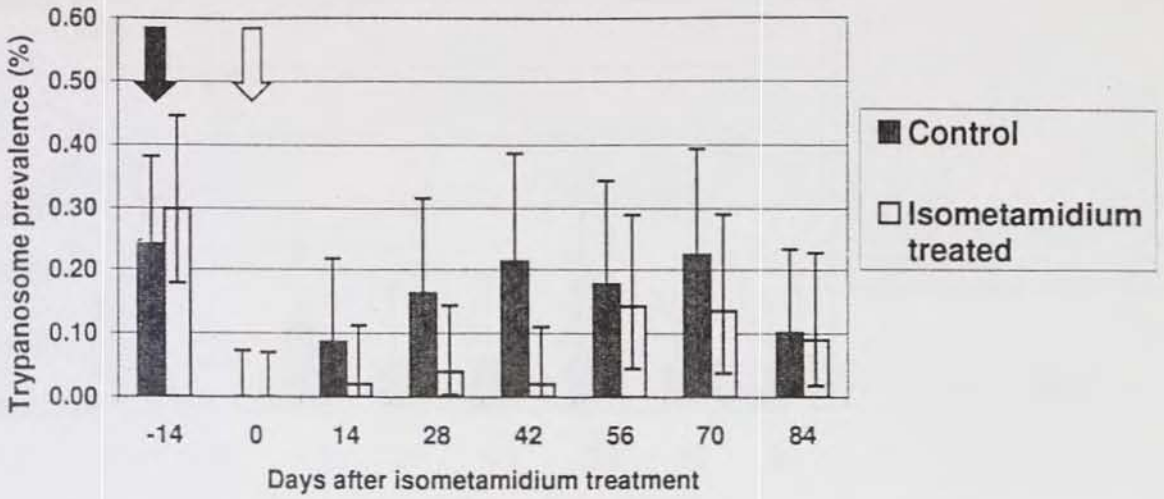

Figure 8. Parasitological results of the control and isometamidium (1 mg/kg bw) treated group of cattle at Cheleleki, in Kone settlement area, western Ethiopia (March/April 2001). Fourteen days prior to isometamidium chloride block treatment (white arrow), all animals were treated with diminazene aceturate (dark arrow) at 7 mg/kg bw. Cattle parasitaemic after day 0 were treated with diminazene aceturate (7mg/kg).

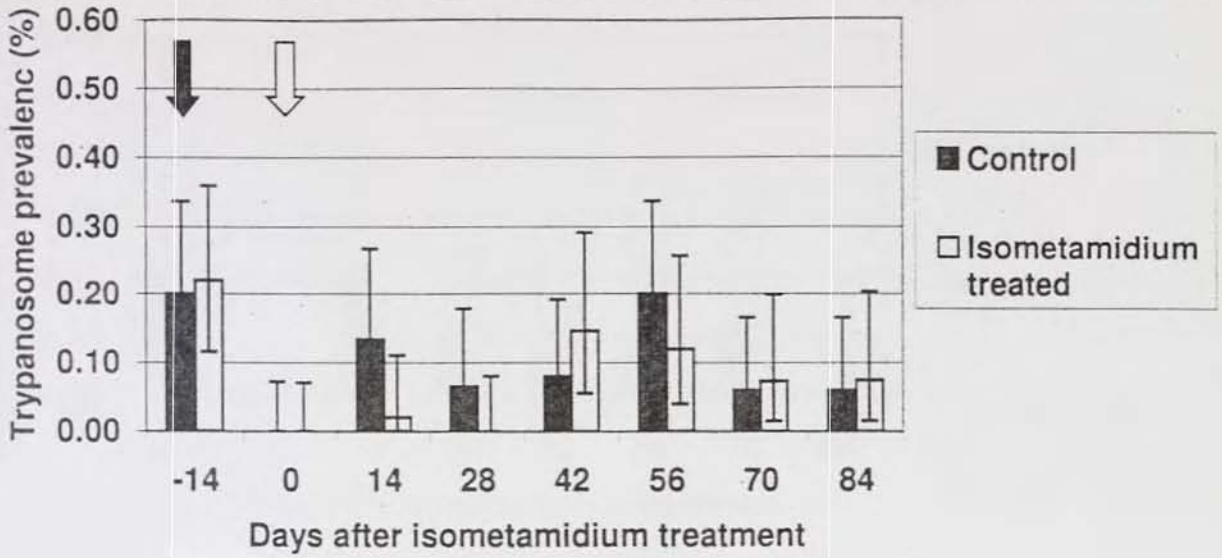

Figure 9. Parasitological results of the control and isometamidium (1 mg/kg bw) treated group of cattle at Kolu, in Kone settlement area, western Ethiopia (April 2001). Fourteen days prior to isometamidium chloride block treatment (white arrow), all animals were treated with diminazene aceturate (dark arrow) at 7 mg/kg bw. Cattle parasitaemic after day 0 were treated with diminazene aceturate (7mg/kg).

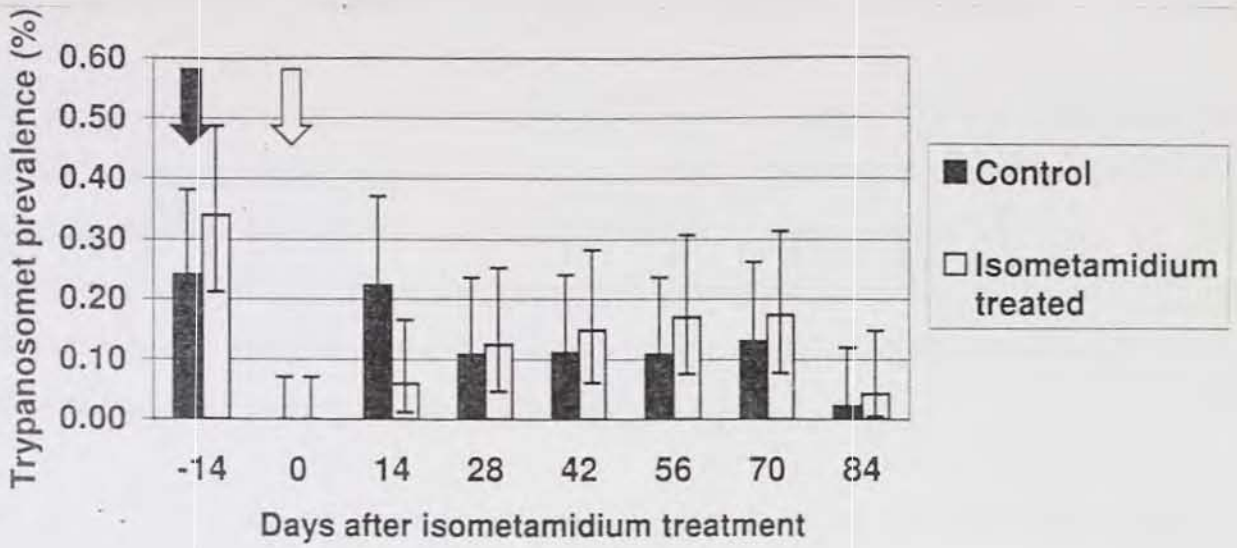

Figure 10. Parasitological results of the control and isometamidium (1 mg/kg bw) treated group of cattle at Burka, in Kone settlement area, western Ethiopia (April 2001). Fourteen days prior to isometamidium chloride block treatment (white arrow), all animals were treated with diminazene aceturate (dark arrow) at 7 mg/kg bw. Cattle parasitaemic after day 0 were treated with diminazene aceturate (7mg/kg).

4.3.1.2. Trypanosome incidence rate

The incidence rate of trypanosome infection at the eighth week of isometamidium block treatment study was calculated as the ratio between the number of new cases of trypanosome infections in each group of cattle and the sum of cattle-days at risk during the eight weeks period. Only new cases were considered when calculating the incidence rate. The incidence rate in the control groups of cattle, during the eight weeks period, at Cheleleki, Kolu and Burka was 1.1%, 0.9% and 1.0%, respectively. In the isometamidium treated groups of cattle it was 0.4%, 0.4% and 0.8% for Cheleleki, Kolu and Burka during this period, respectively. There was a significant difference ($P<0.05$; CI = -0.012, -0.001) between the incidence rates of the isometamidium treated and control group of cattle at Cheleleki. Similarly, the incidence rate ratio of the isometamidium treated to the control group of cattle at Cheleleki was 0.398 ($P<0.05$; CI = 0.174, 0.856). However, neither the incidence rate difference nor the incidence rate ratio of the control and isometamidium treated group of cattle was significant for Kolu and Burka villages.

4.3.1.3. Trypanosome cumulative incidence rate

All animals at each village were not presented at regular intervals. Therefore, the cumulative incidence rate was calculated as the number of animals which contract trypanosome infection at day t and before divided by the number of animals at risk at the start of the study minus half the number withdrawals. When calculating the cumulative incidence, it was assumed that sanative dose of diminazene aceturate clears all the infections diagnosed and as a result, repeated infections of an animal were treated as new cases.

The cumulative incidence rate of trypanosome infection varied over time with village and group of cattle with in a village (Fig. 11, 12 and 13).

Eight weeks after the start of isometamidium block treatment study, the cumulative incidence rate was 42.7%, 36.6% and 50.8% in both groups of cattle at Cheleleki, Kolu and Burka villages, respectively. In the control groups of cattle, the cumulative incidence rate at Cheleleki, Kolu and Burka villages after eight weeks period was 63.8%, 46.5% and 52.1%, respectively. On the other hand the cumulative incidence of the isometamidium treated cattle at Cheleleki, Kolu and Burka during eight weeks time was 22.4%, 26.1% and 49.5%, respectively. The differences in cumulative incidence rates between the control and isometamidium treated groups of cattle, 8 weeks after the start of isometamidium block treatment study, were 0.414, 0.199 and 0.031 for Cheleleki, Kolu and Burka villages, respectively.

At Cheleleki, 43.3% and 40% of the total infections in the control group were due to *T. congolense* and *T. vivax*, respectively. However, each of *T. brucei*, *T. congolense* and *T. vivax* accounted for 27.3% of the infections in the isometamidium treated group of cattle at Cheleleki. On the other hand, 65.2% and 60% of the total infections in the control groups of cattle at Kolu and Burka, respectively, were accounted for *T. congolense* infections. In the treatment groups of cattle at these two villages, *T. congolense* again accounted for 83.3% and 66.6% of the infections, respectively.

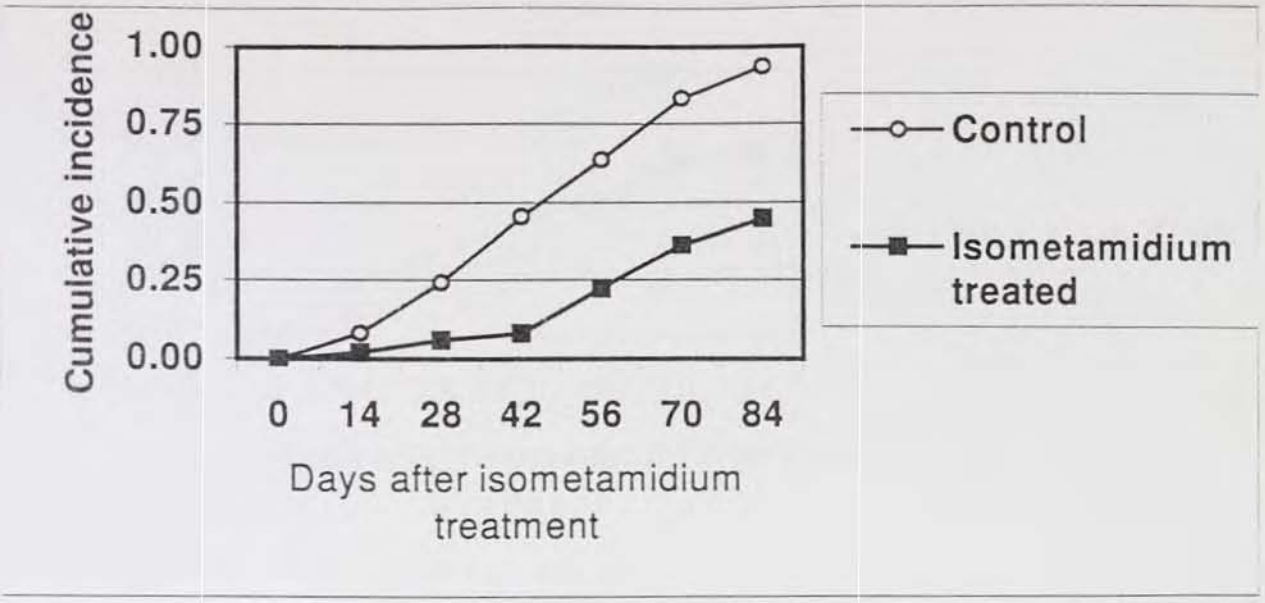

Figure 11. Parasitological results of the control and isometamidium (1 mg/kg bw) treated group of cattle at Cheleleki, in Kone settlement area, western Ethiopia (March/April 2001). Cattle, which became parasitaemic after day 0, were treated with diminazene aceturate (7mg/kg bw).

