EPIDEMIOLOGY OF BOVINE TRYPANOSOMOSIS IN THE ABBY BASIN AREAS OF NORTHWEST ETHIOPIA

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A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in partial fulfillment of the requirements for Degree of Master of Science in Tropical Veterinary Epidemiology

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXES

LIST OF ABBREVIATIONS

AAU	Addis Ababa university
AAT	African Animal Trypanosomosis
BCT	Buffy coat technique
bw	Body weight
°C	Degree centigrade
CI	Confidence interval
DNA	Deoxy ribonucleic acid
ELISA	Enzyme Linked Immuno Sorbant Assay
ESTC	Ethiopian Science and Technology Commission
FAO	Food and Agricultural Organization of the United Nation
FITCA	Farming in tsetse controlled areas of Africa
FVM	Faculty of Veterinary Medicine
GDP	Gross Domestic Product
HCT	Haematocrit centrifugation technique
IBAR	Inter African Bureau of Animal Resources
ICIPE	International Center for Insect Physiology and Ecology
ILCA	International Livestock Center for Africa
ILRI	International Livestock Research Institute
ISCTRC	International Scientific Council for Trypanosomosis Research and Control
ISMM	Isometamidium chloride
NTTICC	National Tsetse and Trypanosomosis Investigation and Control Center
mg	Milligram
MoA	Ministry of Agriculture
mm	Millimeter
nm	Nanometer
m.a.s.l.	Meter above sea level
µl	Microlitre
OAU	Organization of African Unity
PA	Peasant Association
PCV	Packed Cell Volume
PCR	Polymerase Chain Reaction
RBC	Red Blood Cells
SD	Standard deviation
SIT	Sterile insect technique
Spp.	Species
SRVETEP	Southern Rift Valley of Ethiopia Tsetse Eradication Project
T.b	*Trypanosoma brucei*
T.c	*Trypanosoma congolense*
T.v	*Trypanosoma vivax*
US$	United States dollar

ACKNOWLEDGEMENTS

The author would like to thank:

Dr. Arun K. Sangwan and Prof. Getachew Abebe for their guidance and devotion of time in rectifying the thesis. Dr. Nega Ṭewelde, Dr. Bayleyegn Molla, Dr. Bewuket Seraw, Dr. Kassa Teferi, Dr. Gebreyesus Mekonnen, Dr. Legesse T/Giorgis, Dr. Gedion Yilma, Ato Habtamu Kefyalew, Ato Meselu Wagaw, Ato Assefa Sharew, Ato Belistie Esubalew, Ato Belistie Yenalem and Ato Tafere Kerebeh and the farmers at the study area for their help in various ways.

Faculty of Veterinary Medicine of Addis Ababa University, Amhara Region Bureau of Agriculture, Amhara Region Agricultural Research Institute, Bahir Dar Regional Veterinary Laboratory, Dembecha and Jabitehenan weredas Office of Agriculture and Debre Genet Orthodox Church Monastery for their financial, logistic and other supports.

ABSTRACT

Tsetse transmitted animal trypanosomosis is a serious constraint to livestock production and agricultural development in Ethiopia. Part of Abbay basin (Blue Nile) in northwest Ethiopia is tsetse infested where animal trypanosomosis is a serious threat to economic development. The objectives of the study were to generate a base line data on epidemiology of tsetse and trypanosomosis, to assess trypanocidal drug resistance and to know the community awareness regarding the disease and control methods in the area. The study was conducted between September 2003 to April 2004 in Dembecha and Jabitehenan weredas of the Abbay basin areas of northwest Ethiopia. The study methodology was based on questionnaire survey, seasonal cross-sectional studies of tsetse and trypanosomosis and longitudinal study for the assessment of trypanocidal drug resistance in the field.

The questionnaire survey indicated that trypanosomosis is the most important problem affecting the animals and impeding agricultural activity in the area. Entomological survey revealed that *Glossina. m. submorsitans* was the only prevalent tsetse fly along with other biting tabanid and muscid flies. The apparent fly densities were significantly higher ($p<0.05$) in the late rainy season (1.08fly/trap/day, 8.78fly/trap/day and 91fly/trap/day) for *G. m. submorsitans*, tabanids and muscids respectively than the dry season (0.68fly/trap/day, 0.35fly/trap/day and 7.33fly/trap/day) respectively. In the lowland areas (<1600 m. a. s. l.) the apparent density for *G. m. submorsitans* was significantly higher ($p<0.05$) than the midland areas (1600-2000 m.a.s.l.) in the both seasons. The altitudinal distribution limit of *G. m. submorsitans* was upto 1780 m.a.s.l.The proportion of tsetse flies caught was higher in the savanna vegetation type followed by riverine, forest, bush and cultivated lands with maize, teff and horticulture plantations.

In the parasitological survey a total of 1,648 animals, 814 in the late rainy season and 834 in the dry season were examined with buffy coat technique and the prevalence of trypanosomosis was 17.07% and 12.35% respectively with a significant difference ($p<0.05$) between seasons. Higher infection rates found in the lowland areas below 1600 m.a.s.l. (19.87% and 17.62%) than the midland areas ≥1600 m.a.s.l.(13.39% and 6.54%) in the late rainy and dry season respectively with significant difference ($p<0.05$).The mean PCV values (%) of parasitaemic and aparasitaemic animals during the late rainy season were 20.7±3.5SD

and 26.6±4.3SD ($p<0.001$, 95% CI=25.3-25.9) while during the dry season 21.4±3.6SD and 26.6±4.3SD ($p<0.001$, 95% CI=25.4-25.9) respectively. The regression analysis of herd average PCV from herd prevalence indicated that herd average PCV decreased with increasing prevalence of trypanosome infections with a regression coefficient of negative values in both the seasons.

A total of 100 animals were selected for the assessment of Isometamidium chloride (ISMM) and Diminazine aceturate resistance, 50 from each of the high risk villages identified in the area with similar agroecological zones. The selected animals in each village were grouped into 25 in control and 25 in treatment groups and were identified with ear-tags. At day minus 14 of the study all the 100 cattle were treated with Diminazine aceturate at a dose rate of 7mg/kg bw. After two weeks (day 0) the treatment groups were given ISMM at a dose rate of 1mg/kg bw. Both groups of cattle were examined for trypanosome parasite using buffy coat technique every 14 days interval until 84 days. The three indices used in assessing ISMM resistance (the proportion of infection during 8 weeks follow-up period, the 25% survival time and the ratio of mean hazard rates in the control and treatment groups of cattle) provided consistent results across the two villages for the occurrence of ISMM resistant trypanosome infections in the area. There was no significant difference between the Kaplan-Meier survival curve estimates of the control and treatment groups in both villages ($p>0.05$).The results of Diminazine aceturate efficacy showed 16 animals became recurrent infections with *T. congolense* but there was no significant difference between trypanosome incidence rate and trypanosome recurrence rate.

Therefore, trypanosomosis is the most important problem for agricultural activity and animal production in the Abbay basin areas of northwest Ethiopia (Dembecha and Jabitehenan weredas of Amhara Region) and the situation is getting worse as the control and prevention of trypanosomosis is facing a challenge due to limitation of vector control activities and the development of drug resistance in the area.

Keywords: Epidemiology/ Bovine/ Trypanosomosis/ *Glossina m. submorsitans*/ Drug Resistance/ Survival Analysis/ Season/ Altitude/Abbay Basin/ Dembecha/ Jabitehenan/ Northwest Ethiopia.

1. INTRODUCTION

Currently in Ethiopia population is estimated to be 67.2 million with 2.9% growth rate and 90% of the population is engaged in agriculture economy (CSA, 2002). Livestock sector plays a significant role for the economy and has a great potential to assist the economic development by providing meat, milk, other food products, cultivation power, transport, security in times of crop failure and farm yard manure (fertility and energy) and also plays a major role in export commodity. The sector contributes 12% of total GDP and over 30% of the agricultural GDP (MoA, 1998).

The Amhara region-covers an area of 150,123 km^2 with 11 zones and 109 weredas and the population is estimated to be 17.205 million people (CSA, 2002).Three river basins Abbay, Tekeze and Awash and Lakes such as Tana, Zengena, Gudena, Yetilba and Hike are found in the region. Generally the economic situation of the region depends on crop and livestock production. Fogera breed of cattle and Menz breed of sheep found in the region. Although 40% of the Ethiopian livestock is found in this region, the livestock production is low consequently impeding the overall agricultural development. One of the most important constraints for this is trypanosomosis by affecting the health and productivity of livestock in the most fertile and arable land of the country due to the infestation of tsetse fly.

Trypanosomosis is the most important constraint to livestock and mixed crop-livestock farming in tropical Africa. Currently about 3 million livestock die every year due to tsetse fly transmitted trypanosomosis which covers one third of the continent estimated to be 10 million km^2. In this region at least 46 million cattle are exposed to the risk of contracting tsetse-borne trypanosomosis, as are millions of sheep, goats, donkeys, camels and horses (Reid *et al., 1998).* A recent study estimated the direct annual cost of trypanosomosis to be about 1.34 billion US$ (Kristjanson *et al.*, 1999). African livestock producers are administering an estimated 35 million curative and prophylactic treatments annually which costs the producers and the government at least 35 million US$ (Geerts and Holmes, 1998). The direct losses from trypanosomosis in livestock include mortality, morbidity, impaired fertility and the cost of implementing and maintaining tsetse fly and trypanosomosis control operations. Indirect losses stem from farmers responses to the perceived risk of the disease, including the reduction and in some cases, the exclusion of livestock from tsetse-infested grazing lands and reduced crop production due to insufficient animal draught power (ILRAD, 1993).

The host range of trypanosomosis includes domestic and wild animals as well as human beings. The vector includes several species of tsetse flies and biting flies. Tsetse flies are grouped in the three categories: *Glossina morsitans* group (savanna areas), *Glossina fusca* group (forest areas) and *Glossina palpalis* group (river and lake areas). The most important trypanosome species that affect cattle, sheep and goats are: *Trypanosoma congolense, T. vivax* and *T. brucei*.

Tsetse transmitted animal trypanosomosis is a serious constraint to livestock production and agricultural development in Ethiopia. A total of 14.8 million cattle, 6.12 million sheep and goats, 1 million camels and 1.23 million are at risk of contracting trypanosomosis (MoA, 1995). According to this report 1 to 2 million doses of trypanocidal drugs are administered at the cost of 0.5 to1 million US$ per annum in Ethiopia.

There are five 5 species of Glossina in Ethiopia: *G. pallidipes, G. morsitans submorsitans, G. fuscipes, G. tachinoides and G. logipennis.* Several reports made in Ethiopia revealed that tsetse fly occupy over 66,000 km^2 areas (Ford *et al.*, 1976) based on a 1500 m.a.s.l. breeding limit in the southern and southwestern valleys of the country. Langridge (1976) has reported that some 98,000 km^2 area 1600 m.a.s.l. breeding limit in the southern and southwestern parts of Ethiopia. However, due to the advancement of tsetse flies into formerly free areas reaching 130,000 to 150,000 km^2 (Silnbergh, 1992) basedon 1700 m.a.s.l. nd recently 220,000 km^2 areas is estimated to be affected by tsetse flies (NTTICC, 1996) based on 2000 m.a.s.l. breeding limit. These areas follow the Baro, Omo and Abbay valleys of the large rivers in the country. On the other hand these areas possess the most arable land with a high potential for agricultural development due the high annual rain fall (Jemal and Hugh-Jones, 1995).

There are five economically important animal trypanosome species in Ethiopia: *T. congolense, T. vivax, T. brucei brucei, T. evansi* (Langridge, 1976) and *T. equiperdum* (Dagnachew and Shafo, 1981). The most prevalent trypanosome species in tsetse-infested areas of Ethiopia are *T. congolense* and *T. vivax*. Rowlands *et al.* (1993) reported a prevalence rate of 37% for *T. congolense* in southwest Ethiopia. Abebe and Jobre (1996) reported an infection rate of 58.5% for *T. congolense*, 31.2% for *T. vivax* and 3.5% for *T. brucei* in southwest 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*. Different workers (Afewerk, 1998; Tewelde, 2001; Muturi, 1999) indicated a prevalence rate of 17.2%,

21% and 14% in Metekel district, in Upper Didessa Valley and Southern Rift Valley areas of tsetse infested regions respectively and the dominant species was *T. congolense.*

In the western part of the Amhara regional state bordering the Abbay river, one of the north western tsetse belt areas of Ethiopia, tsetse transmitted trypanosomosis is becoming a serious threat for livestock production and agricultural activity in particular. Reports made by Bahir Dar regional veterinary laboratory in 1999 indicated the presence of tsetse transmitted trypanosomosis in three districts of the region (Bure, Jabithen and Ankasha) bordering the Abbay valley areas. A preliminary survey conducted in Dembecha wereda by the ESTC/SRVETEP (2000) indicated a trypanosome prevalence rate of 23% with a dominant species of *T. congolense* and tsetse fly identified was *G. m. submorsitans.*

Earlier works by Langridge (1976) indicated that the tsetse belts extend from the southern part of the Rift valley, around the southern corner of the country and along the western lowlands and escarpment to the Abbay river. There is a suspicion that *G. morsitans* may extend northwards from the Abbay to Tekeze river (Mulligan, 1970). In Ethiopia both *G. morsitans* and *G. tachinoides* have been caught at nearly 1800 m in western Amhara region. Langridge explain this as an exceptional condition as these flies had been forced up to the high level by large grass fires which started in the bottom of the Abbay valley and worked the slopes to the top of the valley walls. According to Langridge (1976) tsetse infested areas of Gojjam are all associated with the Abbay river and *G. m. ugandensis* and *G. tachinoides* are found all along this river system from Beles river valley of Metekel district of the Benshangul-Gumz region upto within 40 km of the bridges on the main highway from Addis Ababa to Debre Markose.

The principle of prevention and control of tsetse-transmitted trypanosomosis depends on minimizing contact between domestic, game animals and tsetse flies. So far the methods used for the control of trypanosomosis in tsetse infested areas include control of tsetse fly numbers, use of curative or prophylactic trypanocidal drugs and use of livestock breeds that tolerate the disease. However, uses of these methods are highly variable.

Trypanocidal drugs remain the principal method of animal trypanosomosis control in most African countries including Ethiopia. With increasing liberalization of veterinary drugs the controls on the use of trypanocides are diminishing, hence there is a growing concern that their future effectiveness may be severely curtailed by widespread drug resistance. In general, in areas where trypanocides have been used intensively drug resistance is more common than

in areas where they have been used less intensively. The problems of drug resistance have been reported from 13 countries in sub-Saharan Africa (Peregrine *et al.*, 1994). Drug resistant *T. congolense* have been reported in Ethiopia by different workers (Scott and Pegram, 1974; Codjia *et al.*, 1993; Wubete *et al.*, 1997; Afewerk *et al.*, 2000; Assefa and Abebe, 2001; Tewelde *et al*, 2004). For the effective way of controlling tsetse transmitted trypanosomosis the knowledge of insect biology and ecology, the status of the disease prevalence and trypanocidal drug efficacy is of paramount importance. As a result the present research proposal is initiated to generate a base line data on the epidemiological status of tsetse and trypanosomosis and to study drug resistance in the Abbay basin areas of northwest Ethiopia

Therefore, the specific objectives of this research are:

- To determine the seasonal apparent density, distribution and species of tsetse flies and other biting flies in the Abbay basin areas of northwest Ethiopia.
- To determine the seasonal prevalence of trypanosomosis in the study area.
- To assess trypanocidal drug resistance (ISMM and diminazine aceturate) in the field cattle in the Abbay basin areas of northwest Ethiopia.
- To assess the community awareness regarding the effect of trypanosomosis and control methods.

2. LITERATURE REVIEW

2.1. Animal trypanosomosis

Animal trypanosomosis is a disease of domestic animals resulting from infection with parasitaemic protozoa of the genus *Trypanosoma* transmitted primarily by tsetse fly and also by other haematophagous flies. Trypanosome parasitizes all classes of vertebrates including human beings and it is predominantly a parasite of blood. Animals affected with trypanosomosis become anaemic and weak; lose weight and body condition, reduced productivity and often mortality rates are high (Urquart *et al.*, 1995).

Trypanosomes are unicellular microscopic elongated spindle-shaped protozoa ranging from 8.0 -39.0 μm long. All possess flagellum which arises at the posterior end of the trypanosome from a basal body at the foot of the flagellar pocket. The flagellum runs to the anterior end of the body and is attached along its length to the pellicle to form an undulating membrane. In stained specimens, a single centrally placed nucleus can be seen, and adjacent to the flagellar pocket, a small structure the kinetoplast which contains the DNA of the single mitochondria is observed.

Animal trypanosomosis occurs widely in the tropics and subtropics and is well recognized, having local names such as Nagana, Samore, or tsetse fly disease in sub-Saharan Africa (typical African trypanosomosis); Malde Caderas in equines of central and South America; and Surra in equines, cattle, buffaloes and camels in India. Human trypanosomosis occurs as "Chagas" disease in central and South America and as Sleeping Sickness in Africa.

Based on difference in the course of development in their vectors, *Trypanosoma* is divided into Stercoraria and Salivaria. Species of the first group complete development in the terminal gut and are transmitted in the feces of the vector. Species belonging to the Salivaria group complete their development in the anterior part of the digestive tract and are transmitted via the vectors saliva. The pathogenic trypanosomes belong to the latter group. The main pathogens in Salivaria group categorized into four sub genera: Duttonella (species: *T. vivax; T. uniformes*); Nanomonas (species: *T. congolense; T. simae*); Pycnomonas (*Trypanosoma suis*) and Trypanozoon (species: *T. brucei; Trypanosoma rhodesiense*; *Trypanosoma gambiense; T. evansi; T. equiperdum*) (Mulligan, 1970).

Trypanosomosis in Africa is mainly restricted to areas in which the vector, tsetse fly (*Glossina* species) can survive. The disease is also found outside the tsetse belt areas transmitted mechanically by biting flies of the genus *Tabanus*, *Hematopota*, *Chrysops*, and *Stomxys*. This type of transmission have caused the spread of *T. evansi* and *T. vivax*, outside tsetse infested areas. The most pathogenic trypanosomes are *T. congolense* and *T. vivax*. *Trypanosoma congolense* is responsible for the most important form of animal trypanosomosis in domestic animals. Report from tsetse-infested areas of Ethiopia indicated that *T. congolense* is the most prevalent trypanosome species (Codjia *et al.*1993; Abebe and Jobre, 1996).

2.2. Epidemiology

The epidemiology of African animal trypanosomosis is highly dependent on the parasite, vector and host factors. Trypanosome species occur in a variety of genotypes with different strains, virulence, immunogenecity and response to chemotherapeutic agent. The severity of the disease also depends on the species and strain of trypanosomes involved. Since the parasite infects a wide range of animals including wild animals which constitute the reservoirs of the disease, the epidemiology of trypanosomosis extremely complicated. The degree of risk to which domestic animals are exposed to the trypanosomosis depends on the species and density of tsetse present, infection rate in tsetse, species and strain of trypanosomes, source of infection (wild or domestic animals) and feeding preference of the flies (MacLennan, 1970).

2.2.1. Transmission and distribution

The transmission of the disease is either cyclically by tsetse flies or mechanically by haematophagous flies. Transmission by tsetse fly is a complex mechanism in which the fly remains life long carrier. In the vector trypanosome changes through several morphological distinct stages (amastigote, promastigote and epimastigote) until it reaches trypomastigote (metacyclic stage) which is infective for mammals (Stephan, 1986; Urquart *et al.*, 1995). Tsetse flies occur only in Africa south of the Sahara and north of the temperate climate in the south of the continent that covers between 15^0N and 25^0S latitude (Hoar, 1957). Animal trypanosomosis cause huge economic losses that are greatest in the vast tsetse infested tracts of sub-Saharan Africa where cattle production is affected in approximately 10 million km^2 areas. There are 31 species and subspecies of tsetse flies identified at present (Leak, 1999).

2.2.2. Biology and distribution of tsetse fly

The most distinctive feature of the life history of tsetse flies, shared with only a few other small families of Diptera, is retention of the single egg in the uterus of the female, where it hatches to a larva and nourished by the products of a pair of modified accessory glands. This method of reproduction is referred to as adenotrophic viviparity (Langley and Weidehaas, 1986; Jordan, 1993 and Leak, 1999).

This form of reproduction involves cyclical production of eggs, which hatch in the uterus and the insect does not feed from the time it leaves the female fly as a mature larva until the adult emerges from the pupa (Phelps and Lovemore, 1994; Leak, 1999). Females are receptive to males as soon as they start seeking food and often mate when taking their first blood meal or soon after. They usually mate once but sometimes more than once mating can occur. Male flies may not mate soon after emergence from the pupa and they are not fully fertile until they are a few days old. Active and viable sperms can remain in the spemathecae, nourished by a secretion of layers of cells, which surrounds the cuticular lining of the lumen of each spermathecae, through out the life of the female. This is the basis for the need to mate not more than once in the female. The whole pregnancy cycle takes about 9 days, although the rate of development of each stage is temperature dependent. By the ninth day the third instar larva with its two conspicuous black polypneustic lobes at the posterior end is deposited through the vagina (Larviposition) on the ground (Jordan, 1993; Nagel, 1995; Leak, 1999). The successful burrowing in the soil by the deposited larva depends on various factors, for instance, soil particle size, moisture content of the soil, and possibly the soil temperature are the most important ones.

Under favourable environmental conditions (temperature and moisture of the soil) newly deposited larva is transformed, within a few hours, in to a hard almost black larva and moults to form the prepupa, but remains within the third cuticle, which then harden to form the puparium within an hour of larviposition. Thirty days later adult fly emerges from the puparium with the sex ratio of 1:1. The puparial period is highly dependent on temperature for example; Jordan (1993) indicated that at a minimum temperature of 20^0C the duration of puparium period is about 47 days while at 30^0C it is about 20 days only. At temperature below 17^0C and above 32^0C there are insufficient fat reserves within the puparium and development can not be successfully completed. The optimum temperature for the puparium

development is about 25^{0}C (Leak, 1999) and at this temperature males emerge after 27 days (Jordan, 1993).

Both sexes of tsetse flies feed exclusively on blood of vertebrates (mainly from mammals but some species take the meal from reptiles and birds). They usually search for hosts and food when they are active. It has been noted that female flies live longer than males. As a result of this, there are always more females than males in any tsetse population. A female fly may produce about 8-10 offspring in her life time. Consequently the rate of reproduction is much lower than in any oviparous insects and in fact resembles that of small mammals (Langley and Weidehaas, 1986; Leak, 1999) that is why the sterile insect technique (SIT) control method is facilitated. Leak (1999) noted that eventhough more precise limits of distribution, particularly in low densities, are not known, the general distribution of tsetse flies is determined principally by climate and influenced by altitude, vegetation and the presence of suitable host animals.

