ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
AND
FREIE UNIVERSITAT BERLIN
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FIELD INVESTIGATION ON THE APPEARANCE OF DRUG-RESISTANT POPULATIONS OF TRYPANOSOMES IN METEKEL DISTRICT, NORTH-WEST ETHIOPIA

YOHANNES AFEWERK

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ADDIS ABABA UNIVERSITY
Faculty of Veterinary Medicine

FREIE UNIVERSITÄT BERLIN
Fachbereich Veterinärmedizin

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YOHANNES AFEWERK

Board of Examiners:

Dr. Yilma M. _____

Prof. Dr. Hörchner _____

_____

Academic Advisors:

Dr. Clausen _____

Dr. Getachew T. _____

_____

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BW	Body weight
CCT	Capillary Centrifugation Technique
CNS	Central Nervous System
CD	Curative Dose
DIIT	Drug Incubation Infectivity Test
ED	Effective Dose
EDTA	Ethylenediamine Tetra Acetate
ELISA	Enzyme Linked Immuno Sorbent Assay
FAO	Food and Agricultural Organisation of the United Nations
HCT	Haematocrit Centrifugation Technique
I. V.	Intra Venous
I. P.	Intra Peritoneal
ILRI	International Livestock Research Institute
g	Gram
m	micro
NGO	Non Governmental Organisation
m-AECT	Miniature Anion Exchange Centrifugation Technique
MCD	Minimum Curative Dose
MOA	Ministry of Agriculture
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PSG	Phosphate Buffer Saline Glucose
RBC	Red Blood Cells
SAS	Statstical Analysis System

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ABSTRACT

The objectives of the present study were (1) to determine the prevalence rate of trypanosome infections in the village cattle populations of Metekel district, North-west Ethiopia, (2) to assess the prophylactic activity of isometamidium chloride to natural trypanosome infections and (3) to monitor the trypanocidal activity of diminazene aceturate and isometamidium chloride in mice experimentally infected with trypanosome field isolates.

In order to obtain information on the prevalence rate of trypanosome infections in the area and to identify cattle herds highly suspected to be infected with drug resistant trypanosome populations, questionnaire surveys and cross sectional studies were carried out from March to October 1997 in four villages. The results of these studies showed that trypanosomosis is a major constraint to livestock production in the area with an overall prevalence rate of 17.2% (95% CI: 14, 20.8). *Trypanosoma congolense* was the dominant species accounting for 47.6% of the overall infections. The overall mean PCV value of the total cattle tested was found to be 24.9 ± 0.20%. Parasitaemic cattle had significantly lower mean PCV values (21.6 ± 0.47) than aparasitaemic cattle (25.5±0.21) ($p<0.05$). Moreover, there is indiscriminate use of trypanocidal drugs and exposure of cattle in the area to subcurative doses of trypanocidal drugs and farmers complaine about failure of treatment to cure infections in cattle.

Based on the results of the cross sectional study and the questionnaire, two villages were selected for longitudinal field study on the occurrence of drug resistant trypanosome populations. From these villages 50 Zebu cattle naturally infected with trypanosomes, 52.4% of the infections being due to *T. congolense*, were selected and treated with a prophylactic dose (1 mg/kg bw) of isometamidium chloride (Trypamidium ®, Lot No. U6962/E, Rhone Merieux). The findings in the field demonstrated 6 (13%) relapse/breakthrough infections, all of them being *T. congolense*, within 4 weeks of treatment. In the same study 18 (37.9%) and 25 (50%) relapse/breakthrough infections were recorded within 8 and 12 weeks of treatment, respectively. The result also indicated that 20 cases (80%) of the overall relapse/breakthrough infections were due to *T. congolense*.

Field isolates were obtained from relapse/breakthrough infections one, two and three months after treatment and injected in mice. To confirm the field results and to study the therapeutic activity of diminazene aceturate in experimental animals, three field isolates of *T. congolense* were randomly selected from the relapse populations. Investigations were conducted on the sensitivity of these isolates to isometamidium chloride (Trypamidium ®, Rhone Merieux) and diminazene aceturate (Berenil ®, Hoechst). Mice infected and treated with ranges of doses of isometamidium chloride and diminazene aceturate were followed for relapse infections. Isometamidium chloride at doses of 0.5 to 4 mg/kg bw failed completely to cure *T. congolense* infections. Similarly, diminazene aceturate at doses of 3.5 to 28 mg/kg bw did not clear the parasites in all of the mice infected. There was a correlation of drug-dosage used and the time of relapse; mice treated with lower doses showed relapses earlier than mice treated with higher doses.

Based on these studies it is concluded that the duration of prophylactic activity of isometamidium chloride (1 mg/kg bw) to trypanosome populations circulating in the study cattle of Metekel region is less than 1 month. *Trypanosoma congolense* field isolates expressed resistance to both isometamidium chloride and diminazene aceturate in mice. However, it was not known whether this double resistance was expressed by individual

trypanosome populations or by two different populations each of which expressing resistance to one of the drugs.

The results indicated that there is an urgent need to extend and intensify field and laboratory works to monitor the development of drug resistance of pathogenic trypanosomes and its impact on livestock productivity in Metekel region in particular and across the tsetse infested zone of Ethiopia in general.

1. INTRODUCTION

Trypanosomosis transmitted by tsetse flies (*Glossina* spp.) is believed to be the most important infectious disease holding back development of livestock production in Africa. At present it is estimated that approximately 60 million cattle are at risk of infection (FAO, 1991). Economic losses due to trypanosomosis are estimated to US $ 2 billion per year (ILRI, 1996). Ten million square kilometres of the continent are infested with tsetse flies of which approximately two thirds of the land could otherwise be suitable for livestock and/or mixed agricultural development (World Animal Review, 1983; ILRI, 1995), and approximately 30% of the 150 million cattle in this area are at risk of infection (Holmes, 1991). Thus, if control of trypanosomosis could be achieved there would be the potential, even at the current low rates of production, to increase meat supply of the region by approximately 16% and milk supply by 17%, of the current production levels (De Haan and Bekure, 1991). In addition to the impact of trypanosomosis on livestock production, 45 million people in Africa are estimated to be at risk of sleeping sickness (UNDP/World Bank/WHO, 1983). Sleeping sickness or human African trypanosomosis is still present in many African countries. An alarming upsurge in sleeping sickness has been reported recently in sub-Saharan Africa where at least 250,000 people are estimated to be carriers of trypanosomes (WHO, 1994). It is these considerable social and economic repercussions that make control of this disease a priority operation for the development of a large part of the African continent. However, the cost of tsetse control, the lack of a field vaccine, the limited prospects of any new trypanocidal drugs appearing in the future and the relatively small numbers of trypanotolerant livestock, make reliance on the currently available trypanocidal drugs unavoidable necessity.

In Ethiopia, trypanosomosis is one of the most important diseases which contribute to the direct and indirect economic losses on livestock production. According to ILRI (1995) at least 6 million of the 30 million cattle of the country are exposed to the disease. In the tsetse infested areas of the country the most prevalent trypanosome species is *T. congolense* followed by *T. vivax*. Rowlands *et al.* (1993) reported a prevalence rate of 37% for *T. congolense* in South-west Ethiopia. A recent report by Abebe and Jobre (1996) indicated an infection rate of 58.5% for *T. congolense*, 31.2% for *T. vivax* and 3.5% for *T. brucei* in the tsetse infested areas of South-west Ethiopia. In the same report it is also indicated that 8.71% prevalence rate was recorded in the highlands (tsetse free areas) of which 99% is due to *T. vivax*.

In Ethiopia, escalating costs and other problems of initiating and maintaining tsetse control campaigns led the livestock sector to be completely reliant on the use of trypanocidal drugs to both prevention and control of the disease. According to the report of the Ministry of Agriculture (1995) 1 to 2 million doses of the trypanocidals are administered at the cost of some US $ 0.5-1 million per annum (excluding illegal and NGO drug imports), although the demand is recorded to be much higher than this supply.

Treatment and prevention of animal trypanosomosis relies essentially on three drugs, namely: Homidium (Homidium chloride-Novidium® and Homidium bromide-Ethidium®), diminazene aceturate (Berenil®) and isometamidium chloride (Samorin®, Trypamidium®). However, almost all of these trypanocides are gradually losing their efficacy due to drug resistance (Williamson, 1970). Experimental studies have demonstrated the occurrence of resistance in trypanosomes to both diminazene (Jones-Davies, 1967; Garber, 1968; Mwambu and Mayende, 1971; Gitatha, 1979; Ainanshe *et al.*, 1992) and isometamidium (Pinder and

Authie, 1984; Schönefeld *et al.*, 1987; Clausen *et al.*, 1992). This has also been confirmed by studies carried out in the Ghibe Valley, Ethiopia (Codjia *et al.*, 1993; Leak *et al.*, 1993; Peregrine *et al.*, 1994; Rowlands *et al.*, 1993). However, trypanosomes are usually not resistant to both diminazene and isometamidium at the same time. Thus, these two compounds have been termed as sanative pair (Whiteside, 1960), and in instances of resistance to one drug the application of the other drug of this sanative pair will usually control the disease. However, recent reports on multiple drug resistance in Burkina Faso (Clausen *et al.*, 1992) and Ethiopia (Codjia *et al.*, 1993) suggest that the concept of sanative pairs might no longer be valid. This indicates a very serious development which adversely affects the future of trypanosomosis control by a chemotherapeutic approach using the trypanocides available. Therefore, the distribution and degree of drug resistance has to be carefully monitored so as to work out the best possible therapeutic strategies and/or alternative control measures. As a prerequisite for such undertaking, it is necessary to first obtain information on the occurrence of infection and the disease in the field.

The objectives of this study are to

I. determine the trypanosome prevalence rate in cattle in the villages of the Metekel district, Ethiopia,
II. assess the presence of trypanosome populations resistant to isometamedium chloride in the villages of the Metekel district, North-west Ethiopia,
III. monitor the trypanocidal activity of diminazene aceturate and isometamidium chloride in mice experimentally infected with *T. congolense* field isolates and to
IV. define appropriate measures for controlling drug resistance in the field.

2. LITERATURE REVIEW

2.1 African Trypanosomosis

African trypanosomes are protozoan parasites causing trypanosomosis in animals and man. They are predominantly parasites of the blood but may exist in other sites of the body such as the skin, lymph nodes or the central nervous system (CNS), where they can give rise to the distinctive sequel of trypanosome infections.

Trypanosomes belong to the genus *Trypanosoma*. Based on their difference in the course of development in their vectors this genus is divided into Stercoraria and Salivaria. Species of the first group complete development in the terminal gut and are transmitted in the faeces of the vector. Species belonging to the salivarian group complete their development in the anterior part of the digestive tract and are transmitted via the vectors' saliva. The pathogenic African trypanosomes belong to the later group.

The main pathogens in this group fall into four subgenera: *Duttonella* (species: *Trypanosoma (Duttonella) vivax; T. (D.) uniforme*); *Nannomonas* (species: *Trypanosoma (Nannomonas) congolense; T. (N.) simiae*); *Pycnomonas* (represented by single species *T. suis*) and *Trypanozoon* (species: *Trypanosoma (Trypanozoon) brucei; T. (T.) rhodesience; T.(T.) gambiense; T.(T.) evansi; T.(T.) equiperdum)* (Mulligan, 1970).

Trypanosomosis in Africa is mainly restricted to areas in which the vector, the tsetse fly (*Glossina* spp.) can survive. In the vector the trypanosome changes through several

morphological distinct stages until it reaches the metacyclic stage which is infective for mammals. However, trypanosomosis is also found in areas outside the habitat of the tsetse fly including countries outside Africa. In these areas the disease is mechanically transmitted by biting flies of genus *Tabanus, Haematopota, Chrysops, Pangonia* or *Stomoxys* species. This type of transmission has caused the spread of *T. evansi* but may also play a role in the transmission of *T. vivax,* outside the tsetse belt.

The most pathogenic trypanosomes for ruminants are *T. congolense* and *T. vivax*. *T. congolense* is responsible for the most important form of animal trypanosomosis in domestic mammals such as cattle, horses, sheep, goats, camels and pigs, as well as dogs. The manifestations of the disease vary according to the strain and host of the parasite, being generally characterised by fever, anaemia and cachexia. *T. congolense* occurs throughout the tropical regions of Africa wherever tsetse flies are present. *T. vivax* is pathogenic to various domestic ungulates (cattle, sheep, goats, horses and camels). The disease is characterised by fever, anaemia and cachexia, as well as paralysis of the hind-quarters. *T. vivax* is widely distributed throughout tropical Africa wherever its tsetse-vectors are found. This species has also been introduced into distant countries outside Africa (Mauritius, West Indies, Central and South America) (Mulligan, 1970).

Report from the tsetse infested area of Ethiopia indicated that *T. congolense* is the most prevalent trypanosome species (Abebe and Jobre, 1996; Rowlands *et al.,* 1993).

Sleeping sickness or human African trypanosomosis is present in many African countries. The chronic form caused by *T. (T.) b. gambiense* is mainly found in West and Central Africa while the acute form caused by *T. (T.) b. rhodesiense* predominantly occurs in East Africa. Neither *T. (T.) b. gambiense* nor *T. (T.) b. rhodesiense* can morphologically be distinguished from *T. (T.) b. brucei* which is not infective to man but only to animal (Schares and Mehlitz, 1996).

2.1.1 Morphology

2.1.1.1 *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, 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*. (Molyneux and Ashford, 1983).

2.1.1.2 *Trypanosoma (Duttonella) vivax* Ziemann, 1905

T. (D.) vivax Ziemann, 1905, have a mean length of 20-26 mm, a long free flagellum and a large terminally placed kinetoplast (Vickerman, 1978), distinguishing it from the other pathogenic salivarian trypanosomes. *T. vivax* is a very mobile and "lively" parasite. "Dashing

around the field of the microscope with such rapidity that it makes it impossible to follow its movements" mentioned Bruce *et al.* (1910).

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

The blood forms of *T. brucei* measure from 11-39 mm in total length. They are typically polymorphic, being represented by three forms: (a) slender forms (mean lengths 14-39 mm) 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 mm) 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 mm) 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 (Mulligan, 1970).

2.1.1.4 *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 (Mulligan, 1970).

2.1.2 Epidemiology

The epidemiology of African animal trypanosomosis is highly dependent of the parasite, vector and host factors.

Trypanosoma species occur in a remarkable variety of genotypes with differing strains virulence, immunogenity and response to chemotherapeutic agents. The severity of the disease also depends on the species and strain of trypanosomes involved. For instance, *T. vivax* and *T. congolense* are known to have high virulence in cattle. Certain isolates of *T. vivax* in East Africa cause severe, rapidly fatal disease with characteristic haemorrhagic syndrome, while *T. brucei* has high virulence in horses, donkeys and dogs.

The fact that the parasite infects not only cattle but also and even to a greater extent wild animals which constitute the reservoirs of the disease, makes the epidemiology of animal trypanosomosis extremely complicated. The animal hosts differ in their response to trypanosome infection depending on the species, breed and individual animals. The level of animal husbandry practices, nutritional status, work load and stress exacerbate the severity of the disease.

The degree of risk to which domestic animals are exposed depends on the species and density of the tsetse present, infection rate in the tsetse, species and strains of trypanosomes, sources of these infections (wildlife or domestic animals) and feeding preferences of the flies. The

extent to which the flies transmit the disease depends on the species of livestock which is their source of food (MacLennan, 1970).