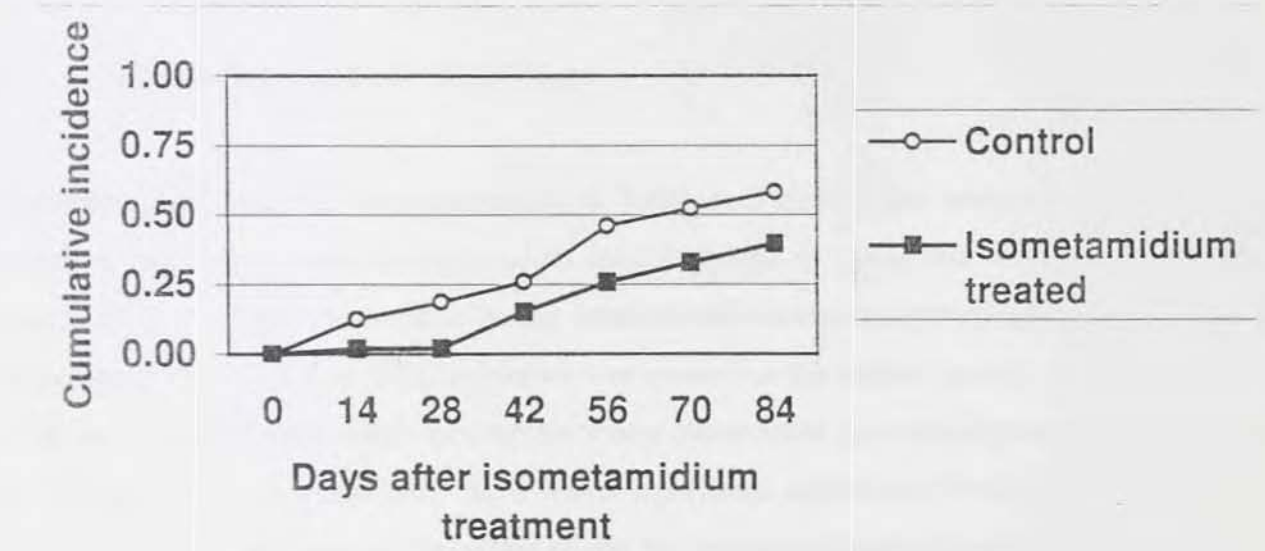

Figure 12. Parasitological results of the control and isometamidium (1 mg/kg bw) treated group of cattle at Kolu, in Kone settlement area, western Ethiopia (April 2001). Cattle, which became parasitaemic after day 0, were treated with diminazene aceturate (7mg/kg bw).

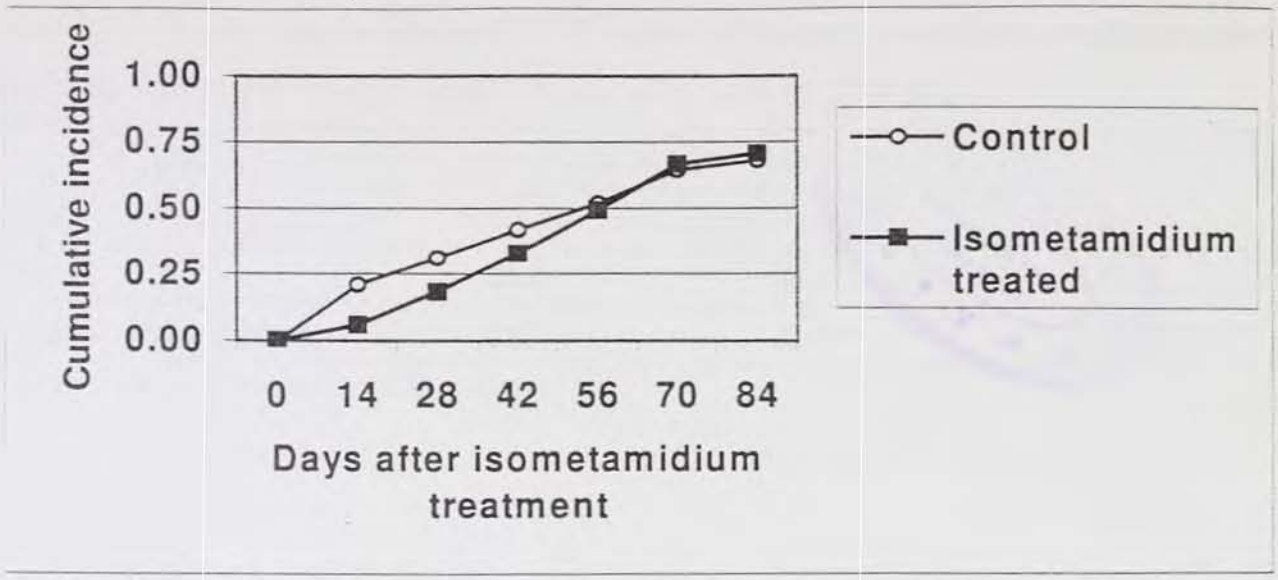

Figure 13. Parasitological results of the control and isometamidium (1 mg/kg bw) treated group of cattle at Burka, in Kone settlement area, western Ethiopia (April 2001). Cattle, which became parasitaemic after day 0, were treated with diminazene aceturate (7mg/kg bw).

4.3.2. Haematological findings

Haematological findings are summarized in Table 4, 5 and 6. The mean slope of the PCV values of the control and isometamidium treated groups of cattle was used to compare the change in PCV values over time. In the isometamidium treated groups of cattle, the mean slope was a positive value. Similar picture was noticed in the control groups of cattle found at Kolu and Burka villages. However, contrary was observed in the control group of cattle found at Cheleleki. On the other hand, there was a significant difference ($P<0.05$; CI = -0.0778, -0.0039) between the mean slope values of the two groups of cattle found at Cheleleki. There was no significant difference between these values in the two groups of cattle found at Kolu and Burka.

Table 4

Mean PCV (%) and mean slopes of PCV (%) values of the control and isometamidium treated group of cattle at Cheleleki, in upper Didessa valley, western Ethiopia.

	Mean PCV		
Group	Day 0	Day 56	Mean slope[1]
Control	25.1	25.2	-0.02551
Isometamidium treated	25.7	26.3	0.01531

[1]95% CI for difference between slopes (-0.0778, -0.0039)

Table 5

Mean PCV (%) and mean slopes of PCV (%) values of the PCV (%) values of the control and isometamidium treated group of cattle at Kolu, in upper Didessa valley, western Ethiopia.

	Mean PCV		
Group	Day 0	Day 56	Mean slope[1]
Control	22.9	24.5	0.03029
Isometamidium treated	23.2	25.9	0.03322

[1]95% CI for difference between slopes (-0.0340, 0.0279)

Table 6

Mean PCV (%) and mean slopes of PCV (%) values of the PCV (%) values of the control and isometamidium treated group of cattle at Burka, in upper Didessa valley, western Ethiopia.

	Mean PCV		
Group	Day 0	Day 56	Mean slope[1]
Control	23.9	25.9	0.04665
Isometamidium treated	23.2	27.0	0.06000

[1]95% CI for difference between slopes (-0.0382, 0.0116)

4.3.3. Survival analysis

Twenty five percent survival time was determined as the time by which 25% of the animals become parasitaemic as a result of trypanosome infection (Table 7, 8 and 9). At Cheleleki and Kolu, the 25% survival time of the control groups of cattle was 42 days. It was only 28 days for this group of cattle at Burka. Similarly, the 25% survival time of the isometamidium treated groups of cattle at Cheleleki and Kolu was 70 days. Where as at Burka, the time was 56 days. There was no overlapping in the 95% CI of the 25% survival time between the control (CI = 28, 42) and isometamidium treated (CI = 56, 84) group of cattle at Cheleleki. However, there was overlapping in the 95% CI of the 25% survival times between these groups of cattle at Kolu and Burka villages.

On the other hand, 22.9% (11/48), 20.0% (10/50) and 36.0% (18/50) of the isometamidium treated groups of cattle at Cheleleki, Kolu and Burka, respectively, were found infected with trypanosomes by day 56 (8^{th} week) of the isometamidium block treatment study.

The mean hazard rate was calculated as the ratio between the number of cattle infected with trypanosomes (failures) and the total time at risk from day 0 up to day 56 of the isometamidium block treatment study (Table 7, 8 and 9). The mean hazard rates of the control and isometamidium treated group of cattle for Cheleleki were 0.011 (22/2016) and 0.004 (11/2534), respectively. For Kolu, the rates were 0.009 (18/1974) and 0.004 (10/2324), respectively. The mean hazard rates for Burka were 0.010 (19/1820) and 0.008 (18/2352), respectively.

The hazard ratio was simply the ratio of the mean hazard rate of the control to the isometamidium treated groups of cattle. Accordingly, the hazard ratio for Cheleleki and Kolu villages for the 8-week study period were 2.75 (0.011/0.004) and 2.25 (0.009/0.004), respectively. As for the Burka village, the ratio was 1.25 (0.010/0.008).

Table 7

Mean hazard rate and 25% survival times of control and isometamidium treated group of cattle up to day 56 since the start of isometamidium block treatment study at Cheleleki, in upper Didessa valley, western Ethiopia.

Group	No. of subjects[1]	No. of failures	Time at risk	Mean hazard rate[2]	25% Survival time[3]	95% CI	
Control	46	22	2016	0.011	42	28	42
Treatment	48	11	2534	0.004	70	56	84
Total	**94**	**33**	**4550**	**0.007**	**56**	**42**	**56**

[1]Total number of cattle presented at day 14 when the first case(s) diagnosed

[2]The ratio between the number of cattle infected with trypanosome parasites (failures) and the total time at risk from day 0 up to day 56 of the isometamidium block treatment study

[3]The time by which 25% of the animals become parasitaemic as a result of trypanosome infection

Table 8

Mean hazard rate and 25% survival times of control and isometamidium treated group of cattle up to day 56 since the start of isometamidium block treatment study at Kolu, in upper Didessa valley, western Ethiopia.

Group	No. of subjects[1]	No. of failures	Time at risk	Mean hazard rate[2]	25% Survival time[3]	95% CI	
Control	45	18	1974	0.009	42	14	56
Treatment	50	10	2324	0.004	70	42	-
Total	**95**	**28**	**4298**	**0.007**	**56**	**42**	**70**

[1]Total number of cattle presented at day 14 when the first case(s) diagnosed

[2]The ratio between the number of cattle infected with trypanosome parasites (failures) and the total time at risk from day 0 up to day 56 of the isometamidium block treatment study

[3]The time by which 25% of the animals become parasitaemic as a result of trypanosome infection

Table 9

Mean hazard rate and 25% survival times of control and isometamidium treated group of cattle up to day 56 since the start of isometamidium block treatment study at Burka, in upper Didessa valley, western Ethiopia.

Group	No. of subjects[1]	No. of failure:	Time at risk	Mean hazard rate[2]	25% Survival time[3]	95% CI	
Control	46	19	1820	0.010	28	14	42
Treatment	50	18	2352	0.008	56	28	56
Total	**96**	**37**	**4172**	**0.009**	**42**	**28**	**56**

[1]Total number of cattle presented at day 14 when the first case(s) diagnosed

[2]The ratio between the number of cattle infected with trypanosome parasites (failures) and the total time at risk from day 0 up to day 56 of the isometamidium block treatment study

[3]The time by which 25% of the animals become parasitaemic as a result of trypanosome infection

Kaplan-Meier survival curves (Stata Corporation, 2000) were plotted for the control and isometamidium treated groups of cattle found at Cheleleki, Kolu and Burka villages. The equality of the survivor functions during eight weeks after the start of isometamidium block treatment study was tested using Log-rank and Wilcoxon (Breslow) tests (Stata Corporation, 2000).

Accordingly results showed that the probability to survive, eight weeks after the start of isometamidium block treatment study, varied between villages and between cattle groups within villages. Significant differences were obtained between the Kaplan-Meier survival estimates of the control and isometamidium treated cattle at Cheleleki ($P<0.01$) and Kolu ($P<0.05$) (Fig. 14 and 15). However, there was no significant difference ($P>0.05$) between the survival estimates of the control and isometamidium treated cattle at Burka (Fig. 16).

Figure 14. Kaplan-Meier survival estimates and statistical test for the equality of the survivor functions of the control and isometamidium treated group of cattle at Cheleleki, in Kone settlement, upper Didessa valley, western Ethiopia. The equality of the survivor functions of the control and isometamidium treated cattle, eight weeks after the start of isometamidium block treatment study, was tested using the Log-rank and Wilcoxon (Breslow) tests (Stata Corporation 2000). Both tested indicated that the survival functions of the control and isometamidium treated cattle differ significantly from each other ($P<0.01$).

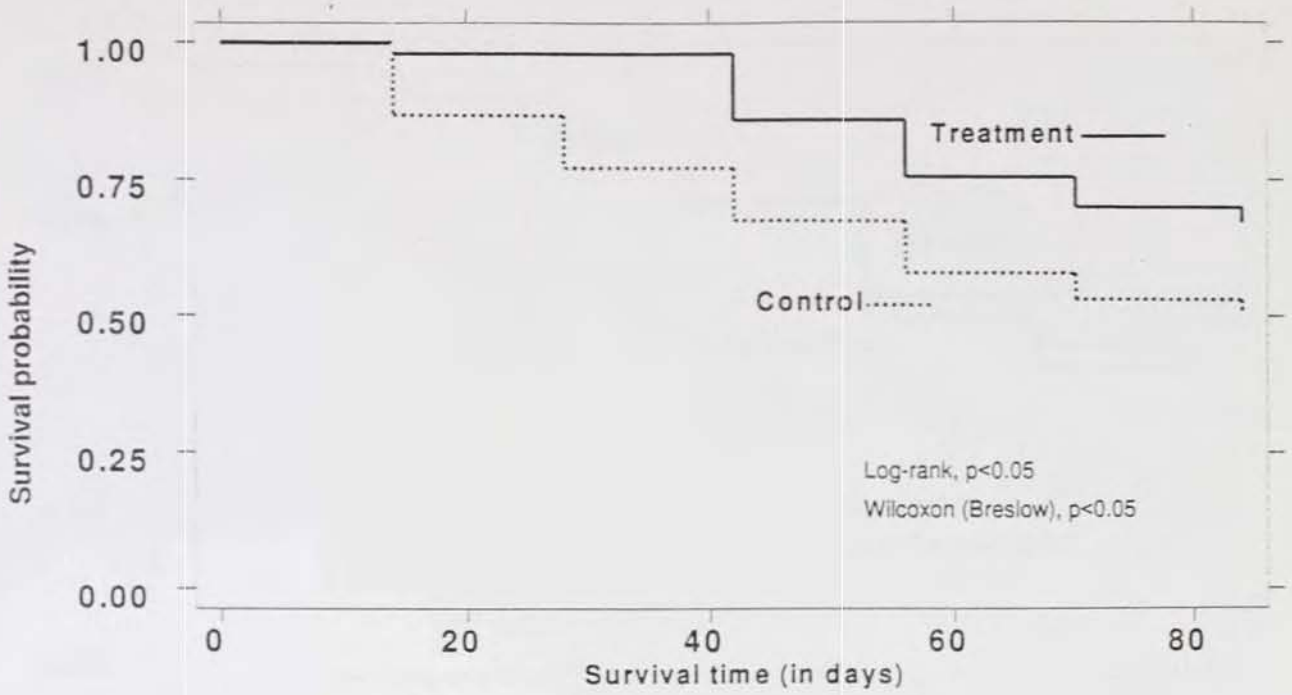

Figure 15. Kaplan-Meier survival estimates and statistical test for the equality of the survivor functions of the control and isometarnidium treated group of cattle at Kolu, in Kone settlement, upper Didessa valley, western Ethiopia. The equality of the survivor functions of the control and isometamidium treated cattle, eight weeks after the start of isometamidium block treatment study, was tested using the Log-rank and Wilcoxon (Breslow) tests (Stata Corporation 2000). Both tested indicated that the survival functions of the control and isometamidium treated cattle differ significantly from each other ($P<0.05$).

