# ADDIS ABABA UNIVERSITY

**FACULTY OF VETERINARY MEDICINE**

# EPIDEMIOLOGICAL INVESTIGATION OF MECHANICALLY TRANSMITTED TRYPANOSOMOSIS (*TRYPANOSOMA VIVAX*) OF DOMESTIC ANIMALS IN THREE DISTRICTS BORDERING LAKE TANA, ETHIOPIA.

**BY**

**ALEKAW SINSHAW TEGEGNE**

**JUNE, 2004**

**DEBRE ZEIT, ETHIOPIA**

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AAU | Addis Ababa University |
| Ab-ELISA | Antibody- Enzyme Linked Immuno Sorbent Assay |
| Ag-ELISA | Antigen-Enzyme Linked Immuno Sorbent Assay |
| ANOVA | Analysis of variance |
| ANRS | Amhara national Regional State |
| ARARI | Amhara agricultural research institute |
| BCS | Body condition score |
| BCT | Buffy coat technique |
| BW | Body weight |
| C.A.B.I | Commonwealth Agricultural Bureau International |
| CATT | Card Agglutination Test for *Trypanosoma* |
| CI | Confidence interval |
| CIREDS | International de recherche-dèveloppement sur l'èlevage en zone sub-humide |
| DIM | Diminazine Aceturate |
| DNA | Deoxy Ribonucleic Acid. |
| DVM | Doctor of Veterinary Medicine |
| FVM | Faculty of Veterinary Medicine |
| HCT | Hematocrit technique |
| IFAT | Indirect Fluorescent Antibody Test |
| IgG | Immunoglobulin G |
| IgM | Immunoglobulin M |
| IICA | Inter-American institute for cooperation on agriculture |
| ILCA | International Livestock Center for Africa |
| ILRAD | International Laboratory for Research of Animal Diseases |
| ILRI | International Livestock Research Institute |
| ISCTRC | International Scientific Council for Trypanosomosis Research and Control |
| ISMM | Isomethamidium Chloride |
| KETRI | Kenyan Trypanosomosis Research Institute |

| | |
|---|---|
| NBM | Non-Biting Muscidae |
| OAU | Organization of African Unity |
| PA | Peasant association |
| PATTEC | Pan African Trypanosomosis and Tsetse Eradication and Control |
| PCR | Polymerase Chain Reaction |
| PCV | Packed Cell Volume |
| PLITM | Prince Leopold Institute of Tropical Medicine |
| SIT | Sterile insect technique |
| SPSS | Statistical package for social sciences |
| STATA | Statistics/data analysis |
| STRC | Scientific and Technical Research Council |
| TA | Tropical Agriculture |

**ACKNOWLEDGEMENTS**

I would like to express my sincere and heart felt appreciation to Prof. Getachew Abebe, my advisor, and for his earnest guidance, supply of literature materials, correction of manuscripts, and suggestion of ideas during the study period and I extend my deepest gratitude to Dr Marc Desquesnes, my advisor at CIREDS, Burkina Faso for accepting fly samples, corrections and sending of materials. My genuine gratefulness goes to Dr. Bayileyegn Molla, Associate Dean for Research and Graduate Studies for his unreserved cooperation and zealous coordination of the course and research programs.

My sincere compliment is to Prof. Feseha Gebreab and Dr Keith Paul of the Donkey Health Project, for their willingness and sending of fly samples to South Africa. I am faithfully owed to Dr Temesgen Alemu of the SIT project for the supply of traps. FVM (AAU), government of the ANRS, ARARI and BOA (ANRS) are worthy of great acknowledgement for the financial input, permission of study and logistics. Here, countless thanks goes to Dr Legesse T/Giorgis, former head of Bahir Dar Regional Veterinary Laboratory, with out his cheerful provision of vehicle, travel to far-away districts would have been impossible. Dr. Gete Zeleke (head, ARARI), Ato Dereje Biruk and Ato Teshome Walle (head and deputy head, BOA), Dr. Bewiket Siraw and Dr. Kassa Teferi (department head and team leader, BOA) are duly praised. Ato Bukayaw Delele (the driver) and Ato Abeje Menberu (laboratory technician) of Bahir Dar regional laboratory and Dr. Anuar Nuru of Fogera agricultural department are highly admired in that they were interested, friendly, and persistent through out the field activity. Thanks also go to Ato Mulat Kabeta, laison officer of FVM and Dr Gedlu Mekonen for their utter cooperation.

I am highly indebted to my devoted friend Menkir Hailu for his diversified support and then concomitantly Yayehyirad Shiferaw for his flattering. The encouragement and backing of Dr Mulualem Eneyew is highly regarded. I also present a considerable credit to my brother Demissie Sinshaw, his wife Emebet Edesa, Bitew Workineh and their family cohort, Etihun Sintayehu for taking care of my daughter while I was away for two years and Kidist, my daughter is esteemed for her serenity. Finally, I am obliged to W/o Senait Gela, executive secretary of the BOA, ANRS, Bahir Dar for the treatment of my family and transfer of my salary on time.

## DEDICATION

This paper is dedicated to my father *Metoaleka* Sinshaw Tegegne and my mother W/o Likina Mengistie, whom in their 45 years of happy marriage life, caressed their children in freedom and still holds close to me as if I am a lad.

**ABSTRACT**

An epidemiological investigation of mechanically transmitted trypanosomosis was undertaken at the three districts (Bahir Dar Zuria, Dembia and Fogera) bordering lake Tana that are located in Amhara National Regional State, Ethiopia. The study was conducted with the objectives of determining the prevalence of trypanosomosis in cattle, small ruminants and equines, identifying biting flies and investigating the presence of drug resistance to Isomethamidium chloride and Diminazine aceturate in the study areas.