Climate: the effect of climate on tsetse distribution is often through its effect on vegetation. Buxton (1955) discussed in detail the relationship between tsetse flies and different climatic factors and effect of temperature on the ecology of tsetse flies is through its effect on the interval and puparial duration and also the influence on the activity of the flies. In temperature below 15^{0}C tsetse fly are inactive and above 35^{0}c they seek refuge in rot-holes in the trees and animal burrows and deep tissues in the barks, where they remain inactive (Phelps and Lovemore, 1994). Humidity is also important factor both for pupal and adult fly development (Nagel, 1995). Cummulative effect of long rainy season or dry season are thought to have been important in influencing advances and recession in tsetse population (Leak *et al.*, 1999). Humidity has also an important effect in relation to the behavior of the flies. Tsetse flies use light for searching food and most of them are active during day time (Buxton, 1955).The effect of altitude on tsetse distribution is through its effect on climate, mainly temperature. As temperature fall with increasing altitude the geographic limitations of different species may be due to their inactivity in lower temperature (Vreyesen *et al.*, 1999).

Vegetation: different species of tsetse flies require particular vegetation type that would provide an optimal condition for growth and survival, and vegetation is also important that provides shelter for their hosts (Buxton, 1955; Leak, 1999). The highest catches of *G. pallidipes* were in bushes and wooden grass land in the Southern Rift Valley of Ethiopia

(Vreyesen *et al.*, 1999). Vegetation types like wooded grassland, forest, riverine forest, bush land, grassland and cultivated land are seen in tsetse infested areas.

Host animal: the presence of wide different types of host animals is essential component of tsetse fly distribution. The distribution and abundance of some species of tsetse flies such as *G. morsitans* and *G. pallidipes* which are often known as game tsetse flies are closely related to the number and habitats of certain wild animals. Nagel (1995) also described that the highest densities of certain tsetse fly species are reported from areas with very high densities of wild animals and low human population areas.

2.3. Pathogenesis of trypanosomosis

African trypanosomosis (Nagana) in all species is a progressive and often fatal disease. The pathogenesis of trypanosomosis depends on the pathogenicity of the strain; the animal host's breed, genotype, age, sex, skin type etc.; and most importantly, on the method by which the infection was induced i.e. natural or artificial (Leak *et al.*, 1987). Once the metacyclic trypanosomes are injected into the host by the fly during feeding, they multiply at the subcutaneous site provoking a local skin reaction called a chancre which is most pronounced in a fully susceptible host and may be slight or absent with some strains or species of trypanosomes. Within a chancre, metacyclic parasites change to trypomastigote form and enter the blood stream directly or through the lymphatic.

The appearance of chancre, follow detectable parasitaemia in a few days, is accompanied by the development of fever and marked enlargement of draining lymph nodes. As the lesion i.e. chancre decreases in size increased numbers of mature plasma cells, macrophages, eosinophils, and mast cells are found, and these compositions of cells within the chancre suggest an initial immune response. This behaviour largely depends on the species of trypanosomes. *Trypanosoma. vivax* usually multiplies rapidly in blood and is evenly dispersed throughout the cardiovascular system, whereas *T. congolense* tends to aggregate in small blood vessels and capillaries of the heart, brain and skeletal muscle from where a small proportion of parasites enter the blood circulation. *Trypanosoma brucei* and rarely *T. vivax* have the added capability of passing out of the capillaries into the interstitial tissues and serous fluids of body cavities where they continue to multiply (Abebe,1991; Luckins *et al.*, 1994).

Trypanosoma vivax and *T. congolense* exert their effect mainly by causing sever anaemia and mild to moderate organ damage. In very acute infections as seen with highly susceptible exotic animals infected with *T. vivax* or in pigs infected with *T. simae*, there is disseminated intravascular coagulation. Trypanosomes can also pass through the placenta and into the fetus in pregnant animals. As a result some cows abort and some calves are born before birth time. A cerebral form of the disease occurs with *T. brucei* alone or in mixed infections with the other species (Stephan, 1986). Murray (1979) stated that the onset and severity of the anaemia is directly related to the appearance of the parasite in the blood and to the level of the parasitaemia. The rapid decline in the hemoglobin concentration, red blood cell number and PCV and the clear clinical sign of pallor of the mucus membrane in infected animal leave as no doubt that anaemia is a very important part of the pathogenesis of trypanosomosis.

When an animal is infected with trypanosomes, antibodies against the surface coat are produced. The problem is that these trypanosomes have multiple genes, which code for different surface proteins; this allows organisms with a new coat glycoprotein to elude the immune response. This process is called antigenic variation and results in the persistence of the organism and this prevents the development of vaccine and permits re-infection when animals are bitten by tsetse flies carrying trypanosomes with surface coat glycoproteins of new antigenic type. Genetic resistance to animal trypanosomosis has been attributed to certain breeds of livestock, e.g. West African N'Dama. This resistance is manifested by ability to withstand the adverse effects of trypanosomes by regulating parasite growth; their ability to prevent or reduce the rate and degree of development of anaemia (Murray, 1988; Seifert, 1996).

2.4. Diagnostic methods

Diagnosis of trypanosomosis in tsetse, humans or domestic livestock is a basic requirement for epidemiological studies as well as for planning and implementing chemotherapy and for monitoring vector control operations. Accurate diagnosis of trypanosome infection in livestock is required for a proper appreciation of the epidemiology of the disease in any geographical locality. Besides clinical diagnosis, direct (parasitological) and indirect (serological) diagnostic methods with varying degrees of sensitivity and specificity are available for trypanosomosis.

2.4.1. Clinical diagnosis

In general, diagnosis of trypanosome infection based on clinical signs alone is rather difficult, but haematological parameters like PCV could be reliable indicators of the progress of the disease. Intermittent fever can be observed due to the variation in parasitaemia and if the animal survives, the disease becomes chronic and there is development of anaemia and emaciation. Therefore, fever, anaemia and loss of body condition are important parameters used routinely for tentative diagnosis of trypanosomosis in areas where this disease is endemic and laboratory service are not available.

2.4.2. Parasitological diagnosis

Parasitological diagnosis is the direct demonstration of the parasite in blood or less frequently in other body fluids (using a microscope). The scarcity of the parasites and the fluctuating nature of the parasitaemia limit 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 increases the chance of trypanosome detection.

2.4.2.1. Dark ground or phase contrast buffy coat technique

The buffy coat zone prepared in a microhaematocrit capillary tube filled with 70µl of blood and centrifuged for 5 minute at 10,000 revolutions is examined for trypanosomes by cutting the capillary tube to include 1mm of erythrocytes and 1cm of the plasma. The buffy coat is poured on a slide and covered with a 22x22mm coverslip. The preparation is examined using a microscope with a phase contrast and dark ground illumination. The use of 10x eyepiece 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 (Murray *et al.*, 1977). In addition, species identification based on size and movement is easier (Paris *et al.*, 1982). Trypanosomes can be identified and the level of parasitaemia estimated using a scoring system (Murray *et al.*, 1983). The PCV is measured before examination of the blood for parasitaemic detection.

2.4.2.2. Haematocrit centrifugation technique (Woo, 1970)

A microhaematocrit capillary tube containing 70 µl of blood is centrifuged for 8 minute at 10,000 revolutions as for measurement of PCV. Two rectangular pieces of glass from a standard microscope slide (1.2 mm thick) are 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 fills the space between the capillary tube and the two pieces of glass, thus reducing the effect of light diffraction. By slowly rotating the tube the Buffy coat plasma junction is examined using a long working distance (6.7mm) objective which allows considerable depth of focus through the capillary unlike the standard objective where the average working distance is approximately 0.5mm. Depending on the trypanosome species the analytic sensitivity for this method is 1-5 $x10^2$ trypanosome per ml of blood.

2.4.2.3. Capillary concentration technique

Because of the tendency of *T. congolense* to be retained amongst red blood cells (RBC), this technique was designed to create a large differential density between the RBC and the parasite. This was achieved by mixing infected blood with a strongly hypertonic nontoxic medium (Walker solution). On centrifugation, the denser red cells separate from the trypanosomes, which display normal motility. Equal volume of diluents and blood are mixed on a micro titer 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. The capillary tubes are placed on a clean microscope slide and the Buffy coat zone covered with a few drops of diluents beneath a coverslip. This technique is more sensitive than haematocrit centrifugation test in the detection of *T. congolense*. However, it needs more time to prepare the samples and PCV cannot be measured at the same time (Walker, 1972).

2.4.3. Serological diagnosis

Serological methods are playing an increasingly important role in the diagnosis and epidemiological assessment of trypanosomosis. Antibody ELISA and antigen ELISA are the methods widely used currently for the diagnosis of cattle trypanosomosis.

2.4.3.1. Antibody ELISA

Luckins and Mehlitz (1976) used micro plate-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.* The serological tests in current use suffer from a lack of well-defined antigens necessary for designing simple and accurate tests that are easily adaptive for field use. Secondly, the detection of antitrypanosomal antibodies in serum can not distinguished between an active infection and a past infection (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).

2.4.3.2. 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 undergoing trypanocidal drug therapy during a period at which it is not possible to isolate parasite from the peripheral circulation. Species-specific monoclonal antibodies, produced against procyclic forms of *T. congolense, T. brucei* and *T. vivax* were used to develope antigen-captured enzyme–linked immunosorbant assay (Ag-ELISA) for the diagnosis of bovine trypanosomosis (Nantulya *et al.*, 1989). Ag-ELISA is no more in use due to problem associated with *T. brucei.* In almost all study areas there were high prevalence rate of *T. brucei* which is not as such really when using parasitological technique. The cross reaction of *T.b. brucei* and *T. vivax* in *T. evansi* and *T. congolense* infections is the limiting problem of this test.

2.4.4. Polymerase chain reaction

Polymerase chain reaction (PCR) with the amplification of DNA samples has been developed as a diagnostic test for a number of parasites both in tsetse and cattle using PCR and DNA probes. In an evaluation of a PCR for detecting *T. vivax* in cattle, sensitivity was not greater than direct technique and gave false negative results when parasitaemia was high. This was thought to be due to the presence of a component in the test sera that inhibited the PCR reaction (Desquesnes, 1997).

2.5. Control of trypanosomosis

Prevention and control of tsetse-transmitted trypanosomosis depends on minimizing contact between domestic livestock, game animals and tsetse fly. There are a number of control measures directed to the parasite, vector and host. However, uses of these methods are highly variable. The methods include reducing tsetse fly population with different techniques, 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 expensive, environmental pollution, drug resistance and poor availability. Vector control may play a role by reducing the level of tsetse challenges to livestock which will in turn encourage the development of land use practices involving livestock. Insecticides in the form of ground spraying and aerial spraying have been employed but the recent development of insecticide impregnated, odour-baited traps and targets which attract and kill tsetse flies offer the prospect of cheaper alternatives with less damage to the environment (Jordan, 1986). Application of deltamethrin pour-on to cattle against tsetse flies has proved to be very efficient in controlling tsetse fly vectors in the pastoral zones of Samorogouan, Burkina Faso (Bauer *et al.*, 1995). Clausen *et al* (1992) indicated 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.

Sterile Insect Technique (SIT) in which artificially sterilized males competes with wild tsetse males for mating with females (Dame and Schanidt, 1970). However this is considered to be very expensive and moreover has the potential of increasing the trypanosomosis risk in the affected area because the sterile male have been found to be as capable as normal male tsetse fly in transmitting the disease (Moloo,1982).

A safe and cost effective vaccine against trypanosome would be a much more effective and sustainable way of controlling the disease. However, because of the ability of trypanosomes to undergo antigenic variation immunization of cattle against trypanosomosis has been unsuccessful (Doyle, 1977). The attempts of vaccine of trypanosomosis failed and now it is recognized that a major obstacle to immunization lies in the phenomenon of antigenic variation in trypanosomes. The phenomenon of antigenic variation and its genetic control have been described and it has been concluded that vaccines based on immune responses to the variant surface coat are unlikely to have any impact on trypanosomosis control. Therefore,

looking for molecular biology of cells component and products responsible for the development of disease is the current research area. This approach could lead to the development of vaccines based on immune responses to invariant molecules such as cysteine proteases or cyclophilins produced by trypanosomes (Teale, 1993).

The introduction and keeping of trypanotolerant West African taurine cattle breeds seem to be an alternative biological method for preventing clinical trypanosomosis and thus economic losses for the animal holders. Trypanotolerance is a feature of both West African long horn and short horn *Bos taurs* breeds such as the N'Dama and Baoule breeds. These breeds of animals possess an increased titer of resistant factors (lysozyme, hemolytic complement C9 and the third complement component C3) and are better able to stabilize the balance of the host-parasite relation ship 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 *et al.*, 1988).

2.5.1. Chemotherapy and chemoprophylaxis

Trypanocidal drugs remain the principal method of animal trypanosomosis control in most African countries (Geerts and Holmes, 1998). The chemotherapy and chemoprophylaxis of animal trypanosomosis relies essentially on three drugs, namely: Homidium (Homidium chloride-Novidium; and Homidium bromide-Ethidium), Diminazine aceturate (Berenil) and Isometamidium chloride (Samorin, Trypamidium). All the three compounds are closely related chemically and have been available for at least 35 years and it is estimated that about 35 million doses per year are currently used in Africa (Budd, 1999). Chemoprophylaxis has been used to protect livestock under low to medium trypanosome challenge using Isometamidium at a dose range of 0.5 to 1.0 mg/kg body weight depending on the trypanosome challenge. 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 (Pinder and Authie, 1984).

2.5.2. Trypanocidal drug resistance

The repeated use of chemicals as pesticides or chemotherapeutic agents inevitably leads to the development of resistance in the target organism (Geerts and Holmes, 1998). Drug resistance is defined as a loss of sensitivity by a strain of an organism to a compound to which it had

been previously susceptible (Uilenberg, 1997). Because of misuse of trypanocidal drugs and lack of essential information dissemination at all levels, the effectiveness of trypanocidal drugs is often limited and this is mainly due to the development of drug resistance (Conner, 1992).

Resistance systematically occurs within approximately ten years following the introduction of antimicrobials, insecticides and anthelmentics to the market (reviewed by Geerts and Holmes 1998). This also occurred with trypanocidal drugs, such as ISMM, the Homidium salts and Diminazine aceturate, which were introduced during the 1950s; the first reports of acquired resistance were published during 1960s. Quinapyramine was marketed earlier, but withdrawn in 1976 because of resistance and toxicity problems. It was later reintroduced for use in camels and horses and may still be used in error in cattle in some locations (Ndoutamia *et al.*, 1993).

2.5.2.1. Current situation of resistance against trypanocidal drug

So far, resistance to one or more of the three trypanocidal drugs used in cattle has been reported in at least 13 countries in sub-Saharan Africa such as Burkina Faso, Chad, Cote d'Ivore, Ethiopia, Kenya, Nigeria, Somalia, Sudan, Uganda, Zimbabwe, Tanzania, Central African Republic and Zambia (Finelle and Yvore, 1960; Peregrine, 1994). This is probably an underestimation of the true situation, because in several countries surveys for resistance have not yet been carried out or cases of resistance have not been published. In eight of the13 countries, multiple resistances have been reported. Most of the currently available information on drug resistance, however, is derived from limited numbers of case reports and does not give any indication of the prevalence of resistance in a region or a country as systematic surveys have not been conducted. There is an urgent need for surveys in which representative numbers of trypanosome isolates are examined for drug resistance.

At present, the most widely used trypanocidal drugs for *T.congolense and T.vivax* infection in Ethiopia are ISMM and Diminazine aceturate. The occurrence of drug resistant trypanosome across Ethiopia is not well known. Scott and Pegram (1974) described the occurrence of Homidium-resistant population of *T. congolense* in Didessa and Angar Valley in Wollega province. The current situation on the phenomenon of trypanocidal resistance particularly against *T. congolense* infection is well documented in the Ghibe Valley (Codjia *et al.*, 1993; Mulugeta *et al.*, 1997). Recent investigation in Metekel district of northwestern Ethiopia,

North Omo Zone of southern Ethiopia from donkey and cattle isolates, and western Ethiopia indicates the occurrence of drug resistant *T. congolense* infections (Afewerk *et al.*, 2001; Assefa and Abebe, 2001; Ademe and Abebe, 2001; Tewelde *et al.*, 2004) respectively.

2.5.2.2. Detection of drug resistance

Several methods have been described to identify drug resistance in trypanosomes (reviewed by Geerts and Holmes, 1994). At present, three types of techniques are commonly used to identify drug resistance: tests in ruminants; tests in mice; and *in vitro* assays. None of these is, however, an ideal test and other tests are still in the phase of development or validation. The use of Trypanocidal ELISA is becoming a promising method of detection.

2.5.2.2.1. Test in ruminants

Tests in ruminants provide direct information from studies in ruminants 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 the animals are parasitaemic, treating them with various levels of trypanocide. The animals are then regularly monitored over a prolonged period (up to 100 days) to determine the effective dose (ED), i.e. the dose that clears the parasites from the circulation, and the curative dose (CD), i.e. the dose that provides 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 re-infection during the study. A useful indication of the level of resistance can be obtained from studies in ruminants by recording the length of time between treatment and the detection of breakthrough populations of trypanosomes. Most trypanosome isolates of cattle are able to grow in these hosts and that the data obtained are directly applicable to the field. If only one isolate per animal is tested it is usually impractical and too expensive to examine a large number of isolates.

2.5.2.2.2. Test in mice

After expansion of an isolate in a donor mouse, groups of 5 or 6 mice are inoculated with trypanosomes, 24 hours later or at the first peak of parasitaemia each group except the control group is treated with a range of drug doses. Thereafter, the mice should be monitored three

times a weak for 60 days. The ED_{50} or ED_{95} (the effective dose that gives temporary clearance of the parasites in 50 or 95 percent of the animals, respectively) can be calculated, as can the CD_{50} or CD_{95} (the curative dose that gives complete cure in 50 or 95 percent of animals, respectively). Sones *et al.* (1988) used groups of five mice, which allowed an easy calculation of ED_{80} and CD_{80} values (1 out of 5 mice was not cleared or cured). These figures should be compared with those obtained using reference sensitive trypanosome strains. The advantage of mouse assay is that it is cheaper than the test in cattle. There are several disadvantages, however: 1) most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice; 2) higher doses of drug must be used in mice (normally 10x higher) in order to obtain comparable results to those obtained in cattle because of the vast difference in metabolic size (Sones *et al.*, 1988); 3) precise assessment of the degree of resistance needs a large number of mice per isolate, which makes it a labor intensive test identification of a discriminatory dose, and 4) it takes as long as 60 days to evaluate the drug sensitivity of an isolate.

2.5.2.2.3. *In vitro* assays

Further progress has been made in the field of in vitro assays (Kaminsky et al., 1990). The advantage of this technique is that large number of isolates can be examined; tests with metacyclic trypanosomes correlate well with field observations. The disadvantages of this technique are: *in vitro* cultivation of blood stream forms is only possible using pre-adapted lines and not using isolates directly from naturally infected animals in addition it is expensive; require good laboratory and well-trained staff.

2.5.2.2.4. Trypanocidal drug ELISA

The use of trypanocidal drug ELISA in combination with parasite detection tests has given promising results for the detection of resistant trypanosomes. The test is both sensitive, detecting subnanogramme concentrations, and specific. It allows the monitoring of drug levels over extended periods and the evaluation of factors influencing drug disappearance rates from the plasma. One interesting finding has been that the drug disappears more rapidly in animals challenged and becoming infected with drug-resistant trypanosome isolates than in those challenged but protected against infection with sensitive trypanosomes (Eisler *et al.*, 1994). Observations showed that the presence of trypanosomes in animals with an ISMM concentration of > 0.4 ng/ml suggests resistance; the higher the drug level detected the greater

the degree of resistance that could be inferred (Eisler *et al.*, 1997). The advantage of the ISMM-ELISA is that large numbers of sera can be tested within a relatively short time. The disadvantage is that the correlation between protection against tsetse challenge with various trypanosome populations and the ISMM concentration in the serum is not understood. It is not yet possible to draw firm conclusions on the sensitivity or resistance of a trypanosome population at the level of individual animal. The ELISA should, however, some indications of the resistance situation at the level of the herd.

2.5.2.2.5. Field methods for rapid assessment of trypanocidal drug resistance

To overcome constraints associated with laboratory tests, field methods to assess resistance of trypanosomes to trypanocides in cattle herds under natural tsetse challenge, have been proposed. Rowlands *e al.* (1993, 2001) developed a model to distinguish new and recurrent infections in order to determine if the high infection rates observed in cattle in Ghibe valley, south west Ethiopia following treatment of *T. congolense* infections with Diminazine aceturate were due to the tsetse challenge or if they were instead due to relapse of infections following treatment. An infection was defined as a new infection if it was preceded by two previous months in which monthly collected samples had a PCV $\geq$ 26% and were not detected with trypanosomes. Eisler *et al.* (2000) have developed a method for the assessment of trypanosomosis risk and the level and prevalence of resistance to ISMM, utilizing cattle populations under natural challenge in the field. This protocol compares new trypanosome infections in a group of cattle treated with ISMM to untreated group. The rate at which new infections occur in the two groups is assessed by a comparison of their survival curves over an 8-12 weeks period. This provides a rapid and accurate assessment of ISMM resistance and the impact of drug use relative to no treatment (Eisler *et al.*, 2000). The main difference between Rowlands and Eisler approaches is that the former addresses the issue of resistance to curative trypanocide Diminazine aceturate, where as the later addresses resistance to ISMM which is first used primarily as a prophylactic drug. This method proposed by Eisler *et al.* (2000) was applied in southwest Ethiopia by Tewelde *et al.* (2004).

3. MATERIALS AND METHODS

3.1. Study area

The present study was conducted in 8 Peasant Associations (PAs) in Dembecha and Jabitehenan weredas of West Gojjam Zone in Amhara Regional State, northwest Ethiopia located about 380 km northwest of Addis Ababa and 220 km southeast of Bahir Dar the capital city of Amhara region. West Gojjam Zone is located at 10^0 30' North latitude and 37^0 29' East longitude. The climatology alternates with long summer rainfall (June-September) and winter dry season (December-March) with mean annual rain fall of 1200-1600 mm. The mean temperature is between 10-20^{0}C and the altitude ranges from 1400-2300 m.a.s.l. The river valleys altitude level ranges from 1700 m.a.s.l. from the main road of Addis Ababa to Bahir Dar to 1300 m.a.s.l. to the lower valley of Abbay.

The study areas includes 8 PAs six from Dembecha wereda namely: Yezeleka, Enewende, Gedebe, Kendamo and Egzabeherab bordering Temechaen and Bir rivers which join together before entering the main river Abbay and two PAs from Jabitehenan wereda namely: Weynema and Adankegne bordering Bir river valley. Ponds and marshy areas are commonly found in the lowland areas of the majority of the study PAs. The different vegetation types such as savanna grassland, forest, riverine and bushlands along with the recently expanded cultivated lands are found. These vegetation types are mainly found in areas below 1700 m.a.s.l. whereas above this altitude the land is occupied by cultivated lands and small areas left for grazing purposes.