2.1.2 1 Transmission and distribution

In animals, trypanosomes are either transmitted acyclically by haematophagus flies of the genus *Tabanus* (Surra, Mal de Caderas, South American trypanosomes) or cyclically by the tsetse flies. Mechanical transmission of African animal trypanosomosis may be important in some localities. Acyclically, however, the pathogen can only be carried over a short distance since it will survive only for a short time on and in the proboscis of the *Tabanidae*. In contrast, the transmission by tsetse flies is a complex mechanism in which the tsetse fly remains a lifelong carrier. Seifert (1996) summarised the transmission and interchange of hosts of African trypanosomosis which is transmitted by the tsetse flies as follows:

- The tsetse fly gets infected with the trypomastigote blood from which loses its surface coat in the goitre of the fly and, while remaining there for at least one hour, restructures its mitochondrium
- The trypanosomes enter the mid gut where they transform through lengthwise division into the epimastigote form in the cardia
- The trypanosomes penetrate the haemocoel via the peritroph membrane and the midgut epithelium and move from there to the salivary glands of the tsetse fly where they develop into the metacyclic infectious trypomastigote form which has now got its surface coat. Because of the complicated development of the trypanosomes within the tsetse fly, only about 0.1-0.4 % of the flies are infected and thus are potential vectors of trypanosomosis
- After the vertebrate host has been infected by the tsetse fly, syngamy takes place; the trypanosomes become haploid and multiply through lengthwise division.

The distribution of the tsetse-transmitted African trypanosomes is governed by that of their tsetse vectors, i.e. roughly between 15° N and 25° S latitude in tropical Africa (Hoare, 1957). Apart from cyclical transmission through *Glossina* spp., *T. vivax* has the capacity to be transmitted mechanically by other blood sucking diptera such as horse-flies (*Tabanidae*) and stable-flies (*Stomoxys* spp.). Mechanical transmission explains the occurrence of *T. vivax* infections outside Africa; and it has been reported from the island of Mauritius (Adams, 1935), the Caribbean islands of Guadeloupe and Martinique and the South American main land (Wells, 1984) and in cases of *T. vivax* infection in Africa in areas outside the tsetse belt (Roeder *et al.*, 1984). This spread in distribution has come about by movement of infected cattle and the ability of *T. vivax* to be transmitted by biting insects other than tsetse (Hoare, 1972).

2.1.2.2 Pathogenesis and host susceptibility

Infected tsetse flies inoculate metacyclic trypanosomes into the skin of animals where the trypanosomes grow for a few days and cause localised swelling. They enter the lymph node, and then the blood stream, where they divide rapidly by binary fission. The trypanosomes exert their effect by multiplying rapidly in the blood stream, causing disseminated intravascular coagulation and then entering and blocking capillaries causing ischemia and anaemia. In *T. congolense* infection, the organisms bind to endothelial cells and localise in the capillaries and small blood vessels (Blood *et al.*, 1989).

Trypanosoma congolense is the most important pathogenic trypanosome affecting cattle in Africa. There are many different strains of this organism and they vary considerably in their virulence. In West Africa, *T. congolense* trypanosomosis in cattle is generally regarded as being a relatively chronic disease whereas in East Africa it may be seen as an acute, subacute or chronic disease. The development of these trypanosomes in tsetse flies takes place in the midgut and proboscis (Hoare, 1970).

Practically all domestic mammals are susceptible to infection with *T. congolense*, the effects of which vary considerably according to the strain of the parasite and the physical condition of the host. Thus, in most ungulates (cattle, sheep, goats, equines and camels), as well as in dogs, the disease may be acute, chronic or mild, while in pigs it usually runs a mild course (Hoare, 1970). *T. congolense* is infective to laboratory rodents which are therefore commonly used for the detection of infection in domestic animals. However, as in the case of natural infections, there is much variation in the virulence of different strains for laboratory animals (Hoare, 1970).

There is a general understanding that *T. vivax* from East and West Africa differ in their pathogenicity and 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; Hoare, 1972; Anosa, 1983). 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 (Mwongela *et al.*, 1981; Wellde *et al.*, 1983).

Trypanosoma brucei invade tissues and result in tissue damage in several organs. *T brucei* is also belonging to human trypanosomes causing sleeping sickness in human beings. The immune response is vigorous and immune-complexes cause inflammation, which contribute to the signs and lesions of the disease (Blood *et al.*, 1989).

When an animal is infected with trypanosomes, antibody against the surface coat is made and the trypanosome is killed. The problem is that these trypanosomes have multiple genes, which code for different surface proteins; this allows organisms with a new surface coat glycoprotein to elude the immune response. This process is called antigenic variation and results in the persistence of the organism. The number of antigenic types of glycoprotein that can be made is unknown, but exceeds several hundred. Antigenic variation has thus far prevented development of a vaccine and permits reinfection when animals are bitten by tsetse flies carrying trypanosomes with surface coat glycoproteins of a new antigenic type (Blood *et al.*, 1989).
Genetic resistance to animal trypanosomosis has been attributed to certain breeds of livestock, most notably to the indigenous West African N'Dama (Murray *et al.*, 1979). This resistance is manifested by the N'Dama's ability to withstand the adverse effects of trypanosomosis by regulating parasite growth; their ability to prevent or reduce the rate and degree of development of anaemia (Murray, 1988). There is evidence that trypanotolerance has a genetic basis and may be inherited as dominant trait (Trail *et al.*, 1989). A better understanding of the mechanisms involved in trypanotolerance could aid in the research for genetic markers that could be used for the selective breeding of resistant cattle.

2.2 Diagnostic methods

Accurate diagnosis of trypanosome infections in livestock is required for a proper appreciation of the epidemiology of the disease in any geographical locality and for assessing the need for, and efficacy of treatment. However, high parasitaemia are usually evident only in early infections, and in the chronic phase of the disease parasites may apparently be absent from the blood for long intervals.

Further, since the sensitivity of the different trypanosome species to the available chemotherapeutic agents often varies (Williamson, 1970; Leach and Roberts, 1981), and mixed infections in the field are common (Hill and Esuruosu, 1976), diagnostic methods with high degree of sensitivity (preferably detecting active infections rather than host responses) and specificity are required. Beside clinical diagnosis, direct (parasitological) and indirect (serological) diagnostic methods with varying degrees of sensitivity and specificity are available.

2.2.1 Clinical diagnosis

In general, diagnosis of trypanosome infection on the basis of clinical signs alone is rather difficult, but haematological parameters like PCV could be reliable indicators of the progress of the disease. The disease shows a variety of clinical manifestations which are also common to other diseases. The fact that the disease may run an acute, chronic or sub-clinical course further complicate the diagnosis of trypanosome infections on the basis of clinical signs. In general, fever can be observed which may be intermittent due to the variation in parasitaemia, and if the animal survives the disease becomes chronic and there is development of anaemia and emaciation (Blood *et al.*, 1989). This, therefore, makes fever, anaemia and body condition important parameters which are routinely used for the tentative diagnosis of trypanosomosis in areas where this disease is endemic and laboratory services are not available. Definitive diagnosis of the disease is ultimately dependent on the detection of the trypanosome in blood samples from infected animals.

2.2.2 Parasitological diagnosis

Parasitological diagnosis is the direct demonstration of the parasites in blood or less frequently in other body fluids (using a microscope). The scarcity of parasites and the fluctuating nature of the parasitaemia limited the use of laboratory tests based on demonstration of trypanosomes in accessible body tissues such as the peripheral blood (Doyle, 1977). Therefore, several techniques for the concentration of blood trypanosomes have been developed, which increase the chance of trypanosome detection.

2.2.2.1 Haematocrit Centrifugation Technique (HCT, Woo, 1970, modified by Mehlitz, 1978)

A microhaematocrit capillary tube containing 70 µl of blood is centrifuged for eight minutes at 10,000 g as for measurement of PCV. Two rectangular pieces of glass from a standard microscope slide (1.2 mm thick) were fixed 1.5 mm apart on a microscope slide. The prepared capillary tube is then placed in the slot and a drop of immersion oil put on top of the capillary tube. The oil filled the space between the capillary tube and the two pieces of glass thus reducing the effect of light defraction. By slowly rotating the tube the buffy coat plasma junction is examined using a long working distance (6.7 mm) objective which allows

considerable depth of focus through the capillary unlike the standard objective where the average working distance is approximately 0.5 mm. Depending on the trypanosome species the analytic sensitivity for this method is 1-5 x 10^2 trypanosome/ml of blood.

2.2.2.2 Capillary Concentration Technique (CCT, Walker, 1972)

Because of the tendency of *T. congolense* to be retained amongst red blood cells, this technique was designed to create a large differential density between the red blood cells and the parasites. This was achieved by mixing infected blood with a strongly hypertonic non-toxic medium (Walker solution). This made it possible to alter the specific gravity of the erythrocytes. On centrifugation the denser red cells separate from the trypanosomes which display normal motility. Equal volumes of diluent and blood are mixed on a micro titre titration plate. After being allowed to stand for a minimum of 15 minutes plain capillary tubes are three quarter filled from the wells, sealed and spun for 2 minute in a microhaematocrit centrifuge. On centrifugation, the denser red blood cells separate from trypanosomes which display normal motility. The capillary tubes are placed on a clean microscope slide (up to 12 at a time) and the buffy coat zone covered with a few drops of diluent beneath a coverslip. The capillaries are examined with the same microscope set up in HCT. This technique is more sensitive than HCT in the detection of *T. congolense*. However, it needs more time to prepare the samples and PCV can not be measured at the same time unlike with HCT.

2.2.2.3 Dark ground/phase contrast buffy coat technique (Murray *et al.* 1977)

The buffy coat zone prepared in a microhaematocrit capillary tube filled with 70 µl of blood and centrifuged for 5 minute at 10,000 g is examined for trypanosomes by cutting the capillary tube to include 1 mm of erythrocytes and 1 cm of the plasma. The buffy coat is poured on a slide and covered with a 22 x 22 mm coverslip. The preparation is examined using a microscope with a phase contrast and dark ground illumination. The use of 10x eye piece in combination with a 25x objective gives optimal viewing, by allowing large visual fields and sufficient magnification for ready identification of trypanosomes. This technique is the most sensitive of the parasitological tests for the detection of *T. congolense* and *T. vivax*, detecting trypanosomes to an estimated level of just over 10^2 parasites per ml. In addition, species identification on the basis of size and movement is easier to assess (Paris *et al.*, 1982).

2.2.2.4 Miniature-anion exchange Centrifugation Technique (m-AECT, Lumsden *et al.*, 1977, 1979)

Anion-exchange columns can be prepared using 2 ml disposable syringes filled with DEAE-cellulose (DE 52) as described by Lanham and Godfrey (1970). The column is primed by filling to the brim with phosphate saline glucose buffer (PSG) and allowing to drain twice. The PSG with an ionic strength of 0.145 was considered appropriate for Bovine blood (Lanham and Godfrey, 1970). The blood sample (100 µl) is then discharged from a capillary tube and then drained into the DEAE-cellulose bed. Four drops of PSG (i.e. a Pasteur pipette containing approximately 2.5 ml PSG connected to the column through a perforated syringe piston). The elute is collected in a prepared centrifuge tube. When drainage stopped this tube is centrifuged at 525 g for 5 min. The drawn out tip is examined microscopically for trypanosomes under a coverslip with PSG using the optical system employed for the HCT and CCT. The analytic sensitivity for m-AECT is 10 trypanosomes/ml of blood, depending on the

trypanosome species. The principle of this technique is that red blood cells have a strong negative charge on the surface and the trypanosomes have a lesser negative charge. Cellulose is positively charged. When the red blood cells and the trypanosomes are passed through the cellulose column, the RBCs are retained and the trypanosomes with a lesser negative charge pass through the column into the pasture pipette. Phosphate which is negatively charged is used to reduce the strength of the positive charge on the cellulose, so that it can allow the trypanosomes to go through but retaining the red blood cells.

2.2.3 Serological diagnosis

Serological methods are playing an increasingly important role in the diagnosis and epidemiological assessment of trypanosomosis. Paris *et al.* (1982) stressed that the serodiagnostic techniques that were in use depended on the demonstration of circulating antibody and as a result they could not by themselves confirm an active infection. Antibody ELISA and antigen ELISA are the methods widely used currently for the diagnosis of cattle trypanosomosis.

2.2.3.1 Antibody ELISA

This test has been used in the diagnosis of trypanosomosis and is based on the detection of anti-trypanosomal antibodies in the sera of diseased animals. Luckins and Mehlitz (1976) used microplate-ELISA system in their study of Bovine trypanosomosis and found that cattle developed positive ELISA values after infection, but it was not possible to differentiate between *T. vivax, T. congolense, T. brucei* or *T. rhodesiense*. Moreover, the serological tests in current use suffer from a lack of well defined antigens necessary for designing simple and accurate tests that are easily adaptable for field use. Secondly, the detection of anti-trypanosomal antibodies in serum cannot distinguish between an active infection and a cured one (Voller, ,1977). The length of time taken for antibodies to disappear from circulation after a successful therapy of cattle is not yet clear. Thirdly, the present serological tests are not sufficiently specific to reveal conclusively the identity of the infecting trypanosomal species (Nantulya, *et al.*, 1987).

2.2.3.2 Antigen ELISA

The detection of circulating trypanosomal antigens may be a more sensitive means of practical diagnosis and could increase the reliability of detection of current infection in animals. It may also be more useful since it does not require access to freshly collected blood as it is necessary for the classical direct detection methods. The test may also offer means by which it would be possible to detect the relapse infections in animals undergoing trypanocidal drug therapy during a period at which it is not possible to isolate parasite from the peripheral circulation (Rae and Luckins, 1984).

Species-specific monoclonal antibodies, produced against procyclic forms of *T. congolense, T. brucei* and *T. rhodesiense*, were used to develop antigen-capture enzyme-linked immunosorbent assays (antigen ELISA) for the diagnosis of bovine trypanosomosis (Nantulya *et al.*, 1989). Such species-specific, sensitive, simple and practical antigen detection tests could be used for large scale screening of animals and thus, were of tremendous benefit to sero-epidemiological investigations. Nantulya *et al.* (1989) showed that circulating antigens

tend to decline with the institution of effective chemotherapy. However, some of the animals showed a sudden rise in antigen levels several weeks after treatment. It is highly probable, despite the absence of detectable parasitaemia in the peripheral blood, that the antigen rise was due to relapse of infection. Thus, antigen-trapping assays are likely to be useful tools for use in the follow up of treatment to assess the efficacy of the drugs used (Nantulya *et al.*, 1989).

2.3 Control of Trypanosomosis

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

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

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

A biological method of control is the sterile male release technique in which artificially sterilised males compete with wild tsetse for mating with females (Dame and Schmid, 1970). However, this is considered to be very expensive (Roclants and Williams, 1982) and moreover has the potential of increasing the trypanosomosis risk in the affected area because the sterile males have been found to be as capable as normal male tsetse in transmitting the disease (Moloo, 1982).

A safe and cost effective vaccine against trypanosomosis would be a much more effective and sustainable way of controlling the disease. However, because of the ability of trypanosomes to

undergo antigenic variation immunisation of cattle against trypanosomosis has been unsuccessful (Doyle, 1977). However, field studies in cattle have provided evidence that animals previously exposed to trypanosomosis may be less susceptible to subsequent challenge.

Based on actual experience in the field, the introduction and keeping of trypanotolerant West African taurine cattle breeds seem to be an alternative biological method to preventing clinical trypanosomosis and thus economic losses for the animal holders. Trypanotolerance is a feature of both West African longhorn and shorthorn *Bos taurus* breeds such as the N'Dama and Baoule breeds. These breeds of animals posses an increased titre of resistant factors (lysozyme, haemolytic complement C_9 and the third complement component C_3) and are better able to stabilise the balance of the host-parasite relationship known as premunity (Seifert, 1996). Trypanotolerance is manifested by the ability of the trypanotolerant animals to regulate parasite growth and to prevent or reduce the rate and degree of development of anaemia (Murray, 1988). However, these breeds of animals are relatively small in number and are limited in West Africa.