The methodology followed was that a total of 1509 cattle, 798 small ruminants and 749 equines were considered for the prevalence study using parasitological methods (buffy-coat method) and the PCV of each animal was measured using hematocrit techniques. A total of 86 traps (66 NGU and 20 Monoconical) were deployed for the purpose of fly survey. At the Fogera district 48 cattle (in the late rainy season) and 19 cattle (in the early dry season) positive for *Trypanosoma vivax* after treatment with Isomethamidium chloride and Diminazine aceturate respectively were followed for 3 months and 24 days, respectively.

The results indicated that the overall prevalence of trypanosomosis in cattle was 6.1% (92/1509). Prevalence was significantly ($t = -3.5$, $P<0.001$.) higher during the late rainy season 9.6% (57/592) than the early dry season 3.6%(22/609) at Fogera district where the two seasons were compared. Prevalence at the district level has significantly varied ($p<0.01$) from 9.6% (57/592) at Fogera district to 4.5%(6/133) at Bahir Dar and 4% (7/175) at Dembia. Prevalence at a peasant association level has significantly ($p<0.01$) varied from 0% (0/54) (Sebatamit, Bahir Dar) to 15.5% (37/239) (Shina, Fogera). Among small ruminants only one sheep 0.8% (1/122) and one goat 0.15% (1/676) were found positive for *Trypanosoma* species and none of the equines were positive. All the trypanosomes encountered in cattle belong to a single species of *T. vivax.* However the *Trypanosoma* species in sheep and goats though it seemed *T. vivax* from the movements of buffy-coat smear, it was not possible to get with in the thin smear preparation made from the buffy-coat which may be due to the very low number of parasites in the blood (single parasites per preparation for each species at the buffy coat). The PCV for each species of animal was with in the normal range available in literature. The PCV of *T. vivax* infected cattle (PCV=21.6, 95%CI=20.9-22.3) was

significantly ($p<0.001$) lower than the negatives (PCV=25.4, 95%CI=25.2-25.5) and PCV values were also significantly and positively associated with body condition score ($p<0.001$), and pregnancy ($p<0.01$), and negatively associated with lactation ($p<0.05$) and parity ($p<0.01$) in cattle. There was also a significant variation in PCV among the three districts ($p<0.01$) and between the two seasons ($p<0.01$) in cattle.

A total of 71,273 flies were caught of which 49,353(69.2%) belong to *Stomoxys*, 15,875(22.3%) to non-biting *Muscidae*, 4,715(6.6%) to horse flies and 1,330(1.9%) to *Chrysops* and there was no tsetse fly. The overall apparent density was 276.3 flies/trap/day. The NGU trap had a significantly ($P<0.05$) high catch of horse flies than the Monoconical where as the Monoconical trap had a significantly high catch of *Stomoxys* ($p<0.0001$) over the NGU trap. However, there was no significant difference in catchments between the two traps for non-biting *Muscidae* (NBM) and *Chrysops* species. Seasonal comparison at Fogera district has revealed that there was a statistically significant variation ($p<0.001$)) of high fly catchments in the late rainy season than the early dry season for each type of traps alone and together for *Stomoxys,* horse flies and *Chrysops* species. However, there was no significant seasonal variation for the non-biting *Muscidae*. Fly species identified include *Atylotus agrestis, Chrysops streptobalia, Stomoxys calcitrans, Stomoxys nigra, S. pulla, S. pallida, S. sataiens, S. taieniata* and *Hippobosca variegata.* A single specimen of the genus *Tabanus* and *Hematopota* were also encountered.

Of cattle subjected to both Isomethamidium chloride and Diminazine aceturate drug sensitivity trial, none of the animals were positive for trypanosoma during the follow up period and at the end of the trail mean PCV has significantly improved by 3.6% for Isomethamidium chloride ($t= -8.48$, $P<0.0001$) treated group (22.7, 95% CI=21.8-23.61 at day zero and 26.3, 95% CI=25.4-27.3 at day 90 post treatment) and 2.6% for Diminazine aceturate ($t = -4.9$, $P < 0.001$) treated group (20.1, 95% CI=19.0-21.2, at day zero and 22.7, 95% CI=21.6-23.7 at day 24 post treatment) respectively.

In general, the present study has indicated that trypanosomosis due to *T. vivax* is relatively important in cattle, and small ruminants would get the infection. Infection with *T .vivax* has negatively affected the PCV of cattle. Various biting flies of veterinary-medical importance are present and tsetse flies were not encountered during the study period. This indicates that *T .vivax* infection in the study area is transmitted mechanically mediated by biting flies. *T. vivax* infection in cattle of the study districts is sensitive to both Isomethamidium chloride (at 1

mg/kg BW dose rate) and Diminazine aceturate (3.5 mg/kg BW) as a prophylactic and normal curative doses respectively. Therefore, a particular attention towards *T. vivax* infection in cattle is essential to control the impact of the disease on productivity. Development of control options that could minimize biting flies especially in seasons of high vector population is another task. Treatment of *T. vivax* positive cattle with Diminazine aceturate would be effective and economical till problems of drug resistance might arise due to Diminazine aceturate. Finally, further studies on biting flies (species behaviors, breeding habitat, host preference, control alternatives, relative importance with regard to *T. vivax* transmission and other relevant aspects) are recommended.