Figure 16. Kaplan-Meier survival estimates and statistical test for the equality of the survivor functions of the control and isometamidium treated group of cattle at Burka, in Kone settlement, upper Didessa valley, western Ethiopia. The equality of the survivor functions of the control and isometamidium treated cattle, eight weeks after the start of isometamidium block treatment study, was tested using the Log-rank and Wilcoxon (Breslow) tests (Stata Corporation 2000). Both tested indicated that the survival functions of the control and isometamidium treated cattle do not differ significantly from each other (P>0.05).

4.3.4. Efficacy of diminazene aceturate treatment

At day 14 prior to the start of the isometamidium block treatment study, all 300 cattle in the three villages were treated with diminazene aceturate at a dose rate of 7mg/kg bw. Starting from day 0 up to day 84 of the study, all cattle which were found infected with trypanosomes were treated with diminazene aceturate at a dose rate of 7mg/kg bw. Therefore, the efficacy of diminazene aceturate treatment was assessed on the basis of whether trypanosome infections followed each diminazene aceturate treatment at day 0, 14, 28, 42, 56, 70 and 84.

At day 0, which was 14 days after diminazene aceturate treatment of all animals, there was no trypanosome infection diagnosed in cattle at any of the three villages (Annex II and III). Two weeks later (at day 14), 25 animals were parasitaemic out of which 5 were at Cheleleki, 7 at Kolu and 13 at Burka. On the other hand, 44% and 56 % of the total parasitaemias were due to *T. congolense* and *T. vivax*, respectively. All the parasitaemic animals were then treated with diminazene aceturate at a dose rate of 7mg/kg bw. Fourteen days later (at day 28), 22 of the animals which were parasitaemic at day 14 were diagnosed as aparasitaemic. The remaining three animals (tag no. 278, 261 and 237) which were all from Burka and had *T. congolense* in the previous infection were again parasitaemic with the same species of trypanosome parasite. At day 28, three of the *T. congolense* infected animals, together with other 21 new infections, were again treated with the same dose of diminazene aceturate (Table 10). At day 42, all the 23 animals were diagnosed aparasitaemic but one animal from Burka (tag no. 278) was again parasitaemic with *T. congolense*. Despite treatment with same dose of diminazene aceturate, the animal remained parasitaemic with *T. congolense* up to day 56. On the other hand, two animals from Kolu (tag no. 154 and 131) were parasitaemic with *T. congolense*, one at day 42 and the other at day 56. The two animals were treated with diminazene aceturate at a dose rate of 7mg/kg bw. Two weeks later, they were again diagnosed parasitaemic with *T. congolense*. There were no cattle from Cheleleki, which were diagnosed parasitaemic two weeks after treatment with diminazene aceturate. Apart from this, there were no cattle in any of the three villages which showed *T. vivax* infection two weeks after treatment with diminazene aceturate. In general, parasitaemia was diagnosed in the rest of the cattle, at least, four weeks after treatment with diminazene aceturate. Comparisons of trypanosome incidence rate and trypanosome infection recurrence rate showed that there was no significant difference ($P>0.05$) between the two rates at each of Kolu and Burka (Table 11).

Table 10

Isometamidium block treatment study: cattle parasitaemic after treatment with diminazene aceturate at dose rate of 7mg/kg bw.

Village	Group[1]	Animal ID	Days after isometamidium block treatment[2]						
			0	14	28	42	56	70	84
Burka	Treatment	284	Neg.	Neg.	Neg.	TC	Neg.	TC	Neg.
Burka	Control	282	Neg.	Neg.	TB	Neg.	TV	Neg.	Neg.
Burka	Treatment	278	Neg.	TC	TC	TC	TC	Neg.	TC
Burka	Control	277	Neg.	TC	Neg.	TC	Neg.	Neg.	Neg.
Burka	Control	267	Neg.	Neg.	TB+TV	Neg.	TV	Neg.	TV
Burka	Control	263	Neg.	Neg.	TB+TV	Neg.	TV+TB	Neg.	Neg.
Burka	Treatment	261	Neg.	TC	TC	Neg.	Neg.	Neg.	Neg.
Burka	Treatment	237	Neg.	TC	TC	Neg.	Neg.	Neg.	Neg.
Burka	Control	226	Neg.	Neg.	TV	Neg.	TV	Neg.	Neg.
Burka	Control	211	Neg.	TV	Neg.	TB+TV	Neg.	TV	Neg.
Burka	Control	208	Neg.	TV	Neg.	TC	Neg.	TV	Neg.
Kolu	Treatment	194	Neg.	TC	Neg.	TC	Neg.	Neg.	Neg.
Kolu	Control	182	Neg.	Neg.	TV+TC	Neg.	TC	Neg.	Neg.
Kolu	Control	162	Neg.	Neg.	TC	Neg.	TC	Neg.	Neg.
Kolu	Treatment	154	Neg.	Neg.	Neg.	TC	TC	Neg.	Neg.
Kolu	Control	153	Neg.	TC	Neg.	Neg.	TC	Neg.	TC
Kolu	Treatment	131	Neg.	Neg.	Neg.	Neg.	TC	TC	Neg.
Kolu	Control	123	Neg.	Neg.	Neg.	TC	Neg.	TC	Neg.
Kolu	Control	121	Neg.	Neg.	TV	Neg.	TV	Neg.	Neg.
Kolu	Control	109	Neg.	Neg.	TC	Neg.	TC	Neg.	Neg.
Cheleleki	Control	100	Neg.	Neg.	TV	Neg.	TC+TB	Neg.	TC
Cheleleki	Control	80	Neg.	TC	Neg.	TV+TC	Neg.	TV	Neg.
Cheleleki	Control	72	Neg.	Neg.	TV	Neg.	TV	Neg.	Neg.
Cheleleki	Control	70	Neg.	Neg.	TC	Neg.	TC	Neg.	Neg.
Cheleleki	Control	62	Neg.	TV	Neg.	TV	Neg.	TV+TB	Neg.
Cheleleki	Control	40	Neg.	Neg.	Neg.	TC	Neg.	TC	Neg.
Cheleleki	Control	32	Neg.	Neg.	Neg.	Neg.	TV	Neg.	TC
Cheleleki	Treatment	17	Neg.	Neg.	Neg.	TV+TB	Neg.	TV+TB	Neg.

[1]Treatment groups were cattle which received isometamidium chloride at 1 mg/kg bw at day 0 and control groups were those which were left untreated with isometamidium chloride

[2] Fourteen days prior to start of the block treatment study, all cattle at all villages were treated with diminazene aceturate at dose rate of 7mg/kg bw. Cattle which were found parasitaemic starting from day 0 onwards, were treated with diminazene aceturate at a dose rate of 7mg/kg bw.

Table 11

Comparison between trypanosome incidence rate and trypanosome infection recurrence rate in cattle which were sampled every two weeks for a total of 12 weeks, at each of Kolu and Burka villages, Kone settlement area, western Ethiopia.

Day	Kolu			Burka		
	Trypanosome incidence rate	Trypanosome infection recurrence rate[2]	p-value[1]	Trypanosome incidence rate	Trypanosome infection recurrence rate	p-value[1]
0	0/99	0		0/100	0	
14	7/93	0		13/95	0	
28	3/90	0		11/94	3/13	0.37 NS
42	10/91	0		12/92	1/11	1.00 NS
56	15/92	1/10	1.00 NS	13/93	1/12	1.00 NS
70	6/91	1/15	1.00 NS	14/92	0	
84	6/90	0		3/90	0	

[1]Fisher's exact test; NS= not significant

[2]The proportion of animals (cattle) which were found infected with the same species of trypanosome among the total number of animals treated with diminazene aceturate two weeks back

NB: Fourteen days prior to start of the block treatment study, all cattle at all villages were treated with diminazene aceturate at dose rate of 7mg/kg bw. Cattle, which were found parasitaemic starting from day 0 onwards, were treated with diminazene aceturate at a dose rate of 7mg/kg bw.

4.4. Trypanocidal drug resistance testing in mice

Blood from nine parasitaemic cattle at Cheleleki (tag No. 53 and 87), Kolu (tag No. 131, 154 and 194) and Burka (tag No. 237, 261, 277 and 284) of the isometamidium treated groups were subinoculated into mice. Out of the nine infected blood samples inoculated, three (33.3%) were able to grow in mice. The isolates were coded according to WHO (1986) as MBOI/ET/01/BURKA 261, MBOI/ET/01/BURKA 237 and MBOI/ET/01/KOLU 154. After the isolates were sub-passaged three times in mice, one isolate from Burka (MBOI/ET/01/BURKA 261) was used for drug sensitivity testing in mice using a single discriminatory dose of isometamidium chloride (1mg/kg bw) and diminazene aceturate (20mg/kg bw) (Eisler *et al.*, 2001). The three trypanosome isolates were then stabilized in liquid nitrogen in case if the study requires further investigation.

Results obtained showed that five out of six mice in each of the diminazene aceturate and isometamidium chloride groups relapsed 11.6±1.34 and 11.0±3.0 days post infection,

respectively (Table 12). Six mice, infected but not treated, served as control groups and similarly, five out of the six mice became parasitaemic 8.6±3.05 days post infection.

Table 12

Drug sensitivity of *T. congolense* (MBOI/ET/01/BURKA 261) in mice treated with diminazene aceturate or isometamidium chloride using a single discriminatory dose (Eisler *et al.*, 2001)

Drug	Dose (mg/kg bw)	Number of mice relapsed/treated	Mean relapse interval in days (+/- SD)
Diminazene aceturate	20	5/6	11.6±1.34
Isometamidium chloride	1	5/6	11.0±3.0

Tsetse control operation is very limited in scope in Ethiopia. It covers only 14% of the total tsetse infested area of the country (NTTICC, 1996). Therefore, trypanosomosis control by using imported trypanocidal drugs is the most widely applied technique of controlling the disease in the country. However, multiple trypanocidal drug resistance have been reported in the Abay/Didessa tsetse belt in Metekel district, North-west Ethiopia (Afewerk, 1998; Afewerk *et al.*, 2000) and in the Ghibe/Omo tsetse belt which is adjacent to the upper Didessa river valley (Codjia *et al.*, 1993; Rowlands *et al.*, 1993; Leak *et al.*, 1993; Mulugeta *et al.*, 1997; Ademe and Abebe, in press). Assefa and Abebe (2001) have also reported multiple drug resistant *T. congolense* in naturally infected donkeys in North Omo Zone, southern Ethiopia.

The main objectives of the present study were to assess the trypanocidal activities of diminazene aceturate and isometamidium chloride and the impact of isometamidium chloride prophylaxis relative to no prophylaxis in selected areas of the FITCA project using cattle populations under natural challenge in the field. Accordingly, questionnaire survey and cross sectional study were conducted in selected areas of the FITCA project, namely, Kone settlement, Loko, Shobe, Village I settlement, Ego Kofele settlement, Sore settlement and Darimu. Based on the results of both surveys, isometamidium block treatment study was conducted in three villages of Kone settlement area, namely, Cheleleki, Kolu and Burka.

5.1. Questionnaire survey

Overall results of the questionnaire survey showed that 95.5% of the interviewees reported that trypanosomosis was a serious problem to keep livestock in their areas. In a questionnaire interview with farmers in Metekel district, northwest Ethiopia, Afewerk (1998) also reported that trypanosomosis was the first most important livestock disease in the area.

In the present finding, all the farmers interviewed reported that trypanosomosis cases occur either in the dry season or start of the rainy seasons or during both seasons. Afewerk (1998) also reported similar findings.

In the present finding, 55% of the farmers replied that they used trypanocidal drugs for the last 20 years. Moreover, 95% of the interviewees responded that they used isometamidium chloride and diminazene aceturate to treat their animals against trypanosomosis. This situation may indicate the severity of the problem of trypanosomosis in keeping livestock in the study areas. Absence of any tsetse control activities in the areas could be one of the main reasons why farmers depend on trypanocidal drugs in order to control trypanosomosis. However, 45% and 40% of the farmers reported that diminazene aceturate and isometamidium chloride were used below the recommended dose rates, respectively. On the other hand 40% and 48% of the farmers reported that they did not have any idea on the dose rates of both drugs, respectively. Afewerk (1998) also reported that more than 40% of the interviewees responded that they used trypanocidal drugs below the recommended dose rate whilst below 20% of the respondents did not have any idea on the doses of trypanocidal drugs. In the present finding, 85% of the interviewees responded that trypanocidal drug were applied only to sick animals. Van den Bossche (2000) had found similar results during questionnaire survey in two areas in Zambia. In similar surveys conducted in West Africa (Bauer, 2001), trypanocidal drugs were used in more than 90% of all cases without a reliable diagnosis.

In the present finding, 57% of the trypanocidal drug treatments were reported to be applied through farmers themselves and other uncertified individuals. Afewerk (1998) had also reported about 43% of it to be applied in the same manner in village cattle of Metekel district, northwest Ethiopia. One of the reasons for trypanocidal drug misuse could be related to the inefficiencies of Government services in Ethiopia to discharge their responsibilities in remote areas. In most of the occasions, clinical service is provided in the country only to those owners who are able to present sick animals to the nearest clinic or health post and provision of mobile clinic to remote areas is neither financially viable nor sustainable (PARC, 1998).