The middle Abbay river valley where the present work was undertaken is adjacent to the upper Didessa valley and to the upper most limit of tsetse transmitted areas of the Metekel district where multiple drug resistance of *Trypanosoma congolense* reported by (Codjia, 1993) and Afewerk *et al.*(2000) respectively. The presence of tsetse fly and tsetse transmitted trypanosomosis was also reported by Langridge (1976) in the area.

3.1.1. Socioeconomic situation and farming system of the study area

Agriculture is the mainstay of the livelihood of people with a mixed farming system and livestock plays an integral role for agricultural activity. Livestock also provide meat, milk, cash income and transportation purposes. The livestock species reared include; cattle, sheep, goat and equines. Animals kept in communal grazing system with herds locally they called it "Sheha / Akata". The herd is owned by 7-10 owners or household levels with the average number of animals per herd are estimated to be 43 for cattle. The herds are managed in outdoor system for 14-21 days by close supervision of one owner and animals at night kept in barn /"berete" system in cultivated land to fertilize the land. The crops commonly produced are maize, teff, sorghum, wheat, pepper, pulses, groundnut, etc.

3.1.2. Constraints of livestock production in the study area

According to the report of Amhara region BoA (1999) animal health problems such as infectious diseases, internal and external parasitic diseases, and protozoan diseases are the main constraints of livestock production and agricultural development in the region. Trypanosomosis is commonly found in the western part of the region where the present study area is located and is becoming a serious threat for livestock production and utilization of the fertile and arable land.

A survey conducted by ESTC/SRVETEP in October(2000) indicated that tsetse transmitted trypanosomosis is the most important problem for agricultural activity and livestock production in Dembecha wereda and the problem was also commonly observed in Jabitehenan, Bure, Ankasha and Dangela weredas of West Gojjam Zone of Amhara region. For instance much of the trypanocidal drugs supply from the Bureau of Agriculture is delivered to West Gojjam Zone and intensive usage of these drugs by local farmers is a long history in the mentioned weredas.

Figure 1. Map of Amhara Regional State showing the study weredas (Dembecha and Jabitehenan).

3.2. Study design

The study was based on questionnaire survey, vector studies (entomological survey), parasitological studies and field assessment of trypanocidal drug resistance. It was an epidemiological cross-sectional study covering two weredas with lowland and midland agroecological zones in two seasons of the year. The area was stratified in to two based on altitude levels that are below 1600 m.a.s.l. and 1600-2000 m.a.s.l. The vegetation types were classified into five (bush land, cultivated land, forest, riverine and savanna) according to the wereda office of agriculture, ESTC/SRVETEP (2000) survey and our observation during the field work. Bush land: if the habitat is dominated by trees and shrubs with the total canopy cover greater than 20% and the single canopy is not more than 10 meters. Cultivated land: if a habitat dominated by man-made cultivated crops. Forest: a habitat that consists of evergreen and some semidecidous trees usually with a closed canopy. Riverine forest: forest or bush habitat along the river banks. Savanna: if the typical savanna grass lands are dominant in an area found.

3.2.1. Questionnaire survey

To assess the perception of farmers on the occurrence of tsetse and trypanosomosis, livestock constraints, socioeconomic status, herd composition, use and source of trypanocidal drugs as well as delivery of the drug for treating their animals and other control methods of trypanosomosis and tsetse fly, a questionnaire survey was undertaken. A total of 80 farmers were selected randomly in the study area for this purpose. The questions included during the interview are shown in Annexe 1.

3.2.2. Entomological survey

To assess the apparent density, species of tsetse fly and other biting flies in relation to season, altitude levels, trap and vegetation types, sampling were done in selected sites of the study area. The altitude levels and vegetation types were recorded during the sampling period.

3.2.2.1. Collection of tsetse and other biting flies

Entomological data were collected twice during the study period; in the late rainy season in October 2003 and during the dry period in February 2004. The flies were caught with Monoconical, Biconical and NGU traps baited with acetone and three week old cow urine (Brightwell *et al.*, 1987). In the selected sites of the study area about 142 traps, 70 in late rainy season and 72 in dry season (From December to April) were deployed before sun rise in the morning and kept in position for 72 hours. During trapping acetone was dispensed from open vials through an approximately,'O'- sized hole while cow urine from open bottles into which a quarter of tissue paper was used. All odours were placed on the ground about 30 cm upwind of the trap. Late rainy season is the time period just after the main rainy season from September to November while dry season is the time from December to April and sampling of tsetse and other biting flies carried out in October and February in this study protocol respectively.

The different fly catches in each trap were counted, identified and analysed; the species of tsetse fly was identified based on the characteristic morphology (Langridge, 1976; Ford *et al.*, 1976, Leak *et al.*, 1993) and for other biting flies according to their morphological characteristics such as size, color, wing venation structure and proboscis at the genus level (Walle and Shearer, 1997). Average aging of tsetse was done by categorizing the degree of wear of wings on scales of 1-6 using wing fray method described by (Jackson, 1946; Challier, 1965). Mean wing fray was calculated as the sum of each category total divided by the sum of fly number for each category and find the corresponding number from tables. Sexing were done just by observing the posterior end of the ventral aspect of the abdomen by microscopic lenses as a result male flies easily identified by enlarged hypophgeum in the posterior ventral part of the abdomen. Tsetse fly apparent density is the mean catches in traps deployed, expressed as the number of tsetse catch/trap/day (Leak *et al.*, 1987).

3.2.3. Parasitological survey

To determine the seasonal prevalence of trypanosomosis and to assess the risk factors associated with trypanosomosis snapshot cross-sectional studies were conducted twice during the study period; in the late rainy season (September 25, 2003 to November 5, 2003) and during dry season (February 10, 2004 to March 10, 2004) in 8 PAs of the study area.

3.2.3.1. Study animals

The study animals constituted about 600 herds of 30,000 cattle in 8 PAs of the Abbay basin areas of Dembecha and Jabitehenan weredas.

3.2.2.2.. Sampling method and sample size determination

The sampling strategy was cluster sampling method (Martin, 1987) and herds were considered as clusters. The sample size was determined based on the expected prevalence rate of 20% and absolute desired precision of 4% at confidence level of 95%. As a result a total of 384 animals were needed to be sampled (Thrusfield, 1995). But in case of cluster sampling the subjects are not independent and hence larger sample is required. Therefore, as rule of thumb double the number of animals required for simple random sample are needed for cluster sampling (Martin, 1987). So the optimum sample size for this study was about 800 and actually 814 and 834 samples were taken during the late rainy season and in the dry season respectively.

One stage clustering was considered on herds in the study area. Herd (locally called "Sheha/Akata") is defined as a group of cattle owned by peoples living together in a village and their animals share the same barn at night and the same grazing area and watering points. About 600 herds were estimated to be present in the 8 PAs of the sudsy area out of which 19 herds (814 cattle) and 18 herds (834 cattle) were sampled during the late rainy season and in the dry season respectively.

3.2.2.3. Sample collection and parasitological examination

Paired blood samples were collected from auricular vein of each animal using two haematocrite capillary tubes that filled 3/4 of the height and sealed with Cristaseal. The capillaries were also used to measure the PCV values for the determination of anaemia and comparison of infected animals with non-infected animals. The capillary tube was cut 1mm below the buffy coat to include the top layer of red cells. The content of the capillary tube was expressed onto a clean slide, mixed and covered with a 22x22mm coverslip.Then the slide examined for trypanosomes based on the type of movement in the microscopic field. Confirmation of trypanosome species by morphological characteristics were done after staining with Giemsa by examination with oil immersion microscopy with 100x power of

magnifications (Murray *et al.*, 1977). During sampling age, sex, herd number, PA, wereda, altitude and previous history of treatment with trypanocidal drugs were recorded. Altitudes were stratified into two, lowland (<1600 m.a.s.l.) and midland (1600-2000 m.a.s.l.). The altitude range of the area was 1400-2000 m.a.s.l. The age was categorized into three (<1year, 1-3 year and >3 year) groups.

3.2.4. Assessment of trypanocidal drug resistance (Isometamidium block treatment study)

Based on the results of the trypanosome prevalence rate Melkachaba village from Gedebe PA of Dembecha wereda and Ergib village from Weynema PA of Jabitehenan were selected for ISMM block treatment using purposive sampling method because it had a prevalence rate of trypanosome above 20% and the complain of drug failure mentioned by the interviewed people in these PAs were suggestive too. This study was conducted from December 10, 2003 to March10, 2004. The grazing area and watering points as well as the husbandry systems of the study animals were similar. Diminazine aceturate and ISMM were the trypanocidal drugs used in the study area.

A total of 100 animals 50 from each village were selected with a simple random method. All these animals were treated with Diminazine aceturate (Diminazine™, Lot No.9687, exp. 07-2006, Farvet Laboratories B.V., Bladel-Holland) at a dose rate of 7mg/kg body weight to eliminate the existing trypanosome infection while parasitological examination was done to determine the initial prevalence of trypanosomosis. Cattle of each village were then randomized into 25 ISMM treatment group and 25 control (sentinel) groups following the methods proposed by (Rowlands, 2001; Eisler *et al.*, 2000). Animals in each group were ear tagged using yellow plastic tags, which allow easy identification of animals during each visit for parasitological examination.

After two weeks of the blanket treatment (day 0), one group was treated with ISMM (Trypamidium™, Lot/Batch: W391971 Aut. Av/Exp.:06/2008, MERIAL -17, rue Bourgelat 69002 LYON - France) at a dose rate of 1mg/kg body weight and the other group was left as untreated control group. Body weight of each animal was estimated using heart girth measuring tape (Arora *et al.*, 1981). Blood examination of animals for trypanosome infection was conducted every two weeks starting from day of blanket treatment upto 12 weeks following ISMM block treatment i.e. day 0, 14, 28, 42, 56, 70 and 84. Date of treatment, dosage of trypanocidal drugs, PCV values and trypanosome infections were recorded during

each examination. In each group, cattle which were found to be infected with trypanosomes were treated with diminazine aceturate at a dose rate of 7mg/kg body weight.

Survival analysis of time was done to ascertain the occurrence of ISMM resistance. Survival time is the time to first detection of trypanosome upto 8 weeks post treatment with ISMM at 1mg//kg body weight. Survival analysis of time provides a rapid and accurate assessment of ISMM resistance in the field and impact of ISMM prophylaxis relative to no prophylaxis and moreover applicable to all species and strains that are pathogenic to cattle (Eisler *et al.*, 2000). The efficacy of diminazine aceturate treatment was assessed on the basis of whether or not parasitaemia followed within two weeks after each treatment of any cattle on day 0 to 84 (Rowlands, *et al.*, 2001).

Survival analysis was used to answer the following three practical questions which cattle keepers are facing (Eisler *et al.*, 2000):

1. Was challenge sufficient to warrant prophylaxis in the study village?
2. Was there evidence of ISMM resistance in each village?
3. If resistance was suscepected, had the drug nevertheless sufficient effect to make its use worthwhile?

To resolve these questions three measures were proposed (Eisler *et al.*, 2000):

1. The time after prophylaxis by which 25% of the herd becomes infected
2. The proportion of-cattle becoming infected by 8 weeks following prophylaxis
3. The ratio of the mean hazard rate for the control and prophylaxis herds over 8 weeks period.

Interpretation of survival data (Eisler *et al.*, 2000):

If fewer than 25% of control cattle become infected within 8 weeks of exposure, then the challenge was insufficient to warrant ISMM prophylaxis, which would be undesirable on grounds of cost, possible side effects and unnecessary drug pressure tending to develop drug resistance. If more than 25% ISMM treated cattle become infected within 8 weeks of exposure, this was strongly suspicious of the occurrence of drug resistant trypanosome, provided there was good evidence that the drug was administered correctly.

Where there was evidence of drug resistance on the grounds of the number of ISMM treated cattle becoming infected within 8 weeks of exposure it may nevertheless be worth continuing prophylaxis in situations where the ratio of the mean hazard rate for the control and prophylaxis herds over 1-8 weeks greater than 2. Accordingly, 25% survival time: the time by which 25% of the animals were parasitaemic as a result of trypanosome infection after the

start of ISMM block treatment study, was determined using a soft ware (Stata corporation 2000) as follows (Klein and Moeschberger, 1997).

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

Where g is the Greenwood point wise standard error estimate for s(tp), and f(tp) is the estimated density function at the p^{th} percentile. The upper confidence limit for the p^{th} percentile was defined as the 1^{st} time at which the upper confidence limit s(t) (based on a Ln (-Ln s(t) transformation) is less than or equal to p , and similarly, the lower confidence limit was defined as the 1^{st} time at which the lower confidence limit of s(t) is less than or equal to p. 95% confidence interval was used to analyze the difference between the 25% survival time of the control and ISMM treated cattle. The proportion of cattle becoming infected 8 weeks after ISMM treatment was calculated as number of cattle infected (failure) during 8 weeks after the start of ISMM block treatment study divided by the total number of cattle presented at day 14 when the 1^{st} case diagnosed in ISMM block treatment study. Mean hazard rate and hazards ratio of the control and ISMM treated groups of cattle over 8 weeks period were calculated (Stata Corporation, 2000) as follows (Frankena and Graat, 1997).

$$\text{Mean hazard rate (hi)} = \frac{\text{no. become infected (failure)}}{\text{Sum of ti (total time at risk)}}$$

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

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

The efficacy of diminazine aceturate treatment was assessed on the basis of whether parasitaemia followed within two weeks after treatment of cattle with drug at a dose rate of 7mg/kg body weight. To analyze data on the efficacy of diminazine aceturate, trypanosome incidence rate and trypanosome infection recurrence (Rowlands, *et al.*, 2001) at each village were compared using Fisher's exact test (Stata Corporation, 2000). Trypanosome infection recurrence rate was defined as the proportion of cattle which were found infected with same species of trypanosome among the total number of animals which were treated with diminazine aceturate a dose rate of 7mg/kg body weight before two weeks.

3.3. Data analysis

For the management, analysis and interpretation of data collected based on the study methodology statistical softwares were employed. Stata version 7.0 Software and Excel were used. The following parameters were analysed: The apparent fly catches in relation to variables measured (season, altitude level, and vegetation and trap types) were analyzed using Kruskal Wallis test. The prevalence of trypanosomosis in different variables (altitude levels, season, sex and age) were compared by χ^2-test. A multivariate computation was conducted using logistic regression analysis in order to establish the effects of different risk factors (age, sex, altitude and season) compared with odds ratio for each risk factors. Student's t-test and ANOVA were employed to compare the mean PCV of the parasitaemic and aparasitaemic animals and the effect of altitude on PCV values in the two seasons. The relationship between herd prevalence of trypanosome infections and herd average PCV were examined by regression analysis using herd average PCV as the dependent variable and the prevalence of trypanosome infections in a herd as independent variable.

Data from ISMM block treatment studies were used to calculate parameters such as trypanosome prevalence; incidence rate and cumulative incidence rate. Mean trypanosome prevalence was calculated to compare study villages during 8 weeks period as the mean of the two weekly trypanosome prevalence during 0-56 days. Since all animals at each village were not presented at regular intervals, the cumulative incidence rate was calculated as the number of animals' contract trypanosome infection at day t and before divided by the number of animals at risk at the start of the study-1/2 the number of withdrawals. Mean slope of PCV and 95% confidence interval were used to assess for any change in PCV values of the ISMM treated and control groups of cattle over 8 weeks in each village.

Survival analysis was done during the 8 weeks time to determine the 25% survival time and 95% CI, hazard ratio and proportion of animals infected during 8 weeks time. Log-rank and Wilcoxon (Breslew) tests employed for the equality of survivor function curves between the control and treatment groups of cattle. The difference in the trypanosome incidence rate and trypanosome infection recurrence rate was compared by Fisher's exact test for the assessment of resistance against diminazine aceturate. Data on the questionnaire were summerised using frequency distribution and percentages (proportions). Significant level was determined at $p<0.05$ for all statistical results.

4. RESULTS

4.1. Questionnaire survey

History of farmers settlement

A total of 80 villagers were interviewed, of which 5 were monks and the remaining 75 were farmers. The interviewees were selected randomly from the study area. All the interviewed people responded to the prepared questionnaire format. Twenty percent of the respondent started to live in the present settlement area in 1940s, 40% in 1960s, 20% in 1970s, 15% in 1980s and 5% in 1990s. The farmers still settle in the lowland areas from highland weredas of West Gojjam Zone and from the same wereda by adjusting their own indigenous social and cultural management system. The peoples in the monastery started to live in Kendamo PA in one of the study area since 1969 and established as a monastery in 1970s for male and female monks.

Socioeconomic status

Above 97% of the respondents livelihood is depends on mixed crop livestock production systems. Livestock are integral part of agricultural activity and are used as food, source of income and for transport purposes. The average number for cattle was 5cattle/household and the cultivated land was about 3 hectar/household level.

Livestock management

Livestock is reared primarily for draught purposes where oxen, donkeys and sometimes cows used for this purpose in order of importance. Livestock also used for milk, meat, source of income and transportation. The composition of livestock species in the lowland was cattle (75%), small ruminants (15%) and equines (10%) area while in the midland it was 60%, 35% and 5% respectively. The average cattle herd size was 43 and each herd included cattle from several owners (average 7). Each herd was kept together for grazing and watering as well as at night in the barn ("Beret"). Each herd "Akata" or "Sheha" was managed by one of the owner or Kenjja (partner in herding) for 2-3 weeks in his own supervision at the grazing area and at night in his own "Beret" in the cultivated land for the purpose of fertilizing the crop land to

increase crop productivity. Milking is carried out in the "Beret" at 6 AM in the morning and 7 PM by individual owners. Majority of activities are carried out by the males in the study area. Each herd (Akata) has one responsible person elected by the agreement of all the people of the Akata member for managerial and decision requirements for the husbandry and health care practice.

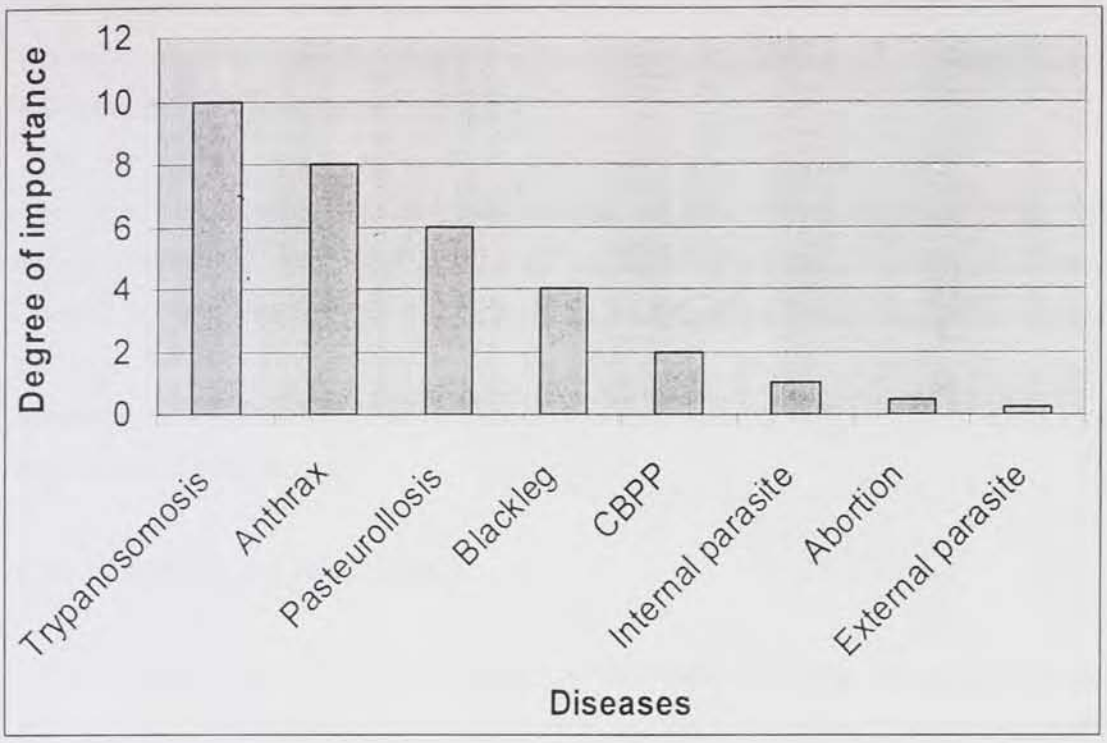
The grazing and watering points are far away from their locality (90% of the respondents) during the dry period. Draught animals travel more long distance than non-draught animals from midland areas to lowland during the rainy season for cultivation purposes. Crop residues and grasses were not preserved for feed source in the lowland area while in the midland area preservation of crop residues and hay for dry period shortage of feeds is practiced.

Livestock rearing including cattle and donkeys were practiced in the monastery but stopped due to livestock diseases. As a result the monks reported the problem to the ESTC and the Amhara Region Bureau of Agriculture. Based on the complaint the ESTC/SRVETEP carried out a survey of trypanosomosis and tsetse fly and implement control activities in and around the monastery for a limited period of time with the assistance of the FAO regional office for Africa.

Livestock constraints

According to the respondents the main livestock constraints in the study area include livestock diseases, lack of grazing land and watering points and scarcity of modern veterinary service. Based on the interview result the main livestock diseases in order of importance are; trypanosomosis, anthrax, pasteurollosis, blackleg, contagious bovine pleuro pneumonia (CBPP), internal parasites, abortion and external parasites as shown in Figure 2.

Figure 2. Major livestock diseases in the Abbay basin areas of northwest Ethiopia.



The status of trypanososmosis

Trypanosomosis as a disease of livestock local name "Mich"or Ghendi is the most important and the first problems affecting livestock productivity and agricultural activity as per 95% of the respondents. The disease had a history of 30-40 years of occurrence as per 60% of the respondent. Twenty five percent of the respondents indicated that they knew trypanosomosis at the start of settlement in their locality and 15% informed that the disease appeared 5-10 years after settlement. Almost 100% of the respondents consider trypanosomosis is mainly a disease of cattle followed by equines and then followed by small ruminants. The main clinical signs of trypanososmosis as known by the interviewed people included; ruffled hair coat, diarrhea, coughing, constipation, emaciation, weakness, reluctant to move, isolated from the herd, depression, abortion, inappetance etc. The impacts of trypanosomosis explained by the respondents in the following sequence: loss of draught power, under cultivation, abortion, reduced fertility, cost of treatment, mortality, loss of milk and meat production, etc.

The questions about transmission of trypanosomosis was responded as follows; 80% of the respondents indicated that the transmitter and cause of the disease is the environment, 15% of the respondent believe biting flies locally called 'Lesso' and "Wegie" their equivalent are

tabanids and muscids flies, and other flies they characterize small in size brown in color biting their animals where the animals move to the forest and savanna vegetation types their equivalent might be tsetse fly. Only 5% of the interviewed people didn't know any about the cause and transmitter of trypanosomosis.