**Keywords**: *Trypanosoma vivax*, biting flies, isomethamidium, diminazine, season, cattle, small ruminants, equines and districts bordering lake Tana

## 1. INTRODUCTION

Trypanosomosis in domestic livestock causes a significant negative impact in food production and economic growth in many parts of the world, particularly in Sub-Saharan Africa (Taylor, 1998) and it has greatly hampered people and animals settlement in a considerable part of the world (Tekle and Abebe, 2001). Trypanosomosis that occurs across more than a third of Africa is arguably the most significant disease (ILRAD, 1994) and therefore remains as the major important constraint to livestock production on the continent. The wide occurrence of this disease in people and their livestock retards agricultural and economic development in Africa and 30% of the continent's cattle population, estimated to be 160 million and comparable numbers of small ruminants are at risk from trypanosomosis (ILRAD, 1994).

Though, the role of mechanical vectors in the transmission of African livestock trypanosomes has always been controversial relative to tsetse flies, their cyclical vectors (Desqesnes and Dia, 2003), it is documented that non-tsetse transmitted trypanosomosis is a potential threat to livestock in many parts of the world (ILRAD, 1991), and the existence of non-cyclical transmission in affecting livestock productivity is available in various literature (Roeder *et al.*, 1984; Stephen, 1986; Urquhart *et al.*, 1987; ILRAD, 1991; Macpherson, 1994; Siefert, 1996; Mare, 1998). There are a large number of cases of trypanosomosis in a variety of domestic animals outside the tsetse belt of Africa as well as in the Americas (Molyneux and Ashford, 1983).

In recent experimental works it was successfully demonstrated that mechanical transmission of *T.vivax* to cattle was effected by African tabanids, *Atylotus agrestis* at the rate of 63% (Desqesnes and Dia, 2003) and that of *Atylotus fuscipes* at a rate of 75% (Foil, Desqesnes and Dia, 2004). Hence, tsetse can transmit *T. vivax* in Africa, consequently the epidemiology of trypanosomosis in cattle is also tabanid dependent and the eradication of tsetse flies will not necessarily lead to eradication of *T. vivax* (Desqesnes and Dia, 2003).

Now a days, in the context of the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), it is expected that more and more areas might be cleared of tsetse. In some areas favorable to mechanical vectors, progressive eradication of tsetse flies will lead to the installation of mechanically transmitted trypanosomosis, in such places, African

veterinary epidemiologists should prepare to observe this new epidemiological aspect of a well-known disease with the right tools and methodology (Desqesnes and Dia, 2004).

Creation of tsetse free zones in Africa will generally lead to the disappearance of *T. congolense*, *T. brucie*, and most often *T. vivax* as well, however, in areas where *T. vivax* can be mechanically transmitted, clearance of tsetse will not be sufficient to eradicate livestock trypanosomosis (Desqesnes and Dia, 2003)

This was previously stressed by Stephen, (1986) that the subject of mechanically transmitted trypanosomosis in animals in Africa and elsewhere is getting more important, but African countries have spent large sums of money and are still spending in the expectation that eradication of tsetse fly would get ride their countries of the scourge of trypanosomosis in their domestic animals and sleeping sickness amongst their peoples. However, Desqesnes and Dia, (2003) indicated that in the mixed situations in Africa, where cyclical and mechanical vectors are found together, it will be difficult to unravel the relative role of both vectors until more refined epidemiological data are obtained for unique situations. PATTEC not only aims at eradication of tsetse flies but also of trypanosomosis, it will then be necessary to consider the role of tabanids and other hematophagous biting insects such as *Stomoxys* for trypanosomosis eradication. Although it is accepted that tsetse are responsible for maintenance of enzootic *T. vivax* in Africa, it is demonstrated that tabanids play a significant role in the transmission of *T. vivax* and could, by themselves, maintain infections in cattle populations

Ethiopia, as part of the African continent shares a substantial loss from the disease. Apart from the cyclical transmission of trypanosomosis by the *Glossina* species, it is highly considered that mechanical transmission is a potential threat to livestock productivity in many parts of the world including Ethiopia (Abebe and Jobre, 1996). However, information on prevalence of non-cyclically transmitted trypanosomosis in domestic animals, the vectors involved and the drug sensitivity of the trypanosome species in Ethiopia is scanty and sufficient data in a compiled form is not available.

In the current Ethiopia, trypanosomosis is one of the most important diseases which contribute to direct and indirect economic losses on livestock productivity and the extent of the disease and the need to control in tsetse free areas (highlands) of the country is strongly emphasized and it is indicated that no attempt whatsoever has so far been made to address the

problem regarding highland (mechanically transmitted) trypanosomosis (Abebe and Jobre, 1996).

In Ethiopia, the presence of mechanically transmitted trypanosomosis outside the documented tsetse belt is previously recognized and the disease in domestic animals is reported in localities far from tsetse area as early as the 1970's (Langridge, 1976; Roeder *et al.*, 1984).

This non-tsetse transmitted animal trypanosomosis that occurs in Ethiopia and the general situation prevailing is found to be serious and of great national concern which requires to be adequately addressed (Lemecha, 1994). For instance, the trypanosomosis of cattle generally known as "NAGANA" (local vernacular Amharic "GENDI") can be found in many provinces where it has greatly hindered development (Langridge, 1976).

In Ethiopia, the prevalence of mechanically transmitted *T. vivax* and *T. evansi* reported by the various workers has indicated their wider distribution in the country and the disease impact due to both parasites is substantial. The presence of mechanical vectors, existence of reservoir hosts, and the involvement of wider host range in parasites, the various agro-climatic zones and the poor veterinary infrastructure would undoubtedly ensure the existence of both *T. vivax* and *T. evansi* in Ethiopia. Therefore, an extensive information about mechanically transmitted trypanosomosis, such as inclusion in research activities and a due consideration in policy-decision making of the control programs in areas where tsetse fly are not present will help to alleviate existing loss of productivity due to trypanosomosis of livestock in Ethiopia.