2.4 Chemotherapy and Chemoprophylaxis

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

Homidium bromide/chloride

Homidium (Watkins and Woolfe, 1952) belongs to the phenanthridine class of compounds and is manufactured as both bromide and chloride salts, which are equally active *in vivo* (Leach and Roberts, 1981). Both salts are generally recommended for use as therapeutic agents at a dose of 1.0 mg/kg bw. However, the same dose, in cattle, has been shown to have prophylactic activity varying from 2 to 19 weeks against field challenge (Dolan *et al.*, 1992). Variation in homidium susceptibility, and the level of trypanosome challenge, are thought to be the primary factors determining the duration of prophylaxis (Dolan *et al.*, 1992).

Diminazene aceturate

Diminazene (Jensch, 1958) is an aromatic diamidine and is marketed in combination with phenyldimethyl pyrazolone (antipyrine) (44.5:55.5 w/w), a stabiliser that prolongs the activity of the compound in solution (Fairclough, 1963). Sensitive populations of *T. congolense* and *T. vivax* are eliminated by intramuscular treatment of the host with diminazene aceturate at a dose of 3.5 mg/kg bw. However, higher doses may be required for infections with *T. brucei* (Fusgänger and Bauer, 1958). Diminazene is now probably the most commonly used therapeutic agent for trypanosomosis in livestock in sub-Saharan Africa (Geerts and Holmes, in press). This is due to a number of factors: activity against trypanosomes that are resistant to most other trypanocides and a higher therapeutic index, in most animal species, than other trypanocides (Williamson, 1970).

Isometamidium chloride

Isometamidium (Berg *et al.*, 1961) is a phenanthridine-aromatic amidine, formed by combining homidium with the diazotized p-aminobenzamide moiety of diminazene, and is marketed as both a therapeutic and prophylactic agent. In the dose range recommended for

prophylactic purposes (0.5-1.0 mg/kg bw), the compound has been used successfully to maintain the productivity of Zebu cattle exposed to tsetse challenge in both village and ranch management systems in East Africa (Moloo *et al.*, 1987). However, considerable variation in prophylactic activity has been observed in that a dose of 1.0 mg/kg bw has been shown to confer prophylaxis to cattle for 2-22 weeks (Kirby, 1964; Pinder and Authie, 1984; Peregrine *et al.*, 1991). Variation in drug susceptibility between different trypanosome populations appears to be the major factor determining the duration of prophylaxis (Peregrine *et al.*, 1991).

All three compounds are closely related chemically and have been available for at least 35 years. After the introduction of isometamedium in 1961 (Berg *et al.*, 1961) the development of new trypanocidal drugs has made little progress. Recently, however, quinapyramin sulphate (Antrycide) has been reintroduced because of the need to especially combat camel trypanosomosis.

Chemoprophylaxis has been used to protect livestock under low to medium trypanosome challenge using isometamidium at dosage ranges of 0.5 to 1.0 mg/kg bw depending the trypanosome challenge. Prophylaxis of between 14 and 36 weeks has been reported in field studies at these dose levels (Fairclough, 1963; Kirby, 1964), although prophylaxis of less than 12 weeks has been reported (Pinder and Authie, 1984). Such variation in prophylactic activity appears to be independent to both the level of trypanosome challenge and the presence or absence of infection at the time of treatment (Peregrine *et al.*, 1988). Variation in susceptibility between different trypanosome populations appears to be the major factor determining the duration of prophylaxis (Peregrine *et al.*, 1991). Although these drugs have effectively controlled the disease when rationally used in the field, the prevalence of resistance to each of these compounds appears to be increasing.

2.5 Chemoresistance

Drug resistance in trypanosomes has been reported from many parts of Africa (Jones-Davies, 1967; Garber, 1968; Mwambu and Mayende, 1971; Lewis and Thomson, 1974; Williamson, 1970; Authie, 1984; Pinder and Authie, 1984; Moloo and Kutuza, 1990; Clausen *et al.*, 1992). In East African drug resistant *T. congolense* strains have been described in Ethiopia (Scott and Pegram, 1974; Codjia *et al.*, 1993; Rowlands *et al.*, 1993; Leak *et al.*, 1993) and in Kenya (Gitatha, 1979). Drug resistant *T. vivax* strains have been identified in Kenya (Schönefeld *et al.*, 1987). Clausen *et al.* (1992) examined 20 Zebu cattle naturally infected with *T. congolense* and/or *T. vivax* in a fly-proof stable and found that diminazene aceturate at 7.0 mg/kg bw cured infections of *T. vivax*, but was ineffective against *T. congolense*. Additionally they also proved in the same animals that isometamidium chloride at a dose rate of 1 mg/kg bw and homidium bromide at a dose rate of 1 mg/kg bw were ineffective. These authors then conducted a corresponding chemotherapeutic trial in previously unexposed Zebu bulls and Sahelian goats infected with one primary *T. congolense* isolate from Samorogouan which demonstrated a high level of resistance to diminazene aceturate (7 mg/kg in cattle and 17.5 mg/kg bw in goats), isometamidium chloride (1 and 2 mg/kg bw i.v. in goats) and quinapyramin sulphate at 5 mg/kg bw in goats. As a result they emphasised the urgent need for new chemical substances as trypanocidal drugs and the increasing importance of efficient vector control because of the appearance of multiple-drug resistant strains of *T. congolense*. Chitamo and Arakaw (1992) described that clones derived from the Mumbwa isolate were resistant to isometamidium, with Minimum Curative Doses (MCD) of 4 mg/kg bw isometamidium, and to diminazene, with MCD of 14 to 28 mg/kg. Isometamidium and

diminazene are usually prescribed as a "sanative pair" in the control of bovine trypanosomosis (Whiteside, 1960). Development of multiple-drug resistant strains of *T. congolense* isolated in the Bobo-Dioulasso region of Burkina Faso (Authie, 1984; Sones *et al.*, 1988; Moloo and Kutuza, 1990; Clausen *et al.*, 1992) and Ethiopia (Codjia *et al.*, 1993; Mulugeta *et al.*, 1997), however, suggest that the concept of sanative pairs might no longer be valid in certain regions.

The basic mechanism of drug resistance and the factors contributing to the appearance, maintenance and disappearance of drug resistant trypanosome population in cattle is not fully understood. The repeated use of chemicals as pesticides or chemotherapeutic agents inevitably leads to the development of resistance by the target organisms. Data from experimental induction of drug resistance to various trypanocides in mice and rats indicate that many situations exist supporting the generation of drug resistance in trypanosomes. Prolonged and excessive use of Berenil and Trypamidium on a routine herd basis is a common feature in African livestock husbandry practices. Resistance to diminazene generally appears to arise under three different conditions: repeated subcurative treatment of infected animals with the drug (Hawking, 1963); cross-resistance associated with the development of resistance to the trypanocide, quinapyramine (Ndoutamia *et al.*, 1993); and innately, i.e., without prior exposure to the compound (Williamson, 1960). Since resistance to diminazene is extremely difficult to induce in cattle using repeated subcurative treatments (Whiteside, 1963), and quinapyramine resistance appears to be associated with cross-resistance to isometamidium (Ndoutamia *et al.*, 1993), the factors responsible for development of diminazene resistance by the *T. vivax* population is unclear.

Although it is generally assumed that uncontrolled infections will have a severe impact on both survival and productivity only few studies have been conducted to accurately assess the impact of drug resistant trypanosomes on livestock productivity. Recently, a study was carried out in this regard in the Ghibe valley of Ethiopia, where a high prevalence of multiple drug resistance was reported (Codjia *et al.*, 1993). Rowlands *et al.* (1994) followed more than 300 East African Zebu calves from birth to 3 years of age together with their dams in this region (between 1986 to 1992). During most of this period animals which were parasitaemic and with a PCV below 26% or animals with clinical signs of trypanosomosis were treated with diminazene aceturate at 3.5 mg/kg bw, although resistance against this drug was known to occur. Some effects on the growth rate of parasitaemic calves were observed which were temporary. The authors concluded that regular trypanocidal therapy might have helped to maintain health and productivity of young cattle. Although calf mortality was rather high, growth rates were compared favourably with those in other village-managed systems in Africa. Similarly, reasonable levels of reproduction in terms of calving interval and age at first calving were maintained under regular trypanocidal therapy in the cows which were monitored over the same period (Rowlands *et al.*, 1994). Similar studies should be carried out in other regions with different resistance problems and under different management conditions.

Drug resistance in trypanosomes will continue to remain a critical challenge to the control of the disease until the basic mechanism of resistance is known. While the problem of drug resistance has encouraged much research into alternative methods of disease control, for example immunological approaches and the use of trypanotolerant animals, it is becoming evident that such methods, to be successful, will have to be used in conjunction with chemotherapy. Therefore, urgent methods need to be taken to maintain the efficacy of the existing drugs.

At present the most widely used trypanocidal drugs in *T. congolense* and *T. vivax* infections in Ethiopia are isometamidium (Samorin®, Trypamidium®) and diminazen aceturate (Berenil®, Hoechst AC). The occurrence of drug resistance in trypanosomes across Ethiopia is unknown. Scott and Pegram (1974) described the occurrence of Homidium-resistant populations of *T. congolense* in Didessa and Angar valleys in Wollega province. In their findings repeated thick and thin blood film examinations after treatment with 1 mg/kg bw of homidium revealed that 25 per cent of the animals were again infected within 30 days and the majority of infections were due to *T. congolense*. Moreover, laboratory experiments using mice confirmed a high incidence of resistance to homidium bromide. The current situation on the phenomenon of trypanocidal resistance particularly in *T. congolense* infections is well documented in the Ghibe valley (Codjia *et al.* 1993) but requires more careful investigation in other parts of the country. The work done in Ghibe valley, Ethiopia, described the presence of multiple-drug resistant strains of *T. congolense* species (Codjia *et al.*, 1993). These authors inoculated 12 stabilates, isolated in the field, into individual Boran (*Bos indicus*) calves, under fly-proof conditions, and characterised for their sensitivity to diminazene aceturate (Berenil®), isometamidium chloride (Samorin®) and homidium chloride (Novidium®). All 12 stabilates produced infections which were shown to be *T. congolense* that were resistant to treatment with diminazene aceturate at a dose of 7.0 mg/kg bw. Eleven of the infections were also resistant to isometamidium chloride at a dose of 0.5 mg/kg bw and homidium chloride at a dose of 1.0 mg/kg bw. Then five clones were derived from one of the isolates which expressed a high level of resistance to all three trypanocides; each clone expressed high level of resistance to all three trypanocides. Thus, the multiple-drug resistance phenotype of the parental isolate was associated with expression of multiple-drug resistance by individual trypanosomes. Moreover, recent field observations in Ethiopia based on cloned populations showed that the drug resistant phenotype of *T. congolense* had not altered over a period of 4 years (Mulugeta *et al.*, 1997). Because of the high level of multiple-drug resistant infections, and because this appeared to be expressed at the level of individual trypanosomes, it was concluded that chemotherapeutic agents *per se* would not control trypanosomosis at Ghibe on a long term basis. Thus, an integrated control program, involving both tsetse-fly control and chemotherapeutic agents, was implemented to control trypanosomosis at the site (Peregrine *et al.*, 1994)

To demonstrate that drug resistant trypanosomes are present it is necessary first to discount other problems such as inadequate drug preparation, handling and administration, and secondly to demonstrate the response of the trypanosomes to treatment under carefully controlled conditions. Ideally this should be carried out in the host species of interest, i.e. *Bos indicus* cattle for strains isolated from East African Zebu cattle. Rowlands *et al.* (1993) showed that the application of a computer model to parasitological data collected over a long period on a monthly basis allowed the incidence of new infections to be distinguished from recurrent infections. This analysis showed that the mean prevalence of diminazene resistant infections in the Ghibe valley, Ethiopia, increased from 6% in 1986 to 14% in 1989. However, in many situations it is necessary to use small ruminants or laboratory animals because of limited finance and facilities (Sones *et al.*, 1988). Standardisation of the existing tests should receive high priority, especially the assays in mice and in definitive hosts, because these can be carried out in less well equipped laboratories.

Underdosing is one of the major causes of resistance development. Subtherapeutic drug concentrations exert a strong selective pressure for the emergency of resistant clones that pre-exist in the trypanosome population. Given the fact that in many countries unskilled persons are allowed to administer drugs, undoubtedly many errors occur in calculating the correct doses for the treatment of the animals. Furthermore, as the drugs are relatively expensive there

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is a temptation to overdilute the drug and hence underdosage. The use of improved formulation of existing drugs can be a possible means to avoid subtherapeutic concentrations. Controlled release devices which provide more stable drug concentrations and a sharper cut off at the end of the release period might have particular advantages in this respect (Geerts and Holmes, in press).

The reduction of selection pressure by the drugs, i.e. decreasing the number of treatments, is considered as the most efficient way to delay the development of drug resistance. This is important particularly in high tsetse challenge areas, which are commonly associated with frequent treatments in a short period (Whiteside, 1960). Drug resistance often emerges in such situations as a result of increased frequency of drug usage. Additionally, systematic mass treatment could hasten the development of resistance. Therefore, in well monitored situations limiting treatments to individual clinical cases may be important. In such situations drug resistance problems can be minimised and acquired immunity encouraged (Scott and Pegram, 1974).

2.6 Assessment of Chemoresistance

"*In vivo*" techniques:

This is one of the methods used in the assessment of the sensitivity of trypanosome isolates to trypanocides and the standard mouse test and tests in ruminants are used. When using mice, they are infected with trypanosomes and subsequently treated after 24, 48 or 72 hours with the drug under investigation. The mice are then screened for trypanosome infections for 30-60 days. The ratio of number treated to number infected indicates drug sensitivity (Kaminsky *et al.*, 1990; Elrayah and Kaminsky, 1991). The ED_{50} or ED_{95} (effective dose, which gives temporary clearance of the parasites in 50% or 95% of the animals) as well as the CD_{50} or CD_{95} (curative dose which gives complete cure in 50% or 95%) of the animals can be calculated. Sones *et al.* (1988) used groups of 5 mice, which allowed an easy calculation of ED_{80} and CD_{80} values (one out of 5 mice not cleared or cured). These figures should be compared with those obtained using reference sensitive trypanosome strains.

The advantage of the mouse test is that it is cheaper than the tests in cattle. The problem with this type of assessment is that the results of a mouse sensitivity test can not necessarily be extrapolated to ruminants (Hawking, 1963; Sones *et al.*, 1988), Since differences in the pharmacokinetics of trypanocides in different host species have been described (Ali and Hassan, 1984). The other limitations of this test include failure of most *T. vivax* and some *T. congolense* isolates to grow in mice, the large number of mice required per isolate in order to assess the degree of drug resistance and the relatively long period (up to 60 days) required to evaluate the drug sensitivity of an isolate.

The tests in ruminants can be of direct relevance to the field situation, because the test is conducted in natural hosts using recommended doses of trypanocidal drugs. The tests commonly consist of infecting a group of cattle or small ruminants with the isolate under investigation and later when they are parasitaemic treating them with various levels of trypanocidal drugs. The animals are regularly monitored over a long period (up to 100 days) to determine the curative dose, i.e. the dose of drug able to provide a permanent cure (Sones *et al.*, 1988). For these studies the cattle or small ruminants must be kept in fly-proof accommodation or in a non-tsetse area in order to eliminate the risk of reinfection during the study. Ainanshe *et al.* (1992) used the technique which is described below to examine a group of isolates from a district in Somalia. A single recipient calf was inoculated with blood from a

group of infected cattle and later when it was parasitaemic treated with trypanocidal drugs at the recommended dose. Then after another groups of calves and mice were inoculated from a breakthrough infection to determine the level of drug resistance. This technique is useful in situations where laboratory facilities are very limited but it only allows a qualitative assessment and does not indicate how many of the isolates inoculated into a single calf were resistant. The level of resistance can be indicated from studies in ruminants and mice by recording the length of time between treatment and the detection of breakthrough populations of trypanosomes. The shorter the period the greater the level of resistance (Ainanshe *et al.*, 1992). The advantages of the study in ruminants are that all trypanosome isolates from cattle are able to grow in these hosts and the data obtained are directly applicable to the field. The limitations are the long follow-up period and the costs of purchasing and maintaining large number of ruminants.