Over 70% of the farmers reported that each animal was treated for trypanosomosis at intervals ranging from 2.5 - 3.5 months. This shows that there is either high pressure of trypanosomosis or treatments were not very effective in curing sick animals. The duration in the present result was, however, shorter than the one reported in Afewerk (1998). In similar interviewes with farmers in Metekel district, northwest Ethiopia, Afewerk (1998) reported that farmers treated their animals every 4 months and above. The number of treatments over a year reflects the magnitude of trypanosomosis challenge in an area (Uilenberg, 1997).

5.2. Cross sectional study

In the present finding, the highest prevalence of trypanosome infection was recorded in Kone and Village I settlement areas. However, trypanosome prevalence was not recorded in cattle raised along the escarpments of Shobe, Sore and Darimu. The prevalence of the disease was also very low in cattle along the valley floors in Loko and Ego Kofele areas. When compared with previous reports, the present findings showed that the prevalence of trypanosomosis in cattle seems to decline in the study areas. Before some 15 years, settlers in Kone settlement area were devoid of cattle and they had to rely on small ruminants (NTTICC, 1989). Therefore, the settlers introduced cattle into the area as a result of, probably, two main reasons. The first reason could have been because of the possible decline in cases of trypanosomosis over years and the other could be as a result of the likely use of massive trypanocidal drug treatments. In Village I settlement, trypanosome prevalence obtained in the present finding was also lower than that obtained by the NTTICC (1998) in the area (55%). Likewise, surveys conducted by the NTTICC (1989) showed a trypanosome prevalence of 29% along the escarpments of the upper Didessa valley. Jemal and Hugh-Johns (1995) had also reported a trypanosome cumulative incidence rate of 11.5-14.1% in an area adjacent to Loko and within Loko area itself. In Ego Kofele, a trypanosome prevalence of 47.6% was reported by the NTTICC (1998).

In Kone and Village I settlement areas where there was high trypanosome infection rate in cattle in the present finding, *T. congolense* was the most predominant species of trypanosome which accounted for 75% and 93% of the cases, respectively. This may indicate that tsetse flies are the main vectors of trypanosomosis in the two study areas. However, the present findings were slightly higher than the reports of Afewerk *et al.* (2000) in northwest Ethiopia (47.6%), Rowlands *et al.* (1993) in southwestern Ethiopia (37%) and Abebe and Jobre (1996) for tsetse infested areas of the country (58.5%).

In the present findings, an animal with a PCV value of less than 26% was considered anemic. Work done at Ghibe in southwestern Ethiopia showed that few animals were detected parasitaemic when the PCV value was greater than or equal to this value (Rowlands, 2000). Trypanosome infection and mean PCV values obtained in the present study in the parasitaemic and aparasitaemic animals were related. Rowlands *et al.* (2001) also observed at Ghibe valley in southwestern Ethiopia that as PCV increased the proportion of samples detected parasitaemic decreased. Therefore, average PCV could be better indicator of the health status of cattle herds. One disadvantage of the reliance on mean PCV value could be

that it is affected by many factors other than trypanosomosis. However, these factors are likely to affect both trypanosomosis positive and negative animals (Van den Bossche and Rowlands, 2001).

5.3. Isometamidium block treatment study

Fourteen days prior to the start of isometamidium block treatrnent study, results obtained at Cheleleki and Kolu villages showed that *T. congolense* was the most predominant species of trypanosome involved. Results were, therefore, closer to that reported by Afewerk (1998), Rowlands *et al.* (1993) and Abebe and Jobre (1996).

At day 0 which was 14 days after treatment of all animals with diminazene aceturate (7mg/kg bw), there were no cattle parasitaemic with trypanosome parasites. Therefore, there was a good response to treatment with this drug at the specified time. Diminazene aceturate was reported to maintain blood therapeutic levels until 22 days post treatment (Jordan, 1986).

The mean prevalence of trypanosome infection in the isometamidium treated groups of cattle during the 8-week period was the lowest at Cheleleki and highest at Burka. Kolu was falling in between the two villages. There was a moderately strong correlation between overall trypanosome prevalence and recurrent infection prevalence in cattle in southwest Ethiopia exposed to a high challenge of drug resistant trypanosomes (Rowlands *et al.*, 2001).

The difference in the incidence rates of the control and isometamidium treated groups of cattle at Cheleleki and Kolu was associated with the differences in the total time at risk. The total time at risk of the control and isometamidium treated groups of cattle was higher at Cheleleki as compared to the same groups of cattle at Kolu. The incidence rate ratio of the isometamidium treated to the control group of cattle at Cheleleki was less than one and it shows that isometamidium treatment was a protective factor (Frankena and Graat, 1997).

Similarly, the cumulative incidence rate of trypanosome infection in the isometamidium treated groups of cattle at the three villages was consistent with the findings of the prevalence data. In the present finding, multiple infections of an animal were treated as new cases and animals found infected with trypanosomes were treated with diminazene aceturate and included in the next data. Therefore, cumulative incidence rate data at each particular reference point in days was dependent upon what has happened in the previous days. As a

result, it was not possible to apply statistical tests to evaluate for any significant difference between control and isometamidium treated groups of cattle since cumulative incidence rate data, in the present finding, were not independent to each other. However, simple mathematical differences between the control and isometamidium treated groups of cattle at day 56 (8^{th} week) was used and it showed that the difference was the lowest at Cheleleki followed by Kolu and then Burka. The present results were in agreement with similar studies conducted in Burkina Faso (McDermott, 1999) which had discovered a trypanosome cumulative incidence of 7-57% eight weeks after isometamidium prophylaxis.

Results of the mean slope of the PCV values have shown that there was an improvement of PCV over time in the isometamidium treated and a decrease in the control groups of cattle at Cheleleki. On the other hand, the control as well as isometamidium treated groups of cattle at both Kolu and Burka villages showed an improvement in mean slope of the PCV values over time.

The 25% survival time showed that 25% of control group of cattle at Cheleleki and Kolu were infected with trypanosome parasites by day 42 of the isometamidium block treatment study. Equal proportions (25%) of the control group of cattle at Burka were infected with trypanosomes by day 28 of the study. This may show that the tsetse challenge was sufficiently high at all the villages and a prophylactic regimen is more indicated if trypanosomosis is to be controlled efficiently. According to Eisler *et al.* (2000), challenge was insufficient to warrant isometamidium prophylaxis when fewer than 25% of sentinel cattle became infected within eight weeks of exposure. This was justified on the grounds of cost, possible side effects and unnecessary drug pressure tending to develop drug resistance.

The difference between the 25% survival times of the control and isometamidium treated groups of cattle at Cheleleki and Kolu was, similarly, associated with the differences in the total time at risk of the control and isometamidium treated groups of cattle across both villages.

On the other hand, less than 25% isometamidium treated group of cattle at Cheleleki (22.9%) and Kolu (20%) become parasitaemic with trypanosomes after eight weeks of treatment using isometamidium chloride. Therefore, this may show that the occurrence of resistance to isometamidium chloride was insignificant and isometamidium chloride could still be used to protect cattle against trypanosomosis at Cheleleki and Kolu. At Burka, more than 25% of the

isometamidium treated groups of cattle were parasitaemic (36%) eight weeks following isometamidium block treatment and this may suggest that there is an indication of resistance of trypanosomes, due to *T. congolense*, against isometamidium chloride. Resistance against this drug was strongly suspected when more than 25% the isometamidium treated cattle became parasitaemic within eight weeks of exposure (Eisler *et al.*, 2000).

The ratio of the mean hazard rate for Burka was lower than two and it could be of no value to continue using isometamidium at the village. Where there is evidence of drug resistance on the grounds of the number of isometamidium treated cattle becoming infected within eight weeks of exposure, it may nevertheless be worth continuing prophylaxis in situations where the ratio of the mean hazard rate for the sentinel and prophylaxis herds over weeks one to eight is greater than two (Eisler *et al.*, 2000). In general, Eisler *et al.* (2000) has suggested that whenever there are indications for drug resistance, chemotherapy has to be combined with other methods such as vector and other integrated control methods.

The Kaplan-Meier survival curves were also in agreement with what were reported so far in the present findings. In general, the probability to survive was much higher in the isometamidium treated compared to the control group of cattle at Cheleleki. It was significantly greater at Kolu whilst isometamidium treatment had insignificant effect on survival time at Burka. Isometamidium treatment had minimal effect on survival times in high drug resistance situation in coastal Kenya (Eisler *et al.*, 2000).

The results of the assessment of the efficacy of diminazene aceturate showed that there was no significant difference between the trypanosome incidence rate and trypanosome infection recurrence rate at each of Kolu and Burka villages. This indicates that they were all new cases and diminazene treatment was still effective at all the study villages. There was strongly significant difference between trypanosome incidence rate and trypanosome infection recurrence rate in southwest Ethiopia where drug resistant trypanosomes against diminazene aceturate was reported (Rowlands, 2000).

5.4. Trypanocidal drug resistance testing in mice

Isometamidium chloride and diminazene aceturate administered i.p. at doses of 1mg/kg and 20mg/kg bw, respectively, failed to cure mice infected with *T. congolense*. In the present study, *T. congolense* expressed a level of resistance to both drugs when examined in mice.

Findings were, therefore, in consistent with results of both Afewerk *et al.* (1998) and Assefa and Abebe (2001). In the report of Afewerk *et al.* (2000), three clones derived from a parental isolate expressed high levels of resistance to diminazene aceturate (3.5-28.0mg/kg bw) and isometamidium chloride (0.5-4.0mg/kg bw). However, in the present study, mice results were not conclusive, as it is difficult to get detailed information on individual isolate using the single discriminatory dose. The main aim of the single discriminatory dose was to compare the level of resistance between geographic areas but due to logistic, financial and technical reasons it was done only on one isolate originated from Burka village.

6. CONCLUSION AND RECOMMENDATION

In the present finding, questionnaire results have revealed that trypanosomosis was a serious problem in the study areas.

The questionnaire survey had also indicated that there was a serious problem of under-dosing of cattle with trypanocidal drugs in the study areas.

In Ethiopia, provision of animal health service through public sector is almost completely based upon expensive and fixed-point delivery system. Therefore, the private veterinary sector need to be considered as an option for a positive contribution towards the development of low cost and village (community) based tsetse and trypanosomosis control system in remote areas.

Data collected in this work, particularly from the escarpments, using the questionnaire survey should be treated with caution. According to the results of the present cross sectional study and other available background information (Keno, 2001), the prevalence of trypanosomosis seems to decline in many of the study areas. Therefore, an urgent investigation of the situation may help to design better ways of combating the problem of tsetse and trypanosomosis over these areas.

In the present finding, survival analysis of time had indicated the significance of isometamidium chloride (1mg/kg bw) in protecting cattle against trypanosomosis at Cheleleki and Kolu. However, there was a strong evidence of isometamidium chloride resistant trypanosomes due to *T. congolense* in cattle herds at Burka village. Therefore, study should be conducted in great detail in order to investigate the epidemiology of drug resistant trypanosomes in other areas of the FITCA project. This will help to develop an appropriate strategy to control the tsetse and trypanosomosis problem in the area.

There were no significant differences between trypanosome incidence rate and trypanosome infection recurrence rate in cattle, which were treated with diminazene aceturate within two weeks interval, at each of Kolu and Burka villages. Therefore, this indicates that all cases were new infections and diminazene treatment was still effective at all the study villages.

Isometamidium chloride and diminazene aceturate administered i.p. at doses of 1mg/kg bw and 20mg/kg bw, respectively, failed to cure mice infected with *T. congolense*. However, in the present study, mice results were not conclusive, as it is difficult to get detailed information on individual isolate using the single discriminatory dose.

Regardless of the presence of drug resistance at Burka, there was an improvement of PCV in both the control and isometamidium treated groups of cattle at the village.

It was concluded that where there are indications for drug resistance in the study areas, the use of trypanocidal drugs should be supervised, the principle of sanative pairs has to be applied and chemotherapy needs to be integrated with other methods like vector (tsetse fly) control.

Abebe, G. and Y. Jobre (1996): Trypanosomiasis: a threat to cattle production in Ethiopia. *Rev. Med. Vet.* **147**: 897-902.

Ademe, M. and G. Abebe (in press): Field study on drug resistant trypanosomes in cattle (Bos indicus) in Kindo Koysha wereda, Southern Ethiopia. *Bull. Anim. Hlth. Prod. Afr.*.

Afewerk, Y. (1998): Field Investigations on the Appearance of Drug Resistant Populations of Trypanosomes in Metekel District, North-west Ethiopia. MSc thesis, Addis Ababa, University and Freie Universität Berlin.

Afewerk, Y., Clausen, P.-H., Abebe, G., Tilahun, G. and D. Mehlitz (2000): Multiple-drug resistant *Trypanosoma congolense* populations in village cattle of Metekel district, north-west Ethiopia. *Acta Trop.* **76**, 231–238.

Allsopp, R. (1991): A practical guide to aerial spraying for tsetse control. Aerial Spraying Research and Development Project. Final Report, Volume 2. EC Delegation, Harare, Zimbabwe.

Arora, V. K., Sharma, R. C., Singh, B. P. and N. S. Tomar (1981): A note of body measurements in Hariana cows. *Vet. Res. J.* **4**, 180-182.

Assefa E. and G. Abebe (2001): Drug-resistant *Trypanosoma congolense* in naturally infected donkeys in North Omo Zone, southern Ethiopia. *Vet. Parasitol.* **99**, 261-271.

Baldry, D.A.T., Everts, J., Roman, B., Boon Von Ochsee, G.A. and C. Laviessiere (1981): The experimental application of insecticides from helicopters for the control of riverine populations of *Glossina tachinoides* in West Africa. Part VIII: the effects of two spray applications of OMS-570 (endosulfan) and of OMS-1998 (decamethrin) on *G. tachinoides* and non-target organisms in Upper Volta. *Pest. Manag. Sci.* **37**, 83-110.