In the future, considering the increase in under-nutrition and malnutrition, drug resistance to both *T. vivax* and *T. evansi*, and the phenomenon of antigenic variation in trypanosomes, an increase both in magnitude of spread and economic impact of mechanically transmitted *T. vivax* and *T. evansi* is expected to be high. To alleviate existing problems and prevent future productivity losses brought about by mechanically transmitted trypanosomosis; information on parasite distribution, biology and bio-chemical characteristics of both *T. vivax* and *T. evansi*, investigations on the biology, epidemiology and habitats of biting flies in different agro ecological zones, assessing the presence of drug resistance to *T. vivax* and *T. evansi*, particular attention towards *T. vivax* and *T. evansi* infection control in areas where tsetse fly is apparently absent, and economic assessment of the impact of both *T. vivax* and *T. evansi* on livestock productivity are some of the points usually mentioned as a prerequisite.

In the Amhara region, the problem imposed by trypanosomosis in districts especially adjacent to lake Tana and the near by Blue Nile river is considered to be the first disease in limiting cattle productivity. In this region in areas away from the documented tsetse fly belt, some prevalence studies have been conducted on bovine trypanosomosis. But information for other species of domestic animals, the extent of mechanical vectors and possible vectors present (including identification at a species level, their seasonality, abundance, habitat, distribution, etc) and the presence of drug resistance to trypanosomes is not known. Nevertheless, such information is essential to control the disease, vectors, and improve the economic return of livestock in the area. Therefore, this study was undertaken with the objectives of:

1. Investigating the distribution of mechanically transmitted trypanosomosis of domestic animals (cattle, small ruminants, and equines) in three districts adjacent to lake Tana, ANRS, Ethiopia.

2. Assessing the distribution of mechanical vectors (biting flies) and identify at a species level in three districts adjacent to lake Tana, ANRS, Ethiopia.

3. Determining the presence of drug resistance to trypanosomes in the study areas

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# 2. LITERATURE REVIEW

## 2.1. Mechanical transmission of trypanosomes

### 2.1.1. Definition

By definition, in mechanical transmission there is no development or multiplication of the agent in the vector (Foil, 1996). If the trypanosomes are passed directly from animal to animal with out the development cycle in the vector, such transmissions are referred to as “acyclical”. The acyclical transmission occurs when blood-sucking insects have an interrupted feed on an infected host and fly off to resume feeding on another. The parasite can be carried in the blood remaining in the proboscis and is then injected when the next bite is made, thus making a sort of syringe passage from animal to animal. Acyclical transmissions are also called “mechanical” or “direct” transmissions (Langridge, 1976).

This method of dissemination depends on the movement of a fly with *Trypanosoma* contaminated mouthparts to a new host with in a short time, so the parasites in the mouthparts remain infective. Such transmission depends on an interrupted feed, as it is necessary for the fly to continue to probe and feed when it has moved to another host. Thus the shorter the interval between feeds, the greater the chance of transmission (Molyneux and Ashford, 1983). This “mechanical transmission” is difficult to study and there is still little information on it. However, this phenomenon undoubtedly plays a role in the dispersion and growth of disease (Clair, 1988).

Seifert (1996) indicated that acyclically, the pathogen could only be carried over a short distance since it will survive only for a short time in the proboscis of the mechanical vectors and the trypanosomes ingested by such vectors from an infected host survive only 15 minutes inside the hypostome. Therefore, the infection will not be carried over long distances.

Stephen (1986) mentioned “we use the term mechanical transmission of trypanosomes with very little understanding of what actually took place”. It is generally assumed that the infecting trypanosomes originates in the peripheral blood of the infected host and passes by some means over the distance separating the latter from the new host and enters the blood stream of the recipient.

### 2.1.2. Agents or vectors involving in mechanical transmission

The role of mechanical transmission of biting flies other than tsetse is already stressed (ILRAD, 1994). It is explained in Stephen (1986), that there are areas where *Glossina* do not occur and yet the trypanosome succeeds in passing from infected to clean animals.

Trypanostomatidae, infecting animals are transmitted from host to host mainly by hematophagous insects and this mechanical transmission occurs by the hematophagous flies of *Tabanidae* (mainly the genus *Tabanus*), *Culicidae*, *Muscidae*, *Hematopoba*, *Leperosia*, *Stomoxys*, and *Chrysops* (Urquhart *et al.*, 1987; Clair, 1988; Seifert, 1996; Mare, 1998,). Similarly, Itard (1989) explained that the most frequent vectors of mechanical transmission are *Tabanidae,* and *Stomoxynae*. *Hipposcidae* are also said to involve some times.

In Africa, *Tabainidae* and *Hippoboscidae* flies principally transmit *T. vivax*. Where as, the vector of *T. vivax* in the Western hemisphere remains unknown but several species of hematophagous (especially *Tabanid* and *Hippoboscid*) are believed to serve as mechanical vectors. Of all the biting flies, the most important mechanical vectors are flies of the genus *Tabanus* (Molyneux and Ashford, 1983; Mare, 1998).

The transmission of *T. vivax* and *T. evansi* in areas outside the tsetse belt is ensured by *Tabanidae*, *Stomoxynae* and some times *Hipposcidae*. Horse flies (*Tabanus*) are essentially the main vectors of *T. evansi* and *T. vivax* outside of the tsetse belt and the African continent. Since 1930, there has been speculation on the role of *Stomoxys* in transmitting *T. evansi* in Indonesia, India, Mauritius, and other parts of the world (Itard, 1989).