"*In vitro*" techniques:

In seeking alternatives, "*in vitro*" techniques have been examined for their usefulness in assessing drug susceptibilities (Elrayah and Kaminsky, 1991). Antitrypanosomal activity of drug can be observed in "*in vitro*" culture conditions as reported (Borowy *et al.*, 1985; Brun and Kunz, 1989).

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.

The Drug Incubation Infectivity Test (DIIT) is a combination of *in vitro* and *in vivo* techniques. It can even be established in only moderately equipped cell culture laboratories: 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).
However, to date, all tests mentioned only have been carried out for a limited number of well defined laboratory trypanosome stocks. For all tests it remains to evaluate whether there always is a clear relationship between the drug sensitivity observed *in vivo* and *in vitro*. In addition, it still has to be evaluated for all methodologies whether always consistent results can be achieved when uncloned field isolates are used.

The limitation of the "*in vitro*" test is similar to the "*in vivo*" test in that the results obtained 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.

2.7 Assessment of the Therapeutic Effectiveness

When trypanosome infected animals are treated successfully, circulating drug levels are by definition adequate to protect against trypanosome challenge and therefore breakthrough infections may only occur where drug levels are inadequate, possibly as a result of inadequate dosage regime. All relapses are not due to drug-resistance. Trypanosome species of the *Trypanozoon* group could reach tissues (CNS, eye etc.) where trypanocidal drugs could not reach (Whitelaw *et al.*, 1985). Alternatively, infections may occur where the challenge population of trypanosomes express drug resistance (Holmes and Torr, 1988). Both causes have important implications for the management of cattle in tsetse infested areas, but their relative importance may be difficult to evaluate in field situations.

The use of trypanocidal-ELISAs in combination with parasite detection tests has given promising results for the detection of resistant trypanosomes (Geerts and Holmes, in press). A competitive ELISA which allowed the detection of small amounts of isometamidium in serum of cattle has been validated in cattle under experimental and field conditions (Eisler *et al.*, 1994). The test is both sensitive and specific and it allows the monitoring of drug levels over extended periods and the evaluation of factors influencing drug disappearance rates from the plasma. The drug disappears more rapidly in animals infected with drug resistant trypanosome isolates than in those infected with sensitive trypanosomes (Eisler *et al.*, 1994). Preliminary observations showed that the presence of trypanosomes in animals with an isometamidium concentration of >5 ng/ml strongly suggests resistance. Further research is necessary, however, in order to confirm these results in a larger number of animals. Whereas drug levels can be measured in the sera of treated animals (Eisler *et al.*, 1994), it is equally important to have diagnostic techniques with very high sensitivity in order to assess the effectiveness of therapy under field conditions and these techniques must be designed such that they can detect very low parasitaemia or even cryptic infections. Large numbers of sera can be tested within 12 to 24 hours using this isometamidium-ELISA and this might give some indication of the resistance situation at the level of the herd. However, 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 (Geerts and Holmes, in press).
The identification of genetic markers for isometamidium resistance, which might be developed into reagents for the identification of resistant trypanosomes using PCR would be a more promising approach in the long term (Geerts and Holmes, in press).

3. MATERIALS AND METHODS

3.1 Field studies at Metekel district, North-west Ethiopia

3.1.1 Study area

The Tana Beles resettlement project is located in the Metekel Administrative Region, 550 km North-west of Addis Ababa. The area lies within the altitude of 1000-1150 m above sea level

with an average annual rainfall of 1270 mm. The project area comprises of Lake Tana and upper, lower and middle Beles river, a tributary of the Blue Nile with swampy plains having many tributaries passing through the project areas. The rainy season lasts from April/May to October and the rainfall is mostly occurring between June and September.

The dominant vegetation types of the area are scattered trees (*Combritum, Terminalia*) and tall savanna grasses with bamboo occupying a significant part of the area. Tall and evergreen trees with brooded leaves cover both sides of the river Beles and its main tributaries namely Changur, Endsa, Buhl, with its tributaries Zara and Ketem, Chanko and others. Buffaloes, bushbucks, dickdicks, hippopotamus, warthogs, baboons, crocodiles, hyenas are some of the wild games in the area.

The 1984/85 horrific drought and famine enforced the Ethiopian government to launch resettlement scheme with the basic rationale of alleviating the sufferings of the people. As a result, the emergency resettlement scheme in the country dislocated several peasants. The Beles-Valley conventional resettlement scheme, situated in the basin of the Beles river located around 550 km away North-west of Addis Ababa, is one of the biggest emergency schemes that hosted two kinds of resettlers: those who were famine victims and those who suffered from land shortage. Currently, over 75,000 settlers who come from drought-stricken highlands of Ethiopia, particularly from parts of Wello, Tigray, Gojjam and Shewa, live in 48 villages located along the Beles river. Additionally, a good number of self-motivated farmers and their families have also moved to the area in search of fertile and arable land (Tilahun *et al.*, 1997). These dislocated peasants were relocated to different physio-biotic and socio-cultural environments far away from the original homelands. The policy basis for the massive emergency resettlement program was to attain higher agricultural productivity in the 'un- or under-utilised' parts of the country in the new locations through mechanisation and agro-industrial development of the area. However, the termination of the project rendered the program unsustainable and as a result animal traction became the only means to crop production. This increased the importance of ploughing oxen to a great extent. Currently, farmers in the area are predominantly keeping animals for traction and harvest of crops. Therefore, an increase in livestock productivity in the area is not only more livestock outputs but also increase in crop production through additional drought power, extra manure, cash to purchase agricultural equipment and improved human health through adequate supply of animal protein and other requirements. However, the continuous threat from tsetse and trypanosomosis on the livestock population in the area complimented with the problem of malaria to human beings may end up in total abandonment of this fertile settlement area and may redislocate hundreds of families unless control measures are instituted.

Trypanosomosis has been identified as one of the major livestock diseases in the area. In 1989, for example, 59% and 40% of prevalence rates were recorded in different villages of the project area (Beyene, 1993). Various trypanosomosis and tsetse fly surveys carried out in the area since 1988 indicated that *G. tachinoides* is the only species of tsetse fly identified within the project areas.

Some field case studies showed many animals repeatedly being detected parasitaemic despite treatment with diminazene aceturate at a dose of 3.5 mg/kg bw. Furthermore, the percentage of infections which relapsed following treatment increased from time to time (Beyene, 1993).

3.1.2 Cattle

The cattle in the area are indigenous East African Zebu breeds. They are kept under traditional extensive husbandry systems with communal herding. During the day, cattle are herded together and looked after by herdsmen. Male cattle over 3 years of age are used as ploughing oxen. Animals work usually during the morning, particularly during the wet season, and graze the rest of the day. They are watered in small and big rivers. Cattle are returning to their owner's homestead each evening. According to 1995 census, the total cattle population in villages 12, 13 and 24 are estimated to be 1236 heads (Tilahun *et al.*, 1997).

Figure 1 Map of Ethiopia showing location of the study area (Metekel/Benshangul region) and Beles and Blue Nile (Abay) rivers.

3.1.3 Questionnaire

During the first 2-3 weeks of March 1997, field investigations were carried out with the major objective of identifying cattle herds highly suspected to be infected with drug resistant trypanosome populations. A total of 105 farmers and 10 groups of farmers were interviewed from three different villages with the aid of a questionnaire about herd structure, diseases, usage of trypanocidal drugs etc. (see Annex 1). The questionnaire was tested in the field before administered to the study population. The questionnaire was administered in person to individuals and groups (5-10 people per group) of livestock owners.

3.1.4 Cross Sectional study

In order to determine the prevalence rate of trypanosome infections in the study population a cross-sectional study was carried out in March 1997. Four different villages were selected. Villages were selected by purposive sampling on the basis of prior information on the problem, farmers co-operation and accessibility. The sampling method used in each of the villages was simple random sampling. The sample size for the prevalence study was determined using the EPI INFO program, with an estimated trypanosome prevalence rate of roughly 20%, a precision level of 5% and a 95% confidence interval. Representative number of animals were sampled from each village.

A total of 484 cattle from four different villages were sampled to determine the prevalence rate of trypanosome infections in different villages. Parameters like age, sex, breed and previous history of treatment were recorded.

Dark ground/ phase contrast buffy coat method (Murray *et al.*, 1977) was employed to study the prevalence rate of trypanosome infections. Two capillary tubes were filled for each blood sample. Blood to be examined were added to the capillary tubes by capillary attraction until the tubes are filled 3/4 way. Each capillary tube was sealed at one end using placticin. After centrifugation at 10,000 revolutions per minute for 10 minute, the capillary tubes were cut 1 mm above and 1 cm bellow the buffy coat, expressed on microscope slides and examined using a microscope with 40 x objective. Thin blood smears stained with giemsa were used to confirm the species of trypanosomes in case of parasitaemia. Consequently, the PCV of each sample was estimated using a Hawksley micro-haematocrit reader (Hawksley and Sons, Lancing, UK).

3.1.5 Longitudinal Study

Assessment of the therapeutic and prophylactic activity of isometamedium chloride in the field

In order to carry out a preliminary study on the efficacy of isometamidium chloride 50 naturally trypanosomes infected Zebu cattle were selected from villages 24 and 26. Village 26 was selected because of high prevalence rate of trypanosome infections (34%) during the cross sectional study and because the result of questionnaire suggested the occurrence of drug resistance in this village. Village 24, with low prevalence rate of trypanosome infections (10%) during the cross sectional study, was selected purposely because of its closeness to village 26 and availability of veterinary facilities. The proportion of infected animals with *T. congolense*, *T. vivax* and *T. brucei* were determined prior to treatment with the parasitological methods described before. Additionally, PCV values of each cattle before treatment were determined. Each of the 50 animals were ear tagged using plastic tags numbered from 51 to 100 so that they could be easily identified during the field visits. Parameters like sex, age, date of treatment and dosages used in each case were recorded (see Annex 2). This longitudinal study was carried out between May 1997 and July 1997.

Treatment

The 50 parasitologically positive animals were treated intramuscularly with isometamidium chloride (Trypamidium ®, Lot No. U6962/E; Rhône Mérieux, France) at a dose of 1 mg/kg bw. For calculating the treatment dose, the body weights of each of the animals was estimated before treatment, using tapes for measuring heart girz (Arora *et al.*, 1981) and body condition scoring (Nicholson and Sayers, 1987) methods.

Monitoring

Blood samples were collected from the ear vein and examined for Packed Cell Volume (PCV) and parasitaemia was determined with the phase contrast buffy coat method (Murray *et al.*, 1977). Cattle were monitored every 30 days for a period of 90 days post isometamidium block treatment. Consequently, the PCV of each sample was determined using a Hawksley micro-haematocrit reader. When relapse/breakthrough infections are recorded parasitaemic animals were treated again with 7.0 mg/kg bw of diminazene aceturate (Berenil ®).

Field isolation of trypanosomes

T. congolense field isolates were harvested from parasitaemic cattle before isometamidium treatment by collecting 5 ml of blood into potassium ethylenediamine tetra acetate (EDTA) treated vacutainers, stabilated in liquid nitrogen and transferred to laboratory at Debre Zeit. At Debre Zeit, the isolates were expanded in mice. Relapse/breakthrough infections were inoculated i.p. into mice in the field and thereafter transferred to Debre Zeit for further studies. The stabilates were prepared using the protocol indicated in Annex 5.

3.2 Drug Sensitivity Studies of trypanosomes in Mice

3.2.1 Experimental animals

Swiss white mice, with age range of 8-10 weeks and weighing 20-25 g, were obtained from the breeding colony of the Ethiopian Health and Nutrition Research Institute and maintained on a commercial pelleted ration and water *ad libitum*. They were housed in a fly-proof stable at the Faculty of Veterinary Medicine, Debre Zeit.

3.2.2 Experimental design

Six drug sensitivity studies were conducted, three using isometamidium chloride (Trypamidium ®, Rhône Mérieux) and the other three with diminazene aceturate (Berenil ®, Hoechst).

In each of the studies 25 mice were divided randomly into five experimental groups of 5 mice each (I-V). The first four groups (Group I-IV) formed the infected and treated groups with differing doses of trypanocidal drugs. The fifth group (Group V) served as untreated control (Tables 1 and 2).

Table 1 Isometamidium chloride (Trypamidium ®) treatment groups

Group	Number of Mice	Infection with *T. congolense*	Isometamidium treatment at a dose of (mg/kg)
I	5	+	0.5
II	5	+	1
III	5	+	2
IV	5	+	4
V(untreated control)	5	+	–

Table 2 Diminazene aceturate (Berenil ®) treatment groups

Group	Number of Mice	Infection with *T. congolense*	Diminazene treatment at a dose of (mg/kg)
I	5	+	3.5
II	5	+	7.0
III	5	+	14.0
IV	5	+	28.0
V(untreated control)	5	+	–

Infection

Three stocks of *T. congolense* isolated from three different animals in the field and expanded in mice were used to infect the experimental mice in group I-V. *T. congolense* isolates MBOI/ET/97/Pawe73 and MBOI/ET/97/Pawe87 were obtained from animal number 73 and 87 respectively, which showed relapse/breakthrough infections two months after treatment with 1 mg/kg bw isometamidium chloride (see Annex 2). These isolates were used for the drug sensitivity studies in mice after two passages. Whereas, *T. congolense* isolate MBOI/ET/97/Pawe77 was obtained from animal number 77 which showed relapse/breakthrough infections one month after treatment with 1 mg/kg bw isometamidium chloride. It was used for the drug sensitivity test after four passages in mice. The mice were infected with 10^6 trypanosomes/ml blood by intraperitoneal inoculation with blood from infected mice taken at the first parasitaemic peak.

Treatment

Diminazene aceturate (Berenil ®, Lot No. 518W742; Hoechst, Germany) and isometamidium chloride (Trypamidium ®, Lot No. U6962/E; Rhône Mérieux, France) were used for drug sensitivity studies in mice. Mice were treated 24 hours post infection. Mice were numbered using cupric acid and weighed on a flat pan balance prior to administration of trypanocidals for calculation of drug dosages.

Calculation of treatment doses

Mice were weighed on a flat pan balance prior to administration of trypanocidals for calculation of drug dosages. The majority of mice weighed 25g. The body weight for each mouse was assumed 25g to calculate the dosage of each trypanocidal drug. The steps used for calculating treatment doses are shown in Annex 4.

Monitoring

After treatment mice were monitored every other day for the presence of trypanosomes (wet smear, tail blood).

3.3. Data analysis

The prevalence rate of trypanosome infection is calculated as the number of parasitologically positive animals as examined by dark ground phase contrast buffy coat method (Murray *et al.*, 1977) divided by the total number of animals investigated at that particular time.

Confidence intervals (95%) for the prevalence rates of trypanosome infections in different villages were calculated. Chi-square test was used to compare the prevalence rates of trypanosome infections in different villages (SAS, 1987). Students t-test was utilised to

compare the mean PCV of the parasitaemic cattle with that of the aparasitaemic cattle (SAS, 1987).

The prevalence rate of relapse infections in cattle after treatment was calculated as the number of animals with relapse/breakthrough infections on the day of monitoring divided by the total number of animals examined at that particular day. Once an animal showed relapse/breakthrough infection it was excluded from the calculation in the following monitoring days. Least Square Repeated measure analysis of variance was used to show the effect of isometamedium therapy on PCV on 0, 30, 60 and 90 days after treatment (SAS, 1987).