Bauer, B. (2001): Improved strategies for sustainable trypanosomosis management within the context of primary animal health care. In: proceedings of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Mombasa, Kenya 1999, OAU/STRC, Publication No. **120**, pp. 123-130.

Baylis, M. and P. Stevenson (1998): Trypanosomiasis and tsetse control with insecticidal pour-ons - fact and fiction? Applications. *Parasitol. Today* **14**, 2, 77-82.

Bengaly, Z., Kasbari, M., Desquesnes, M. and I. Sidibe (2001): Validation of a polymerase chain reaction assay for monitoring the therapeutic efficacy of diminazene aceturate in trypanosome-infected sheep. *Vet. Parasitol.* **96**, 101-113.

Berhanu, B. (1998): Consultancy report on quantifying the impact of tsetse control on farming. Food and Agriculture Organization of the United Nations, Addis Ababa, Ethiopia.

Boun, D. and M. Scott (1978): The successful use of work oxen in agricultural development of tsetse infested land in Ethiopia. *Trop. Anim. Hlth. Prod.* **10**: 191-203.

Brun, R. and Kunz, C. (1989): *In vitro* drug sensitivity test for *Trypanosoma brucei* subgroup blood stream trypomastigotes. *Acta Trop.* **46**, 361-368.

Budd, L. T. (1999): DFID-Funded Tsetse and Trypanosome research and development since 1980. Volume 2, Economic Analysis. DFID Livestock Production Programme, DFID Animal Health Programme, Natural Resources Systems Programme.

Clausen, P.-H., Adeyemi, I., Bauer, B., Breloeer, M., Salchow, F. and C. Staak (1998a): Host preferences of tsetse (Diptera: Glossinidae) based on bloodmeal identifications. *Med. Vet. Entomol.* **12**, 169-180.

Clausen, P.-H., Leendertz, F.H., Blankenburg, A., Tietjen, U., Mehlitz, D., Sidibe, I. and B. Bauer (1999a): A drug incubation glossina infectivity test (DIGIT) to assess the susceptibility of *Trypanosoma congolense* bloodstream forms to trypanocidal drugs (Xenodiagnosis). *Acta Trop.* **72**, 111-117.

Clausen, P.-H., Schares, G., Patzelt R.J, Poetzsch, C.J, Scheer, A., Kakaire, D., Olila D., Peregrine, A.S., McDermott, J. and D. Mehlitz (2001): PCR and DNA-probe hybridization to assess the efficacy of therapeutic and prophylactic isometamidium treatment in *Trypanosoma* spp.-infected dairy cattle in peri-urban Kampala, Uganda. In: proceedings of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Mombasa, Kenya 1999, OAU/STRC, Publication No. **120**, pp. 429-434.

Clausen, P.-H., Sidibe, I., Kabore, I. and B. Bauer (1992): Development of multiple drug resistance of *Trypanosoma congolense* in Zebu cattle under high natural tsetse fly challenge in the pastoral zone of Samorogouan, Burkina Faso. *Acta Trop.* **51**, 229-236.

Clausen, P.-H., Waiswa, C., Katunguka-Rwakishaya, E., Schares, G., Steuber, S. and D. Mehlitz (1999b): Polymerase chain reaction and DNA probe hybridization to assess the efficacy of diminazene treatment in *Trypanosoma brucei*-infected cattle. *Parasitol. Res.* **85**, 206-211.

Clausen, P.-H., Wiemann A., Patzelt R., Kakaire, D., Poetzsch, C., Peregrine A. and D. Mehlitz (1998b): Use of a PCR assay for the specific and sensitive detection of *Trypanosoma* spp. in naturally infected dairy cattle in peri-urban Kampala, Uganda. *Ann. N. Y. Acad. Sci.* **849**, 21-31.

Codjia, V., Mulatu, W., Majiwa, P.A.O., Leak, S.G.A., Rowlands, G.J., Authie, E., d'Ieteren, G.D.M. and A.S. Peregrine (1993): Epidemiology of bovine trypanosomiasis in the Ghibe valley, South-west Ethiopia. 3. Occurrence of population of *Trypanosoma congolense* resistant to diminazene, isometamidium and homidium. *Acta Trop.* **53**, 151-163.

Connor, R.J. (1989): Final report of the regional trypanosomosis expert. Regional tsetse and trypanosomosis control programme, Malawi, Mozambique, Zambia and Zimbabwe. FGU-Kronberg Consulting and Engineering GmbH, Germany, p. 123.

Connor, R.J. (1992): The diagnosis, treatment and prevention of animal trypanosomiasis under field conditions. FAO, *Anim. Prod. Hlth. Pap.* **100**, 1-35.

Dagnachew, Z. and K. Shafo (1981): An investigation of Dourine in Arsi administrative region. *Eth. Vet. Bull.* **4**: 3-9.

d'Ieteren, G.D., Authié, E., Wissocq, N. and M. Murray (1998): Trypanotolerance an option for sustainable livestock production in areas at risk from trypanosomiasis. *Rev. Sci. Tech.* **17**, 154-175.

Eisler, M.C., Arowolo, R.O.A., Gault, E.A., Moloo, S.K., Holmes, P.H. and A.S. Peregrine (1994): Isometamidium concentrations in the serum of treated cattle: correlation with prophylaxis against tsetse-transmitted *Trypanosoma congolense*. *Acta Trop.* **56**, 39-50.

Eisler, M.C., Brandt, J., Bauer, B., Clausen, P.-H., Delespaux, V., Holmes, P.H., Ilemobade, A., Machila, N., Mbwambo, H., McDermott, J., Mehlitz, D., Murilla, G., Ndung'u, J.M., Peregrine, A.S., Sidibe, I., Sinyangwe, L. and Geerts, S. (2001): Standardised tests in mice and cattle for the detection of drug resistance in tsetse-transmitted trypanosomes of African domestic cattle. *Vet. Parasitol.* **97**, 171-182.

Eisler, M.C., Gault, E.A., Moloo, S.K., Holmes, P.H. and A.S. Peregrine (1997): Concentration of isometamidium in the sera of cattle challenged with drug-resistant *Trypanosoma congolense*. *Acta Trop.* **63**, 89-100.

Eisler, M.C., Gault, E.A., Peregrine, A.S., Smith, H.V. and P.H. Holmes (1993): Evaluation and improvement of an Enzyme-linked Immunosorbent Assay (ELISA) for the detection of isometamidium in bovine serum. *Ther. Drug Monit.* **15**, 236-242.

Eisler, M.C., Lessard, P., Moloo, S.K., Masake, R.A. and A.S. Peregrine (1998): Sensitivity and specificity of antigen-capture ELISAs for diagnosis of *Trypanosoma congolense* and *Trypanosoma vivax* infections in cattle. *Vet. Parasitol.* **79**, 187-201.

Eisler, M.C., McDermott, J.J., Mdachi, R., Murilla, G.A., Sinyangwe, L., Mubanga, J., Machila, N., Mbwambo, H., Coleman, P.G., Clausen, P.-H., Bauer, B., Sidibe I., Geerts, S., Holmes, P.H. and Peregrine, A.S. (2000): Rapid method for the assessment of trypanocidal drug resistance in the field. In: proceedings of the 9th

Symposium of the International Society for Veterinary Epidemiology and Economics (ISVEE 9), Breckenridge, Colorado, August 6-11, 2000.

Elrayah, I. E. and R. Kaminsky (1991): The effect of diminazene aceturate and isometamidium chloride on cultured procyclic forms of susceptible and drug resistant *Trypanosoma* congolense. *Acta Trop.* **49**, 201-213.

Erkelens, A.M., Dwinger, R.H., Bedane, B., Slingenbergh, J.H.W. and W. Wint (2000): Selection of priority areas for tsetse control in Africa; a decision tool using GIS in Didessa valley, as a pilot study. In: proceedings of the 25th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, 27 Sep.- 1 Oct. 1999, Mombasa, Kenya.

FAO (1992): Use of attractive devices for tsetse survey and control. Training manual for tsetse control personnel, Food and Agriculture Organization of the United Nations, Rome Vol. **4**, pp. 140-141.

FITCA (1998): OAU/IBAR Regional Eastern Africa Programme, Farming in Tsetse Control Areas, Ethiopia - Component, Federal Democratic Republic of Ethiopia, Ministry of Agriculture, project proposal for EU funding, Addis Ababa, Ethiopia.

Frankena, K. and E.A.M. Graat (1997): Analysis of time at risk (survival) data. In: Application of Quantitative Methods in Veterinary Epidemiology. ed. 1, the Netherlands, Wageningen Pers, Wageningen.

Gall, Y., Clausen, P.-H., Tanja, W., Bauer, B., McDermott, J., Sidibe, I. and D. Mehlitz (2001): PCR to monitor the efficacy of drug treatrnent in cattle naturally infected with *Trypanosoma* spp. in the province of Kénédougou, Burkina Faso. 26th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), 1-5 October 2001, Ouagadougou, Burkina Faso, Abstract No. 508, pp108.

Gardiner, P. R. and A. J. Wilson (1987): *Trypanosoma (Duttonella) vivax. Parasitol. Today* **3**, 49-52.

Geerts, S. and P.H. Holmes (1999): Drug management and parasite resistance in animal trypanosomiasis in Africa. Gestion medicamenteuse et resistance parasitaire. In: proceedings of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), Maputo, Mozambique, OAU/ISTRC Publication No. **119**, pp. 371-385.

Geerts, S., Diarra, B., Eisler, M.C., Brandt, J., Lemmouchi, Y., Kageruka, P., De Deken, R., Ndao, M., Diall, O., Schacht, E., Berkvens, D., Speybroeck, N. and P.H. Holmes (1999): Extension of the prophylactic effect of isometamidium against trypanosome infections in cattle using a biodegradable copolymer. *Acta Trop.* **73**, 49–58.

Geerts, S., Ndungu's, J.M., Murilla, G.A., Mbwambo, H., Sinyangwe, L., Machila, N., Delespaux, V., Brandt, J., Peregrine, A.S., McDermott, J.J., Holmes, P.H. and M.C. Eisler (2000): *In vivo* tests for the detection of resistance to trypanocidal drugs: tests in mice and ruminants. In: proceedings of the workshop on drug delivery and resistance in the context of integrated disease management, 31 May – 4 June 1999, Nairobi, Kenya. News letter on Integrated Control of Pathogenic Trypanosomes and their Vectors (ICPTV), No. **2**, pp. 6-12.

Gu, Y., Gettinby, G., McKendrick, I., Murray, M., Peregrine, A.S. and C. Revie (1999): Development of decision support system for trypanocidal drug control of bovine trypanosomiasis. *Vet. Parasitol.* **87**, 9-23.

Guelmbaye, N., Shamshudeen, K. M., Noel B.M. and A.S. Peregrine (1993): Derivation and characterization of a quinapyramine-resistant clone of *Trypanosoma congolense*. *Antimicrob. Agents Chemother.* **37**, (5), 2-5.

Hursey, B.S. and J. Slingenbergh (1995): The tsetse fly and its effect on agriculture in sub-Saharan Africa. *World Anim. Rev.* **84/85**, 67-73.

Jemal, A. and M.E. Hugh-Johns (1995): Association of tsetse control with health and productivity of cattle in the Didessa Valley, western Ethiopia. *Prev. Vet. Med.* **22**: 29-40.

Jones-Davies, W.J. (1967): The discovery of Berenil® resistant strains of trypanosomes in cattle in North Nigeria. *Bull. Epizoot. Dis. Afr.* **14**, 65-72.

Jordan, A.M. (1986): Trypanosomiasis control and African rural development. Longman, UK & USA, 357 pp.

Kaminsky, R., Gumm, I. D., Zweygarth E. and F. Chuma (1990): A drug incubation infectivity test (DIIT) for assessing resistance in trypanosomes. *Vet. Parasitol.* **34**, 335-343.

Klein, J.P. and M.L. Moeschberger (1997): Survival analysis: Techniques for censored and truncated data. New York, Springer-Verlag, pp. 341.

Keno, M. (2001): Impact of forced land use on tsetse distribution in FITCA Ethiopia project area. 26th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), 1 - 5 Oct. 2001, Ouagadougou, Burkina Faso, Abstract No. 611, pp 143.

Keno, M. and M. Mengistu (1995): The control of *Glossina morsitans submorsitans* by the application of deltamethrin 1% pour on to cattle in an area of south western Ethiopia. In: proceedings of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC). 24th meeting, OAU/STRC, Publication No. **118**, pp. 288-290.

Krafsur, E.S., Madsen, M., Wohlford, D.L., Mihok, S. and N.T. Griffiths (2000): Polpulation genetics of *Glossina morsitans submorsitans* (Diptera Glossinidae). *Bull. Entomol. Res.* **90**: 329-335.

Kristjanson, P.M., Swallow, B.M., Rowlands, G.J., Kruska, R.L. and P.N. de Leeuw (1999): Measuring the cost of African animal trypanosomiasis, the potential benefits of control and returns to research. *Agricultural Systems* **59**, 79-78.

Kuzoe, F.A.S. (1991): Perspectives in research on and control of African trypanosomiasis. *Ann. Trop. Med. Parasitol.* **85**, (1), 33-41.

Langridge, W.P. (1976): A tsetse and trypanosomiasis survey of Ethiopia. Ministry of Oversea Development, Britain.

Leak, S.G.A., Woudyalew, M., Authié, E., d'Ieteren, G.D.M., Peregrine, A.S., Roelands, G.J. and J.C.M. Trail (1993): Tsetse challenge and its relationship to trypanosomiasis prevalence in cattle. *Acta Trop.* **53**, 121-134.

Lewis, A.R. and J.W. Thomson (1974): Observation on isometamidium resistant strains in *Trypanosoma congolense* in Rhodesia. *Rhod. Vet. J.* **4**, 62-67.

MacLennan, K.J.R. (1980): Tsetse-transmitted trypanosomiasis in relation to the rural economy in Africa-part 1. *World Anim. Rev.* **36**, 2-17.