The mechanical transmission of *T .evansi* by tabanids has been extensively studied in different countries since the turn of the century and the evidence incriminating tabanids as

vectors is conclusive. *T .vivax* is the only species of tsetse-transmitted trypanosome that has become permanently established outside of Africa (Foil, 1996).

D'Amicus *et al.* (1996) has studied the vector potential of *Stomoxys*. They supported the vector potential of these flies for *T. vivax* in that stable flies were very abundant at the cattle resting site, there was a close interaction between cattle and stable flies in the study site, and they observed a good correlation between the apparent stable fly densities at the resting site and the frequency of *T. vivax* in the cattle. They suggested that independent of the abundance and ecology of each species, these arthropods are good candidates as vectors of *T. vivax* in the Central African Republic. Except that a significant regression occurred between the monthly estimates of *T. vivax* frequency in cattle and stable fly densities, no such relationship was observed for *T. congolense* and *T. brucie*. Contaminated needles, syringes and surgical instruments are also able to transmit *T. vivax* from one animal to another (Mare, 1998).

In South America and perhaps elsewhere in the world where *T. vivax* exists in the absence of tsetse, the non-cyclical transmission by biting flies, cyclical transmission through as yet unidentified vector and maternal transmission together compensate for the lack of the particularly efficient *Glossinidae* (Gardiner, 1989). In Latin America (in Salvador and Paraguay), Camus (1996) confirmed that among the *Tabanidae* species, *Tabanus importunes, Tabanus nebulosus,* and *Tabanus cryptotylus* are the main vectors of *T. vivax* in the absence of *Glossina* species. Here, *Stomoxys* are also indicated.

It is probable that the part played by hematophagous arthropods in American surra has been overshadowed by the undoubted efficiency of the vampire bat, *Desmodus rotundus* as the vector. Clearly, however there is a need to investigate the significance of biting flies, such as the *Tabanidae*, in the epizootiology of surra in this region, particularly under field conditions. The vampire bats (*Desmodus rotundus*) feeds with equal freedom on equine and bovine animals, and they live for a month or more after they acquire an infection with *T. evansi*. During that time they can pass the organism to a new host in the saliva and they continue to feed until a few hours before death, hence they act as both reservoir and vector of the infection. The evidence is strong that the vampire bat is the main vector of *T. evansi* in areas where it is present. Only the common vampire, *Desmodus rotundus*, of the family *Phyllotomatidae* is of economic importance (Stephen, 1986).

Roeder *et al.* (1984) suggested that mechanical transmission is probably facilitated by abnormally high concentration of stock and their close confinement combined with unusually humid conditions favoring an increase in the hematophagous fly population.

Tabanids have been associated with the transmission of over 35 pathogenic agents of animals and the majority of diseases associated with tabanids are mechanically transmitted and this mechanical transmission is important in the epidemiology of many agents of livestock disease (Foil, 1996).

Regarding the epidemiological factors involving in mechanical transmission (Foil, 1996) reported that the titer of infectious agent, the persistence of the agent, and the infectiousness of the agent at the portal of entry are the major ones. Similarly the number and type of insects feeding on hosts is important. The viremia or parasitemia of the donor most often determines the number and types of vectors required to transfer infection. Information on the quantity of blood remaining on the mouthparts of insects after an interrupted meal also can provide a starting point for evaluating the importance of different insects. Certainly, the number of insects and the quantity of blood transferred between hosts is important. The distance between animals has a significant impact on the percentage of mixed feeding. The larger the tabanid, the greater the potential for transfer between two host animals. Smaller tabanids may move between hosts less frequently than larger flies and individually transport less residual blood meal. However differences in population density may change the relative importance of different sized tabanid or other insect vectors.

Apart from disease transmission, Hollander and Wright, (1980) the blood loss caused by tabanids was estimated at more than 200 ml/animal per day in cattle. Stable flies and tabanids cause weight loss due to blood loss and annoyance as well as create feeding lesion sites which may promote contaminative transmission of agents or myiasis, (Foil, 1996).

An extensive work was done on the different aspects of biting flies (species present, behavior, vector potential, habitat, etc) in many parts of the world. In Africa, different workers reported the presence of different genera or species of biting and non-biting flies (Adeyefa, and Dipeolu, 1986; Amoudi, 1989; Amoudi and Leclercq 1988; Amoudi, and Leclercq, 1992; Amoudi and LeClercq, 1993; Amoudi, and Leclercq, 1996; Bowden, 1976; Burg, *et al.*, 1991; Dia, *et al*, 1997; Acapori *et al.*, 2001; Desqesnes and Dia, 2003; Desqesnes and Dia, 2004). However, in Ethiopia, few authors has mentioned the name of some of the biting flies

at a family or genera level such as *Tabanidae*, *Stomoxys*, *Hematopota*, *Pangonium*, and *Chrysops* and information at a species level and related aspects is not available (Enyew and Abebe 1997; Kidane-Mariam, 2000). How ever, Kigaye, and Jiffar (1991) in a survey of ectoparasites of cattle in Harar and Dire Dawa districts, south eastern part of Ethiopia.has reported the prsesnece of 10 species of "stable flies", 7 "housefly" species, one H*ippobosca. variegata*, 2 tabanid species.