The agroecological occurrence of trypanosomosis was high in areas bordering Abbay valley and the tributaries of Abbay such as Bir and Temechan river valleys. 80% of the respondent in the midland areas revealed that their animals contract trypanosomosis from lowland areas of the river valleys when animals move for grazing and draught purposes. The seasonal occurrence of trypanosomosis was indicated by respondents (98%) after the long rainy season and after short rainy season.

Control methods of trypanosomosis

The only control method of trypanosomosis (Mich/Ghendi) was the use of trypanocidal drugs (100%). A recent method of control of trypanosomosis by applying fly traps and mobile targets initiated by monks in the monastery with the assistance by FAO/ESTC and the Amhara Region Bureau of Agriculture was practiced for a limited period. This activity created awareness about the control methods of trypanosomosis and tsetse fly. As a result the midland people decreased their animal movement into the lowland areas particularly after rainy season. Chemotherapy is the main practice for the treatment of animals affected by trypanosomosis for the last 20-30 years. Diminazine aceturate (Berenil) locally known as "Bicha" or German and Isometamidium chloride (Trypamidium) locally known as "Buna" has been used extensively (97% respondent). 90% of the treatment was given to clinical cases and 10% to non clinical cases.

Above 60% of the respondents indicated that the sources of trypanocidal drugs are the private legal and illegal shops while the remaining was from the veterinary clinics. About 70% of the animals treated by farmers got 55% with Berenil and 45% Trypamidium. Forty five percent of the treatment of infected animals was done by the owner himself, 15% by other partner with improved skill and 40% by veterinary post personnel.

Most of the farmers treatment practice didn't follow the proper dosage and site of injection. As a result treatment of clinical and poor body conditioned animals was done 3-4 times per month in the lowland areas and 1-2 times per month in the midland areas after the long rainy

season and after the short rainy season. Later on mixing of Berenil and Trypamidium was started when treatment failure was encountered by most of the interviewed people. The injection sites of trypanocidal drugs known by local peoples were the gluteal muscle and between the last two ribs below the vertebrae on the right side of the body as shown in Figures 3 and 4. On an average 12-16 birr/month/animal were spent for Berenil and 24-50 birr/month/animal for Trypamidium. No traditional method of treatment was being tried for the control of trypanosomosis which is a common practice for other livestock diseases. The curative efficiency of trypanocidal drugs was reported to be poor by 60% of the respondents and good by 30% of the respondents and unknown by 10%. For this reason frequent treatment and mixing of drugs was the conventional solutions accepted by the local farmers.

Figure 3. A farmer injecting trypanocidal drug to an ox on the gluteal muscle.

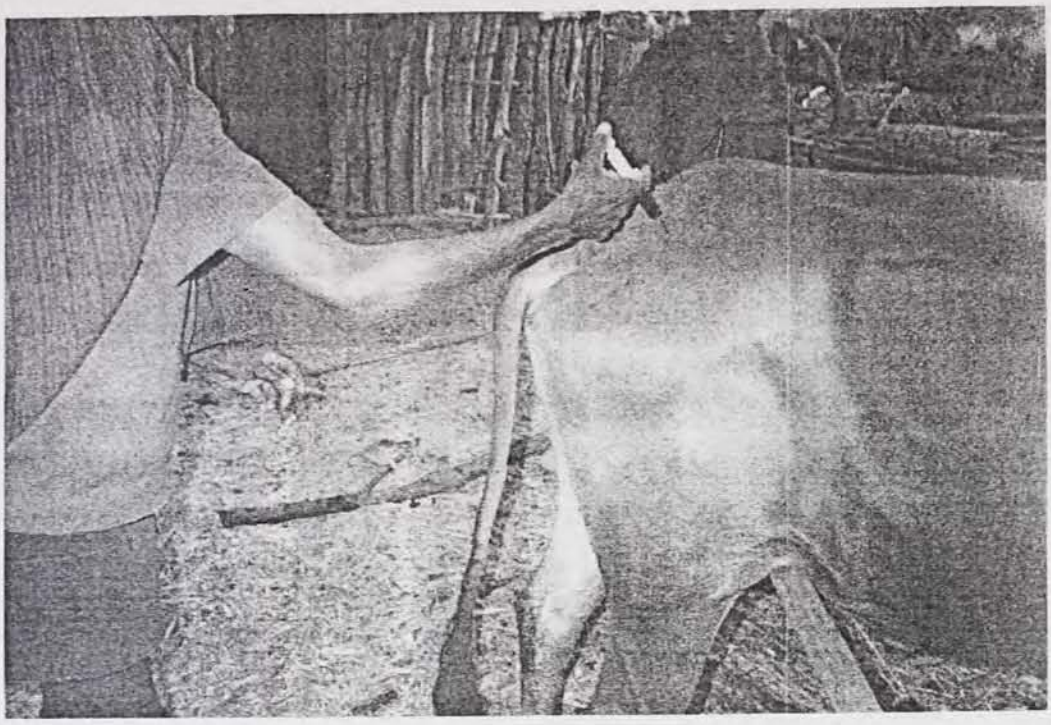
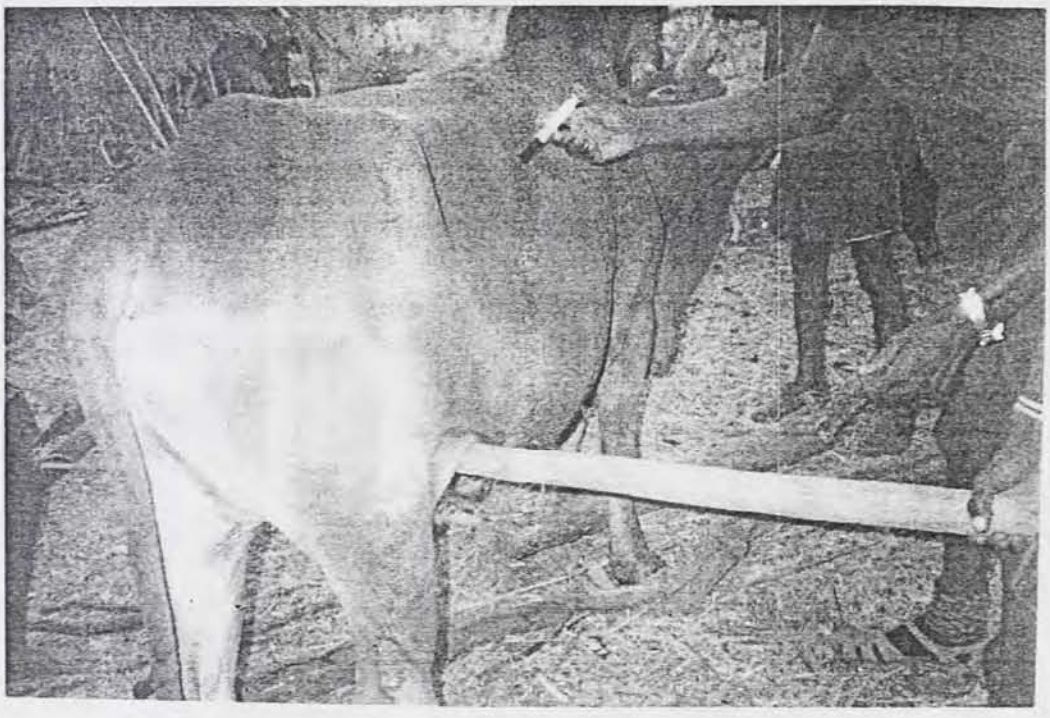


Figure 4. A farmer injecting trypanocidal drug to an ox between the last two ribs below the vertebrae.



4.2. Entomological survey

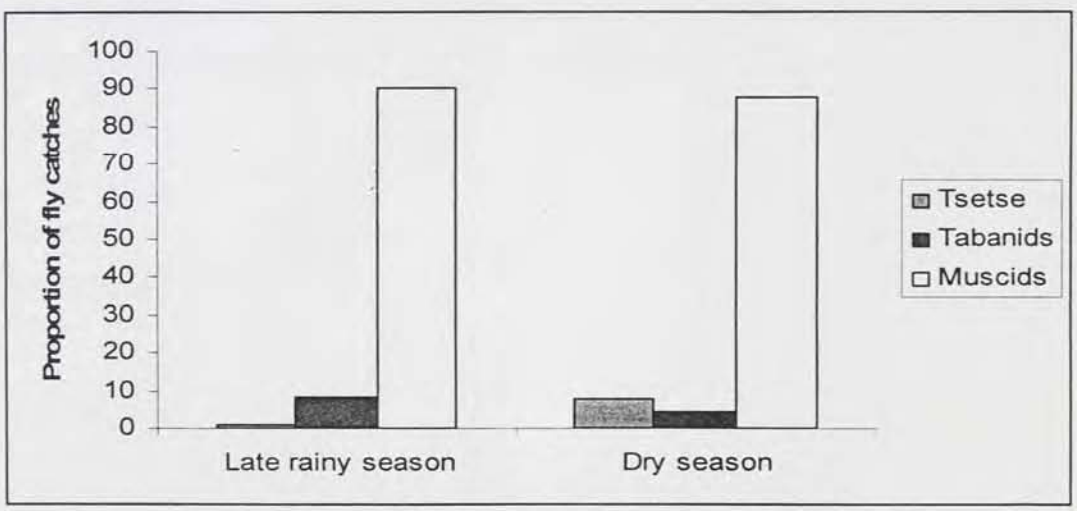
Fly Species

A total of 13,927 flies were caught during the late rainy season and 1,731 flies during the dry season as shown in Table 1. The tsetse fly found during the study period was *Glossina. m. submorsitans.* Tabanid and biting flies of the Muscid groups were caught along the tsetse fly and in areas where tsetse fly were not caught. The tsetse fly accounted for 1.12% of the total fly catches while tabanids account for 8.45% and the rest covers 90.42% during late rainy season and 7.79%, 4.27% and 87.92% during dry season. The tabanid flies included *Tabanus, Haematopota* and *Chrysops* whilst the majority of Muscids were *Stomoxys*. The proportion of fly types caught in the late rainy season and in the dry season is shown in Figure 5.

Table 1. The different fly catches in two seasons in the Abbay basin areas of northwest Ethiopia.

Season	Fly types(no.)			
	Tsetse	Tabanids	Muscids	Total
Late rainy season	157	1176	1258	13927
Dry season	135	74	1522	1731
Total	292	1250	2780	15658

Figure 5. Proportion of fly catches during the study seasons in the Abbay basin areas of northwest Ethiopia.



The mean catches of flies by three different types of traps (Monoconical, Biconical and NGU) during late rainy season are shown in Table 2; Monoconical tap performed the best in the study area was used for determination of apparent density and statistical description.

Table 2. The mean fly catches in three trap types during the late rainy season.

Trap type	Mean catches/trap		
	Tsetse	Tabanid	Muscid
Biconical	2.29	13.76	145.47
Monoconical	3.22	26.36	274.31
NGU	1.51	11.7	131.77

There was significant difference between trap types for the mean catches of flies (Kruskal-Wallis test, $\chi^2 = 8.38$ with 2 d.f. and $p=0.0151$).

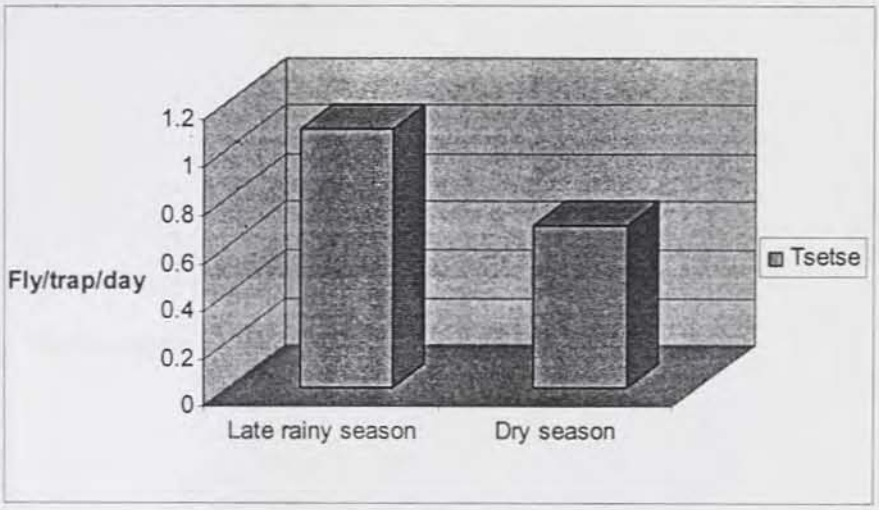
The apparent fly density was found to be 1.08fly/trap/day, 8.78fly/trap/day and 91fly/trap/day for tsetse, tabanids and muscids in the late rainy season and 0.68fly/trap/day, 0.35fly/trap/day and 7.33fly/trap/day in the dry season with a significant difference ($p<0.05$) shown in Figure 6 for tsetse and in Figure 7 for other biting flies. The apparent fly density in five vegetation types indicated in Table 3 showed no significant difference. Altitude has a significant effect on the apparent density of tsetse in both seasons ($p<0.05$) shown in Figure 8. In the late rainy season the lowland areas (below 1600 m.a.s.l.) recorded higher apparent density 1.35fly/trap/day than the midland areas (1600-2000 m.a.s.l.) with 0.66fly/trap/day, and during the dry season the apparent density was 0.95fly/trap/day and 0.15fly/trap/day in the lowland and midland areas respectively. Higher apparent density for biting flies also found in the lowland but not significant.

Table 3. Apparent densities of tsetse and other biting flies in different vegetation types in the Abbay basin areas of northwest Ethiopia during the two study seasons.

Vegetation type	Late rainy season			Dry season		
	Tsetse	Tabanid	Muscid	Tsetse	Tabanid	Muscid
Bushland	1.0	1.33	57.25	0.5	0.3	4
Cultivated land	0.66	17	108.33	0	0.05	15.33
Forest	1.0	17.08	51.91	0.88	0.36	3.86
Riverine	1.88	5.66	227.22	0.49	0.37	4.86
Savanna	1.08	16.8	84.5	1.02	6	12.72

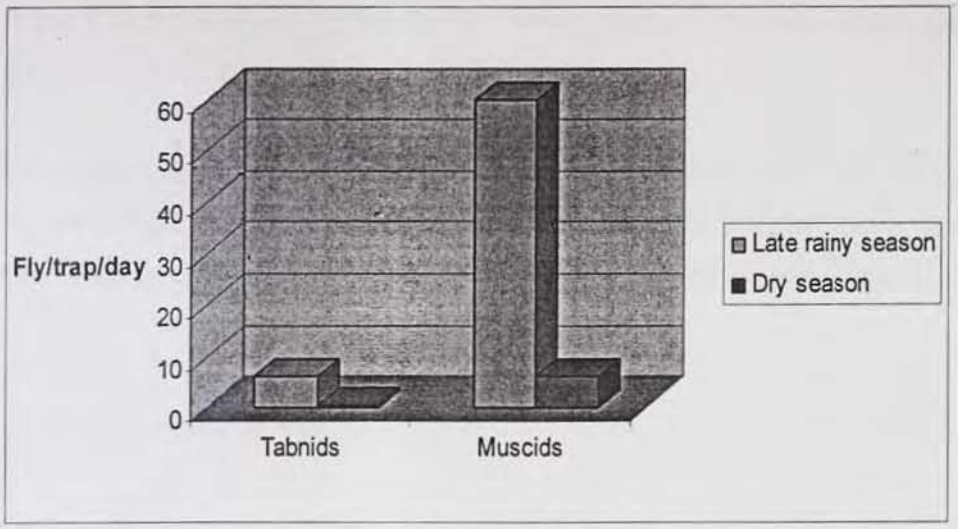
Relatively higher apparent density of tsetse caught in riverine and savanna vegetation types in late rainy season where as in the dry season higher apparent density was caught in savanna and forest areas, in both seasons the lowest was in cultivated lands.

Figure 6. Tsetse apparent density in late rainy season and in dry season in the Abbay basin areas of northwest Ethiopia.



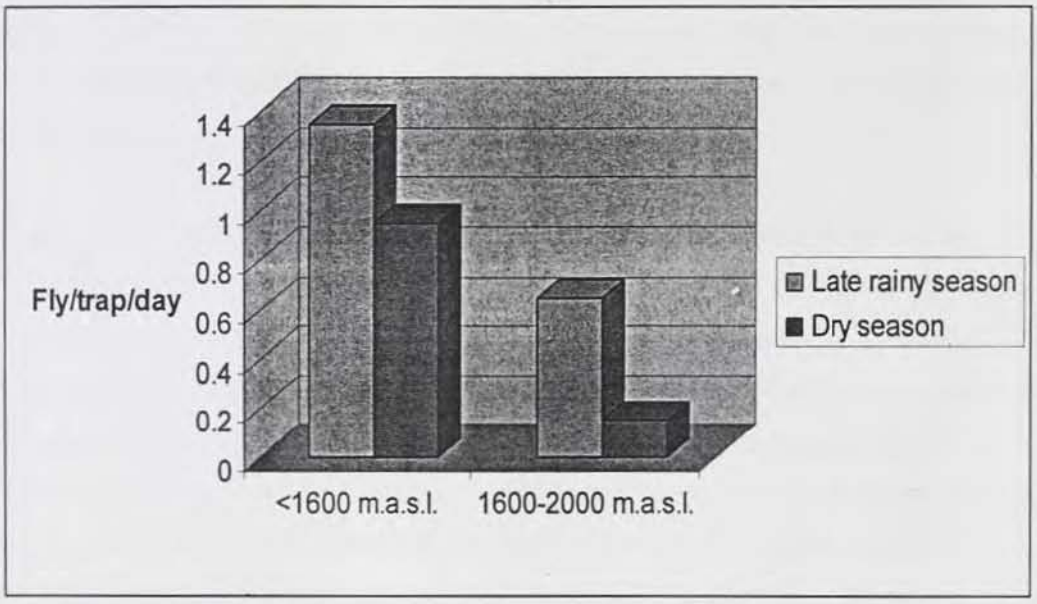
There was significant difference between seasons in the apparent density of tsetse (Kruskal-Wallis test, $\chi^2 = 4.319$ with 1 d.f. $p = 0.0377$).

Figure 7. Biting flies apparent density in late rainy season and in dry season in the Abbay basin areas of northwest Ethiopia.



There was significant difference in apparent fly density between seasons (Kruskal-Wallis test, χ^2 =13.268 with 1 d.f. p =0.0003) for biting flies.

Figure 8. Tsetse apparent density at the different altitudes in late rainy season and dry season in the Abbay basin areas of northwest Ethiopia.



There was significant difference in altitude levels in both seasons (Kruskal-Wallis test, χ^2 =3.65 with 1 d.f. p = 0.0002).

Tsetse fly sexing in the study period indicated that the predominant sex was females (88.78%) during the late rainy season and 53.33% in the dry season. Based on the wing fray analysis the

average age of the population of tsetse fly were 31 days in the late rainy and 26 days during the dry seasons as shown in Annex 2. The mean wing fray age of tsetse fly was slightly higher during the late rainy season than the dry season. Female flies were caught at altitude levels upto 1780 m.a.s.l. while males upto 1650 m.a.s.l.

An attempt was made to detect infection rates in tsetse which were found to be alive during fly identification for sexing and aging. A total 60 flies were dissected and in two (3.3%) male flies "congolense type" infection was found from the mid gut content only.

4.3. Parasitological survey

4.3.1. Trypanosome prevalence

A season based cross-sectional study was carried out during the study period in late rainy season and during dry season. A total of 1,648 animals (cattle) were examined in both seasons i.e. 814 during the late rainy season and 834 during the dry season. The prevalence of trypanosomosis was determined and compared with risk factors such as altitude, season, sex, and age. The herd prevalence was determined with altitude levels and between seasons. The prevalence of trypanosome infection rates between trypanosome species were also assessed at each season.

The prevalence of trypanosomosis during the late rainy season was 17.07% of which 66.9 % (95% CI=0.58-0.74) was *T. congolense* infection where as during the dry season the prevalence was 12.35% of which *T. congolense* infection covered 79.61% (95% CI=0.70-0.86) as shown in Table 4. *Trypanosoma. vivax* infection was 31.65% and mixed infections (*T. congolense* and *T. vivax*) was 1.45% during the late rainy season and 16.5% for *T. vivax* and the remaining infections 1.94% and 1.94% were due to mixed infections (*T. congolense* and *T.vivax*) and *T. brucei* infection at dry season. The risk of infections in the dry season was 0.7 times Odds ratio lower than late rainy season (95% CI=0.53-0.92) with a significant difference.

The prevalence rate of trypanosome infection between sex category (Tables 5 and 6) was 18.45% for males and 13.87% for females in the late rainy season and 12.82% and 11.32% during the dry season but there was no significant difference between sex groups within the

same season or between seasons. There was no significant difference ($p>0.05$) observed in the age groups in the study seasons (Tables 7 and 8). Higher infection rates observed in older animals 17.2% (95% CI=0.14-0.20) and lower in young animals 11% (95% CI=0.04-0.23).The relatively lower infection rates observed during the dry period in calves 2.56% (95% CI=0.001-0.13) and in adult animals 15% (95% CI=0.1-0.15).

The prevalence of trypanosomosis was 19.87% in lowland areas (<1600 m.a.s.l.) and 13.39% in midland areas (≥1600 m.a.s.l.) during the late rainy season while in dry season 17.62% and 6.54% respectively ($p<0.05$) as shown in Tables 9 and 10. In both seasons 0.5 times Odds ratio lower infection rates observed in midland areas than the lowland areas (95% CI=0.35-.65).

Table 4. Prevalence of trypanosome infection in two seasons in the Abbay basin areas of northwest Ethiopia.

Season	Infected	Noninfected	Total	Trypanosome spp.diagnosed				Prevalence rate (%)
				T.c	T.v	T.b	Mixed	
Late rainy season	139	675	814	93	44	0	2	17.07
Dry season	103	731	834	82	17	2	2	12.35
Total	242	1406	1648	175	61	2	4	14.68

T.c=*Trypanosoma congolense*, T.v=*T. vivax*, T.b= T. *brucei*, Mixed =*T. congolense* and *T. vivax*

There was statistical significant difference between seasons ($p=0.007$, $\chi^2 = 7.3$ at 1 d.f). The risk of animals being infected with trypanosome during the dry period was lower than the late rainy season by 0.7 times Odds ratio (95% CI=0.53, 0.92). The prevalence of trypanosome infection in the late rainy season was 17.07% (95% CI=0.14-0.19) and in the dry season 12.35 % (95% CI=0.1-0.14). The overall prevalence rate of trypanosomosis was 14.68% (95% CI=0.13-0.16) and the predominant trypanosome species was *T. congolense* i.e.72.3% (95% CI=0.66-0.77).