In the mice experiment incidence of relapse, mean time of relapse and the correlation between the time of relapse and the dose of drugs were analysed using a statistical software (SAS, 1987).

4. RESULTS

4.1 Field Studies

4.1.1 Questionnaire

The first attempt to introduce cattle in to this area was in 1985/86 when the Ministry of Agriculture brought around 600 head of cattle into the Beles settlement area from the nearby highlands. Since then trypanosomosis remained to be major constraint to livestock production in the area. Many of the farmers in the present study villages responded that they knew the disease trypanosomosis in the lowlands of the Beles-Valley since their resettlement in 1984/85. Although some of the resettlers moved to the new resettlement areas along with their livestock there was a severe loss of livestock up on arrival. Trypanosomosis is claimed to be the major cause for these losses.

Management

Free grazing covers the largest proportion (99%) of the livestock feed. Cattle in the area graze in large herds. Most of the livestock follow the same pattern of grazing. The majority (96%) of the farmers move their cattle for as long as 5 km for grazing. The grazing areas are in most cases very close to livestock watering points. According to the individual testimony 96% of the farmers water their cattle around the grazing field, only few of them move for a longer distance.

Livestock feed is better available during the rainy season (July-September) than the dry season (October-April).

Livestock Diseases

Among diseases affecting livestock in the area, the livestock owners interviewed mentioned trypanosomosis, pasteurellosis, anthrax, and gastro-intestinal helminthiasis as the most important livestock diseases. All the groups ranked trypanosomosis as the first most important disease of livestock followed by pasteurellosis and anthrax. The farmers claimed trypanosomosis to cause reduced appetite, rough hair coat, diarrhoea, emaciation, weakness and as a result a reduction in the working power of their ploughing oxen. Trypanosomosis

affect their cattle both during the rainy and dry season, although their cattle look in a better condition during the rainy season, the group interview responded. All the groups interviewed in village 26 agreed that trypanosomosis is getting worse in the area. In contrast, groups interviewed in village 24 indicated that due to better veterinary services provided during the last two years the disease situation has improved. However, other groups who are receiving the same veterinary service but living in a different village (village 12) stated that trypanosomosis still affects their livestock. Generally, the livestock owners in the area are desperate because of the problem of trypanosomosis on the one hand and that of malaria on the other hand. They stated that if these problems are not solved they may be forced to abandon the area.

Treatment

According to the questionnaire, 35% of the livestock owners are treating their cattle themselves against trypanosomosis. In 30% of the cases treatment is done by the owners and/or by veterinarians or veterinary assistants. When it comes to village 12 and 26 alone some 47% and 42% of the livestock owners are treating their cattle themselves, respectively. Personnel involved in the treatment of livestock is indicated in Figure 2. Livestock owners (farmers) account for some 34% of the treatments and drug smugglers (people who bring trypanocidals and other drugs from towns and sell them to farmers in the area) for 9% of the treatment.

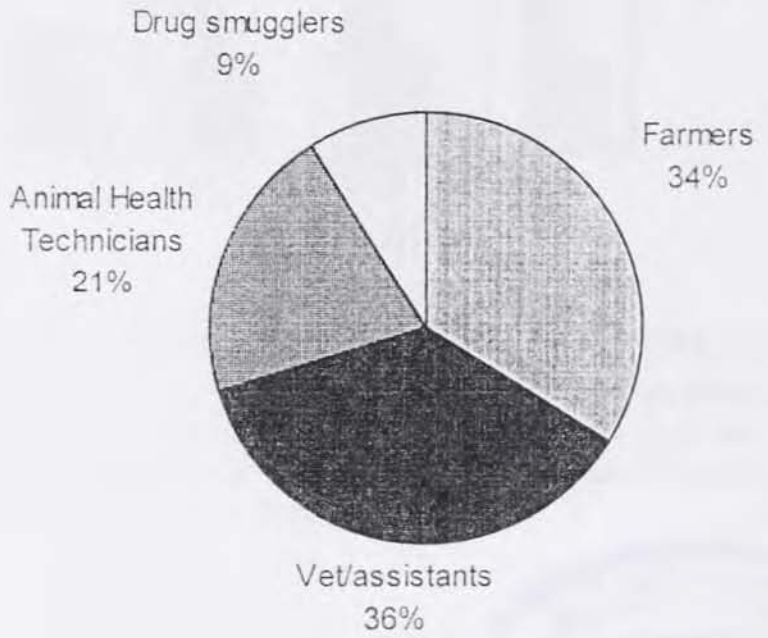

Figure 2 Personnel involved in the treatment of animal trypanosomosis in Metekel district, North-west Ethiopia, as indicated by questionnaire results, March 1997 (Drug smugglers are non-professional people who bring trypanocidals and other drugs from towns and sell them to farmers in the area illegally)

7 (70%) of the groups said that they get trypanocidals from veterinary clinics and open markets and pharmacies. 3 (30%) of the groups utilise veterinary clinics as a major source of trypanocidals. Nevertheless, when these drugs are lacking in the veterinary clinics, drugs available in open markets and pharmacies are used.

The common trypanocidals used in the area are diminazene aceturate, isometamidium chloride and homidium. Some of the farmers could not tell the usual dose of each trypanocidals used to treat their livestock. However, most farmers (particularly in village 26) said that local farmers and the animal health technicians in the village are using one sachet of Berenil (1.05 g) for 2 to 3 cattle and one tablet of Novidium for 2 cattle. Figure 3 shows utilisation of trypanocidals by farmers. Number of treatment of cattle against trypanosomosis since last year as indicated by the interviewee is illustrated in Figure 4.

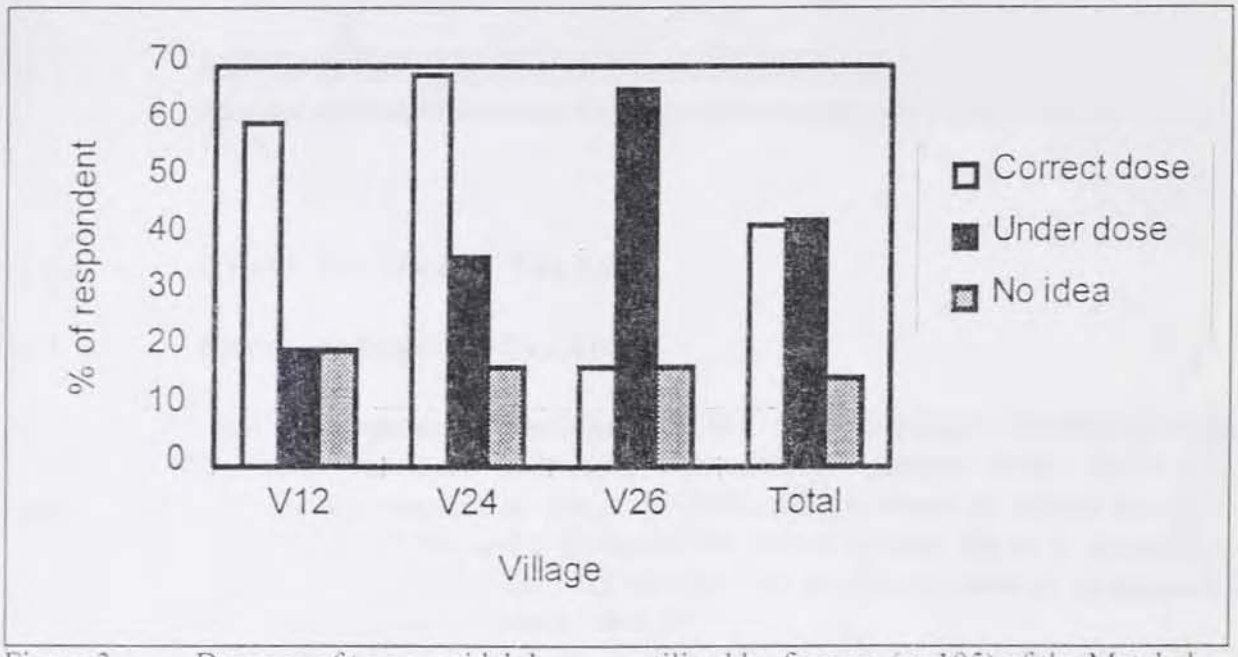

Figure 3 Dosages of trypanocidal drugs as utilised by farmers (n=105) of the Metekel district, North-west Ethiopia, indicated by questionnaire, March 1997. (Correct dose: 1 sachet of Berenil® or 1 tab of Ethidium for 1 adult cow; Under dose: 1 sachet of Berenil® or 1 tab of Ethidium for more than 2 adult cows; No idea: farmers with no idea for this question)

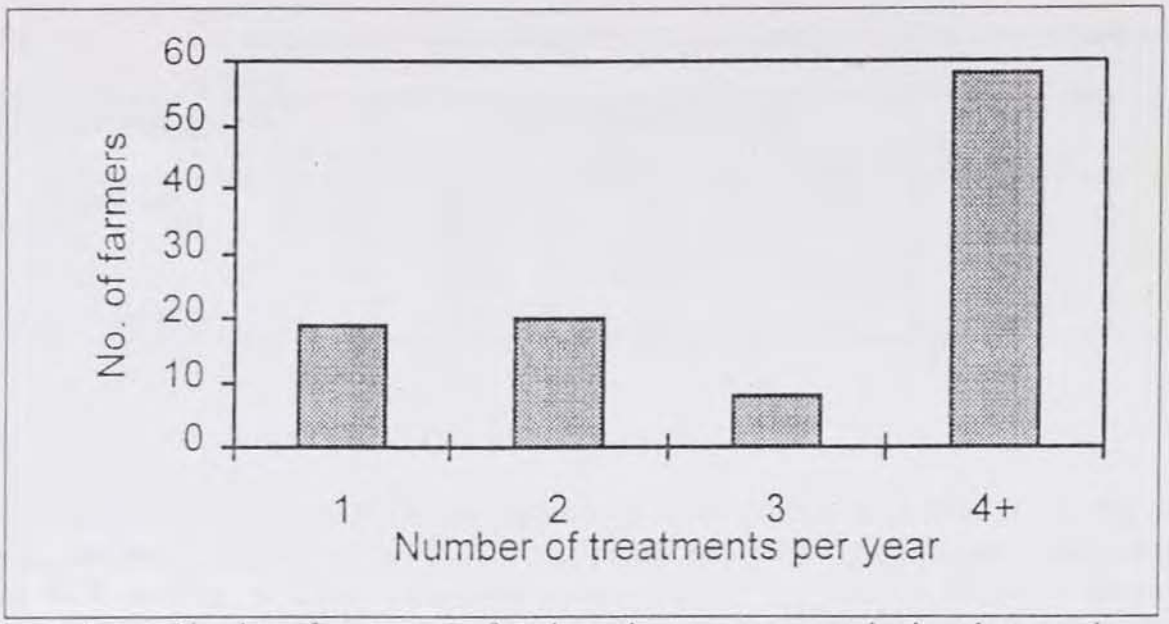

Figure 4 Number of treatments of cattle against trypanosomosis since last year in Metekel district, North-west Ethiopia as indicated by questionnaire, March 1997.

4.1.2 Cross Sectional Study

4.1.2.1 Parasitological Findings

The prevalence rate of trypanosome infections in cattle in the four villages investigated using parasitological methods was found to be 17.2% (Confidence interval: 14.02, 20.78). The highest prevalence rate was recorded in village 26 (34%) and the lowest in village 24 (10%). 40 (47.6%), 33 (39.3%), 9 (10.7%) and 2 (2.4%) of the infections were due to *T. congolense, T. brucei, T. vivax* and mixed infections, respectively. The prevalence rates of trypanosome infections in different villages are shown in Table 3.

Table 3 Prevalence rate and 95% confidence interval (CI) of trypanosome infections in cattle in four villages of the Metekel district, North-west Ethiopia, March 1997.

Village	Number of cattle examined	Trypanosome prevalence rate (%)	95% Confidence interval
12	100	14[a]	(7.2, 20.8)
13	48	13[a]	(3.14, 21.86)
24	216	10[a]	(6.16, 14.24)
26	120	34[b]	(25.71, 42.69)
Total	484	17.2	(14.02, 20.78)

[a, b] Prevalence followed by different superscripts are significantly different (Chi square-Test, $p<0.01$)

Table 4 shows the relative importance (occurrence) of trypanosome species in four different villages. There were differences between villages as regards the most prevalent trypanosome species. At one extreme, 50% of the parasitaemia in village 13 and 58.5% of the parasitaemia in village 26 resulted from *T. congolense* infections, while *T. brucei* was the dominant species in village 12 (42.9%) and village 24 (60.9%).

Table 4 The relative importance of different trypanosome species in four different villages

Trypanosome species	Percentage of infection			
	Village 12	Village 13	Village 24	Village 26
T. congolense	28.6	50	39.1	58.5
T. vivax	21.4	16.7	-	26.8
T. brucei	42.9	33.3	60.9	12.2
Mixed infections	7.1	-	-	2.5

4.1.2.2 Haematological Findings

The mean PCV (%) value of the total number of cattle tested was 24.85±0.20. 83.9% of the total number of cattle tested had PCV values of less than 27%. The frequency distribution of the PCV readings of the overall cattle tested is given in Figure 5. Figure 6 shows the frequency distribution of PCV (%) readings of the parasitaemic cattle only. More than 90% of the parasitaemic cattle had PCV values of less than 27%. Fewer than 10% of blood samples were found to be parasitologically positive when PCV was above 27%.

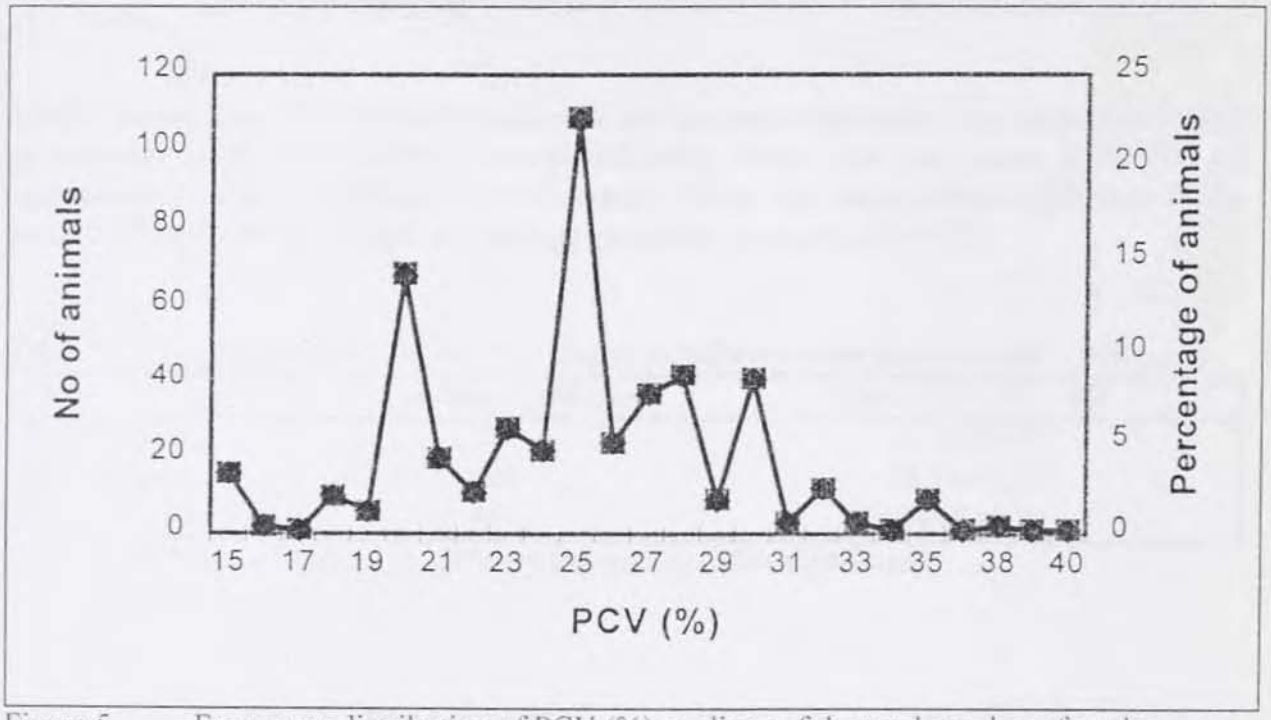

Figure 5 Frequency distribution of PCV (%) readings of the total number of cattle tested in four villages of the Metekel district, North-west Ethiopia, March 1997.