In studies conducted in Mauritania, Guinea (West Africa), Nigeria, Uganda, Burkina Faso, and Turkey various genera and species of *Tabanus, Atylotus, Stomoxynae, Haematobia, and Hippobosca, Haematopota, Chrysops, Haematobosca and Glossina* and several types of non-biting muscidae are reported to present in various composition (Dipeolu, 1975; Iwuala, and Onyeka, 1977; Kangwagye, 1977; Hussein *et al.*, 1991; Dia *et al*., 1998; Kilic, 1999; Acapori *et al.*, 2001; Desqesnes and Dia, 2003).

In studying biting flies, the use of odor attractants developed for tsetse flies was found to be effective by different investigators and so far baiting of traps with 1-octen-3-ol (octenol), acetone, CO2, ammonia, phenols and cow urine baited traps alone or in combination was observed in improving the catch of biting flies than the non-baited traps (Vale, 1982; Tikubet *et. al*., 1988; French and Kline, 1989; Jaenson, *et al.,* 1991; Hribar, *et al.,* 1992; Hayes, *et al*., 1993; Foil and Hribar, 1995; Djiteye, *et al.* 1998; Hall *et al.*, 1998*;* Kristensen, and Sommer, 2000; Ngare and Mwendia, 2001).

Trap design was also important to increase the efficiency of catchments of biting flies. Though there are traps developed for trapping of biting flies, those traps developed for tsetse fly are also helpful in trapping biting flies. This will help especially to rule out the absence of tsetse in preliminary investigations of biting flies other than tsetse. Hence, the NGU trap was better than the Monoconical and bi-conical traps in trapping the horse flies (Amsler and Filledier, 1994a and 1994b; Amsler *et al.* 1994; Foil and Hribar, 1995; Djiteye, *et al*. 1998). For instance, Vale, (1982) demonstrated that traps with larger screens produced relatively small catches of *Stomoxyinae* and non-biting *Muscidae*, where as the small screen produced relatively large catches of these flies.

The seasonal distribution of biting flies is one important factor in assessing their vector potential. In most of the cases, there occurs high abundance of fly population during or immediately following the rainy season (Kangwagye, 1974; Dipeolu, 1975; Bowden, 1976;

Kangwagye; 1977;; Bowden, 1977; Davis and Sanders; 1981; Leprince *et. al*., 1991; McElligott and Galloway, 1991; Gorayeb, 1993; Cilek, *et al.*1994. Dia, *et al*., 1997, McElligott, and Lewis, 1996 ; McElligott, and Lewis, 1998 McElligott, 1998).

Biting flies of the various genera and species were reported to have a specific habitat for multiplication, resting, host seeking and others. Therefore, captures of adult flies will differ in different biotopes like gallery, collar, savanna, forest, bog, fen, natural or artificial ponds, in marshes, in the mud of the banks of irrigation canals, ample water, vegetation, and in pastures (Hafez, *et al.,* 1970; Matthysse, *et al.,* 1974; Dale and Axtell, 1976; Burger*et al.* 1981; Baribeau and Maire, 1983; Lewis, 1987; Lago and Testa, 1990; Dia, *et al*. 1997; Dia, *et al.* 1998; Janzen, and Hunter 1998; Acapori *et al.*2001).

Collection of adult flies is also dependent on larval habitat. Foil, (1996) reported that the larva of tabanids feed on organic debris &/or small invertebrates in a variety of aquatic to semi aquatic habitats. However, stable fly larvae develop in manure-spilled feed and decaying vegetation. Cattle manure on cattle feed lots is an important medium for stable fly larval development.

### 2.1.3 Trypanosome species transmitted mechanically

The existence of mechanical transmission of both *T. vivax* and *T. evansi* by biting flies is documented in various literatures (ILRAD, 1991; ILRAD, 1994; Camus, 1996; Enyew and Abebe, 1997). *T .vivax* is transmitted from herd to herd by infected cattle or sheep, and is transmitted within the herd by biting insects (Desquesnes *et al*., 1996).

Since, *T. vivax* can readily be transmitted mechanically by biting flies other than tsetse (Nyindo, 1992), and it has established itself in the Caribbean, South America, and Central America where the tsetse flies are absent (ILRAD, 1991; Mare, 1998). Similarly, *T. evansi* is widely distributed in livestock in Africa and Asia (Urquhart *et al.,* 1987; ILRAD, 1991) in the apparent absence of tsetse flies.

The transmission of *T. evansi* in an area depends upon the presence of mammalian hosts harboring *T. evansi* in their blood, and some means of transferring the parasite from infected to clean susceptible animals (Stephen, 1986).

## 2.2. *Trypanosoma* (Duttonella) *vivax*

### 2.2.1. Morphology of *T. vivax*

*T.vivax* was named because of the vigor of its activity under the microscope when examined in fresh preparations. The parasite moves across the field of view. The trypanosomes are 18-31μm in total length (a free flagellum of 3-5 μm with a body length of around 15-26 μm). It is characterized by a large kinetoplast usually situated close to the posterior end (Molyneux and Ashford, 1983).

### 2.2.2. Geographic distribution and host range

*T. vivax* is geographically the most extensive pathogenic trypanosome (with the exception of *T. evansi*). *T. vivax* has been found over an enormous area of Africa with livestock movements and mechanical transmission extending its range outside the tsetse belts (Gardiner and Wilson, 1987). *T. vivax* was thought to have been imported into South America with infected cattle from Senegal in West Africa in the 19$^{th}$ century. The propensity of *T. vivax* for mechanical transmission by biting flies other than tsetse and the exportation of livestock have carried this trypanosome to Mauritius, to Central and South America and perhaps to Indonesia (Gardiner, 1989).