Table 5. Prevalence of trypanosomes in male and female cattle during the late rainy season in the Abbay basin areas of northwest Ethiopia.

Sex	Infected	Noninfected	Total	Trypanosome spp.diagnosed				Prevalence rate (%)
				T.c	T.v	T.b	Mixed	
Male	105	464	569	71	32	0	2	18.45
Female	34	211	245	22	12	0	0	13.87
Total	139	675	814	93	44	0	2	17.07

T.c=*Trypanosoma congolense*, T.v=*T. vivax*, T.b= T. *brucei*, Mixed =*T. congolense* and *T. vivax*

Higher prevalence rates of trypanosome infection were observed in male animals 18.45% (95% CI=0.15-0.21) than female animals 13.87% (95% CI=0.09-0.18) but there was not significant difference (p>0.05).

Table 6. Prevalence of trypanosomes in male and female cattle during the dry season in the Abbay basin areas of northwest Ethiopia.

Sex	Infected	Noninfected	Total	Trypanosome spp. diagnosed				Prevalence rate (%)
				T.c	T.v	T.b	Mixed	
Male	73	496	569	60	12	1	0	12.82
Female	30	235	265	22	5	1	2	11.32
Total	103	731	834	82	17	2	2	12.35

T.c=*Trypanosoma congolense*, T.v=*T. vivax*, T.b= T. *brucei*, Mixed =*T. congolense* and *T. vivax*.

The sex groups in the dry season were almost equally affected with the disease as the prevalence was 12.82% (95% CI=0.10-0.15) for male animals and 11.32% (95% CI=0.07-0.15) for female animals (P>0.05).

Table 7. Prevalence of trypanosomes in different age groups of cattle in the late rainy season in the Abbay basin areas of northwest Ethiopia.

Age	Infected	Noninfected	Total	Trypanosome spp.diagnosed				Prevalence rate (%)
				T.c	T.v	T.b	Mixed	
<1 year	6	45	51	5	1	0	0	11
1-3 year	19	84	103	8	11	0	0	18
>3 year	114	546	660	80	32	0	2	17.2
Total	139	675	814	93	44	0	2	17.07

T.c=*Trypanosoma congolense*, T.v=*T. vivax*, T.b= T. *brucei*, Mixed =*T. congolense* and *T. vivax*.

No significant difference was observed in the age groups in the late rainy season while higher infection rates seen in older animals >3year 17.2% (95% CI=0.14-0.20) than calves (<1year old) 11% (95% CI=0.04-0.23).

Table 8. Prevalence of trypanosomes in different age groups of cattle in the dry season in the Abbay basin areas of northwest Ethiopia.

Age	Infected	Noninfected	Total	Trypanosome spp.diagnosed				Prevalence rate (%)
				T.c	T.v	T.b	Mixed	
<1year	1	38	39	1	0	0	0	2.56
1-3year	12	63	75	5	7	0	0	16
>3year	90	630	720	76	10	2	2	15
Total	103	731	834	82	17	2	2	12.35

T.c=*Trypanosoma congolense*, T.v=*T. vivax*, T.b= T. *brucei*, Mixed=*T. congolense* and *T. vivax*.

Lower infection rates were observed during the dry period in calves (<1year) 2.56 % (95% CI=0.01-0.13) than in adult animals (.>3year) 15 % (95% CI=0.1-0.15) but statistically the different was not significant (p>0.05).

Table 9. Prevalence of trypanosomes in cattle at different altitudes during the late rainy season in the Abbay basin areas of northwest Ethiopia.

Altitude (m.a.s.l.)	Infected	Noninfected	Total	Trypanosome spp.diagnosed				Prevalence rate (%)
				T.c	T.v	T.b	Mixed	
<1600 (lowland)	92	371	463	68	24	0	0	19.87
1600-2000 (midland)	47	304	351	25	20	0	2	13.39
Total	139	675	814	93	44	0	2	17.07

T.c=*Trypanosoma congolense*, T.v=*T. vivax*, T.b= T. *brucei*, Mixed =*T. congolense* and *T. vivax*

The prevalence of trypanosome infection varied significantly between the lowland and midland areas (p=0.015, χ^2=5.9205 at 1d.f) and animals in the midland area was at lower risk from lowland area by 0.7 times Odds ratio from lowland areas (95% CI=0.42-0.91).The prevalence of trypanosome in the lowland areas was 19.87% (95% CI=0.16-0.23) and in the midland areas 13.39% (95% CI=0.10-0.17). The predominant trypanosome infection was *T. congolense* at both altitude levels with 73.9% (95% CI=0.63-0.82) and 53.2 % (95% CI=0.38-0.67) in the lowland and midland areas respectively.

Table 10. Prevalence of trypanosomes in cattle at different altitudes during the dry season in the Abbay basin areas of northwest Ethiopia.

Altitude (m.a.s.l.)	Infected	Noninfected	Total	Trypanosome spp.diagnosed				Prevalence rate (%)
				T.c	T.v	T.b	Mixed	
<1600 (lowland)	77	360	437	73	3	1	0	17.62
1600-2000 (midland)	26	371	397	9	14	1	2	6.54
Total	103	731	834	82	17	2	2	12.35

T.c=*Trypanosoma congolense*, T.v=*T. vivax*, T.b= T. *brucei*, Mixed =*T. congolense* and *T. vivax*

There was significant difference in the lowland and midland areas in the dry season (p = 0.000, χ^2 = 23.55 at 1 d.f) as animals in the midland area were at lower risk than animals in lowland areas, 0.3 Odds ratio (95% CI= 0.15-0.47). Prevalence rate of trypanosomosis in the dry season were 17.62% (95% CI=0.14-0.21) in lowland areas and 6.54% (95%CI=0.04-0.09) in the midland areas. The predominant trypanosome infection was *T. congolense* in lowland

areas with 94.8% (95% CI=0.87-0.98) whilst the predominant trypanosome parasite in the midland areas was *T. vivax* 53.8 % (95% CI=0.33-0.73).

The overall prevalence rates of trypanosome infection in sex, age and altitude categories are shown Table 11. Significant difference observed in altitude levels in the overall infection rates ($p<0.05$) with the risk of infection in midland areas was 0.6 times Odds ratio lower than the lowland areas.

Table 11. The overall prevalence of trypanosome in different sex, age and altitude categories during the study period in the Abbay basin areas of northwest Ethiopia.

Variable		Infected	Non infected	Total	Trypanosome spp.diagnosed				Prevalence rate (%)
					T.c	T.v	T.b	Mixed	
Sex	Male	178	960	1138	131	44	1	2	15.37
	Female	64	446	510	44	17	1	2	12.54
Age	<1yr	7	83	90	6	1	0	0	7.77
	1-3yr	31	147	178	13	18	0	0	17.41
	>3yr	204	1176	1380	156	42	2	4	14.78
Altitude	<1600m.a.s.l.	169	731	900	141	27	1	0	18.77
	≥1600 m.a.s.l.	73	675	748	34	34	1	4	9.75
Total		242	1406	1648	175	61	2	4	14.68

T.c=*Trypanosoma congolense*, T.v=*T. vivax*, T.b= T. *brucei*, Mixed =*T. congolense* and *T. vivax*

The relatively higher infection rates were observed in male animals 15.37% (95% CI=0.13-0.17) than female animals 12.54% (95% CI=0.09-0.15) but the difference was not significant ($p>0.05$). There was not significant difference between the age groups of animals as the prevalence in adult animals (>3 year) was 14.78% (95% CI=0.12-0.16) and in calves (<1year) 7.77% (95% CI=0.03-0.15) ($p>0.05$). There was significant difference in trypanosome prevalence rate with altitude levels ($p=0.000$) and risk of trypanosome infection in animals of the midland area was lower by 0.5 times Odds ratio than the lowland areas (95%CI= 0.35 - 0.63). The prevalence rates in the lowland areas was 18.77% (95%CI=0.16-0.21) and in the

midland areas 9.75% (95%CI=0.07-0.12). The predominant trypanosome species in the lowland areas was *T. congolense* 83.43% (95%CI=0.76-0.88) while in the midland altitude levels infection rates were the same for *T. congolense* and *T. vivax* infections 46.56% (95%CI=0.34-0.58). The remaining infections were due to mixed (*T. congolense* and *T. vivax*) and *T. brucei*.

Herd prevalence was determined from a total of 1,648 animals sampled from 37 herds, 19 herds during the late rainy season and 18 during the dry season to assess the effect of trypanosomosis on the herd average PCV values. The herd prevalence of trypanosome in the late rainy season was 16.02% and in the dry season 12.38% ($P<0.05$) and the overall herd prevalence was 14.25%. All the sampled herds were positive for trypanosome infections.

4.3.2. Haematological findings

The mean PCV (%) values of parasitaemic and aparasitaemic animals during the late rainy season was 20.7±3.5 and 26.6± 4.3 ($p<0.001$, 95%CI=25.3-25.9) while during the dry season 21.4%± 3.6 and 26.6%±4.3 ($p<0.001$, 95%CI=25.4-25.9) respectively. The overall mean PCV values were also significantly different between parasitaemic and aparasitaemic animals 25.63±4.5 ($p<0.0001$, 95%CI= 25.4 - 25.8).

The range of PCV values in parasitaemic animals was from 11-35% and in aparasitaemic animals from 14-43% in the late rainy season while in the dry season from 14-32% in parasitaemic animals and in aparasitaemic animals from 16- 44%.. The frequency distribution of PCV values of parasitaemic and aparasitaemic animals in the late rainy season and in the dry season are shown in Figures 9 and 10.

Figure 9. Frequency distribution of PCV values of parasitaemic and aparasitaemic animals during the late rainy season in the Abbay basin areas of northwest Ethiopia.

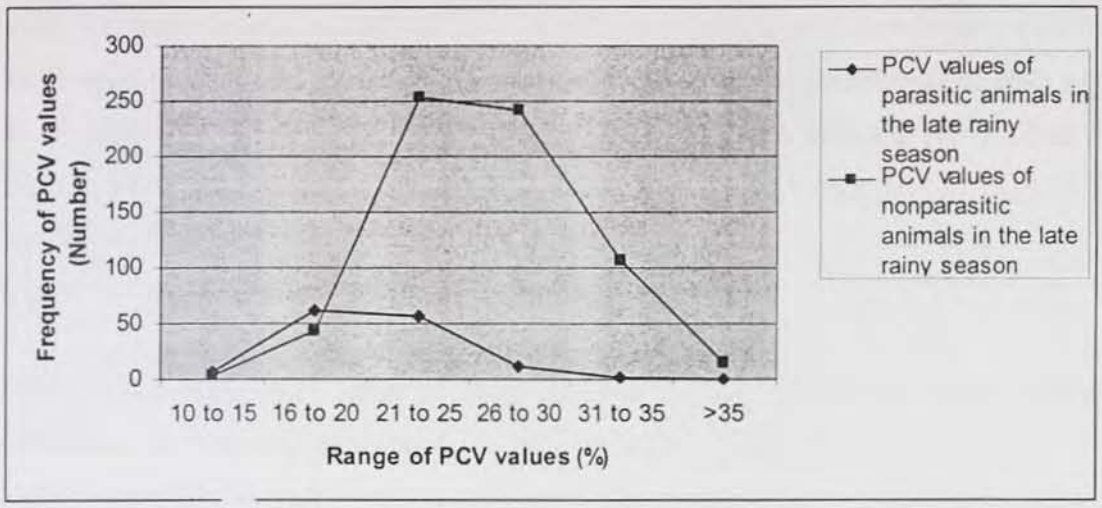
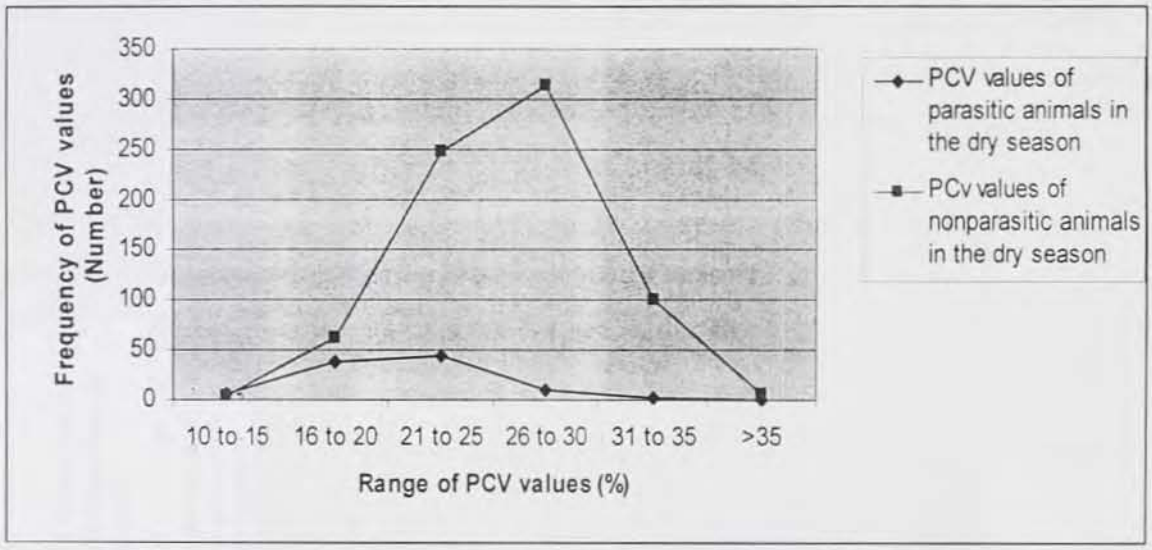


Figure 10. Frequency distribution of PCV values of parasitaemic and aparasitaemic animals during the dry season in the Abbay basin areas of northwest Ethiopia.

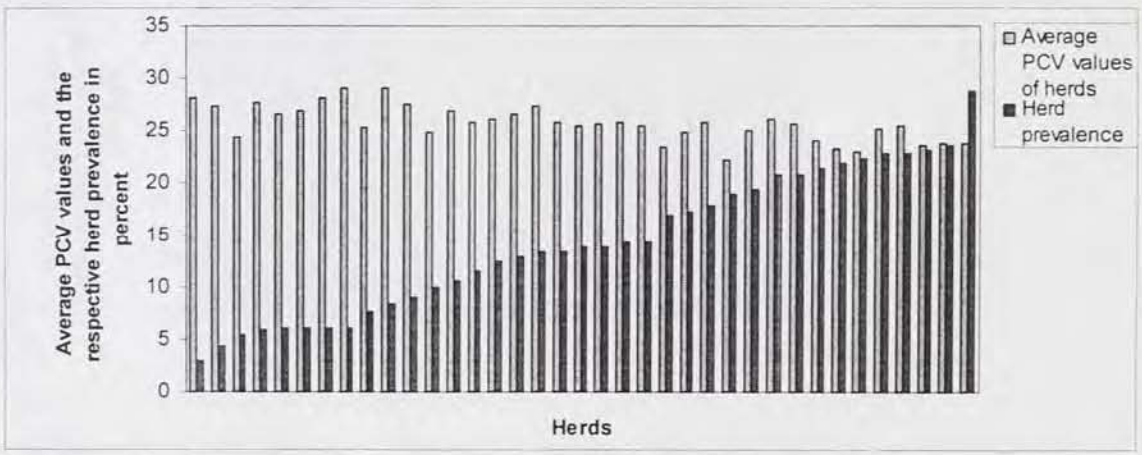


The mean PCV (%) of animals in the lowland area was 24.8±4.8 (95% CI: 24.4-25.2) and in the midland area 26.6±4.4 (95% CI=26.1-27.1) in the late rainy season while in the dry season the mean PCV (%) values was 24.5±4.1 (95% CI=24.1-24.9) in lowland areas and 26.8± 4.1(95% CI=26.5-27.3) in the midland areas. The analysis of variance between mean PCV values in the lowland and midland areas were significantly vary in the two seasons (p=0.000, F=30.8 with 3 d.f.).

The mean PCV (%) values of parasitaemic animals in late rainy season in the lowland areas was 20.3± 3.5 (95% CI=19.5-21.0) and in the midland areas 21.6±3.5 (95% CI=20.5-22.6) while for aparasitaemic animals 25.9±4.4 (95%CI=24.2-26.2) and 27.4±4.0 (95% CI=25.4-28.1) respectively. In the dry season the mean PCV (%) values of parasitaemic animals were 21.4± 4.2 (95% CI=20.5-22.4) in the lowland areas and 21.2±3.6 (95% CI=19.7-22.7) in the midland while for aparasitaemic animals 26.3±3.9 (95% CI=25.9-26.5) and 27.2±3.8 (95% CI=26.8-27.7) respectively. No significant difference found within the parasitaemic animals and aparasitaemic animals between altitude levels in both seasons.

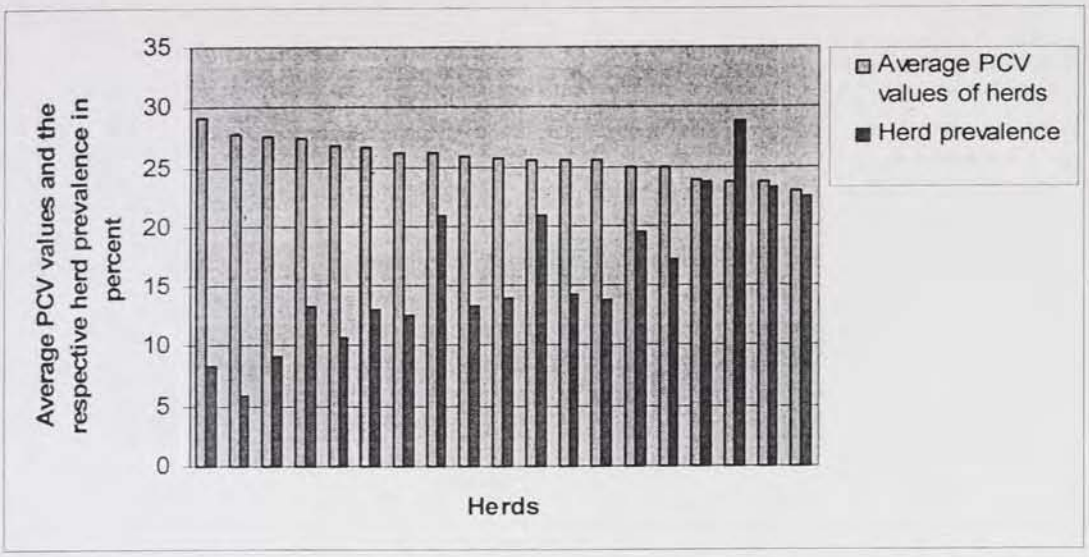
The overall average PCV values of herds (37) was 25.73% significantly varied with the prevalence of trypanosome in herds 14.25% ($p<0.0001$, 95% CI= -0.23 to -0.12) with regression coefficient of -0.17 shown in Figure11. The average PCV values of herds (19) was 25.78% significantly varied with mean prevalence of trypanosome in herds 16.02% ($p<0.002$, 95% CI=-0.29 to -0.15) with a regression coefficient -0.22 during the late rainy season as shown in Figure 12. During the dry season the average PCV values of herds (18) was 25.68% with a significant difference with the mean prevalence of trypanosome in herds 12.38% ($p<0.002$, 95% CI=-0.27 to -0.07) with a regression coefficient of -0.17 as shown in Figure 13. The comparison of herd prevalence rates between seasons were indicated in Figure 14.

Figure 11. The overall averages PCV values and herd prevalence rate of trypanosome in the 37 cattle herds in the Abbay basin areas of northwest Ethiopia.



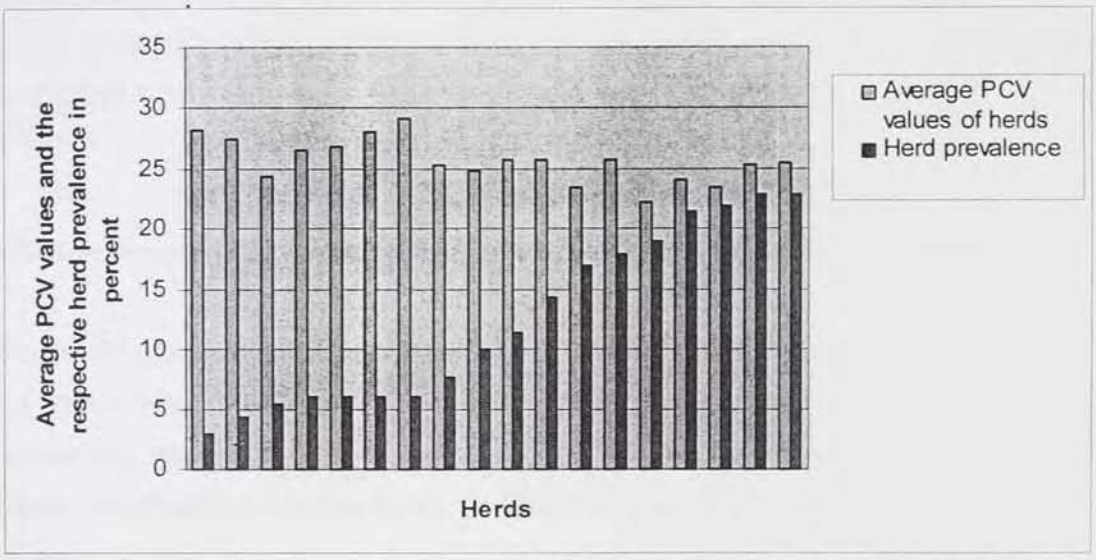
When the mean prevalence of herds increased the herd average PCV decreased.

Figure 12. The average PCV values and herd prevalence rate of trypanosome infection in the 19 cattle herds during the late rainy season in the Abbay basin areas of northwest Ethiopia.



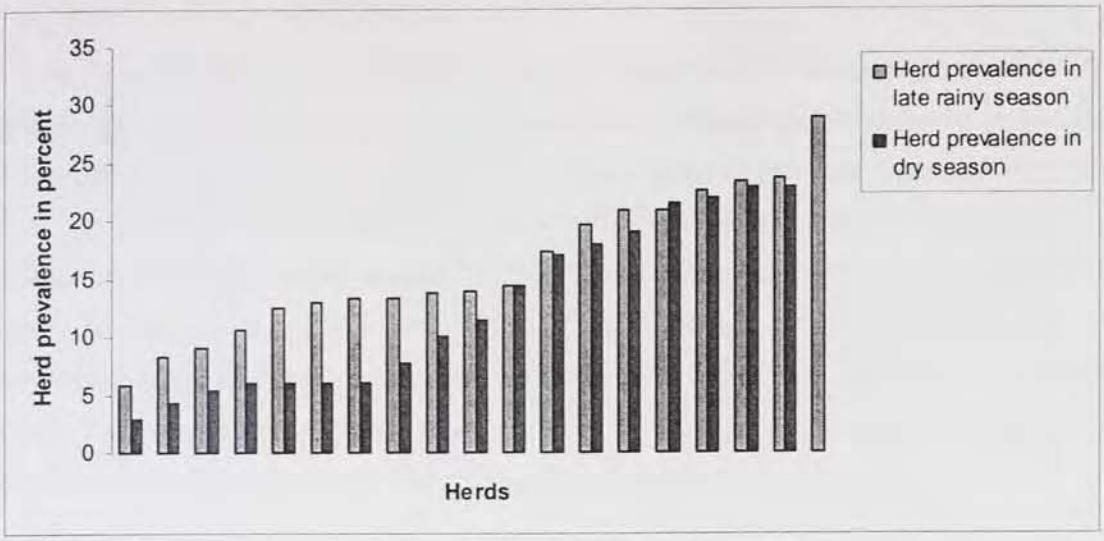
As the average PCV values of the herds decreased the herd prevalence of trypanosome infections increased considerably.