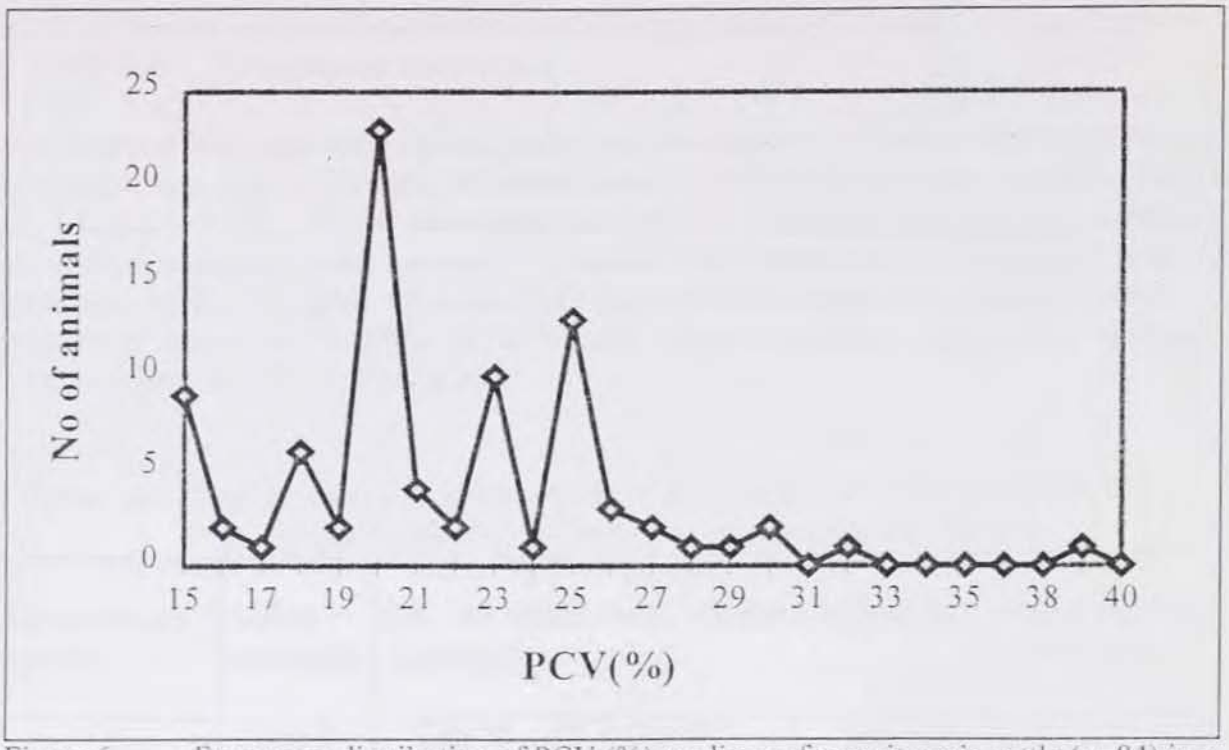

Figure 6 Frequency distribution of PCV (%) readings of parasitaemic cattle (n=84) in a cross sectional study in Metekel district, North-west Ethiopia, March 1997.

Table 5 shows mean PCV (%) of parasitaemic and aparasitaemic cattle. The mean PCV (%) of parasitaemic cattle (21.63±0.47) was significantly lower than the mean PCV (%) of aparasitaemic cattle (25.54±0.21) ($p<0.05$, t-test). There was no significant difference in the mean PCV between age groups, sex and age group-sex interaction ($P>0.05$).

Table 5 Comparison of mean PCV (%) of parasitaemic and aparasitaemic cattle

Cattle	No. of cattle Examined	Mean PCV (%) ±SE
Parasitaemic	84	21.63±0.47 [a]
Aparasitaemic	400	25.54±0.21 [b]
Overall	484	24.85±0.20

a, b Means followed by different superscript differ significantly ($p<0.05$, t-test)

4.1.3 Longitudinal Studies

The interest of this study was to evaluate the therapeutic and/or prophylactic efficacy of isometamidium chloride to natural trypanosome infections. Therefore, 50 infected Zebu cattle were selected from two different villages (village 24 with trypanosome prevalence rate of 10% and village 26 with 34%). Village 26 was selected because of the high prevalence of trypanosome infections during the cross-sectional study and because the result of the questionnaire suggested the occurrence of drug resistance in this village. Village 24, with low prevalence rates of trypanosome infections during the cross-sectional study, was selected purposely because of its closeness to village 26 and availability of veterinary facilities. Stained blood smears, prepared from each animal on the day of treatment, indicated that

52.4% of the cattle showed an infection with *T. congolense*, 20.8% with *T. vivax*, 18.8% with *T. brucei* and 6.3% had mixed infections.

The results of drug sensitivity test in the field are summarised in Table 6. During the 90 days of monitoring a total of 25 cases of relapse/breakthrough infections were recorded (Table 6 and Annex 3). At day 30 post isometamidium treatment 6 cases (13%), all of them being *T. congolense* infections, were recorded. All cattle with parasitaemia were treated with 7.0 mg/kg bw. At day 60, again, 12 animals (27.9%) showed relapse/breakthrough infections. *T. congolense* accounted for 80% of the overall relapse infections. Most cases of relapse infections were recorded in village 26.

Table 6 Drug sensitivity of *trypanosomes* in Zebu cattle (n=50) naturally infected in the field and treated with 1 mg/kg bw isometamidium chloride

Trypanosome species	before treatment	No. of cattle with relapses (days after treatment)			Total number of relapses
	Day 0	Day 30	Day 60	Day 90	
T. congolense	26	6	8	6	20
T. vivax	10	-	-	1	1
T. brucei	9	-	2	-	2
Mixed	5	-	2	-	2
Number examined*	50	46	43	31	
Percentage of relapses		6/46 (13%)	12/43 (27.9%)	7/31 (22.6%)	25/50 (50%)

* number of cattle present and examined at each visits excluding cattle which already showed relapse/breakthrough infections during the previous visits.

PCV readings

There were significant increases in the average PCV readings of the cattle during the first months after treatment (Table 7). However, the development of subsequent relapse infections was followed by falls in haematocrit values (Table 7).

Table 7 Average PCV (%) readings after treatment with 1 mg/kg bw isometamidium chloride and subsequent treatment of each relapse/breakthrough infections with 7 mg/kg diminazene aceturate (Repeated measure ANOVA)

No. examined*	Days after treatment	Mean PCV (%)
39	0	22.3958^{a}
39	30	26.1304^{b}
39	60	26.0625^{b}
39	90	24.5744^{b}

$^{a, b}$ Means followed by different superscripts differ significantly ($p<0.01$)

* number of animals present and examined at all visits.

4.2 Drug Sensitivity Studies in Mice

Three *T. congolense* field isolates were selected randomly from the relapsed population in the field (see Annex 3) and tested for their drug sensitivity in mice.

The results of drug sensitivity tests in mice are summarised in Tables 8, 9 and 10. Isometamidium chloride administered i.p. at doses of 0.5 to 4 mg/kg bw failed completely to cure mice infected with the three *T. congolense* isolates. Likewise, treatments with diminazene aceturate at doses of 3.5 to 28 mg/kg bw did not clear the parasites in all of the mice infected with three different *T. congolense* isolates (Tables 8, 9 and 10). There was a correlation between the time of relapse and the dose of the drugs used such that mice treated at lower doses showed a shorter time to relapse than mice treated at higher doses ($P<0.001$). Most of the mice died of infection 3 to 4 weeks after the infection had relapsed.

Table 8 Drug sensitivity of *T. congolense* (MBOI/ET/97/PAWE 73) in mice treated with diminazene and isometamidium

Drug/dose	Number of mice relapsed/treated	Mean relapse interval in days (±SD)
Diminazene aceturate		
3.5 mg/kg	5/5	5.8±0.45
7.0 mg/kg	5/5	6.0±0.0
14.0 mg/kg	5/5	8.2±2.05
28.0 mg/kg	5/5	15.6±2.5
Isometamidium chloride		
0.5 mg/kg	5/5	6.4±0.89
1.0 mg/kg	5/5	7.4±0.89
2.0 mg/kg	5/5	9.6±0.89
4.0 mg/kg	5/5	16.6±2.1

Table 9 Drug sensitivity of *T. congolense* (MBOI/ET/97/PAWE 77) in mice treated with diminazene and isometamidium

Drug/dose	Number of mice relapsed/treated	Mean relapse interval in days (±SD)
Diminazene aceturate		
3.5 mg/kg	5/5	6.2±1.1
7.0 mg/kg	5/5	5.8±1.8
14.0 mg/kg	5/5	7.8±1.1
28.0 mg/kg	5/5	7.8±1.1
Isometamidium chloride		
0.5 mg/kg	5/5	5.8±1.1
1.0 mg/kg	5/5	7.6±2.2
2.0 mg/kg	5/5	10.2±1.8
4.0 mg/kg	5/5	19.4±6.5

Table 10 Drug sensitivity of *T. congolense* (MBOI/ET/97/PAWE 87) in mice treated with diminazene and isometamidium

Drug/dose	Number of mice relapsed/treated	Mean relapse interval in days (±SD)
Diminazene aceturate		
3.5 mg/kg	5/5	5.2±0.4
7.0 mg/kg	5/5	3.6±1.3
14.0 mg/kg	5/5	5.4±1.3
28.0 mg/kg	5/5	7.6±1.7
Isometamidium chloride		
0.5 mg/kg	5/5	4.2±1.6
1.0 mg/kg	5/5	3.0±0.0
2.0 mg/kg	5/5	9.0±2.0
4.0 mg/kg	5/5	23.4±5.1

5. DISCUSSION

A questionnaire was administered to livestock owners to know whether trypanosomosis is a constraint to livestock production in the area and in that case to identify cattle herds highly suspicious for carrying drug resistant trypanosome populations.

The results of the questionnaire suggested that trypanosomosis occurred in the area from the time of settlement of the farmers and that there is considerable losses due to the disease. If the losses due to trypanosomosis is continuing in the same extent, it may result in total abandonment of the area by the farmers. The results also indicated that there is indiscriminate use of trypanocidal drugs to treat cattle. Treatment of cattle by farmers is a common phenomenon and most farmers are underdosing their cattle. Some of the reasons for underdosing cattle are the ever-increasing prices of trypanocidal drugs, lack of availability of trypanocidal drugs, insufficient veterinary services and lack of knowledge of utilisation of trypanocidal drugs. This result is in agreement with the report of Geerts and Holmes (in press). These authors stressed that many errors occur in calculating the correct doses for the treatment of animals because of the fact that unskilled persons are allowed to administer drugs. Furthermore, as the drugs are relatively expensive there is a temptation to overdilute the drug and hence underdosage. Underdosing is one of the major causes of resistance development. Subtherapeutic drug concentrations exert a strong selective pressure for the emergence of resistant clones that pre-exist in the trypanosome population.

In the present study an overall trypanosome prevalence rate of 17.2% (confidence interval CI: 14.02, 20.78) was recorded. The result disclosed that *T. congolense* with 47.6% of the infection is the most prevalent trypanosome species in the village cattle population of the Metekel district. The finding that *T. congolense* is the most prevalent trypanosome species in the study population is in accordance with earlier reports from other parts of Ethiopia (Rowlands *et al.*, 1993; Abebe and Jobre, 1996). Rowlands *et al.* (1993) reported a prevalence rate in cattle of 37% for *T. congolense* in South-west Ethiopia. A recent report by Abebe and Jobre (1996) indicated infection rate of 58.5% for *T. congolense*, 31.2% for *T. vivax* and 3.5% for *T. brucei* in the tsetse infested areas of Ethiopia.

In village 26, veterinary services are poor and most cattle are treated by livestock owners, whereas in village 12, 13 and 24 the veterinary services provided are more developed. Significantly lower prevalence rates of trypanosome infections were recorded in these villages when compared to village 26. This may be basically due to the better veterinary services provided to these villages and the vector control program which was still active during the study period (Tilahun *et al.*, 1997). At the beginning of this vector control intervention (November, 1995), the prevalence rates of trypanosome infections in villages 12, 13 and 24 were 15.6%, 19.5% and 27.5%, respectively (Tilahun *et al.*, 1997). The results in the present study indicate a decline in the prevalence rate of trypanosome infections particularly in villages 13 and 24, following the intervention under the pilot vector control trial. A drop both in the apparent density of the tsetse flies (*G. tachinoides*) and the prevalence rate of trypanosome infections observed in the three villages during the two years pilot vector control interventions was mainly reflected in *T. vivax* infections (Tilahun *et al.*, 1997). For instance, pre-intervention haematological examination in 1995 showed that in village 24.90.2% of the trypanosome infections were due to *T. vivax*, 6.0% due to *T. congolense* and 3.85% due to *T. brucei* (Tilahun *et al.*, 1997). In this cross-sectional study no *T. vivax* infection was encountered in the same village and the percentage of *T. congolense* and *T. brucei* infections were 60.9% and 39.1%, respectively. One of the factors which could have depressed the incidence of *T. vivax* is the use of drugs. The lower ratio of *T. vivax* to *T. congolense* and *T. brucei* infections in this study supports earlier findings (D'Ieteren *et al.*, 1988) and suggests that cattle may more readily develop immunity to *T. vivax* than *T. congolense* (MacLennan, 1970). In East Africa, *T. vivax* is generally less virulent than *T. congolense* and consequently cattle develop tolerance to *T. vivax* more readily and easily than to *T. congolense*.

In the present cross-sectional study an overall mean PCV value of 24.8% was recorded. More than 90% of the parasitaemic cattle had PCV values of less than 27%. The mean PCV of the parasitaemic cattle (21.63±0.47) was significantly lower than that of aparasitaemic cattle (24.85±0.2). The appearance of parasitologically negative animals with PCV values of less than 27% may be due to the inadequacy of the detection method used (Murray *et al.*, 1977) or delayed recovery of the anaemic situation after current treatment with trypanocidal drugs. Trypanosome infection is characterised by an intermittent increase and drop of parasitaemia. More sensitive diagnostic techniques which can be used in the field are urgently required in order to ascertain the true prevalence of trypanosomes in the field. The appearance of negative results in animals with PCV values of less than 27% may also possibly be attributed to the compounded effects of poor nutrition and concurrent haematophagus helminth infections such as haemonchosis and bunostomiasis. Coprological examination showed that 17.8% of the cattle in village 24 and 10.5% in village 13 were harbouring nematode eggs (Tilahun *et al.*, 1997). The importance of gastrointestinal helminthosis in the area was also underlined by the livestock owners. The appearance of positive animals with PCV of greater than 27% may be explained by recent infections of the animals. In conclusion, the present study revealed that parasitaemia depressed PCV.

Village 26 was selected for the longitudinal field study because of the high prevalence of trypanosomes recorded during the cross-sectional study and based on the results of the questionnaire, which suggested that there is indiscriminate use of trypanocidal drugs to treat cattle, usually by farmers and more complaints of livestock owners and herdsmen in this village about the success of treatment. Additionally, village 24, with lower prevalence rates of trypanosome infections, was included in the study purposely because of better farmers co-operation, availability of some facilities for the study and its closeness to village 26.