McDermott, J.J., Woitag, T., Bauer, B., Sidibe, I., Ouedraogou, D., Kamuanga, J.M.B., Clausen, P.-H., Eisler, M.C., Peregrine, A.S., Zessin, K.H., and D. Mehlitz (1999): Trypanosomiasis risk, tsetse challenge and trypanocide resistance in Kènèdougou province, Burkina Faso. 25th Meeting of the International Scientific Council for Trypanosomiasis Research and Control, 27 Sep.- 1 Oct. 1999, Mombasa, Kenya, Abstract No. 212, pp 37-38.

Mdachi, R.F. (1999): Epidemiological studies into the impact of trypanocidal drug resistance on the control of trypanosomiasis in coastal Kenya. PhD Thesis, University of Glasgow, 352 pp.

MOA (1995): Ruminant Livestock Development Strategy (RLDS). Ministry of Agriculture (MOA), Addis Ababa, Ethiopia.

Mohammed-Ahmed, M.M., Rahman, A.H. and E.I. Abdel Karim (1992): Multiple drug-resistant bovine trypanosomes in South Darfur Province, Sudan. *Trop. Anim. Hlth. Prod.* **24**, 179-181.

Moloo, S.K. and S.B. Kutuza (1990): Expression of resistance to isometamidium and diminazene in *Trypanosoma congolense* in Boran cattle infected by *Glossina morsitans centralis*. *Acta Trop.* **47**, 79-89.

Mulugeta, W., Wilkes, J., Mulatu, W., Majiwa, P.A.O., Masake, R. and A.S. Peregrine (1997): Long-term occurrence of *Trypanosoma congolense* resistant to diminazene, isometamidium and homidium in cattle at Ghibe, Ethiopia. *Acta Trop.* **64**, 205-217.

Murray, M., Murray, P.K. and W.I.M. Mc Intyre (1977): An improved parasitological technique for the diagnosis of African trypanosomosis. *Trans. R. Soc. Trop. Med. Hyg.* **71**, 325-326.

Murray, M., Stear, M.J., Trail, J.C.M., d'Ieteren, G.D.M., Agyemang, A. and R.H. Dwinger (1991): Trypanosomiasis in cattle: prospects for control. Breeding for disease resistance in farm animals (Owen, J.B., Axford, R.F.E., Eds), CAB International, Wallingford, Ch. **12**, 203-223.

Mwambu, P.M. and J.S.P. Mayende (1971): Occurrence of Berenil resistant strains of *T. vivax*. *Trans. R. Soc. Trop. Med. Hyg.* **65**, 254-255.

Ndoutamia, G., Moloo, S.K., Murphy, N.B. and A.S. Peregrine (1993): Derivation and characterization of a quinapyramine-resistant clone of *Trypanosoma congolense*. *Antimicrob. Agents Chemother.* **37**, 1163–1166.

Ndungu, J.M., Murilla, G.A. and R.M. Mdachi (1999): Area-wide appraisal of drug resistance in trypanosomes infecting cattle in East and Southern Africa. 25th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), 27 Sep.- 1 Oct. 1999, Mombasa, Kenya, Abstract No. 215, pp 40.

Nega, T. (1999): The use of antigen ELISA to monitor the effectiveness of a tsetse control campaign in the upper Didessa valley, Western Ethiopia. Animal Trypanosomiasis: Vector and Disease Control Using Nuclear Techniques. In: proceedings of the second FAO/IAEA seminar for Africa, 27 November - 1 December 1995. ISBN 90-5782-048-X. Zanzibar, United Republic of Tanzania, pp. 273-278.

Nega, T., Kebede, A. and A. Tsegaye (1997): The use of antigen ELISA to monitor the effectiveness of a tsetse control campaign in the upper Didessa valley, Western Ethiopia. IAEA-TECDOC-925. In: proceedings of the workshop on epidemiological tools for monitoring trypanosomiasis and tsetse control programmes organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and the IAEA Department of Technical Co-operation held in Addis Ababa, Ethiopia, 17-28 April 1995, pp. 39-43.

NLDP (1997): National Livestock Development Programme. Ministry of Agriculture, Addis Ababa, Ethiopia.

NMSA (1996): Climate classifications of Ethiopia. National Meteorological Services Agency (NMSA), Meteorological Research Report Series, No. 3, Addis Ababa, Ethiopia.

NTTICC (1989): Annual Report. Ministry of Agriculture, National Tsetse and Trypanosomiasis Investigation and Control Centre (NTTICC), Bedelle, Illubabor, Ethiopia.

NTTICC (1996): Annual Report. Ministry of Agriculture, National Tsetse and Trypanosomiasis Investigation and Control Centre (NTTICC), Bedelle, Illubabor, Ethiopia.

NTTICC (1998): Annual Report. Ministry of Agriculture, National Tsetse and Trypanosomiasis Investigation and Control Centre (NTTICC), Bedelle, Illubabor, Ethiopia.

PARC (1998): Consultancy report on cost recovery in delivery of animal health services. Pan African Rinderpest Campaign (PARC), Ministry of Agriculture, Addis Ababa, Ethiopia, pp. 1-2.

Paris, J., Murray, M. and F. McOdimba (1982): A comparative evaluation of the parasitological techniques currently available for the diagnosis of African trypanosomiasis in cattle. *Acta Trop.* **39**, 307-316.

Peregrine, A.S. (1994): Chemotherapy and delivery systems: Haemoparasites. *Vet. Parasitol.* **54**, 223-248.

Pinder, M. and E. Authié (1984): The appearance of isometamidium resistant *Trypanosoma congolense* in West Africa. *Acta Trop.* **41**, 247-252.

Rowlands, G.J. (2000): Detecting resistance of trypanosomal infections to diminazene aceturate-a possible field test? In: proceedings of the workshop on drug delivery and resistance in the context of integrated disease management, 31 May – 4 June 1999, Nairobi, Kenya. News letter on Integrated Control of Pathogenic Trypanosomes and their Vectors (ICPTV), No. **2**, pp. 6-12.

Rowlands, G.J., Leak, S.G.A., Peregrine, A.S., Nagda, S.M., Mulatu, W. and G.D.M. d'Ieteren (2001): The incidence of new and the prevalence and persistence of recurrent trypanosome infections in cattle in southwest Ethiopia exposed to a high challenge with drug-resistant parasites. *Acta Trop.* **79**: 149-163.

Rowlands, G.J., Wudyalew, M., Authie, E., d'Ieteren, G.D.M., Leak, S.G.A., Nagda, S.M. and A.S. Peregrine (1993): Epidemiology of bovine trypanosomiasis in the Ghibe valley, southwest Ethiopia. 2. Factors associated with variations in trypanosome prevalence, incidence of new infections and prevalence of recurrent infections. *Acta Trop.* **53**, 135-150.

Schönefeld, A., Röttcher, D. and S.K. Moloo (1987): The sensitivity to trypanocidal drugs of *T. vivax* isolates in Kenya and Somalia. *Trop. Med. Parasitol.* **38**, 177-189.

Scott, J.M. and R.G. Pegram (1974): A high incidence of *Trypanosoma congolense* strains resistant to homidium bromide in Ethiopia. *Trop. Anim. Hlth. Prod.* **6**, 215-222.

Slingenbergh, J.H.W (1992): Tsetse control and agricultural development in Ethiopia. *World Anim. Rev.* **70/71**, 30-36.

Stata Corporation (2000): Intercooled Stata 6.0 for Windows 95/98/NT. University Drive East College Station, Texas, USA.

Stevenson, P., Okech, G., Mwendia, C. and K.R. Sones (2000): Comparison of isometamidium-based trypanocidal drugs Samorin™ and Veridium™ in cattle under field conditions at Nguruman, Kenya. *Acta Trop.* **77**: 195-201.

Thrusfield, M.V. (1995): Veterinary Epidemiology. 2nd edn. Blackwell Science, Oxford, pp. 183.

Trail, J.C.M., Sones, K., Jibbo, J.M.C., Durkin, J., Light, D.E. and M. Murray (1985): Productivity of Boran cattle maintained by chemo-prophylaxis under trypanosomiasis risk. ILCA Research Report No. **9**, ILCA, Addis Ababa, Ethiopia, pp. 76.

Uilenberg, G. (1997): A field guide for diagnosis, treatment and prevention of African animal trypanosomosis. Adapted from the original edition by W.P. Boyt, FAO, Rome.

Van den Bossche, P., Doran, M. and R. J. Connor (2000): An analysis of trypanocidal drug use in the Eastern Province of Zambia. *Acta Trop.* **75**: 247-258.

Van den Bossche, P. and G.J. Rowlands (2001): The relationship between the parasitological prevalence of trypanosomal infections in cattle and heard average packed cell volume. *Acta Trop.* **78**: 163-170.

Weitz, B. (1963): The feeding habits of *Glossina*. *Bull. World Hlth. Organ.* **28**, 711-729.

Wellde, B. T., Chumo, D. A., Adoyo, M., Kovatch, R. M., Mwongela, G. N. and E. A. Opiyo (1983): Haemorrhagic syndrome in cattle associated with *Trypanosoma vivax* infection. *Trop. Anim. Hlth. Prod.* **15**, 95-102.

Whitelaw, D.D., Bell, I.R., Holmes, P.H., Moloo, S.K., Hirumi, H., Urquhart, G.M. and M. Murray (1986): Isometamidium chloride prophylaxis against *Trypanosoma congolense* challenge and the development of immune responses in Boran cattle. *Vet. Rec.* **118**, 722-726.

Whiteside, E.F. (1960): Recent work in Kenya on the control of drug-resistant cattle trypanosomiasis. In: International Scientific Council for Trypanosomiasis Research and Control, Eighth Meeting, Jos. Nigeria. Publication No. **62**. Commission for Technical Co-operation South of the Sahara, London.

Williamson, J. (1970): Review of chemotherapeutic and chemoprophylactic agents. In: Mulligan H.W. (ed.): The African trypanosomes. London: George Allen and Unwin LTD. pp. 125-221.

WHO (1986): Epidemiology and control of African trypanosomiasis. Report of WHO Expert Committee, World Health Organization Technical Report Series 739, Geneva, Switzerland.

8. ANNEXES

Annex I. Questionnaire for interviewing individual farmers

1. Farmers identity and location

Date.....Name of farmer.....
Age.....Peasant Association.....
Village.....

2. Herd composition

2.1. Do you have any animal? (Yes, No)

2.2. If yes, which species and number?
Cattle.....
Sheep and goats.....
Equines.....
Camels.....
Others.....

3. Major health problems

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

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

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

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

3.5. When did trypanosomosis start to occur in this area?
.....

3.6. Is trypanosomosis getting worse, better or unchanged in this area since you first encountered it in the area?

It is getting worse.....
It is getting better.....
It is the same .....
I do not know .....

3.7. How do you think is trypanosomosis transmitted?

.....

3.8. In which season/month does trypanosomosis most occur?

.....

4. Livestock management

4.1. How do you manage cattle?

Free grazing .....
Tether .....
Stall feed .....

4.2. Where do cattle graze?

.....

4.3. Where is the location of livestock watering point?

.....

4.4. In which season/month of the year is livestock feed most available?

.....

4.5. In which season is it least available?

.....

5. Socio-economic activities

5.1. What are your main sources of income?

.....

5.2. What is the importance of keeping cattle?

For milk production .....
For meat production .....
For draught power .....
For manure production .....
Paying of dowry .....
Othere (please spectify).....

6. Sources and usage of trypanocidal drugs and suspected drug failures

6.1. Where do you commonly treat your animals when they get sick with trypanosomosis?

At home .....
In Veterinary clinic .....

Other (specify) .....

6.2. Where are the common treatment sources?

Veterinary Clinic .....
Local farmers .....
Smugglers .....
Others (specify) .....

6.3 Who is applying the treatment?

You yourself? .....
Veterinarian or assistant veterinarian .....
Animal health technician .....
Drug Smugglers .....
Others(specify) .....

6.4 Which trypanocidal drugs are you commonly using to treat your animals? (names/types/color etc.).....
.....

6.5. What quantity of each trypanocidal drugs do you use to treat your cattle?

6.5.1. If Berenil®

1 sachet for 1 adult cow.....
1 sachet for more than 2 adult cow.....
No idea.....

6.5.2. If Ethidium:

1 tablet for 1 adult cow.....
1 tablet for more than 2 adult cow.....
No idea.....

6.5.3. If Trypamidium:

1sachet for 10 adult cows.....
1 sachet for 15 adult cows.....
1 sachet for 20 adult cows.....
No idea.....

6.6. Since when have you been using each of these drugs?

Since the last 20 years.....
Since the last 10 years.....
Since the last 5 years.....

6.7. How much money do you pay to get a single mature oxen treated?
.....

6.8. How many times did each animals get veterinary treatment against trypanosomosis since last year?

One time only..... .....
Two times..... .....
Three times..... .....
More than three times.....

6.9. Can you tell how much expense is incurred in payment for treatment against trypanosomosis since last year?

.....

6.10. Of the cattle treated last time:

How many are healthy at present?.....

When were these animals lastly treated?.....

Calculate days between treatment..... .....

6.11. How many cattle have you lost due to trypanosomosis since last year?

6.12. Do you have any trypanocidal medicine now in stock? (Yes, No)

If yes, can you show please?..... .....

How many months have elapsed since you acquire it? .....

How do you use it?..... .....

6.13. Which drugs, do you think, are most effective to treat your animals against trypanosomosis?.....

6.14. Which ones are less effective?.....

6.15. When you use trypanocidals on cattle do you usually treat:

All your animals .....

Only sick animals .....

Only mature oxen .....

Only cows in milk.....

Others(specify)..... .....

6.16. Do you think that the problem of trypanosomosis is expanding to new areas? (Ys, No, We do not know)

If yes, what are the new areas affected?

.....

6.17. Was there cure after these treatments? (Ys, No, I do not know)

6.18. If not, what do you think is the reason?

.....

Thank You!

Name of interviewer .....

Date .....

Signature .....

Annex II. Parasitological results

Trypanosome species diagnosed and mean PCV values in the control and isometamidium treated (1mg/kg bw) group of cattle at each Village from day 0 to 84. All cattle were treated with diminazene aceturate (7mg/kg bw) 14 prior to start of isometamidium block treatment study and thereafter whenever found infected at day 0-84.