Figure 13. The average PCV values and herd prevalence rate of trypanosome infection in the 18 cattle herds during the dry season in the Abbay basin areas of northwest Ethiopia.



As the average PCV values of the herds decreased the herd prevalence of trypanosome infections increased significantly.

Figure 14. The comparison of herd prevalence rates of trypanosome infection between seasons in the Abbay basin areas of northwest Ethiopia.



Significantly higher herd prevalence of trypanosome infection observed in the late rainy season.

From 1,648 animals of 37 herds examined, trypanosome infection due to *T. congolense* were detected in 175 (72%) animals of the total trypanosome infections and the remaining 67 were due to *T. vivax* 61 (25.25%), mixed infections (*T. congolense and T. vivax*) 4 (1.65%) and *T. brucei* 2 (0.83%).

4.4. Assessment of trypanocidal drug resistance (Isometamidium block treatment study)

The ISMM block treatment study was conducted in two villages, Melkachaba from Kendamo PA (Dembecha wereda) and Ergib from Weynema PA (Jabitehenan wereda) selected purposively where drug resistance problem was suspected from questionnaire survey and higher parasitological findings during the first prevalence study carried out in the late rainy season.

At the blanket treatment day i.e. day -14 the prevalence of trypanosome infection was 30% (95% CI=0.17-0.44) in Ergib out of these 24% were *T. congolense* infections while in Melkachaba 34% (95% CI=0.21- 0.48) and out of these 28% of infections were *T.*

congolense. The proportion of infection rates in control and treatment groups were 32% and 28% in Ergib and 28% and 40% in Melkachaba villages Annexe 3.

At day 0 of the ISMM block treatment study 4% infection in treatment groups and 12% infection in control group average (7%) in Ergib while 12% infection in treatment groups and 8% infection in control group of Melkachaba village average (10%) were diagnosed. Fourteen days after ISMM block treatment, one animal with *T. congolense* infection in the treatment group and 2 animals with *T. congolense* and *T. vivax* infection in the control group were found in Ergib village where as in Melkachaba 5 animals were parasitaemic with *T. congolense* in the treatment group and 3 animals with *T. congolense* infection in the control group. The trypanosome infection rates and mean PCV values of the control and treatment groups of cattle from day 0 to day 84 is indicated in Annexe 3.

The mean prevalence rate of trypanosome infection during 8 weeks period at Ergib was 7.52% in the treatment group and 13.6% in the control group and in Melkachaba villages 22.4% and 18.12% respectively. The prevalence rates of trypanosome infection in the ISMM block treatment study for two villages were shown in Figures 15 and 16.

Figure 15. Prevalence of trypanosomes in the control and treatment groups of cattle in Ergib village of the ISMM block treatment study in the Abbay basin areas of northwest Ethiopia.

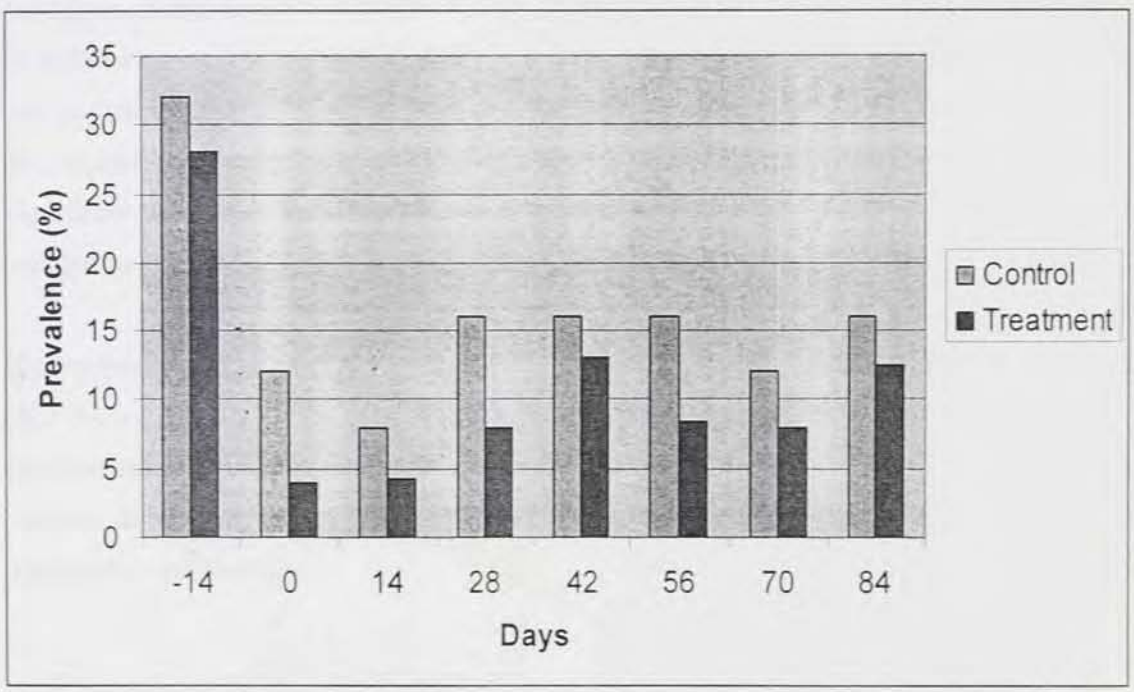
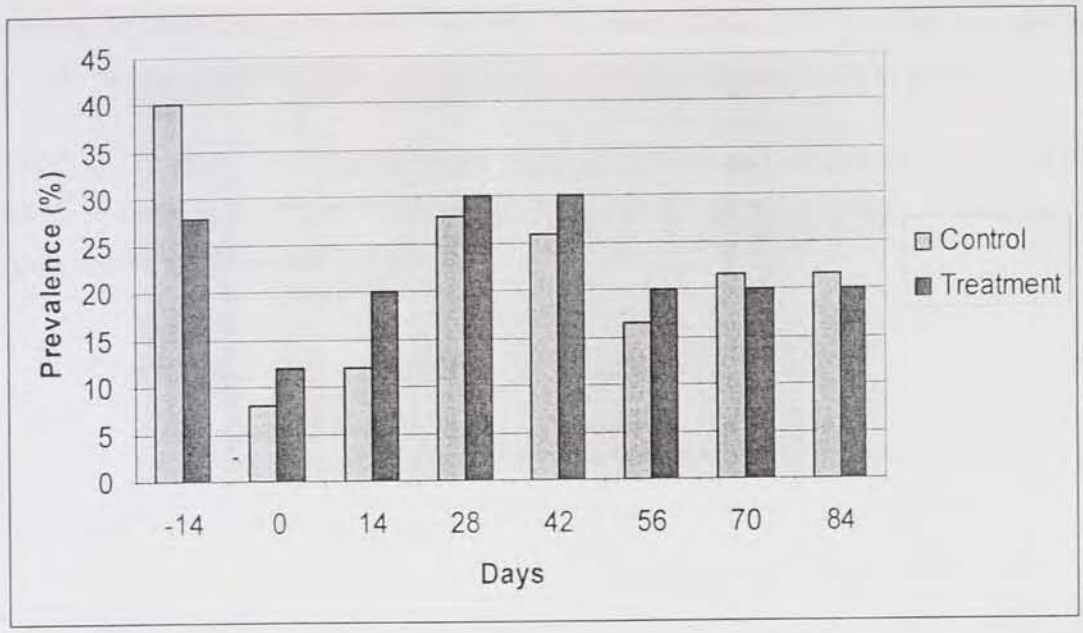


Figure 16. Prevalence of trypanosomes in the control and treatment groups of cattle in Melkachaba village of the ISMM block treatment study in the Abbay basin areas of northwest Ethiopia



Trypanosome incidence rate

Trypanosome incidence rate at 8 weeks of ISMM block treatment study was the number of new cases of trypanosome infection in each group of cattle divided by the sum of cattle days at risk during the 8 weeks period. Only new cases were considered for this study. Incidence rate in control group of cattle in Ergib and Melkachaba villages were 0.0097 and 0.0122 and in the treatment group 0.0056 and 0.0175 respectively (Tables 15 and 16). There was not significant difference in the incidence rate between control and treatment group in both villages ($p>0.05$).

Trypanosome cumulative incidence rate at 8 weeks of ISMM block treatment study were 52.17% and 21.73% for the control and treatment groups in Ergib and 62.22% and 73.91% in Melkachaba respectively. Cumulative incidence rate was calculated as the number of animal contract disease at day t and before divided by number of animals at the start of the day-1/2 the number withdrawals.

Haematological findings

The mean slope of the PCV values of the control and treatment group of cattle was used to compare the change in PCV values over time. The mean slopes of PCV values were positive in all groups of the ISMM block treatment study in both villages as shown in Table 12.

Table 12. The mean PCV (%) and mean slope of PCV values in the control and treatment groups of cattle in Ergib and Melkachaba villages of ISMM block treatment study in the Abbay basin areas of northwest Ethiopia.

Village/group		Mean PCV (%)		Mean slope
		Day 0	Day 56	
Ergib	Control	25.04	25.96	0.8
	Treatment	26.44	27.4	1.18
Melkachaba	Control	25.12	23.33	1.23
	Treatment	23.34	24.04	0.92

There was no significant difference between the mean slopes of the control and treatment group in Ergib ($P>0.05$, 95% CI = -1.42 to 3.40) and also in Melkachaba ($P>0.05$, 95% CI= -0.89 to 3.04). In all groups of the village the slope of mean PCV values were positive.

Survival analysis

Survival analysis of the animals included in the ISMM block treatment study was done. The 25% survival time of the control and treatment group of cattle was determined as the time by which 25% of the animals become parasitaemic as a result of trypanosome infection. The 25% survival time for the treatment and control groups of cattle in Ergib was 42 days while in Melkachaba it was 28 days and there was overlapping in days in both groups at each village, however, the confidence interval varies between groups in each village as shown in Tables 13 and 14.

Table 13. The 25% survival time of the control and treatment groups of cattle upto day 56 of the ISMM block treatment study at Ergib village in the Abbay basin areas of northwest Ethiopia.

Group	No.[a]of subjects	25% survival time(days)	Std. Err	95% CI (days)	
Control	22	42	7.306614	14	56
Treatment	24	42	7.638733	14	70
Total	46	42	7.970852	28	56

a –number of animals examined when the first case of parasitaemic animals detected at day 14

Table 14. The 25% survival time of the control and treatment groups of cattle upto day 56 of the ISMM block treatment study at Melkachaba village in the Abbay basin areas of northwest Ethiopia.

Group	No[a].of subjects	25% survival time (days)	Std. Err	95% C I (days)	
Control	23	28	4.297546	14	42
Treatment	21	28	3.083022	14	28
Total	44	28	3.768138	14	28

a =number of animals examined when the first case of parasitaemic animals detected at day 14

The mean hazard rates of the treatment and control groups of cattle in Ergib were 0.0056 and 0 .0097 (Table 15) while in Melkachaba 0.0178 and 0.0122 (Table 16) respectively. The hazard ratio of control to treatment group of cattle for Ergib was 1.73 and for Melkachaba was 0.68 (Table 1 7). The mean hazard rate is calculated as the number of cattle infected with trypanosome (failure) divided by the total time at risk from day 0 to 56 of the ISMM block treatment study and the hazard ratio is calculated as the ratio of the mean hazard rate of control to treatment group of cattle. More than 25% of the animals at each village of the treatment group were found infected with trypanosome by day 56 (8^{th} week) of the ISMM block treatment study (Table 18). Therefore the three indices in both villages indicated the occurrence of drug resistance to ISMM and the prophylactic failures is strongly appreciated.

Table 15. Mean hazard rate and 25% survival time of control and treatment groups of cattle upto day 56 of the ISMM block treatment study in Ergib village in the Abbay basin areas of northwest Ethiopia.

Group	No.[a] of subjects	No. failure	Time at risk	Mean[b] hazard rate	25%[c] survival time(days)	95%CI (days)	
Control	22	10	1022	0.0097	42	14	56
Treatment	22	6	1064	0.0056	42	14	70
Total	44	16	2086	0.0056	42	28	56

a=Total no. of cattle presented at day 14 when the first case was diagnosed.

b=Number cattle infected (failure) divided by total time at risk from day 0 to 56 of ISMM block treatment study.

c=Time by which 25% of the animal become parasitaemic as a result of trypanosomosis infection.

Table 16. Mean hazard rate and 25% survival time of control and treatment group of cattle upto day 56 of the ISMM block treatment study in Melkachaba village in the Abbay basin areas of northwest Ethiopia.

Group	No.[a] of subjects	No. failure	Time at risk	Mean[b] hazard rate	25%[c] survival time(days)	95%CI (days)	
Control	23	12	980	0.0122	28	14	42
Treatment	21	13	728	0.0178	28	14	28
Total	44	25	1708	0.0146	28	14	28

a=Total no. of cattle presented at day 14 when the first case was diagnosed.

b=Number cattle infected (failure) divided by total time at risk from day 0 to 56 of ISMM block treatment study.

c=Time by which 25% of the animal become parasitaemic as a result of trypanosome infection.

Table 17. Hazard ratio of control and treatment groups of cattle upto day 56 of ISMM block treatment study in Ergib and Melkachaba villages in the Abbay basin areas of northwest Ethiopia.

Village	Group	No.[a] of subjects	No. failure	Time at risk	Mean hazard rate	Hazard[b] ratio
Ergib	Control	22	10	1022	0.0097	1.73
	Treatment	22	6	1064	0.0056	
	Total	44	16	2086	0.0056	
Melkachaba	Control	23	12	980	0.0122	0.68
	Treatment	21	13	728	0.0178	
	Total	44	25	1708	0.0146	

a=Total no. of cattle presented at day 14 when the first case was diagnosed.

b=The ratio of mean hazard rate of control to treatment groups of cattle in the ISMM block treatment study since day 0 upto day 56

Table 18. Proportion of cattle become parasitaemic upto day 56 since day 0 of ISMM block treatment in Ergib and Melkachaba villages in the Abbay basin areas of northwest Ethiopia

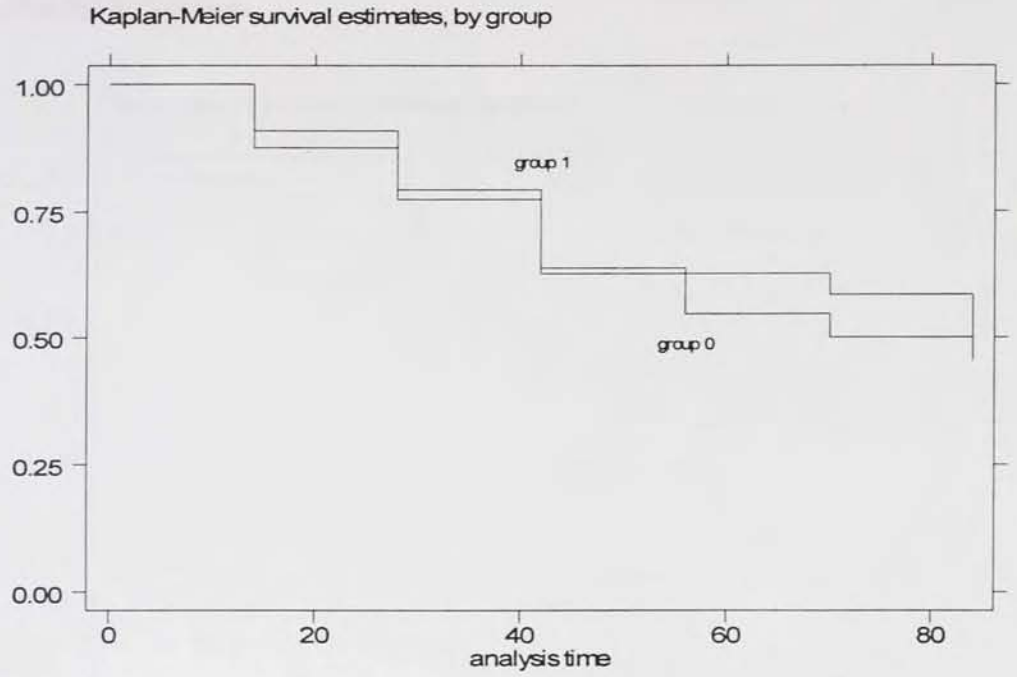
Village	Group	No.[a] of subjects	No. failure	Proportion[b]
Ergib	Control	22	10	0.45
	Treatment	22	6	0.27
	Total	44	16	0.36
Melkachaba	Control	23	12	0.52
	Treatment	21	13	0.61
	Total	44	25	0.56

a-Total no .cattle presented at day 14 when the first case was diagnosed

b- The proportion of cattle becoming parasitaemic during the 8 weeks period of ISMM block treatment study since day 0.

The Kaplan-Meier survival curves (Stata Corporation 2000) were plotted for the control and treatment groups of cattle in each village. The equality of the survivor function during the 8 weeks after ISMM block treatment study was tested using Log-rank and Wilcoxon (Breslew) tests (Stata Corporation 2000). The probability to survive, 8 weeks after the start of ISMM block treatment study varied between village and between cattle. However there was no statistical difference between Kaplan-Meier survival estimates of the control and treatment groups at each village (P>0.05) as shown in Figures 17 and 18.

Figure 17. Kaplan-Meier survival estimates and statistical test for the equality of the survivor function of the control and treatment group of cattle in Ergib village in the Abbay basin areas of northwest Ethiopia.

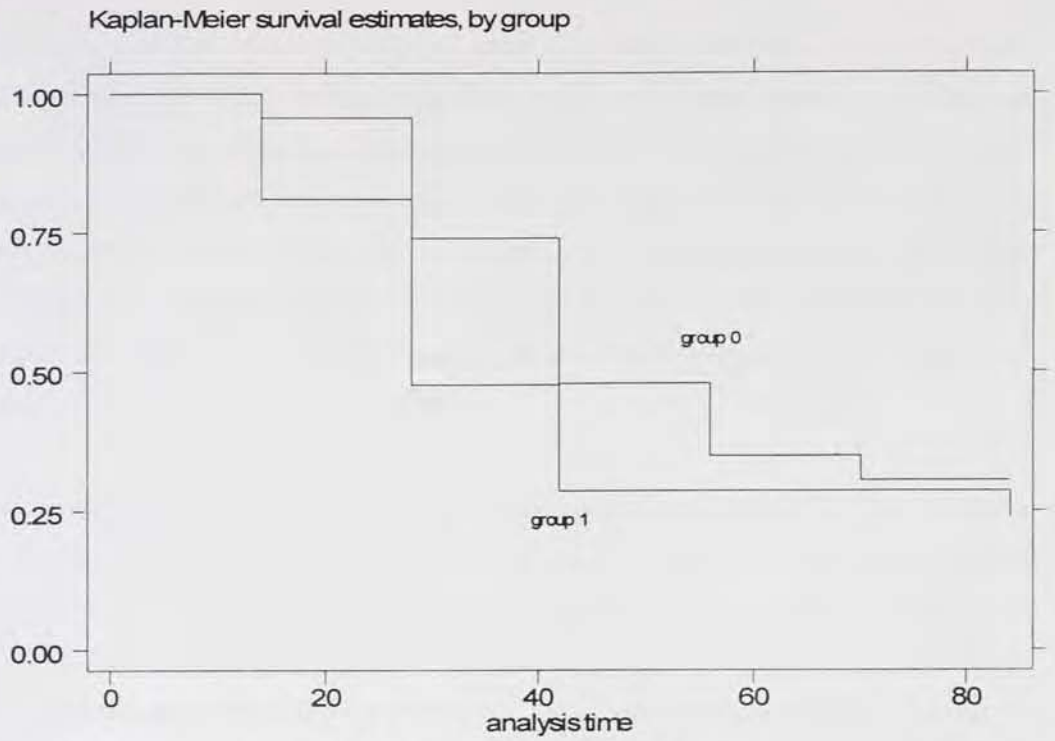


Group 0=Control group in Ergib village in the ISMM block treatment study from day 0 to day 84

Group1=Treatment group in Ergib village in the ISMM block treatment study from day 0 to day 84

Log-rank test (p =0.2503) and Wilcoxon (Breslow) test (p = 0.2815) for equality of survivor functions revealed no significant difference between control and treatment groups.

Figure 18. Kaplan-Meier survival estimates and statistical test for the equality of the survivor function of the control and treatment group of cattle in Melkachaba village in the Abbay basin areas of northwest Ethiopia.



Group 0=Control group in Melkachaba village in the ISMM block treatment study from day 0 to day 84

Group1=Treatment group in Melkachaba village in the ISMM block treatment study from day 0 to day 84

Log-rank test (p=0.2310) and Wilcoxon (Breslow) test (p=0.1159) for equality of survivor functions showed no significant difference between control and treatment groups.

In both villages the-two tests performed on the Kaplan-Meier survivor function curves of the control and treatment groups of cattle showed no significant difference on the probability of surviving from infection by trypanosome pathogen consequently the prophylactic activity of ISMM was not as effective as recommended by drug manufacturers and the level of resistance was high along the previous indices.

Efficacy of diminazine aceturate treatment

The efficacy of diminazine aceturate was assessed on the basis of parasitaemia after each diminazine aceturate treatment on day 0, 14, 28, 42, 56, 70, 84. All the cases found infected were again treated with diminazine aceturate (7mg/kg body weight). At day 0 three animals

from the control group and one animal from the treatment group were parasitaemic in Ergib and two and three animals in Melkachaba respectively. At day 14 two animals were parasitaemic in control group and 1 animal in the treatment group in Ergib and three and five animals in Melkachaba respectively.

In Ergib village a total of 39 cattle (24 from control and 15 from treatment group) were found at least one times parasitaemic in 84 days follow up out of this 26 were during the 8 weeks time and 22(87.5%) were due to *T. congolense* infection. Seven animals from 39 became parasitaemic at least twice during the 84 days of the study, 4 from control group and 3 from treatment group as shown in Table 19. Five animals (2 from treatment group and 3 from control group) were recurrent infections during the 84 days while 3 of 5 recurrent infections were within 8 weeks time. All the recurrent parasitaemia were due to *T. congolense* infections.