The results of the longitudinal drug sensitivity study demonstrated 6/46 (13%) relapse/breakthrough infections in the field within 4 weeks of treatment with the prophylactic dose of isometamidium chloride. Most cases of the relapsed/breakthrough infections (80%) were due to *T. congolense*. The results presented here have shown that the period of prophylaxis conferred by isometamidium against trypanosomes, mainly of *T. congolense*, was very short; less than 1 month. This is further supported by the results of drug sensitivity tests in mice. Similar results have been reported by Rowlands *et al.* (1993) from South-west Ethiopia and by Sutherland *et al.*(1991) under controlled laboratory conditions. Sutherland *et al.* (1991) reported that the period of prophylaxis conferred by 1.0 mg/kg bw isometamidium chloride was less than 28 days in cattle challenged with a clone of *T. congolense* which in therapeutic trials had been shown to be highly resistant to isometamedium chloride.

At present the prevalence of drug resistance in trypanosomes across Ethiopia is unknown. The result presented here is in accordance with earlier reports in South-west Ethiopia (Scott and Pegram, 1974; Codjia *et al.*, 1993). Scott and Pegram (1974) described the occurrence of homidium resistant populations of *T. congolense* in Didessa and Angar valleys in Wollega province. Their field observations showed that 25% of treated cattle developed parasitaemia within 30 days of treatment with 1 mg/kg bw of homidium. The work done in Ghibe valley, Ethiopia, described the presence of multiple-drug resistant strains of *T. congolense* species (Codjia et al., 1993). Moreover, recent field observations in Ethiopia based on cloned populations showed that the drug-resistant phenotype of *T. congolense* had not altered over a period of 4 years (Mulugata *et al.*, 1997). Because high levels of multiple-drug resistant infections appeared to be expressed at the level of individual trypanosomes, it was concluded that chemotherapeutic agents *per se* would not control trypanosomosis at Ghibe on a long term basis (Codjia *et al.*, 1993).

Despite a significant percentage of relapse infections in the present study, treatment with 1 mg/kg bw of isometamidium chloride has shown an improvement in the PCV of the cattle during the first months after treatment. Cattle which showed relapse infections were subsequently treated with 7.0 mg/kg bw diminazene aceturate. This improvement in the PCV readings after treatment may be due to elimination of the sensitive populations of trypanosomes from the animal body. Mulugeta *et al.* (1997) stressed that administration of different drugs to which the subpopulations are sensitive, will eliminate the whole trypanosome population.

The results obtained here in mice confirm the results of the field study. Isometamidium chloride administered intraperitoneally at doses of 0.5 to 4.0 mg/kg bw and diminazene aceturate at doses of 3.5 to 28 mg/kg bw failed completely to cure mice infected with three different *T. congolense* field isolates. Since both drugs failed to cure the infections in all doses tested, the minimum curative dose (MCD) for each of the isolates could not be determined. However, from the results obtained the minimum curative dose (MCD) values for all the three isolates appeared to be greater than 4 mg/kg bw of isometamidium chloride and greater than 28 mg/kg bw of diminazene aceturate. This finding is in agreement with the reports of Chitamo and Arakaw (1992) who described that clones derived from the Mumbwa isolate were resistant to isometamidium, with MCD of 4 mg/kg bw isometamidium, and to diminazene, with MCD of 14 to 28 mg/kg bw diminazene. The same authors used a single-trypanosome derived clonal population from the original Chisamba field isolates as a sensitive control; it was proved to be sensitive to both isometamidium and diminazene, with minimum curative dose (MCD) values (minimum dose necessary to achieve 100% cure in at least 3 mice) of 0.5 mg/kg bw isometamidium and 7.0 mg/kg bw diminazene in mice. The isolates

described in the present work indicated that even 4 fold of the MCD for the sensitive control isolates used in Zambia (Chitambo and Arakaw, 1992), in the case of diminazene, and 8 fold in the case of isometamidium, did not clear the parasites in mice. The present field isolates also had greater MCD when compared to the Zambian resistant isolates described above.

Therefore, the three *T. congolense* field isolates in the present study, besides showing relapse to treatment with 1 mg/kg bw of isometamidium in cattle, expressed level of resistance to both isometamidium and diminazene when examined in mice. It is not known whether the double-resistance phenotype of these stocks is because they contained at least 2 distinct populations, one of which expressed resistance to isometamidium and the other which expressed resistance to diminazene, or because the stocks contained parasites which expressed resistance to both drugs. If the latter was the case, then the use of sanative pairs would be ineffective to treat such infections. Further studies are urgently required in order to verify this dispute by deriving clones from the present stocks and characterising them in mice for their sensitivity to both diminazene and isometamidium.

The drug sensitivity of trypanosome populations can be characterised by using *in vitro* cultivation (Kaminsky *et al.*, 1990; Elrayah and Kaminsky, 1991), mice (Scott and Pegram, 1974; Kaminsky *et al.*, 1990; Elrayah and Kaminsky, 1991) and ruminants. In this study cattle naturally infected in the field were used so that the data can have direct relevance to the field, which is not the case with data obtained in mice or *in vitro* as there is no consistent correlation between the sensitivity expressed in these systems and that expressed in cattle (Sones *et al.*, 1988). These cattle were not kept in fly-proof stables or in a non tsetse area but rather they were left to graze under their natural environment. Therefore, the risk of reinfection during the study was not eliminated. We do not know whether the relapse in the field is from the trypanosome population which was already present in the animals before treatment or from populations after reinfection with drug resistant trypanosomes. However, this technique generates a useful preliminary information as to the efficacy of isometamidium chloride to trypanosome populations present in the area is concerned. Moreover, it can also be used in situations where laboratory facilities are very limited like the case in Metekel region. The method showed that isometamidium chloride at a dose rate of 1 mg/kg bw is no more accomplishing its originally described mission of protecting cattle for a period of 14 to 36 weeks as Metekel region, North-west Ethiopia is concerned. Nevertheless, this method usually requires long follow up period and one can not exclude the possibility of interference of the study animals by the livestock owners. The assay in mice, also used in the present study, has an advantage of cheaper alternatives. However, it suffers from disadvantages that most *T. vivax* and some *T. congolense* isolates do not grow in mice, assessment of the degree of resistance requires a large number of mice per isolate and it usually takes 30-60 days to evaluate the drug sensitivity of an isolate which is quite long. Standardisation of the existing tests should receive high priority, especially the assays in mice and in the definitive hosts, because these can be carried out in less well equipped laboratories. This should allow the establishment of a resistant monitoring system, to compare data on a temporal and spatial basis across Africa.

Drug therapy has been the main strategy used in the past to control trypanosomosis in the study site and throughout Ethiopia (Slingenbergh, 1992). There is a flourishing black market and farmers can purchase a variety of trypanocidal drugs in most village markets (Questionnaire result), although all trypanocidal drugs are supposed to be imported through the Ministry of Agriculture. It is suspected that the widespread use and misuses of drugs contributed to the development of drug resistance in the population of *T. congolense* parasites

in the Metekel region of North-west Ethiopia. Whiteside (1960) recorded that circumstances tending to produce resistant parasite populations in the field include underdosing with anti-trypanosomal drugs, the irregular use of prophylactics or their discontinuation while cattle remain at risk, and the high incidence of trypanosomosis. Generally, the exposure of parasites to subtherapeutic drug concentrations, due to underdosing and uncontrolled use of trypanocidal drugs due to lack of proper diagnosis have been considered as the major reasons for the increasing drug resistance throughout Africa. However, Clausen *et al.* (1992) and Geerts and Holmes (in press) stressed that the prolonged and frequent use of trypanocides in high challenge areas, even when well applied, is likely to select for resistance as well.

The epidemiology of drug resistant populations of trypanosomes is dynamic i.e. once established the incidence is progressively spread within the population. For instance, the incidence of recurrent infection was 7% in 1986 and it increased to 14% in 1989 in the Ghibe valley of Ethiopia (Rowlands *et al.*, 1993). Transmission by tsetse flies does not appear to affect the drug sensitivity of trypanosomes and drug resistant strains remain resistant after passage through tsetse flies (Moloo and Kutuza, 1990). Therefore, this trait is known to be stable for a long time and such stocks can spread to wider areas through cattle movement and/or the spread of tsetse populations. There is therefore an urgent need for detailed experimental work in the field as well as under laboratory conditions to monitor the development of drug resistance in pathogenic trypanosomes and its impact on livestock productivity in Metekel region in particular and across the tsetse infested zone of Ethiopia in general. Furthermore, socio-economic studies should be conducted to identify factors which influence the development of resistance to trypanocidal drugs.

6. CONCLUSIONS

I. This study demonstrated that trypanosomosis is a major constraint to livestock development in selected villages of the Metekel district, North-west Ethiopia. The trypanosome prevalences appeared to be higher in villages where the veterinary service is poor, most cattle are treated by livestock owners and as a result cattle are exposed to subcurative doses of trypanocidal drugs.

II. The general health condition of the cattle population under study, as indicated by PCV, was poor. The mean PCV value of the cattle examined was found to be 24.8%. Parasitaemia depressed PCV significantly.

III. The field study has shown that the period of prophylaxis conferred by 1 mg/kg bw isometamidium chloride in cattle for *T. congolense* infection was less than 1 month.

IV. Despite a significant percentage of relapse/breakthrough infections in the field, treatment with 1 mg/kg bw isometamidium and subsequent treatment with 7.0 mg/kg bw diminazene aceturate has shown an increase of PCV but only during the first month after treatment. However, this treatment regime would certainly not be cost effective and most probably select for drug resistant trypanosome populations.

V. Three *T. congolense* field isolates deriving from the study area showed resistance to both isometamidium and diminazene when examined in mice. Further studies are urgently required in order to verify whether this double resistance is expressed by individual

trypanosome population or by two distinct populations, one of which express resistance to isometamidium and the other to diminazene, by deriving clones and characterising them in mice for their sensitivity to both diminazene and isometamidium.

VI. Given the potential problems associated with drug resistance there is a need for detailed epidemiological work in the field as well as in the laboratory to monitor the development of drug resistance in pathogenic trypanosomes and its impact on livestock productivity in Metekel region in particular and across the tsetse infested zones of Ethiopia in general.

VII. The great agricultural potential of the Metekel region, North-west Ethiopia, can only be exploited if cattle trypanosomosis and the arising appearance of drug resistance is controlled. Therefore, more attention should be given to adopting an integrated disease management strategy, involving the vector as well as the parasite. Such strategies need to be economically feasible, socially acceptable and sustainable and environmentally sound.

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8. ANNEXES

Annex 1

QUESTIONNAIRE SET TO INTERVIEW A GROUP OF FARMERS ABOUT HERD STRUCTURE; DISEASE AND USAGE OF TRYPANOCIDAL DRUGS:

Peasant Association ..... Date .....

Village ..... Code .....

A. Livestock Management

1. When you first settled in this area which livestock did you start keeping?
   a. cattle
   b. small ruminants
   c. others (specify)

2. How do you manage cattle?
   a. Free grazing
   b. Tether
   c. Stall feed

3. If cattle are allotted to free grazing scheme, are they in herd? or in small groups?
.....
.....

4. Where do cattle graze?
.....
.....

5. Where is the location of livestock watering point?
.....
.....

6. Does the stream flow all around the year or it dries up in long dry season?
   Season of stream availability.....
   Season it dries up.....

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

8. How long is the distance of watering point from the cattle barn?
.....
.....

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

.....

.....

10. In which season is it least available?

.....

.....

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

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

B. Livestock Diseases

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

.....

.....

.....

2. Does trypanosomosis occur in this area?
(Yes, No, Other)

3. If yes, would you rank trypanosomosis with regard to cattle loses compared to other diseases?

.....

.....

4. Which livestock does trypanosomosis most affect?
- Cattle (Yes, No, Other)
- Small ruminants (Yes, No, Other)
- Others (specify)

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

.....

.....

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

.....

7. Was trypanosomosis present when you settle in this area?
(Yes, No, Other)

8. If no, when did you first encounter the problem of trypanosomosis in your cattle?
a. 1 year after settlement
b. 5 years after settlement
c. 10 years after settlement

9. Is trypanosomosis getting worse, better or unchanged in this area since you first encountered it in the area?
    a. it is getting worse
    b. it is getting better
    c. it is the same
    d. I do not know

10. Do you know that flies transmit trypanosomosis?
    (Yes, No, Others)
If yes, which flies do you think to transmit trypanosomosis? specify
    .....
    .....

11. Do you know that tsetse (local name) flies transmit trypanosomosis?
    (Yes, No, Others)
If yes, can you identify tsetse flies? (Yes, No, Other)

12. In which season/month are these flies most abundant?
    .....
    .....

13. Where is tsetse population high?
    a. in areas close to the river
    b. in the grazing scheme
    c. in the bush
    d. in savanna areas

C. Treatment

1. Where are the common treatment sources?
    a. Veterinary Clinic
    b. Local farmers
    c. Smugglers
    d. Others (specify)

2. Who is applying the treatment?
    a. Local farmers
    b. Veterinarian, assistant veterinarian, animal health technicians
    c. Smugglers
    d. Others (specify)

3. Which drugs are most commonly used in the area?
(names/types/colour etc.)
    .....
    .....
    .....

4. Since when have you been using each of these drugs?
   a. Since 20 years
   b. Since 10 years
   c. Since 5 years

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

.....

.....

Which ones are less effective?

.....

.....

6. When you use trypanocidals on cattle do you usually treat:
   a. all your animals
   b. only sick animals
   c. only mature oxen
   d. only cows in milk
   e. others (specify)

7. Can you tell the usual doses used per course of treatment per animal of different body weight?

.....

.....

8. Are there traditional treatments or management practices to cure animals from trypanosomosis? If yes, what?

.....

.....

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

If yes, what are the new areas affected?

.....

.....

Thank You!

Name of interviewer .....

Date .....Signature .....

QUESTIONNAIRE SET TO INTERVIEW INDIVIDUAL FARMERS

Name of the farmer ..... Code .....
Village ..... Date .....

A. Livestock Management

1. Which livestock do you keep?
    a. Cattle
    b. Small ruminants
    c. Others (specify)

2. How many cattle are under your management? How many are privately owned by you?
    .....

3. How do you manage cattle?
    a. Free grazing
    b. Tether
    c. Stall feed

4. If cattle are allocated to free grazing scheme, are they in herd or in small groups?
    .....

5. Where do cattle graze?
    .....

6. How long is the distance of grazing land from cattle barn?
    .....
    .....

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

B. Treatment

1. Where do you commonly treat your animals when they get sick with trypanosomosis?
    a. At home
    b. In Vet. Clinic
    c. Others (specify)

2. Who is applying the treatment?
    a. You yourself?
    b. Veterinarian or assistant veterinarian
    c. Animal health technician
    d. Drug Smugglers
    e. Others (specify)

3. Which trypanocidals are you commonly using to treat your animals?

.....

.....

.....

4. What quantity of trypanocidals do you use to treat your cattle? (in tab, sack etc.)

.....

.....

.....

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

.....

.....

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

a. one time only
b. two times
c. three times
d. more than three times

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

.....

.....

8. Of the cattle treated last time:

a. how many are healthy at present?.....
b. when were these animals lastly treated?.....
c. calculate days between treatment.....

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

.....

10. Do you have any trypanocidal medicine now in stock?

(Yes, No, Other)

If yes, can you show please? how many months since you acquired it?

How do you use it?.....

.....

Thank You!

Name of interviewer .....

Date .....

Signature.....