The movement of African cattle to the Caribbean, South America and Mauritius resulted in the dissemination of *T. vivax* where it has persisted in local cattle populations, except in Mauritius where it has been eradicated. Biochemical characterization of mechanically transmitted *T. vivax* should provide interesting information about their origins. However, the problems of the general insusceptibility of laboratory animals to *T. vivax* remains serious (Molyneux and Ashford, 1983).

The hosts of *T. vivax* include cattle, water buffalo, sheep, goats, camels, horses and donkeys. (Stephen, 1986; Urquhart *et al.*, 1987; Gardiner, 1989; Nyindo, 1992).

2.2.3. Disease due to *T. vivax*.

*Trypanosoma* (Duttonella) *vivax* is a major cause of the mortality and morbidity due to trypanosomosis of livestock in West Africa and contributes to the toll that trypanosomosis takes of animal health and productivity in East Africa (Gardiner, 1989). In East Africa, chronic *T. vivax* infections are commonly encountered, but in West Africa, a high parasitemia occurs.The distribution of *T. vivax* has also extended to South America (Nyindo, 1992).

However, there is little comfort to be taken from the often repeated statement that in West Africa *T. vivax* causes an acute form of bovine trypanosomiasis, while in East Africa, the disease may be regarded as a chronic infection (Stephen, 1986) and in Roeder *et al.* (1984), it is concluded that *T. vivax* is capable of causing acute disease in East Africa (Ethiopia) and the characteristic pathological hemorrhagic syndrome of *T. vivax* (Gardiner, 1989) is also a common occurrence. Though, the distribution of *T. vivax* has extended to South America (Nyindo, 1992), it is interesting to note that despite this widespread distribution of the infection, out breaks of severe disease is sporadic (Gardiner, 1989).

*T. vivax* is predominantly a parasite of cattle, sheep, goats, camels, horses, and water buffalo (Bubalus bubalis) can all be infected and may suffer from the disease. However, stocks that are pathogenic in some ruminants may be only poorly infective for others (Gardiner, 1989).

One to two weeks following infection with *T. vivax*, animals that are susceptible to the disease develop intermittent fever and anemia and cattle deteriorate for months before dying (*ILRAD*, 1994). The parasitemia in most infections due to *T. vivax* in domestic animals rises to a peak and then quite rapidly drops to either low levels, or to a level at which no parasites can be found in the peripheral blood. During the early stages of infection the peaks are often very high and the parasitemia can be referred to as massive and the interval between the peaks is a matter of a few days only. As the infection progresses, the peaks tend to become lower and the interval increases. Chronic infection with *T. vivax* may be said to be the normal state of affairs in east Africa and steady declines in parasitemia will be observed (Stephen, 1986;

Nyindo, 1992). Though self-cure is relatively common in *T. vivax* infections, it is unlikely that it results in sterile immunity. The cause of this self-cure may be augmented by the tendency of this parasite to run through its (smaller?) repertoire of variant antigens (Gardiner, 1989).

The pathology of *T. vivax* includes anemia, hemorrhagic syndrome and the effect of infection upon fertility. Mortality and weight loss result directly from bovine trypanosomiasis, but effects on fertility, which are generally more difficult to measure, in to the productivity equation and extend the economic losses experienced by those who attempt to raise cattle in the trypanosomosis belts of Africa and South America (Gardiner, 1989).In Africa, the disease in which *T. vivax* gives rise in bovines is variously described as "nagana" or "soumna" and in South America as "Secadera" (Gardiner, 1989).

In West Africa "nagana" due to *T. vivax* is considered to be economically the most important form of trypanosomosis in cattle. Cattle inoculated with *T. vivax* may develop the peracute, acute, chronic or cryptic types of disease. Inoculated animals develop pyrexia and become lethargic and weak. Anemia is a common feature particularly in chronic infections in which severe emaciation also occurs (Murray et al., 1983; Nyindo, 1992).

Following infection with some strains of *T. vivax*, death may occur in two weeks. The extremely acute disease, produced by *T. vivax* resembles a septicemic condition. The animals are febrile, show sustained high levels of parasitemia and often exhibit massive hemorrhage, particularly into the gastrointestinal tract (Murray *et al.,* 1983). The prepatent period in zebu cattle ranges from 8-17 days. In Eastern and some Central areas of Africa infections of *T. vivax* in cattle are commonly encountered but they are usually regarded as being relatively mild in nature compared with those due to *T. congolense*. In West Africa, *T. vivax* is very wide spread and the infection in cattle usually runs a rapidly fatal course (Stephen, 1986). Many of the animals that survive remain unproductive and the growth of young animals is often stunted. Adult animals may show decreased fertility. In addition, pregnant cows sometimes abort and even when calves are born at full term, they are often small and weak so that neonatal mortality is high. In milking cows, a drop in output is often the first indication of infection (Murray et al., 1983).

Taylor (1998) indicated that anemia persists during the chronic stages of infection when parasitemia is generally quite low, probably because different mechanisms are involved in its genesis during the acute and chronic stages of infection. This suggests that control of

parasitemia and control of anemia is unrelated in the chronic phase when immunity due to infections is depressed and anemia is sustained through dyserythropoiesis.

A variation in mean PCV due to physiological status or productivity in ruminants with respect to *T. vivax* infection was recorded by different workers at the international livestock center for Africa and international livestock research for animal diseases (D' Ieteren et. al. 1988; Defly *et al.* 1988; Mulatu et. al. 1988, Malo *et. al.* 1988; Ordner *et. al.,* 1988).