In Melkachaba village a total of 70 cattle (32 from control group and 38 from treatment group) were found to be at least one times parasitaemic in 84 days follow up out of these 52 were during the 8 weeks time and 47(90.62%) were due to *T. congolense* infection. 21 animals (11 from treatment group and 10 from control group) of 70 became parasitaemic at least twice. On the otherhand 11 (5 from treatment group and 6 from control group) of the 21 multiple parasitaemic cases were recurrent infections. 100% of the recurrent infections were due to *T. congolense*. Eventhough the required doses of diminazine aceturate were given with some animals remains positive for trypanosome infections with *T. congolense* upto day 56 and this was detected in both villages. The comparison of trypanosome incidence rate and trypanosome recurrence infection rate is shown in Table 20 and there was no significant difference between the two rates at each village.

Table 19. ISMM block treatment study: Cattle becoming parasitaemic at least twice after treatment with diminazine aceturate at dose rate of 7mg/kg body weight in Ergib and Melkachaba villages in the Abbay basin areas of northwest Ethiopia.

Village	Group	Tag no. (ID)	Days after ISMM block treatment						
			0	14	28	42	56	70	84
Ergib	Treatment	5	-	-	-	T.c	T.c	T.c	T.c
Ergib	Treatment	18	-	-	-	T.c	T.c	-	-
Ergib	Treatment	25	-	-	T.c	-	-	T.c	-
Ergib	Control	51	T.c	-	-	T.c	-	-	-
Ergib	Control	55	T.c	-	T.c	-	T.c	T.c	T.c
Ergib	Control	58	-	-	-	-	T.c	T.c	T.c
Ergib	Control	66	-	-	-	T.c	T.c	-	T.c
Melkachaba	Treatment	28	T.c	-	T.c	-	-	T.c	-
Melkachaba	Treatment	29	T.c	-	-	T.c	T.c	T.c	T.c
Melkachaba	Treatment	31	-	T.c	-	T.c	-	-	-
Melkachaba	Treatment	33	T.c	T.c	-	T.c	-	-	-
Melkachaba	Treatment	34	-	T.c	-	-	T.c	-	-
Melkachaba	Treatment	36	-	-	-	T.c	T.c	T.c	-
Melkachaba	Treatment	40	-	-	T.c	-	T.c	-	-
Melkachaba	Treatment	41	-	-	T.c	-	-	-	T.c
Melkachaba	Treatment	43	-	-	-	T.c	-	T.c	T.c
Melkachaba	Treatment	44	-	-	T.c	T.c	-	T.c	-
Melkachaba	Treatment	49	-	-	-	T.c	T.c	-	T.c
Melkachaba	Control	76	-	T.c	-	T.c	-	-	T.c
Melkachaba	Control	79	-	-	-	-	-	T.c	T.c
Melkachaba	Control	82	-	-	-	T.c	T.c	-	-
Melkachaba	Control	83	T.c	T.c	T.c	-	-	T.c	T.c
Melkachaba	Control	85	-	-	T.c	T.c	-	-	-
Melkachaba	Control	86	-	-	-	T.c	-	T.c	-
Melkachaba	Control	92	-	-	-	T.c	-	T.c	-
Melkachaba	Control	96	-	-	T.c	-	-	T.c	-
Melkachaba	Control	98	-	-	T.c	-	T.c	-	T.c
Melkachaba	Control	100	T.c	T.c	T.c	-	-	-	-

T.c= *Trypanosoma congolense*, - = Negative from parasitaemia

All the parasitaemic cases were due to *T. congolense* infection in each village during the study period while 75% of the multiple parasitaemic cases were observed in Melkachaba village.

Table 20. Comparison between trypanosome incidence and trypanosome infection recurrence rate in cattle which were examined every 2 weeks interval for a total of 14 weeks in Ergib and Melkachaba villages in the Abbay basin areas of northwest Ethiopia.

	Ergib			Melkachaba		
Day	Trypanosome incidence rate	Trypanosome[1] recurrence rate	P^2-value	Trypanosome incidence rate	Trypanosome[1] recurrence rate	P^2-value
-14	15/50=0.3	0		17/50=0.34	0	
0	4/50=0.08	0		6/50=0.12	0	
14	3/48=0.0625	0/4=0		8/50=0.16	3/6=0.5	NS
28	6/50=0.12	0/3=0		14/48=0.291	0/8=0	
42	7/48=0.1458	1/6=0.166	NS	13/46=0.282	2/14=0.14	NS
56	6/49=0.1224	2/7=0.428	NS	9/49=0.183	4/13=0.30	NS
70	6/49=0.1224	2/6=0.33	NS	10/48=0.208	0/9=0	
84	7/48=0.1458	0/6=0		10/48=0.208	2/10=0.2	NS

1= The number of cattle which were found infected with the same species of trypanosome among the total number of animals treated with diminazine aceturate

2= Fisher's exact value for the comparison of recurrence and incidence of trypanosome infections

NS=Nonsignificant (P>0.05)

The trypanosome incidence rate and trypanosome infection recurrence indicated no significant difference during the 14 weeks observation time with Fisher's exact test (P-value > 0.05). Higher recurrence infection of 68.75% was detected in Melkachaba than in Ergib.

5. DISCUSSION

5.1. Questionnaire survey

The results the questionnaire survey revealed that trypanosomosis is the most important problem for agricultural activity and animal production in the Abbay basin areas of Amhara Region northwest Ethiopia. Above 97% of the interviewed farmer's livelihood depends on mixed agriculture (crop-livestock) farming system which is consistent with the general situation of Ethiopia where above 80% of the population is engaged in mixed farming system. The majority of livestock reared in the study area were Cattle (75%), small ruminants (15%) and equines (10%) in the lowland areas and 60%, 30% and 10% respectively in the high land areas. The assessment of tsetse and trypanosomosis situation and the current initiative and awareness to other modern control methods in the Abbay basin areas of Dembecha and Jabitehenan districts showed livestock diseases, scarcity of grazing land and watering points and lack of veterinary service are bottleneck challenges for development of the area.

The disease trypanosomosis, locally called as 'Mich /Ghendi' was reported to be the most important livestock constraint limiting the overall agricultural activity and livestock productivity by 90% of the interviewed people. The same result reported by (Tewelde, 2001; Afewerk, 1998) in the western and northwestern parts of Ethiopia where tsetse-transmitted trypanosomosis is the primary problem for livestock productivity and agricultural development. The occurrence of trypanosomosis is throughout the year but major infections are observed after rainy season and after short rainy season. Tewelde (2001), Nagaro and Mwenndia (2000) and Afewerk (1998) reported consistent results; however their results indicated the occurrence of trypanosomosis was in all seasons. Absence of tsetse control activity generally makes the farmers prone and dependable on the use of chemotherapy and above 60% of the respondents admitted using trypanocidal drugs for the last 25 years. Diminazine aceturate and Trypamidium drugs are used and 95% of the respondents are familiar with these drugs. About 65% of animals are treated by the farmers themselves and 55% diminazine aceturate and 45% Trypamidium drugs are used. The dosage required for treatment was not properly known by 55% of the respondents and 25 % didn't have any idea. Similar results were also reported by Tewelde (2001) and Afewerk (1998) about 57% and 43% of the drugs applied by the farmers themselves and other uncertified people. In the same

report 48% and 40% of the treatment were given below the recommended dose while 20% and 40% were did not have any idea.

Above 90% of the treatment was given for clinical cases and 10% for nonclinical cases. Similar results in two areas of Zambia and in the upper Didessa valleys of Ethiopia (Van den Bossche 2000; Tewelde, 2001) showed 85% of the treatment was given for clinical cases. Survey conducted in West Africa (Bauer, 2001) indicated that trypanocidal drugs used greater than 90% of all cases without diagnosis of the exact cause of the disease entity. Pan African Rinderpest Campaign (PARC) report (1998) indicated clinical service delivered in clinics and animal health posts for diseased animals and mobile clinic to remote areas is not practical either financially or sustainable way.

Van den Bossche, *et al.* (2000) indicated that the majority of farmers prefer to use diminazine aceturate than ISMM and most of the time treatment is given for clinical cases not only for trypanosomosis and oxen and cows took priority for treatment. In tsetse infested areas cattle owners administered most trypanocides themselves. The frequency interval of treatment given for each animal was 3.0-4.0 per month in the late rainy season and after short rainy season as indicated by 75% of the interviewed people. Tewelde (2001) reported 2.5–3.5 per month and Afewerk (1998) reported treatment was delivered every 4 months. Uilenberg (1997) reported that the number of treatment over a year reflects the magnitude of trypanosome challenge in an area.

5.2. Entomological survey

Glossina m. submorsitans is the only species of tsetse fly found in the Abbay basin areas of northwest Ethiopia with apparent density of 1.08fly/trap/day in the late rainy season and 0.68fly/trap/day in the dry season during the present research work. Biting flies of tabanids and muscids also caught along with tsetse fly and in areas where tsetse were not found. The apparent density was 6fly/trap/day and 91fly/trap/day in the late rainy season and 0.43fly/trap/day and 7 fly/trap/day in the dry season for tabanids and muscids respectively. The result of tsetse fly survey agrees well with the general knowledge on the ecology of tsetse species found in southwest Ethiopia for the *morsitans* group. Typical habitat pattern were found in the study area for the savannah species *G. m. submorsitans* which prefers for savanna grass, riverine, and forest land. The geographical distribution of *G. m. submorsitans* is

concentrated in the lowland area as climatic conditions are more favourable. Some flies, however, were found as high as 1780 m.a.s.l.

Earlier works by (Krug, 1971; Ford *et al.*, 1976; Langridge, 1976) had established the tsetse geographical limit at 1600 m.a.s.l. and later Tikubet and Gemechu (1984) the upper limit reaches to 1700 m.a.s.l. and NTTICC (1996) reported the limit to be 2000 m.a.s.l. while in the present survey the maximum limit was 1780 m.a.s.l. Survey conducted by ESTC/SRVETEP (2000) in this study area indicated that the upper limit was below 1900 m.a.s.l. This is therefore an indication of the advancement of the fly into higher altitudes.

The apparent density of *G. pallidipes* were 2.4 and 0.6 in the wet and dry season and for *G. fuscipes* 0.1 and 0.06 respectively reported by (Msangi, 1999) in southern rift valley of Ethiopia and the mean fly catches of *G. pallidipes* was 1.42 and *G. fuscipes* was 0.29 at Ghibe valley by (Leak *et al.*, 1993). *G. tachinoides* and *G. m. submorsitans* were detected by Langridge (1976) in the Abbay valley areas and Beles river valleys is also incriminated with these species of tsetse fly. While Tikubet and Gemechu (1984) also reported *G. tachinoides and G. m. submorsitans* in the Abbay and Didessa valleys.

Most of the tsetse were caught in the lowland areas so that the apparent density decreases as altitude increases ($p<0.05$). This findings support earlier works by Langridge (1976), Tikubet and Gemechu (1984) and Leak *et al.* (1999) indicated that climate, which is largely dependent (influenced) by altitude has an impact on tsetse population.

The apparent density of the different flies was significantly higher during the late rainy season. Similar results were reported by Msangi (1999), Mohamed-Ahmed and Dairri (1987) and Leak *et al.* (1987). This could suggest an absolute increase in the number of tsetse flies due to favourable environment such as enough moisture, vegetation growth and suitable habitat or spread of flies from the rivers and thickets where they usually inhabit during the dry season, to more open areas during the rains increases relative density in open areas (Brightwell *et al.*, 1992). Leak *et al.* (1993) also cited the latter as possible reason for the high densities of *G. pallidipes* obtained during dry season when the traps were deployed in the Ghibe river valley.

Sex ratio and age composition of the flies were assessed and higher number of female and the adult flies were recorded during the present study. Similar results have been reported by

Msangi (1999), Mohamed-Ahmed and Dairri (1987) and Allosopp *et al.* (1972). Leak (1999) showed that in unbiased sample female would comprise between 70-80% of the mean population. The only host like bait that gave fair representation of the relative abundance of each sex was traps. The mean age of fly population was 31days in the late rainy season and 26 days in the dry season with wing fray analysis. Wing fray analysis for the estimation of the age of fly population was also supported by Msangi (1999) as compared to ovarian dissection provided the flies are collected frequently less than 24 hours. It is more reliable in Savanna species than riverine species. NGU traps are efficient for savanna species (Leak *et al.*, 1987) but in this case monoconical traps was the best of three trap types used during tsetse fly sampling. *Glossina. m. submorsitans* was detected in western Ethiopia Gullele/Tolly (Leak et al., 1989) and they indicated that biconical traps were not efficient while *G. m. submorsitans* was the only species of tsetse than previously *G. tachinoides* found is that the higher efficiency in the transmission of trypanosomosis and the potential occupation of the savanna wood land in the valleys than other morsitans groups (Jordan, 1986).

Slingenbergh(1992), discussing the invasion of *G. m. submorsitans* in to the upper Didessa valley, cited a USAID report (USAID, 1976) which suggested that the invasion of tsetse began during the 1970s and was responsible for an evacuation of the human population from the Didessa valley at that time. The Didessa and Angar rivers are both tributaries of the Abbay river (Blue Nile). Ford *et al.* (1976) reported that 5902 km^2 of the river basin of the Angar, Didessa and Wama valleys were infested by *G. m. submorsitans* and *G. tachinoides*. *G. m. submorsitans* has a more wide spread habitat than *G. tachinoides* and *G. pallidipes* and also an efficient vector of pathogenic trypanosome to domestic livestock. The advance of *G. m. submorsitans* in the Abbay basin areas of northwest Ethiopia as seen in the present study could have great importance regarding the epidemiology of bovine trypanosomosis and human settlement in study area mainly due to high level of drug resistance observed for ISMM.

5.3. Parasitological survey

The highest prevalence of bovine trypanosomosis was found in the low altitude areas along the river valleys of Bir, Temechan and Abbay compared to the mid altitude areas. The seasonal occurrence of the disease is also consistent with the general knowledge of the vectors of trypanosomosis and hence it was higher during the late rainy season. The most prevalent

trypanosome species in tsetse-infested areas of Ethiopia are *T. congolense* and *T. vivax*. Rowlands *et al.* (1993) reported a prevalence rate of 37% for *T. congolense* in southwest Ethiopia. Abebe and Jobre (1996) reported an infection rate of 58.5% for *T. congolense*, 31.2% for *T. vivax* and 3.5% for *T. brucei* in southwest 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*. Different workers (Afewerk, 1998; Tewelde, 2001; Muturi, 2001) reported a prevalence rate of 17.2%, 21% and 17.5% in Metekel district, in Upper Didessa Valley and Southern Rift Valley areas of tsetse infested regions respectively and the dominant species was *T. congolense*.

Higher challenge of trypanosomosis 5 years before as during that time farmers used labour power and equines for cultivation purposes. But due to the awareness of the control methods such as mass treatment as well as increase in the cultivated land reduced the prevalence of trypanosomosis than reported earlier by ESTC/SRETEP (2000) in the five PAs about 23%. Jemal and Johns (1995) indicated, trypanosome cumulative incidence rate of 11.5-14.1% in southwest Ethiopia. Survey conducted by NTTICC (1998) along the escarpment of the upper Didessa valley revealed 29% prevalence. All these results are in agreement to the present finding.

The prevalence of bovine trypanosomosis in North Omo Zone in the dry and wet season were 14.2% and 21.5% (Muturi, 1999) respectively. The dominant trypanosome species was *T. congolense* (66.1%) followed by *T. vivax* (20.8%). The same trend also reported by Rowlands *et al.* (1995) in southern Rift Valley of Ethiopia. In Ghibe their work indicated *T. congolense* (84%) and *T. vivax* (14%). In the northwestern and southwestern parts of Ethiopia (Afewerk, 1998; Abebe and Jobre, 1996; Tewelde, 2001) reported the dominant species was *T. congolense*.

The predominance of *T. congolense* infection in cattle may be due to the high number of serodemes of *T. congolense* as compared to *T. vivax* and the development of better immune response to *T. vivax* infected animals (Leak *et al.*, 1999; MacLennan, 1970). In the midland areas tsetse apparent density for *G. pallidipes* during the dry and wet season were 0.02 and 0.17 and therefore the proportion of *T. vivax* infection would have been expected to be higher than in the lowland where tsetse apparent density during the dry and wet season were 0.6 and 2.9 (Msangi, 1999). But their observation confirms that animals in the midland area are grazing in the lowland areas during the day time. In addition the result agrees to the known

"vivax-ratio" (Ford, 1976) in cattle which is affected by the species of tsetse to which cattle are exposed. *G. morsitans* and *G. pallidipes* are efficient in the transmission of *T. congolense* than *T. vivax* in East Africa (Langridge, 1976). The present finding was in a harmony that *G. m. submorsitans* the species of tsetse caught might increase the infections due to *T. congolense* and most of the animals in the study areas also graze during the day time in lowland areas. The apparent density was also 1.35 in lowland and 0.66 in the midland areas in the late rainy season, and 0.95 and 0.15 in the dry season respectively.

Higher infection rates were observed in male animals in the present study but the difference was not significant. Similar results reported by different workers (Afewerk, 1998; Muturi, 1999; Tewelde, 2001). Other findings indicated that lactation stress results into higher prevalence than nonlactating cows (Rowlands *et al.*, 1995). The possible suggestion to the present findings would be that male animals are more exposed to draught purposes travel long distances for draught in areas where the tsetse challenge is high and as a result the risk of trypanosomosis also high. Female animals are usually not used for draught and milk production was not the primary objectives of local farmers in the study area.

Age was not found to be a risk factor in the present finding but higher infection rates were observed in adult animals and animals above one years of age in both altitude levels and seasons. This could be associated to the fact that animals travel long distance for feed and draught as well as for harvesting crops to tsetse high challenge areas. Rowlands *et al.* (1995) in Ghibe valley indicated that suckling calves did not go out with their dams but graze at homesteads until weaned off. Young animals are also naturally protected to some extent by maternal antibodies (Fimmen *et al.*, 1982). This could result in low prevalence of trypanosome that was observed in calves. *T. congolense* infection was chronic diseases that increase infection rates with age. *T. congolense* infection is usually higher in adult animals than youngs (McDermott and Coelman, 1999). Rowlands *et al.* (1995) found that cows ≥ 9 years had 1.15x higher trypanocidal treatment than the corresponding values in 3 year old animals. This is a reflection of higher risk of trypanosomosis in adults than as compared to young/calves.

The prevalence of trypanosomosis in low altitude areas (lowland) in late rainy season 19.87% and in the dry season 17.62% have been found significantly higher than the mid altitude areas (midland) 13.39% and 6.54% respectively. Odds ratio indicated the risk of trypanosomosis in the midland areas were 0.7 times lower than lowland areas. Similar findings reported by

Muturi (1999) in North Omo Zone significant difference found between lowland and midland areas. 29.9% and 22.2% in lowland areas in wet and dry seasons while in the midland areas 13% and 5.7% respectively with Odds ratio of 3.3x higher risk in the lowland areas compared to midland areas. This might be attributed to the difference in tsetse apparent density in the two altitude levels. It is a known fact that the risk of trypanosomosis is influenced by tsetse apparent density and infection rates in flies. Riordan (1977) demonstrated that a high tsetse apparent density and infection rates of 50% in tsetse results 42% trypanosome prevalence in cattle exposed to tsetse flies. The present study also reveals that the apparent tsetse density varies significantly between altitude levels and concurrently the risk of trypanosomosis also varies as *G. m. submorsitans* was found higher apparent density in the lowlands.

There was significant difference ($p<0.05$) between sampling seasons that higher prevalence of trypanosomosis found in the late rainy season (17.07%) than dry season (12.35%). The risks of trypanosomosis in cattle were 0.5 times lower in the dry season than in the late rainy season. The concurrent tsetse survey at the same time in the same altitude areas revealed that higher apparent density was caught in the late rainy season than the dry season. Similar results reported by Muturi (1999) in North Omo Zone higher prevalence of trypanosomosis found in the wet season than the dry season. The increase in tsetse apparent density during the wet season have been reported in Ethiopia (Msangi, 1999) in Somalia (Mohamed and Dairri,1987) and Cote d'Ivore, Togo, Gabon and Zaire (Leak *et al.*,1988). The increase in apparent tsetse density led to an increase in trypanosome challenge to cattle in the study area resulted into the observed difference in trypanosome prevalence during the two sampling seasons (late rainy season October 2003 and dry season February 2004).

Trypanosome infection and mean PCV obtained between parasitaemic and aparasitaemic animals had significant difference ($p<0.05$). It was in agreement to the work done in Ghibe, southwest Ethiopia indicated PCV less than 26% required treatment, and for animals treatment was given with positive cases. Rowlands *et al.* (2001) in Ghibe observed in an increase in PCV value, the proportion of positivity decreases and hence mean PCV was a good indicator for the health status of herds in an endemic area. The lower mean PCV value in parasitaemic animals than the aparasitaemic animals is reported by several authors (Leak, 1987; Afewerk, 1998; Muturi, 1999; Tewelde, 2001).

Analysis of variance for the mean PCV of animals in the late rainy season were 24.8% in the lowland area and 26.6% in the midland area while in the dry season the mean PCV values

were 24.5% in lowland areas and 26.8%in the midland areas were significant ($p<0.05$). The mean PCV of parasitaemic animals in the lowland areas in late rainy season was 20.3% and in the midland areas 21.6% while for aparasitaemic animals 25.9% and 27.4% respectively. In the dry season the mean PCV of parasitaemic animals were 21.4% and in the midland 21.2% while for aparasitaemic animals 26.3% and 27.2% respectively. The significant change in the mean PCV values between altitude levels may be attributed to the higher infection rates observed in the lowland areas during the study period. Similar finding reported by Muturi (1999) 24.9% and 25.5% in the dry and wet season in lowland areas respectively and in the midland areas the mean PCV were 27.3% and 27.4% in the dry and wet seasons respectively. The mean PCV of parasitaemic cattle in the same study indicated 16.7% and 18% in the lowland and midland areas to 28% and 28.3% in aparasitaemic cattle.

The PCV profile in herd level analysis indicated that the herd average PCV were dependent on herd prevalence that regression coefficient were negatively correlated. The same result reported by Van den Bossche and Rowlands (2001) that regression analysis of herd average PCV of parasitologieally positive herds decreased with increasing prevalence of trypanosomal infection. The development of anaemia is one of the most typical signs of trypanosomosis caused by *T. congolense* in susceptible cattle breeds (Murray and Dexter, 1988). The level of anaemia or the PCV usually gives a reliable indication of the disease status and productive performance of an infected animal (Trail *et al.*, 1991b). Bovine trypanosomosis control aims at reducing the prevalence of infection with a concomitant increase in the herd average PCV (Bauer *et al.*, 1999).Therefore the knowledge of relationship between prevalence of trypanosome infection and herd average PCV could be a useful tool for the assessment of impact of control intervention. However, the herd average PCV is affected by factors other than trypanosomosis (Conner, 1994). These confounding factors are not always identifiable but they are likely to affect both trypanosomosis positive and negative animals.