Annex 2 Experimental animals used for the drug sensitivity test in the field

Village	Animal no Ear tag no.	Sex	Age	Estimated body wt.	Parasitaemia before RX	PCV	Date of RX	Dose in mg/kg	Dose in ml
24	51	M	5	150	T. C	27	11/5/97	1	15
24	52	M	6	250	T. C	20	11/5/97	1	25
24	53	M	6	200	T. C	20	11/5/97	1	20
24	54	M	6	150	T. C	25	11/5/97	1	15
24	55	M	6	200	T. C	30	11/5/97	1	20
24	56	M	8	250	T. B	26	11/5/97	1	25
24	57	M	4	100	T. B	20	11/5/97	1	10
24	58	M	8	250	T. V	28	11/5/97	1	25
24	59	M	4	150	T V	15	11/5/97	1	15
24	60	M	6	200	T. C	23	11/5/97	1	20
24	61	F	5	150	T. V	15	11/5/97	1	15
24	62	M	5	200	T. V	20	11/5/97	1	20
24	63	F	8	100	T. C	24	11/5/97	1	10
24	64	M	6	200	T. C	23	11/5/97	1	20
24	65	M	3	150	T. V	20	11/5/97	1	15
24	66	M	4	150	T. V	27	11/5/97	1	15
24	67	F	2	100	T. B	20	11/5/97	1	10
24	68	M	4	200	T. B	20	11/5/97	1	20
24	69	M	5	150	T. B	20	11/5/97	1	15
24	70	F	5	200	T. C	25	11/5/97	1	20
24	71	M	5	150	T. V+T. B	27	11/5/97	1	15
26	72	M	6	100	T. C	20	11/5/97	1	10
26	73	M	5	150	T. B +T.C	20	11/5/97	1	15
26	74	F	4	150	T. B	24	11/5/97	1	15
26	75	F	8	100	T. C	15	11/5/97	1	10
26	76	M	4	150	T. C	20	11/5/97	1	15
26	77	F	6	100	T. V+T. C	22	11/5/97	1	10
26	78	M	2	100	T. C	27	11/5/97	1	10
26	79	M	8	150	T. C	19	11/5/97	1	15
26	80	M	1	50	T. B	22	11/5/97	1	5
26	81	M	3	200	T. C+T. V	20	11/5/97	1	20
26	82	M	5	150	T. C	19	11/5/97	1	15
26	83	M	6	150	T. C	20	11/5/97	1	15
26	84	F	5	200	T. B	24	11/5/97	1	20
26	85	M	5	150	T. V+T. B	25	11/5/97	1	15
26	86	M	3	100	T. C	25	11/5/97	1	10
26	87	F	7	150	T. C	20	11/5/97	1	15
26	88	M	4	150	T. C	15	11/5/97	1	15
26	89	M	4	200	T. C	23	11/5/97	1	20

Continued (Annex 2)

26	90	M	5m	40	T. B	28	11/5/97	1	4
26	91	M	1	80	T. V	20	11/5/97	1	8
26	92	F	2m	30	T. V	40	11/5/97	1	3
26	93	M	4	150	T. V	21	11/5/97	1	15
26	94	F	4	150	T. C	23	11/5/97	1	15
26	95	F	8	150	T. C	32	11/5/97	1	15
26	96	F	7	150	T. C	15	11/5/97	1	15
26	97	F	11	150	T. C	18	11/5/97	1	15
26	98	F		100	T. C	20	11/5/97	1	10
26	99	M	2	80	T. V	25	11/5/97	1	8
26	100	M	6	200	T. C	23	11/5/97	1	20
26									

Annex 3: Results of longitudinal drug sensitivity studies in trypanosome infected Zebu cattle in the feild

Region: Metekel (Pawe)

Animal Species: Bovine (Local Zebu)

Drug used: Trypamidium(Rhone Merieux), 1mg/kg

Bach number and expiry date: LOT U6962/E, EXP 01 98

No.	Owner's Name	Village	Ear tag No.	Sex	Age (yr.)	Body Wt.	Parasitaemia before RX	PCV (%)	Date of Rx	Dose in mg/kg	Dose in ml	Parasitaemia on days 30	60	90	PCV (%) on day 30	60	90
1	Bezabih Atalay	24	51	M	5	150	T.C	27	11.05.97	1	15				23	21	22
2	Ebabu Y.	24	52	M	6	250	T.C	20	11.05.97	1	25	T.C			25	25	26
3	Wonduante M.	24	53	M	6	200	T.C	20	11.05.97	1	20				27	27	25
4	Assefa Belachc	24	54	M	6	150	T.C	25	11.05.97	1	15	T.C	T.C		25	25	26
5	Abebe Demissi	24	55	M	6	200	T.C	30	11.05.97	1	20				25	28	29
6	Shumet Derso	24	56	M	8	250	T.B	26	11.05.97	1	25					24	25
7	Atnafu Tade	24	57	M	4	100	T.B	20	11.05.97	1	10		T.B		25	22	24
8	Ayichew W.	24	58	M	8	250	T.V	28	11.05.97	1	25				30	30	35
9	Dejen Setyarge	24	59	M	4	150	T.V	15	11.05.97	1	15				27	32	27
10	Amogne Asye	24	60	M	6	200	T.C	23	11.05.97	1	20					22	26
11	Demeke D.	24	61	F	5	150	T.V	15	11.05.97	1	15				25	22	22
12	Ayalnesh B.	24	62	M	5	200	T.V	20	11.05.97	1	20			T.C	27	24	20
13	Melaku Abebe	24	63	F	8	100	T.C	24	11.05.97	1	10		T.C		25	20	15
14	Tadesse W.	24	64	M	6	200	T.C	23	11.05.97	1	20				29	29	
15	Ayele Abitew	24	65	M	3	150	T.V	20	11.05.97	1	15		T.V+T.B		22	24	25
16	Jemberu Abe	24	66	M	4	150	T.V	27	11.05.97	1	15			T.V	30	29	25
17	Admasu Girma	24	67	F	2	100	T.B	20	11.05.97	1	10				25	28	30
18	Melkamu Taye	24	68	M	4	200	T.B	20	11.05.97	1	20				25	26	
19	Tiruye Kassa	24	69	F	5	150	T.B	20	11.05.97	1	15				24	26	25
20	Getachew T.	24	70	M	5	200	T.C	25	11.05.97	1	20		T.V.C.B		26	27	24
21	Lakew Derese	24	71	M		150		27	11.05.97	1	15				28	26	25
22	Abezu Bere	26	72	M	6	100	T.C	20	11.05.97	1	10				20	20	20
23	Amare Beyene	26	73	M	5	250	T.B+T.C	20	11.05.97	1	25		T.C	T.C	25	25	27
24	Mulat Mitiku	26	74	F	4	150	T.B	24	11.05.97	1	15				28	32	29
25	Melak Tsega	26	75	F	8	160	T.C	15	11.05.97	1	16		T.C		29	14	20
26	Wondyifraw Y	26	76	M	4	200	T.C	20	11.05.97	1	20		T.C		25	20	30
27	Deguale Taye	26	77	F		180	T.C+T.V	22	11.05.97	1	18	T.C	T.C	T.C	25	25	25
28	Melak Tsega	26	78	M	2	100	T.C	27	11.05.97	1	10				22	31	20
29	Kasse Zeleke	26	79	M	8	150	T.C	19	11.05.97	1	15			T.C	27	25	20

30	Wondyifraw Y	26	80	M	1	65	T.B	22	11.05.97	1	6.5		T.B		35	35	
31	Birke Kasse	26	81	M	3	200	T.C+T.V	20	11.05.97	1	20	T.C			24	20	30
32	Habtam Abiye	26	82	M	5	150	T.C	19	11.05.97	1	15			T.C	20	27	12
33	Abiye Emiru	26	83	M	6	150	T.C		11.05.97	1	15				23	29	29
34	Degu Beze	26	84	F	5	200	T.B	24	11.05.97	1	20		T.C		25	27	25
35	Mitike Assefa	26	85	M		150			11.05.97	1	15				27	35	25
36	Asmamaw Mu	26	86	M	3	200	T.C	25	11.05.97	1	20		T.C		30	11	30
37	Sewinet Shifer	26	87	M	7	150	T.C	20	11.05.97	1	15				29	27	22
38	Kassaw Yessuf	26	88	M	4	150	T.C	15	11.05.97	1	15	T.C			26	23	22
39	Mizanu Worke	26	89	M	4	200	T.C	23	11.05.97	1	20				27	40	25
40	Asefa Mekones	26	90	M	5m	40	T.B	28	11.05.97	1	4				30	?	25
41	Yitayew Abiye	26	91	M	1	80	T.V	20	11.05.97	1	8				29	40	25
42	Kassa Ejigu	26	92	F	2m	30	T.V	40	11.05.97	1	3		T.C	T.C	30	23	35
43	Fanaw Alemu	26	93	M	4	150	T.V	21	11.05.97	1	15				25	30	15
44	Ayele Workine	26	94	F	4	150	T.C	23	11.05.97	1	15					20	30
45	Sewinet S.	26	95	F	8	150	T.C	32	11.05.97	1	15				25	34	15
46	Awikew Aboye	26	96	F	7	200	T.C	15	11.05.97	1	20	T.C			27	28	34
47	Ayal Limenih	26	97	F	11	150	T.C	18	11.05.97	1	15				23	26	19
48	Kassa Ejigu	26	98	F	4	100	T.C	20	11.05.97	1	10				28		25
49	Wondimu A.	26	99	M	2	80	T.V	25	11.05.97	1	8					25	23
50	Biderse Kassay	26	100	M	6	200	T.C	23	11.05.97	1	20				25	22	22
			Isolates used for the drug sensitivity test in mice														
			Isolates collected and preserved in liquid nitrogen at FVM-AAU and FUB.														

Annex 4 Calculation of treatment doses

Berenil® treatment groups:

The maximum dosage used for diminazene aceturate treatment groups in the present study was 28 mg/kg bw

(a) Treatment group: 28 mg/kg bw

For 1 kg (1000g) bw →28 mg diminazene aceturate

25g mouse $\rightarrow \frac{25g \times 28\ mg}{1000g}$ diminazene aceturate = 0.7 mg

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

1g (1000 mg) Berenil® →445 mg diminazene

0.7 mg diminazene $\rightarrow \frac{0.7\ mg \times 1000\ mg}{445\ mg}$ Berenil

Dosage per mouse→1.57 mg Berenil®/ml distilled water (157 mg Berenil/100 ml distilled water)

- dissolve 157 mg Berenil® in 100 ml distilled water
- mix
- inject 1 ml into each mouse of the fourth treatment group (28 mg/kg bw) intraperitoneally.

(b) Treatment group: 14 mg/kg bw

- to obtain 14 mg/kg bw diminazene aceturate the preparation for 28 mg/kg bw. is double diluted.

i.e. 10 ml (28 mg/kg Berenil®) + 10 ml distilled water

- from this preparation 1 ml was injected into each of the five mice in the third treatment groups (14 mg/kg bw)

(c) Treatment group: 7 mg/kg bw

- to obtain 7 mg/kg bw diminazene aceturate the preparation for 14 mg/kg bw is double diluted.

i.e. 10 ml (14 mg/kg bw Berenil®) + 10 ml distilled water

- from this preparation 1 ml was injected into each of the five mice in the second treatment groups (7 mg/kg bw)

(d) Treatment group: 3.5 mg/kg bw

- to obtain 3.5 mg/kg bw diminazene aceturate the preparation for 7 mg/kg bw is double diluted.

i.e. 10 ml (7 mg/kg bw Berenil®) + 10 ml distilled water

- from this preparation 1 ml was injected into each of the five mice in the second treatment groups (3.5 mg/kg bw)

Trypamidium® treatment groups

(a) Treatment group: 4 mg/kg bw

1 kg (1000 g) → 4 mg
25 g mouse → $\frac{25\text{ g} \times 4\text{ mg}}{1000\text{g}}$ = 0.1 mg isometamidium/mouse (ml)

- mix 100 mg trypamidium with distilled water until it reaches a volume of 1000 ml.
- inject each mouse in this treatment group with 1 ml of this solution i.p.

(b) Treatment group: 2 mg/kg bw

- dilute the preparation for 4 mg/kg bw by 1/2 (i.e. 1 part of isometamidium (4 mg/kg bw) and 1 part of distilled water)
- inject each of the 5 mice in this treatment group with 1 ml of this solution

(c) Treatment group: 1 mg/kg bw

- dilute the preparation for 2 mg/kg bw by 1/2 (i.e. 1 part of isometamidium (2 mg/kg bw) and 1 part of distilled water)
- inject each of the 5 mice in this treatment group with 1 ml of this solution

(d) Treatment group: 0.5 mg/kg bw

- dilute the preparation for 1 mg/kg bw by 1/2 (i.e. 1 part of isometamidium (1 mg/kg bw) and 1 part of distilled water)
- inject each of the 5 mice in this treatment group with 1 ml of this solution

(e) Treatment group: 0 mg/kg bw

- inject each of the 5 mice in this treatment group with 1 ml of distilled water.

Annex 5 Preparation of PBSG solution and cryopreservation

Stock Phosphate-buffered saline solution (PS), PH 8.0

$Na_2HPO_4 \times 2H_2O$		16.90 g
$NaH_2OPO_4 \times 2H_2O$		0.78 g
NaCl		4.25 g
H_2O	to	1000 ml

Phosphate-buffered saline-glucose (PSG) solution, PH 8.0

PS	400ml
H_2O	600ml
Glucose	25g

Glycerol Solution

Three parts of 100% Glycerol solution is mixed with seven parts of PSG to obtain 30% glycerol solution.

Stabilation of trypanosomes (Cryopreservation)

The stabilation procedure consisted of the following steps:
- obtain 5 ml of blood from the jugular vein of an infected animal with EDTA treated vacutainer
- take 0.5 ml of 30% glycerol and 0.5 ml of EDTA blood into serum vials (to have a final glycerol concentration of 15%)
- mix gently
- label according to WHO (1979) recommendation
- leave for 15 minutes to equilibrate at room temperature
- insert the tubes (vials) into a placticin insulated large screw cap plastic bottle. The plasticine covering the tube should be 1 cm thick
- suspend in the vapour phase of the liquid nitrogen container and allow to cool for 2 hours
- after cooling, transfer the test tubes from the cooling device to a storage canister in the liquid nitrogen bath

The following procedures were used to check the stabilates:
- withdraw a tube (vial) and instantly thaw at room temperature and examine for the mobility and degree of viability
- infect mice and check for infectivity.

9.CURRICLUM VITAE

Name:	Yohannes Afewerk Mebratu
Nationality:	Ethiopian
Date and Place of birth:	20, December, 1970, Dire Dawa, Ethiopia
Education:	Elementary and Junior Secondary School, Jijiga, Ethiopia (1976-1983) Secondary School, Jigiga, Ethiopia (1984-1987) University, Addis Ababa University, Ethiopia (1988-1993)
Academic qualification:	Doctor of Veterinary Medicine (DVM)
Professional Experience:	District Veterinary Officer(1993-1995)

Workshops and trainings:

- Advanced level training course in Goat Health by FARM-Africa Dairy Goat Development Project, Dire Dawa Zonal Veterinary Laboratory, Ethiopia., 29 Nov.-14 Dec. 1995, Certificate.

- Project Monitoring and Evaluation (Monitoring und Evaluierung von Projekten (M&E), a Workshop by KAAD (Katholischer Akademischer Ausländer-Dienst), Euskirchen, Germany, Certificate of participation. 26 February-01 March 1996

- 1st Joint Workshop on Veterinary Epidemiology by the Free University of Berlin and the Addis Ababa University, Debre Zeit, Ethiopia, 9 December-20 December, 1996, with Certificate of participation

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 Yohannes Afewerk

Signature

Date of submission 7th January, 1998

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

Dr. Clausen

Dr. Getachew

1998
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Yohannes Afewerk

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