Survey round	Site	Group	No of animals	Infection rate (%)	Trypanosome spp				Mean PCV (%)
					Tv	Tc	Tb	Mixed	
Day 0	Cheleleki	Control	49	0.0	0	0	0	0	25.2
		Treatment	49	0.0	0	0	0	0	25.6
	Total		*98*	*0.0*	*0*	*0*	*0*	*0*	25.4
	Kolu	Control	49	0.0	0	0	0	0	23.0
		Treatment	50	0.0	0	0	0	0	23.2
	Total		*99*	*0.0*	*0*	*0*	*0*	*0*	23.1
	Burka	Control	50	0.0	0	0	0	0	23.9
		Treatment	50	0.0	0	0	0	0	23.2
	Total		*100*	*0.0*	*0*	*0*	*0*	*0*	23.6
Day +14	Cheleleki	Control	46	8.7	2	2	0	0	25.8
		Treatment	48	2.1	1	0	0	0	25.0
	Total		*94*	*5.3*	*3*	*2*	*0*	*0*	25.4
	Kolu	Control	45	13.3	2	4	0	0	25.4
		Treatment	48	2.1	0	1	0	0	26.1
	Total		*93*	*7.5*	*2*	*5*	*0*	*0*	25.7
	Burka	Control	45	22.2	9	1	0	0	24.2
		Treatment	50	6.0	0	3	0	0	26.6
	Total		*95*	*13.7*	*9*	*4*	*0*	*0*	25.5
Day +28	Cheleleki	Control	49	16.3	2	6	0	0	23.5
		Treatment	49	4.1	0	2	0	0	25.6
	Total		*98*	*10.2*	*2*	*8*	*0*	*0*	24.6
	Kolu	Control	46	6.5	1	1	0	1	23.3
		Treatment	44	0.0	0	0	0	0	25.0
	Total		*90*	*3.3*	*1*	*1*	*0*	*1*	24.1
	Burka	Control	46	10.9	1	0	2	2	23.8
		Treatment	48	12.5	0	5	0	1	26.0
	Total		*94*	*11.7*	*1*	*5*	*2*	*3*	24.9
Day +42	Cheleleki	Control	47	21.3	4	2	1	3	23.1
		Treatment	48	2.1	0	0	0	1	25.4
	Total		*95*	*11.6*	*4*	*2*	*1*	*4*	24.2
	Kolu	Control	50	8	0	3	1	0	24.2
		Treatment	41	14.6	0	6	0	0	25.4
	Total		*91*	*11.0*	*0*	*9*	*1*	*0*	24.7
	Burka	Control	45	11.1	1	2	1	1	27.0
		Treatment	47	14.9	0	7	0	0	27.3
	Total		*92*	*13.0*	*1*	*9*	*1*	*1*	27.2
Day +56	Cheleleki	Control	45	17.8	4	3	0	1	25.2
		Treatment	49	14.3	2	1	3	1	26.2
	Total		*94*	*16.0*	*6*	*4*	*3*	*2*	*25.8*
	Kolu	Control	50	20.0	3	7	0	0	24.6
		Treatment	42	11.9	2	3	0	0	25.8
	Total		*92*	*16.3*	*5*	*10*	*0*	*0*	*25.1*
	Burka	Control	46	10.9	4	0	0	1	25.9
		Treatment	47	17.0	7	1	0	0	27.0
	Total		*93*	*14.0*	*11*	*1*	*0*	*1*	*26.5*
Day +70	Cheleleki	Control	49	22.4	3	5	1	2	23.6
		Treatment	44	13.6	4	1	0	1	25.7
	Total		*93*	*18.3*	*7*	*6*	*1*	*3*	*24.6*
	Kolu	Control	50	6.0	0	3	0	0	27.1
		Treatment	41	7.3	0	2	1	0	26.5
	Total		*91*	*6.6*	*0*	*5*	*1*	*0*	*26.8*

Survey round	Site	Group	No of animals	Infection rate (%)	Trypanosome spp				Mean PCV (%)
					Tv	Tc	Tb	Mixed	
	Burka	Control	46	13.0	3	3	0	0	28.2
		Treatment	46	17.4	5	2	1	0	28.5
Total			*92*	*15.2*	*8*	*5*	*1*	*0*	*28.3*
Day +84	Cheleleki	Control	49	10.2	2	3	0	0	23.9
		Treatment	44	9.1	1	3	0	0	25.2
Total			*93*	*9.7*	*3*	*6*	*0*	*0*	*24.5*
	Kolu	Control	50	6.0	0	3	0	0	26.5
		Treatment	40	7.5	1	2	0	0	25.9
Total			*90*	*6.7*	*1*	*5*	*0*	*0*	*26.2*
	Burka	Control	44	2.3	1	0	0	0	26.0
		Treatment	46	4.3	1	1	0	0	27.3
Total			*90*	*3.3*	*2*	*1*	*0*	*0*	*26.7*

Annex III. Isometamidium block treatment study

Control group of cattle at Cheleleki parasitaemic during day 0-84 of isometamidium block treatment study. All cattle were treated with diminazene aceturate (7mg/kg bw) 14 prior to isometamidium block treatment study and, thereafter, whenever found infected with trypanosomes at day 0-84.

Group	Tag No	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84
Control	84	Neg.	NA	Neg.	NA	NA	Neg.	Neg.
Control	78	Neg.	NA	TC	Neg.	Neg.	Neg.	Neg.
Control	82	Neg.	NA	Neg.	Neg.	NA	Neg.	Neg.
Control	52	Neg.	NA	Neg.	TV	Neg.	Neg.	Neg.
Control	98	NA	Neg.	Neg.	NA	NA	Neg.	Neg.
Control	42	Neg.	NA	Neg.	Neg.	Neg.	Neg.	Neg.
Control	86	Neg.	Neg.	NA	NA	NA	Neg.	Neg.
Control	92	Neg.	Neg.	Neg.	NA	NA	Neg.	Neg.
Control	38	Neg.	Neg.	Neg.	Neg.	Neg.	NA	NA
Control	6	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	14	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	16	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	18	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	20	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	24	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	28	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	48	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	76	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	88	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	90	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	30	Neg.	TV	Neg.	Neg.	TV	Neg.	Neg.
Control	66	Neg.	TC	Neg.	Neg.	Neg.	Neg.	Neg.
Control	62	Neg.	TV	Neg.	TV	Neg.	TV+TB	Neg.
Control	80	Neg.	TC	Neg.	TV+TC	Neg.	TV	Neg.
Control	100	Neg.	Neg.	TV	Neg.	TC+TB	Neg.	TC
Control	8	Neg.	Neg.	TC	Neg.	Neg.	Neg.	Neg.
Control	10	Neg.	Neg.	TC	Neg.	Neg.	Neg.	Neg.
Control	36	Neg.	Neg.	TC	Neg.	Neg.	Neg.	Neg.
Control	50	Neg.	Neg.	TC	Neg.	Neg.	Neg.	Neg.
Control	70	Neg.	Neg.	TC	Neg.	TC	Neg.	Neg.
Control	72	Neg.	Neg.	TV	Neg.	TV	Neg.	Neg.
Control	60	Neg.	Neg.	Neg.	TB	Neg.	Neg.	Neg.
Control	4	Neg.	Neg.	Neg.	TB+TV	Neg.	Neg.	TV
Control	40	Neg.	Neg.	Neg.	TC	Neg.	TC	Neg.
Control	94	Neg.	Neg.	Neg.	TC	Neg.	Neg.	Neg.
Control	68	Neg.	Neg.	Neg.	TV	Neg.	Neg.	TV
Control	26	Neg.	Neg.	Neg.	TV	Neg.	Neg.	TC
Control	22	Neg.	Neg.	Neg.	TV+TB	Neg.	Neg.	Neg.
Control	32	Neg.	Neg.	Neg.	Neg.	TV	Neg.	TC
Control	12	Neg.	Neg.	Neg.	Neg.	TC	Neg.	Neg.
Control	54	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Control	96	Neg.	Neg.	Neg.	Neg.	TC	Neg.	Neg.
Control	2	Neg.	Neg.	Neg.	Neg.	Neg.	TV	Neg.
Control	34	Neg.	Neg.	Neg.	Neg.	Neg.	TV+TB	Neg.
Control	44	Neg.	Neg.	Neg.	Neg.	Neg.	TC	Neg.
Control	46	Neg.	Neg.	Neg.	Neg.	Neg.	TC	Neg.
Control	56	Neg.	Neg.	Neg.	Neg.	Neg.	TC	Neg.
Control	58	Neg.	Neg.	Neg.	Neg.	Neg.	TB	Neg.
Control	64	Neg.	Neg.	Neg.	Neg.	Neg.	TC	Neg.
Control	74	Neg.	Neg.	Neg.	Neg.	Neg.	TV	Neg.

Isometamidium treated (1mg/kg bw) group of cattle at Cheleleki parasitaemic during day 0-84 after the start of isometamidium block treatment study. All cattle were treated with diminazene aceturate (7mg/kg bw) 14 prior to isometamidium block treatment study and, thereafter, whenever found infected with trypanosomes at day 0-84.

Group	Tag No	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84
Treatment	47	Neg.	NA	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	31	Neg.	NA	Neg.	Neg.	Neg.	NA	NA
Treatment	89	Neg.	Neg.	NA	NA	NA	Neg.	Neg.
Treatment	15	Neg.	Neg.	Neg.	Neg.	Neg.	NA	NA
Treatment	21	Neg.	Neg.	Neg.	Neg.	Neg.	NA	NA
Treatment	51	Neg.	Neg.	Neg.	Neg.	Neg.	NA	NA
Treatment	63	Neg.	Neg.	Neg.	Neg.	Neg.	NA	NA
Treatment	73	Neg.	Neg.	Neg.	Neg.	Neg.	NA	NA
Treatment	3	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	9	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	13	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	29	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	33	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	35	NA	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	37	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	39	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	41	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	43	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	49	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	55	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	59	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	61	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	65	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	67	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	69	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	71	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	77	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	81	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	83	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	91	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	93	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	99	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	95	Neg.	TV	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	87	Neg.	Neg.	TC	Neg.	Neg.	Neg.	TC
Treatment	53	Neg.	Neg.	TC	Neg.	Neg.	TC	Neg.
Treatment	17	Neg.	Neg.	Neg.	TV+TB	Neg.	TV+TB	Neg.
Treatment	1	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Treatment	5	Neg.	Neg.	Neg.	Neg.	TB	Neg.	Neg.
Treatment	7	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Treatment	19	Neg.	Neg.	Neg.	Neg.	TB+TC	Neg.	Neg.
Treatment	23	Neg.	Neg.	Neg.	Neg.	TB	Neg.	Neg.
Treatment	25	Neg.	Neg.	Neg.	Neg.	Tb	Neg.	Neg.
Treatment	85	Neg.	Neg.	Neg.	Neg.	TC	Neg.	Neg.
Treatment	45	Neg.	Neg.	Neg.	Neg.	Neg.	TV	Neg.
Treatment	57	Neg.	Neg.	Neg.	Neg.	Neg.	TV	Neg.
Treatment	79	Neg.	Neg.	Neg.	Neg.	Neg.	TV	Neg.
Treatment	97	Neg.	Neg.	Neg.	Neg.	Neg.	TV	Neg.
Treatment	27	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	TV
Treatment	11	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	TC
Treatment	75	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	TC

Control group of cattle at Kolú parasitaemic during day 0-84 of isometamidium block treatment study. All cattle were treated with diminazene aceturate (7mg/kg bw) 14 prior to isometamidium block treatment study and, thereafter, whenever found infected with trypanosomes at day 0-84.

Group	Tag No	Day 0	Day 14	Day 28	day 42	day 56	Day 70	Day 84
Control	191	Neg.	NA	NA	Neg.	Neg.	Neg.	Neg.
Control	193	Neg.	NA	Neg.	Neg.	Neg.	Neg.	Neg.
Control	195	Neg.	NA	Neg.	Neg.	Neg.	Neg.	Neg.
Control	198	Neg.	NA	Neg.	Neg.	Neg.	Neg.	Neg.
Control	199	NA	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	200	Neg.	NA	Neg.	Neg.	Neg.	Neg.	Neg.
Control	108	Neg.	Neg.	NA	Neg.	Neg.	Neg.	Neg.
Control	176	Neg.	Neg.	NA	Neg.	Neg.	Neg.	Neg.
Control	187	Neg.	Neg.	NA	Neg.	Neg.	Neg.	Neg.
Control	101	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	102	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	104	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	112	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	122	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	128	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	136	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	142	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	143	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	151	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	152	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	155	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	157	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	158	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	159	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	164	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	165	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	167	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	181	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	183	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	103	Neg.	TC	Neg.	Neg.	Neg.	Neg.	Neg.
Control	115	Neg.	TC	Neg.	Neg.	Neg.	Neg.	TC
Control	127	Neg.	TC	Neg.	Neg.	Neg.	Neg.	Neg.
Control	153	Neg.	TC	Neg.	Neg.	TC	Neg.	TC
Control	160	Neg.	TV	Neg.	Neg.	Neg.	Neg.	Neg.
Control	197	Neg.	TV	Neg.	Neg.	TC	Neg.	Neg.
Control	109	Neg.	Neg.	TC	Neg.	TC	Neg.	Neg.
Control	121	Neg.	Neg.	TV	Neg.	TV	Neg.	Neg.
Control	162	Neg.	Neg.	TC	Neg.	TC	Neg.	Neg.
Control	182	Neg.	Neg.	TV+TC	Neg.	TC	Neg.	Neg.
Control	114	Neg.	Neg.	Neg.	TB	Neg.	Neg.	Neg.
Control	118	Neg.	Neg.	Neg.	TC	Neg.	Neg.	Neg.
Control	123	Neg.	Neg.	Neg.	TC	Neg.	TC	Neg.
Control	139	Neg.	Neg.	Neg.	TC	Neg.	Neg.	Neg.
Control	107	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Control	141	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Control	147	Neg.	Neg.	Neg.	Neg.	TC	Neg.	Neg.
Control	186	Neg.	Neg.	Neg.	Neg.	TC	Neg.	Neg.
Control	161	Neg.	Neg.	Neg.	Neg.	Neg.	TC	Neg.
Control	185	Neg.	Neg.	Neg.	Neg.	Neg.	TC	Neg.
Control	110	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	TC

Isometamidium treated (1mg/kg bw) group of cattle at Kolu parasitaemic during day 0-84 after the start of isometamidium block treatment study. All cattle were treated with diminazene aceturate (7mg/kg bw) 14 prior to isometamidium block treatment study and, thereafter, whenever found infected with trypanosomes at day 0-84.