Further analysis of PCV values revealed that 88.9% of all parasitaemic animals had a mean PCV below 26% which agrees with the work conducted by (Leak *et al.*, 1993; Rowlands *et al.*, 1995; Abebe and Jobre, 1996; Afewerk, 1998; Muturi, 1999). As anaemia is the classical symptom of the disease pathogenicity (Murray *et al.*, 1977; Seifert, 1996) the low PCV in parasitaemic animals could have contributed in reducing the mean PCV for cattle in the lowland area and midland areas.

The resultant low PCV values in infected animals may not only be trypanosomosis as a sole factor, however the difference in mean PCV between parasitaemic and aparasitaemic animals indicating that trypanosomosis involves in reducing the PCV values in infected animals. Other diseases considered to be affecting the PCV values in animals in the study area are helmenthiasis, Tick borne diseases and nutritional imbalances. On the otherhand most of the parasitaemic animals in the lowland areas were in good body condition despite having low PCV%. This could be attributed to the fact that animals in the low altitude were at high plan of nutrition due availability of sufficient pasture.

Conner (1994) indicated anaemia associated trypanosomosis causes weakness, lethargy and lack of stamina which ultimately reduce efficiency of working animals. The consequence of anaemia is one of the most typical signs trypanosome caused by *T. congolense* in susceptible cattle breeds (Murray and Dexter, 1988; Abebe, 1991). Swallow (2000) indicated that animals in tsetse infested area has lower calving rate, milk yield, calf mortality and more treatment with trypanocidal drugs and trypanosusptible animal can be devastated by sudden exposure to high levels of trypanosome risk.

5.4. Assessment of trypanocidal drug resistance (Isometamidium block treatment study)

In the two villages during the blanket treatment day *T. congolense* was the dominant parasite and similar results were reported in tsetse infested areas particularly in savannah species of tsetse (Tewelde *et al.*, 2004; Afewerk *et al.*, 2000). The mean prevalence of trypanosome infection in ISMM treated group during the 8 week was lower in Ergib than Melkachaba. The strong correlation between overall trypanosomosis prevalence and recurrent infection prevalence in cattle were studied in southwest Ethiopia exposed to a high challenge of drug resistant trypanosomes (Rowlands *et al.*, 2001). The difference in the incidence rate of the control and treatment group of cattle in Ergib may be associated to the total time at risk. Cumulative incidence rate of trypanosome infection in the ISMM treated group of cattle at the two villages was consistent with the findings of the prevalence data.

The 25% survival time analysis showed that 25% of the control group of cattle become infected with trypanosome parasite by day 42 in Ergib while equal proportion of cattle in the control group of cattle in Melkachaba were infected with trypanosome by day 28 of the study period. There was a surprising condition that overlapping of the control and treatment group

of cattle in both villages for the 25% survival time but the confidence interval varies between groups. The confidence interval in the treatment group of cattle at Melkachaba was lower than the control group. The result here indicated that the tsetse challenge was sufficiently high at all villages and a prophylactic regimen is more valuable if trypanosomosis is to be controlled efficiently but the prophylactic effect of ISMM was found to be low. However protective level of the treatment groups of cattle were the same in both villages and the prophylactic way of treatment is useless and other approaches should be found.

Based on Eisler *et al.* (2000), challenge was insufficient to warrant ISMM prophylaxis when fewer than 25% of the sentinel (control) cattle become infected within 8 weeks of exposure. This was justified on the grounds of cost, possible side effects and unnecessary drug pressure tending to develop drug resistance. In both villages more than 25% of the ISMM treated groups of cattle were parasitaemic 27% and 61% in Ergib and Melkachaba in 8 weeks following ISMM block treatment study and this might suggest that there is an indication of resistance of trypanosome due to *T. congolense* against ISMM. Resistance against this drug was strongly suspected when more than 25% of ISMM treated cattle became parasitaemic within 8 weeks of exposure (Eisler *et al.*, 2000). The same results reported by Tewelde *et al.* (2004) in one village of southwest Ethiopia from three villages.

The ratio of the mean hazard rate for both villages was lower than 2 and hence it might be no value to continue using ISMM at the villages. In areas with evidence of drug resistance on the grounds of the ISMM treated cattle becoming infected within 8 weeks of exposure, it may nevertheless be worth continuing prophylaxis in situations where the ratio of the mean hazard rate for the sentinel and prophylaxis herds over 8 weeks is greater than 2 (Eisler *et al.*, 2000).Generally Eisler *et al.* (2000) has suggested that whenever there are indicators of drug resistance, chemotherapy has to be combined with other methods such as vector control and other integrated methods.

The Kaplan-Meier survival curves also in agreement with what we reported so far in the present findings. In general the probability to survive was some what higher in the treated group in Ergib than in Melkachaba but in both villages ISMM treatment had insignificant effect on survival time. ISMM treatment had minimal effect on survival times in high drug resistance situation in coastal areas of Kenya (Eisler *et al.*, 2000) and southwest Ethiopia (Tewelde *et al.*, 2004).

The assessment of the efficacy of diminazine aceturate showed no statistical difference, however, the repeated recurrence infection of trypanosome after treatment with the recommended dosage in both villages contradict the results particularly in Melkachaba where 68.8% of recurrent infections recorded. Therefore, the recurrent infections might not be only due to new infections but also resistant strains might be detected. Hence instead of fully depending on treatment by diminazine aceturate the principle of sanative pairs of treatment should be practiced in both villages. The comparisons of incidence rate and trypanosome infection recurrence rate in southwest Ethiopia showed drug resistant trypanosomes against diminazine aceturate was reported by (Rowlands, 2001) using a long-term study.

The period of ISMM prophylaxis in infected cattle in the field was less than 1 month by Afewerk (1998) northwest Ethiopia. Rowlands *et al.* (1993) from southwest Ethiopia under laboratory condition indicated 1mg/kg bw ISMM was less than 28 days in cattle challenged with clone of *T. congolense* which in therapeutic trials had been shown to be highly resistant to ISMM. The present result is in agreement with previous works in southwest Ethiopia (Scott and Pegram, 1974; Codjia *et al.*, 1993; Tewelde *et al.*, 2004), northwest Ethiopia (Afewerk *et al.*, 2000) and in Southern Rift valley of Ethiopia (Muturi, 1999; Ademe and Abebe., 2001). Scott and Pegram (1974) described the occurrence of Homidium resistant *T. congolense* in Didessa and Angar valley in Wellega province. Their field observation showed that 25% of the treated cattle developed parasitaemia within 30 days of treatment 1mg/kg Homidium. Field observation in Ethiopia based on cloned population indicated that the drug resistant phenotype of *T. congolense* had not altered over a period of 4 years (Mulugeta *et al.*, 1997). Because high level of multiple drug resistance infections appeared to be expressed at the level of individual trypanosome, chemotherapeutic agents only did not control trypanosomosis at Ghibe on a long term basis (Codjia *et al.*, 1993).

Whiteside (1960) indicated that under-dosing, irregular use of prophylactics and discontinuation while at risk time and high incidence of trypanosomosis are the root cause of development of drug resistance. Clausen *et al.* (1992) and Geerts and Holmes (1999) stressed that the prolonged and frequent use of trypanocides in high challenge areas resulting high selection pressure for resistance as well. The epidemiology of drug resistance population of trypanosome is dynamic when the incidence is progressively spread within the population. For instance in Ghibe 7% recurrent infection in 1986 increased to 14% in 1989 (Rowlands *et al.*, 1993). Transmission of resistant trypanosome by tsetse do not change the strains resistant

after passage, the trait stable for long time and spread by cattle movement and or spread of tsetse population (Moloo and Kutuzu, 1990).

The method generates useful information on the efficacy of ISMM and diminazine aceturate to trypanosome populations present in the area and laboratory limitations common. Hence 14-36 weeks prophylactic efficacy of ISMM was not recommendable by this result. However the method usually required long follow up and the interference of farmers intervention on the study animals should be considered. Experimental work in the field and laboratory to monitor development of drug resistance in pathogenic trypanosomes and its impact on livestock productivity and studies on factors which influence the development of resistance to trypanocidal drugs have paramount importance.

6. CONCLUSIONS AND RECOMMENDATIONS

The result of the present research work revealed that trypanosomosis is the most important problem for agricultural activity and animal production in the Abbay basin areas of north west Ethiopia (Dembecha and Jabitehenan weredas of Amhara Region) and the situation is getting worse as the control and prevention of trypanosomosis is facing a challenge due to limitation of vector control activities and the development of drug resistance in the area.

Only one species of tsetse *G. m. submorsitans*, the main vector of pathogenic trypanosome in the savanna areas of Africa and biting flies such as tabanids and muscids were caught. *Glossina. m. submorsitans* advanced as high altitude as 1780 m.a.s.l. posing a risk to areas considered tsetse free by earlier studies. It can be said that the control and sampling of *G. m. submorsitans* can be done by monoconical trap in the area instead of NGU trap which were used in the study area for a pilot survey and control programme before.

The prevalence of bovine trypanosomosis was found to be 17.07% in the late rainy season and 12.35% in the dry season ($p<0.05$). The prevalence was higher in low altitude areas compared to mid altitude ($p<0.05$) in both the seasons. The mean PCV values of parasitaemic and aparasitaemic animals had significant difference ($p<0.05$) and the herd average PCV values were also negatively correlated to the herd prevalence. Eventhough the present study did not include the whole year, altitude and seasons have been found to be important risk factors for the apparent density of vectors and the prevalence of trypanosomosis.

The application of rapid prevalence studies followed by 3 months block treatment study indicated the occurrence of ISMM resistant trypanosomes in cattle under natural field conditions. As a result the three indices; the proportion of animals becomes infected in 8 weeks time (>25%), the hazard ratio (<2) and the 25% survival time (<56 days) were consistent in both villages for the occurrence of resistant trypanosome against ISMM. Log-rank and Wilcoxon (Breslew) statistical tests also showed no significant difference ($p>0.05$) between the treatment and control groups of cattle in both villages. The assessment of diminazine aceturate efficacy showed no significant difference between trypanosome incidence rate and trypanosome recurrence rate, however, 68.8% of the recurrence infections were recorded in Melkachaba village.

Thus the research work gave vital information for the epidemiological picture of trypanosomosis and its vector and occurrence of drug resistance.

Therefore, the following recommendations are forwarded:

1. Designing and implementation of control strategies of trypanosomosis focusing integrated approach (vector control and chemotherapy) should be undertaken in the Abbay basin areas of northwest Ethiopia (Dembecha and Jabitehenan weredas of Amhara Region).

2. Proper and strict follow-up of trypanocidal drugs treatment should be done by professionals and supervision of the field personnel by experts should be practiced. The delivery and distribution of trypanocidal drugs need special attention to avoid misuse.

3. Awareness creation about the disease and control methods as well as the risk of trypanocidal drug resistance is required in the area.

4. Further studies on the tsetse challenge, the economic impact of trypanosomosis and drug resistance have essential roles for the overall control of tsetse transmitted trypanosomosis in the Abbay basin areas of northwest Ethiopia.

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8. ANNEXES

Annexe1. Questionnaire format to interview farmers individually in the Abbay basin areas of northwest Ethiopia.

Region_____ Peasant Association_____

Zone_____ Village_____

Wereda_____ Name_____

I. Livestock management and farming system

1. When did you start farming in the area? Specify

2. What type of farming started at that time?

Livestock and crop production

Livestock production

Crop production

Others

3. Which livestock species are commonly found?

Cattle

Sheep and goat

Equines

Others

4. What are the major types of crop produced in the area?

Teff

Maize

Barely

Pulses

Others

5. What are the main inputs required for agricultural activity?

Livestock

Fertilizers

Land

6. Which livestock species used for agricultural activity?

Cattle

Equines

Others

7. What is the management system of cattle?

Communal and free grazing; live at the out side of the farmers' house in beret system

Private and free grazing; live at the out side of farmers' house

Tether

Stall feed

If the cattle managed in communal and free grazing type, are they in small group or in herds?

8. Where is the grazing and watering point?

Around the locality of farmers

Long distance away from the farmers' residential area

9. Are the watering points perennial or seasonal?

If it is perennial; Rivers and ponds

If it is seasonal; Rivers, ponds and streams

10. In which season does the availability of water and forage scarcity occurs?

After rainy season

During dry season

During the rainy season

11. What are the major types of forages/feeds/season

II. Major constraints of livestock and agricultural activity

1. Feed shortage

Lack of grazing land

Drought

Lack of cultivated land

2. Livestock diseases

2.1. What are the most common livestock diseases affecting your cattle?

2.2. Does trypanosomosis occur in this area?

Yes No I don't know exactly

2.3. What do you call it by local name?

2.4. What is the importance of this disease compared to other diseases?

2.5. Which livestock species is affected most by trypanosomosis?

Cattle Small ruminants Equines

2.6. What are the main clinical signs observed when an animal affected by trypanosomosis?

2.7. In which season does the disease occur commonly?

2.8. Since when did you know the problem of trypanosomosis in this area?

a. Just when you started to live here

b. After 5 years of settlement

c. After 10 years of settlement

d. After 20 years of settlement

e. After 50 years of settlement

f. Before settlement

2.9. What is the status of the disease once you know in this area?

It is getting better

It is getting worse

Nothing is changed

I don't know.

2.10. What is the transmitter (vector) of this disease?

Flies

Ticks

Others

I don't know

2.11. If flies are the transmitter of the disease, which flies do you think and specify it characteristically?

2.12. What is local name of that fly that transmitter of trypanosomosis?

2.13. In which season does this flies are most abundant?

2.14. Where this fly population is very high?

a. In grass land areas

b. In cultivated land

c. In bush land

d. In savanna areas

e. In areas close to river and watering points

2.15. What are the main control measures of trypanosomosis?

Treatment of affected animals

Feeding well of affected animals

Resting the animal from working conditions

Others

2.16. Where are the common drug sources?

Veterinary clinics

Private legal and illegal drug shops

Others

2.17. Who are giving the treatment?

Veterinarians, Animal health assistant, Animal health technicians

Local farmers

The owner himself

Others

2.18. Which drugs are most commonly used in the area? (name, types, color, etc.)

2.18. When did you start treatment of animals with trypanocidal drugs? Specify in years

2.19. When do you treat animals and how many times treatment per year. Specify

2.20. Is treatment effective or not effective?

If it is not effective what is the reason behind that?

2.21. Are there traditional method of treatment and management practices for controlling and prevention of trypanosomosis?

Thank you!

Name of interviewer_____

Signature_____

Date_____

Annexe 2. Wing fray analysis for the estimation of average age of the tsetse population in the late rainy seasòn and in the dry season in the Abbay basin areas of northwest Ethiopia.

Wing fray number	No. of flies for the category	
	Late rainy season	Dry season
1	6x1=6	9x1=9
2	10x2=20	12x2=24
3	12x3=36	28x3=84
4	20x4=80	34x4=136
5	27x5=135	25x5=125
6	35x6=210	10x6=60
Mean wing fray (days)	4.4=31days	3.7=26 days

Key

1=perfect wing with no damage.

6= highly damaged wing with excessive wearing

4.4=was calculated from sum of each category product divided by the sum of actual number of flies, 31days= the equivalent average age of the tsetse population read from tables of wing fray analysis.

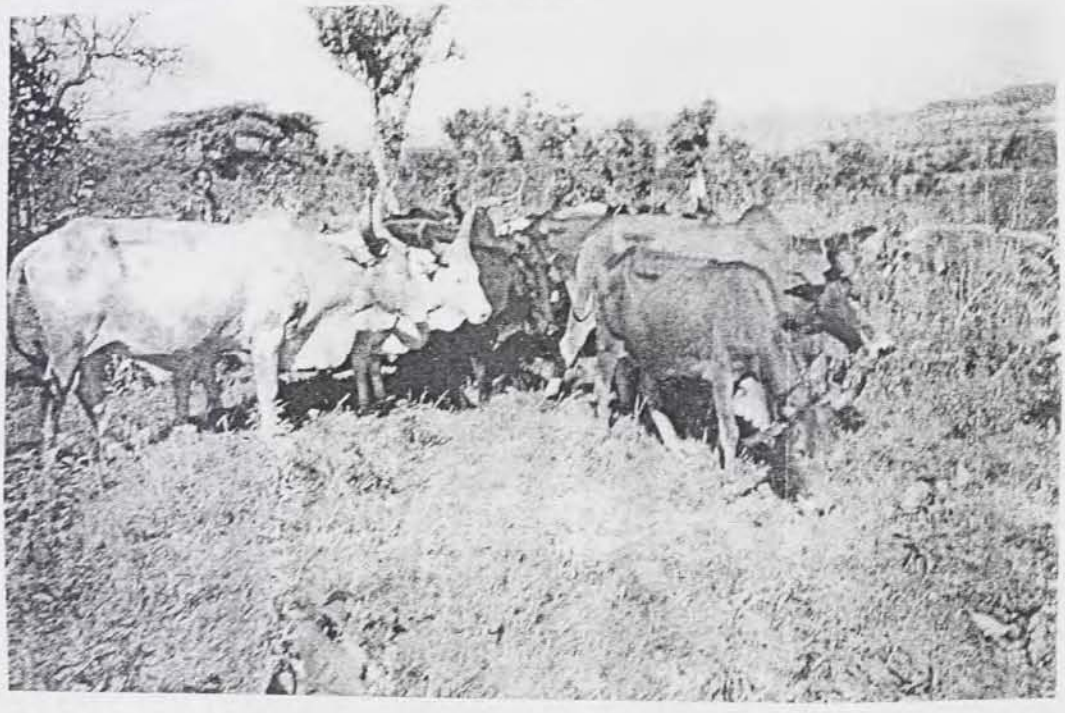
3.7= was calculated from sum of each category product divided by the sum of actual number of flies, 26 days= the equivalent average age of the tsetse population read from tables of wing fray analysis.

Annexe 3. Parasitological results in the ISMM block treatment study from day 0 to day 84: Trypanosome species diagnosed and mean PCV in control and treatment group at each village from 0-84 days.

Day	Village	Group	No. animal	Infection rate (%)	Trypanosome spp. diagnosed		Mean PCV
					T.c	T.v	
0	Ergib	Control	25	12	3	0	25.04
		Treatment	25	4	1	1	26.44
		Total	50	8	4	1	25.74
	Melkachaba	Control	25	8	2	0	25.12
		Treatment	25	12	1	0	23.34
		Total	50	10	3	0	24.23
14	Ergib	Control	25	8	1	1	24.48
		Treatment	23	4.3	1	0	26.25
		Total	48	6.15	2	1	25.36
	Melkachaba	Control	25	12	3	0	26.4
		Treatment	25	20	5	0	24.16
		Total	50	16	8	0	25.25
28	Ergib	Control	25	16	3	1	25.76
		Treatment	25	8	2	0	28.6
		Total	50	12	5	1	27.18
	Melkachaba	Control	25	28	6	1	23
		Treatment	23	39	7	0	23.86
		Total	48	29	13	1	23.43
42	Ergib	Control	25	16	4	0	24.4
		Treatment	23	13	3	0	24.5
		Total	48	14.5	7	0	24.45
	Melkachaba	Control	23	26	6	0	23.66
		Treatment	23	30	7	0	23.18
		Total	46	28	13	0	23.42
56	Ergib	Control	25	16	3	0	25.96
		Treatment	24	8.3	2	1	27.4
		Total	49	12.15	5	0	26.68
	Melkachaba	Control	24	16.6	4	0	23.33
		Treatment	25	20	5	0	24.04
		Total	49	18.3	9	0	23.68
70	Ergib	Control	25	12	3	0	25.44
		Treatment	25	8	2	0	26.35
		Total	50	10	5	0	25.89
	Melkachaba	Control	23	21.7	5	0	24.33
		Treatment	25	20	5	0	22.96
		Total	48	20.85	10	0	23.65
84	Ergib	Control	24	16	4	0	25.81
		Treatment	24	12.5	3	0	25.85
		Total	48	14.25	7	0	25.83
	Melkachaba	Control	23	21.7	5	0	26
		Treatment	25	20	5	0	23.48
		Total	48	20.85	10	0	24.74

Annexe 4. A farmer practicing preparation of trypanocidal drugs for treatment of their animals in Melkachaba village of the Abbay basin areas of northwest Ethiopia.

Annexe 5. A herd of cattle sampled in Melkachaba village of the Abbay basin areas of northwest Ethiopia during the study period



9. CURRICULUM VITAE

1. Personal identification

Name	Shimelis Dagnachew Nigatu
Address	Ebinat wereda, South Gondar Zone
Nationality	Ethiopian
Language	Amharic: Speaking, Reading and Writing
	English: Speaking, Reading and Writing
Sex	Male
Date of birth	March 5, 1975
Place of birth	Neftegna Selassie, Hulte-Eju Enesse, East Gojjam Zone
Marital status	Single
Religion	Orthodox
Profession	Veterinarian
Occupation	Field veterinary practitioner

2. Educational back ground

Year of education	Place
1982- 1986	Tekeldengaye Elementary School, Hulte-Eju Ensesse, East Gojjam (Certificate)
1987-1989	Seddie Junior Secondary School, Hulte-Eju Ensesse, East Gojjam (Certificate)
1990-1993	Motta Seinior Secondary School, Hulte-Eju Ensesse, East Gojjam (ESLCE)
1994-1999	Addis Ababa University, Faculty of Vetrinary Medicine (DVM)
2002-2004	Addis Ababa University, Faculty of Vetrinary Medicine (MSc)

3. Work Experience

Nov.1999- Sep.2002 Veterinary practioner in Ebinat Wereda, South Gondar Zone, Amhara Region.

4. Paper Writing

1. Incidence rate of major postpartum problems and estimation of uterine involution in Welayta Soddo dairy farms (Jersey, Holstein and Cross Breed Cattle). DVM Thesis, AAU, FVM, Debre Zeit, Ethiopia. June, 1999.

2. The Impact of Stress on Hormonal Regulation and Immune Function in Domestic Animals. Seminar on current topics in livestock production and development. AAU, FVM, Debre Zeit, Ethiopia, 1998.

3. Bovine trypanosomosis in the Tekeze basin of Ebinat wereda, northwest Ethiopia with a projected assisted by ORDA, Ebinat-Belssa IFSP, GAA, South Gondar, Amhara Region Bureau of Agriculture, 2000.

4. Bovine trypanosomosis and drug resistance in tsetse infested areas of Africa. Seminar on current topics on basic sciences in MSc course work, 2003.

5. Epidemiology of bovine trypanosomosis in the Abbay basin areas of northwest Ethiopia. MSc thesis, AAU, FVM Debre Zeit, 2004.

5. Membership to the Scientific Associations

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10. SIGNED DECLARATION SHEET

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

Name Shimelis Dagnachew

Signature

Date of submission 02/07/2004

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

1.

2.

Epidemiology of Bovine Trypanosomosis in the Abby Basin Areas of Northwest Eth.

Shimelis Dagnachew