Group	Tag No	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84
Treatment	106	Neg.	Neg.	NA	NA	NA	NA	TV
Treatment	126	Neg.	Neg.	NA	NA	NA	NA	NA
Treatment	129	Neg.	Neg.	NA	NA	NA	NA	Neg.
Treatment	140	Neg.	Neg.	NA	NA	Neg.	NA	Neg.
Treatment	146	Neg.	Neg.	NA	NA	NA	NA	NA
Treatment	190	Neg.	Neg.	NA	NA	NA	NA	NA
Treatment	135	Neg.	Neg.	Neg.	NA	NA	NA	NA
Treatment	163	Neg.	Neg.	Neg.	NA	Neg.	NA	TC
Treatment	170	Neg.	Neg.	Neg.	NA	Neg.	Neg.	NA
Treatment	116	Neg.	Neg.	Neg.	Neg.	NA	Neg.	NA
Treatment	171	Neg.	Neg.	Neg.	Neg.	NA	Neg.	NA
Treatment	117	Neg.	Neg.	Neg.	Neg.	Neg.	NA	Neg.
Treatment	172	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	NA
Treatment	188	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	NA
Treatment	105	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	119	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	120	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	124	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	125	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	130	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	133	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	138	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	144	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	145	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	148	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	149	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	150	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	166	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	168	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	169	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	173	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	174	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	178	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	179	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	184	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	192	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	196	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	194	Neg.	TC	Neg.	TC	Neg.	Neg.	Neg.
Treatment	113	Neg.	Neg.	Neg.	TC	Neg.	Neg.	Neg.
Treatment	132	Neg.	Neg.	Neg.	TC	Neg.	Neg.	Neg.
Treatment	134	Neg.	Neg.	Neg.	TC	Neg.	Neg.	NA
Treatment	154	Neg.	Neg.	Neg.	TC	TC	Neg.	Neg.
Treatment	180	Neg.	Neg.	Neg.	TC	Neg.	Neg.	Neg.
Treatment	111	Neg.	Neg.	Neg.	Neg.	TC	Neg	Neg.
Treatment	131	Neg.	Neg.	Neg.	Neg.	TC	TC	Neg.
Treatment	156	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Treatment	175	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg
Treatment	137	Neg.	Neg.	Neg.	Neg.	Neg.	TB	Neg.
Treatment	189	Neg.	Neg.	Neg.	Neg.	Neg.	TC	Neg
Treatment	177	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	TC

Control group of cattle at Burka parasitaemic during day 0-84 of isometamidium block treatment study. All cattle were treated with diminazene aceturate (7mg/kg bw) 14 prior to isometamidium block treatment study and, thereafter, whenever found infected with trypanosomes at day 0-84.

Group	Tag No	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84
Control	291	Neg.	NA	Neg.	Neg.	Neg.	Neg.	Neg.
Control	297	Neg.	NA	Neg.	Neg.	Neg.	Neg.	Neg.
Control	299	Neg.	NA	NA	NA	NA	NA	NA
Control	300	Neg.	NA	Neg.	Neg.	Neg.	Neg.	Neg.
Control	230	Neg.	Neg.	NA	NA	NA	NA	NA
Control	290	Neg.	Neg.	NA	NA	Neg.	Neg.	NA
Control	273	Neg.	Neg.	Neg.	NA	NA	NA	NA
Control	276	Neg.	Neg.	Neg.	Neg.	NA	NA	NA
Control	202	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	205	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	207	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	213	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	224	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	233	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	234	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	246	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	249	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	250	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	253	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	254	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	257	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	259	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	265	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	271	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	280	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	287	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	289	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	294	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	298	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Control	208	Neg.	TV	Neg.	TC	Neg.	TV	Neg.
Control	209	Neg.	TV	Neg.	Neg.	Neg.	Neg.	Neg.
Control	211	Neg.	TV	Neg.	TB+TV	Neg.	TV	Neg.
Control	216	Neg.	TV	Neg.	Neg.	Neg.	Neg.	Neg.
Control	223	Neg.	TV	Neg.	Neg.	Neg.	Neg.	Neg.
Control	241	Neg.	TV	NA	NA	Neg.	Neg.	NA
Control	252	Neg.	TV	Neg.	Neg.	Neg.	Neg.	Neg.
Control	264	Neg.	TV	Neg.	Neg.	Neg.	TV	Neg.
Control	270	Neg.	TV	Neg.	Neg.	Neg.	TC	Neg.
Control	272	Neg.	TV	Neg.	Neg.	Neg.	Neg.	Neg.
Control	277	Neg.	TC	Neg.	TC	Neg.	Neg.	Neg.
Control	214	Neg.	Neg.	TB	Neg.	Neg.	Neg.	Neg.
Control	226	Neg.	Neg.	TV	Neg.	TV	Neg.	Neg.
Control	263	Neg.	Neg.	TB+TV	Neg.	TV+TB	Neg.	Neg.
Control	267	Neg.	Neg.	TB+TV	Neg.	TV	Neg.	TV
Control	282	Neg.	Neg.	TB	Neg.	TV	Neg.	Neg.
Control	235	Neg.	Neg.	Neg.	TB	Neg.	Neg.	Neg.
Control	279	Neg.	Neg.	Neg.	TV	Neg.	Neg.	Neg.
Control	262	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Control	256	Neg.	Neg.	Neg.	Neg.	Neg.	TC	Neg.
Control	260	Neg.	Neg.	Neg.	Neg.	Neg.	TC	Neg.

Isometamidium treated (1mg/kg bw) group of cattle at Burka parasitaemic during day 0-84 after the start of isometamidium block treatment study. All cattle were treated with diminazene aceturate (7mg/kg bw) 14 prior to isometamidium block treatment study and, thereafter, whenever found infected with trypanosomes at day 0-84.

Group	Tag No	Day 0	Day 14	Day 28	Day 42	Day 56	Day 70	Day 84
Treatment	219	Neg.	Neg.	NA	NA	Neg.	Neg.	NA
Treatment	243	Neg.	Neg.	NA	TC	NA	NA	NA
Treatment	244	Neg.	Neg.	Neg.	NA	Neg.	Neg.	Neg.
Treatment	269	Neg.	Neg.	Neg.	NA	Neg.	NA	Neg.
Treatment	218	Neg.	Neg.	Neg.	Neg.	NA	NA	NA
Treatment	242	Neg.	Neg.	Neg.	Neg.	NA	NA	NA
Treatment	201	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	203	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	206	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	212	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	215	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	217	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	221	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	229	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	231	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	232	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	239	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	251	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	255	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	258	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	268	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	275	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	281	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	285	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	292	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	296	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Treatment	237	Neg.	TC	TC	Neg.	Neg.	Neg.	Neg.
Treatment	261	Neg.	TC	TC	Neg.	Neg.	Neg.	Neg.
Treatment	278	Neg.	TC	TC	TC	TC	Neg.	TC
Treatment	220	Neg.	Neg.	TC	Neg.	Neg.	Neg.	Neg.
Treatment	227	Neg.	Neg.	TC	Neg.	Neg.	TC	Neg.
Treatment	295	Neg.	Neg.	TB+TV	Neg.	Neg.	TV	Neg.
Treatment	222	Neg.	Neg.	Neg.	TC	Neg.	Neg.	Neg.
Treatment	245	Neg.	Neg.	Neg.	TC	Neg.	Neg.	Neg.
Treatment	266	Neg.	Neg.	Neg.	TC	Neg.	Neg.	Neg.
Treatment	283	Neg.	Neg.	Neg.	TC	Neg.	Neg.	Neg.
Treatment	284	Neg.	Neg.	Neg.	TC	Neg.	TC	Neg.
Treatment	204	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Treatment	225	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Treatment	228	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Treatment	238	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Treatment	247	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Treatment	288	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Treatment	293	Neg.	Neg.	Neg.	Neg.	TV	Neg.	Neg.
Treatment	210	Neg.	Neg.	Neg.	Neg.	Neg.	TB	Neg.
Treatment	240	Neg.	Neg.	Neg.	Neg.	Neg.	TV	Neg.
Treatment	248	Neg.	Neg.	Neg.	Neg.	Neg.	TV	Neg.
Treatment	274	Neg.	Neg.	Neg.	Neg.	Neg.	TV	Neg.
Treatment	286	Neg.	Neg.	Neg.	Neg.	Neg.	TV	Neg.
Treatment	236	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	TV

9. CURRICULUM VITAE

Name	:	Nega Tewelde Tikue
Address	:	Ministry of Agriculture, Animal & Fisheries Resource Development Animal Health Technology and Regulatory Department; Animal Health Technology and Regulator Team, P. O. Box 62347, Addis Ababa, Ethiopia
Nationality	:	Ethiopian
Sex	:	Male
Date of birth	:	May 1962
Place of birth	:	Adwa, Tigray
Marital status	:	Maried

Education:

1970-75 Elementary school Queen Sheba Elementary School Adwa (Tigray)

1976-81 High school 7th-11th Queen Sheba Comprehensive Secondary School, Adwa (Tigray) and 12th Koladuba Sec. School (Koladuba, Gondar)

Qualification and training:

1982-87 University Graduate (DVM), Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia

International Group Training Course on Tsetse Integrated Management for Tropical Developing World, ICIPE, Nairobi, Kenya, 2-28 November 1992.

Regional Training Course on Field Data Management in Tsetse and Trypanosomosis Control, GIS and Global Positioning System (GPS) FAO/SADC, Lusaka, Zambia, 22 March to 6 April 1995.

Joint FAO/IAEA Epidemiology Workshop on the Monitoring of Trypanosomosis and Tsetse Control Programme, ILRI, Addis Ababa, Ethiopia, 17-28 April 1995.

Personal training plan in ELISA & Related Topic: Enzyme Immunoassays; Card Agglutination Test (CATT); Fluorescent Antibody Tests, Immunoblotting including SDS-PAGE and Western Blotting at the University of Edinburgh, Centre for Tropical Veterinary Medicine, Edinburgh, Scotland from 1st April to 21st June 1996.

Work experience:

From June 1 – January 31 1999, worked as National Project Co-ordinator of the Farming in Tsetse Control Areas (FITCA), a 5.6 million EURO and five-year project of the EU (an East African Regional Programme involving Ethiopia, Kenya, Uganda and Tanzania and coordinated through the OAU/IBAR in Nairobi, Kenya).

1997-99 worked as a task force in the formulation of the FITCA project.

Since February 1998, Senior Veterinarian at the Ministry of Agriculture, Animal Health Technology and Regulator Team, Addis Ababa.

I worked at the Epidemiology Unit of the Federal Veterinary Services Team as an expert from August 07/08/97 up to 21/10/1997.

1993 to January 1998 Technical Coordinator of the NTTICC and Chief Scientific Investigator of the Research Contract and TC project 7820/R1/NL; ETH/5/010 of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (Bedelle)

1989-92 Junior Research Officer (NTTICC, Bedelle)

1988 Provincial Veterinary Officer (Gore, Illubabor)

Paper Writing & Publication:

The use of Ag-ELISA to monitor the effectiveness of a tsetse control campaign in the upper Didesa valley, Western Ethiopia. Proceedings of the workshop on epidemiological tools for monitoring trypanosomosis and tsetse control programmes organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and the IAEA Department of Technical Cooperation held in Addis Ababa, Ethiopia, 17-28 April 1995.

An overview of the activities of the NTTICC. Proceedings of the 8th Conference of the Ethiopian Veterinary Association, Addis Ababa, 1994.

Unpublished research works:

Productivity of sheep and goat under natural tsetse challenge in the upper Didessa valley, Western Ethiopia.

Study on the ecology of tsetse and epidemiology of trypanosomosis along Arjo escarpment in the upper Didessa valley, Western Ethiopia.

Efficiency test on odour baited and unbaited biconical traps in the upper Didessa valley, Western Ethiopia. A method for tsetse control. Paper presented to the Addis Ababa University, Faculty of Veterinary Medicine in partial fulfilment of the requirement for DVM degree, June 1987.

Consultations:

I have consulted the following NGOs on the possibility of tsetse and trypanosomosis control on various occasions from 1989-97. SIM (South Omo), Irish *CONCERN* (Keto, Western Wollega), FHI (Western Shoa), SOS Sahel (North Omo), ORA (Western Wollega), Agriservice (North Omo).

Advice and technical assistance was provided to undergraduate students of the Faculty of Veterinary Medicine attached to the NTTICC from 1991-97 to conduct their research work as partial fulfilment of the requirement for DVM degree.

Computer skills:

Microsoft (Word, Excel, Access, Power Point, Outlook, Binder), Quatro Pro for Win, Minitab for Win, Map Info for Win, IDRISI, Epi Info 6, Epi Map, Epi Stat, Panacea, Network Administration (LAN), Programme installation & troubleshooting, Computer parts replacement (CDROM Drive, Floppy Disk Drive, Tape Drive, Network card, Sound card, RAM).

10. SIGNED DECLARATION SHEET

I the under signed, declare that the thesis is my original work and has not been presented for a degree in any university.

Name NEGA TEWELDE TIKUE

Signature .....

Date of submission 21.12.2001,

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

Dr. Peter-Henning Clausen .....

Dr. Getachew Abebe .....

Dr. Yohannes Afewerk .....

2001
NEG/1737

Stduy on the occurrence of drug Resistant Trypanosomes in cattle in cattile in the farming in tsetste control Areas(Fitca)

Nega Tewelde

C-1