Cattle and especially sheep infected with *T.vivax* have variable clinical manifestations. Depending on the management of the farm, clinical signs may vary from nil to significant loss of weight and condition and even abortion in ewes. If the management level of a farm is satisfactory, clinical signs may be absent and the infection inapparent even if the trypanosome is circulating. Sheep and cattle can act as reservoirs of *T.vivax* for several years (Desquesnes *et al*., 1996).

Sheep and goats are susceptible to inoculation with *T. vivax* and acute to chronic forms of the disease have been described. Affected animals develop progressive anemia (Nyindo, 1992). In goats (Soulsby, 1982), both acute and chronic forms of the disease may occur. Emaciation and splenomegaly are reported and causes substantial mortality in goats and sheep in Africa. In an experimental infection of *T. vivax* to goats, Van Dam et al. (1997), has observed the development of intermittent fever and anemia, a reduction in gross energy and metabolizable energy intake in infected goats than the controls. Here, PCV has gradually decreased in all infected animals with time after infection to an average 17% in week four-post infection (control animals has a mean PCV of 38%). All infected animals showed parasites in the blood, but parasitemia followed an irregular course towards the end of the infection period some animals had undetectable parasite levels.

Stephen (1986) has documented that goats and Abyssinian sheep were susceptible to *T. vivax* often dying in twelve days. In these species most infections end fatally but wide variation occurs particularly in sheep. In tsetse infested part of southwestern Ethiopia, 27/533 (5.1%) small ruminants were positive for trypanosoma. Of which, only four in sheep and three in goats were due to *T. vivax* infections (Dinka, and Abebe, in press).

Horses, donkeys and mules are susceptible to inoculation with *T. vivax*. Animals become anemic and progressively become emaciated. Edema of the legs, scrotum, lower abdomen and

the subcutaneous tissue is a common finding (Soulsby, 1982; Nyindo, 1992). When *T. vivax* was sub-inoculated in to horses and donkeys, the infection was more chronic, resulting in death in a year and five months respectively. Camels are also susceptible (Stephen, 1986). Dogs, cats, monkeys, pigs, rats, mice, guinea pigs, and rabbits are refractory to infection (Stephen, 1986; Urquhart *et al*., 1987; Nyindo, 1992).

After an experimental infection of *T. vivax* to goats, gross and microscopic examination has revealed the presence of hyperplasia and a plasma cellular reaction of lymph nodes and hyperplasia of the spleen in all of the infected animals. Mononuclear infiltration of kidneys, brain and heart was also observed (Van Dam *et al*., 1997).

Gardiner (1989), has reviewed that immunosuppression has been regularly demonstrated in infections of ruminants. Stressing previously infected but aparasitemic animals, appears to allow recrudescence of parasitaemia. Parasite control mechanisms are obviously involved in the self-cure phenomenon, which may be augmented by the tendency of this parasite to run through its (smaller?) repertoire of variant antigens. At present there are no correlates of host preference, virulence or the pathological consequences of the infection host animals with particular *T. vivax*.

### 2.2.4. Status of *T. vivax* in Ethiopia

In Ethiopia, *T. vivax* is one of the commonest trypanosome species and has been found in all provinces (Langridge, 1976). This trypanosome species is most prevalent that infect cattle in tsetse free areas (Abebe and Jobre, 1996). When sample were taken from livestock that were not in contact with tsetse, *T. vivax* was the dominant trypanosome species encountered (Table 1). Similarly, Bekele (1980) indicated that when “Nagana” occurs in places far away from tsetse, the non-cyclical trypanosomes are predominantly *T. vivax*.

TABLE 1. PREVALENCE OF *T.VIVAX* AND *T. CONGOLENSE* IN RELATION TO THE DISTANCE FROM TSETSE BELT IN ETHIOPIA

| Trypanosoma species | Tsetse infested (n=1,398) | Tsetse free (n=6,711) | Source |
|---|---|---|---|
| *Trypanosoma congolense* | 60 | 14 | Langridge (1976) |
| *Trypanosoma vivax* | 31 | 85 | |
| Mixed and other infections | 9 | 1 | |

The effect of *T. vivax* infection on packed cell volume (Table 2) and body condition score of cattle of the Ethiopian highlands have been reported by various workers (Hassen, 1988; Enyew and Abebe, 1997; Aklilu, 2002).

TABLE 2. PCV OF PARASITIC AND NON-PARASITIC CATTLE INFECED WITH *T. VIVAX*.

| Parasitemic | Non-parasitemic | Source |
|---|---|---|
| 22.1 | 26.1 | Getinet, 1994 |
| 22.0 | 26.2 | Mihiret, 1995 |
| < 20 (60 %) | - | Abebe and Jobre, 1996 |
| 21.7 | 26.4 | Enyew and Abebe, 1997 |
| 21.2 | 25.2 | Terefe and Abebe, 1999 |
| 22.8 | 27.8 | Aklilu, 2002 |

In Ethiopia, the work of Abebe and Jobre (1996) indicated that although the hemorrhagic syndrome was not observed, the degree of anemia caused by infection of *T. vivax* in the highland indigenous zebu cattle was found to be severe. Here, the results revealed that about 60% of *T. vivax* infected cattle in the highland showed anemia below a PCV of 20% compared to 50% of *T. congolense* and *T. vivax* infected zebu cattle in the lowland.

These findings of *T. vivax* from the tsetse free areas highlights the importance of infection. Except for the few cases where *T. congolense* infections are detected, the scenario is almost 100 % dominated by *T. vivax.* Interestingly, trypanosomosis or "Ghendi" is not regarded as an important disease in most parts of the highlands of Ethiopia. However, upon routine blood

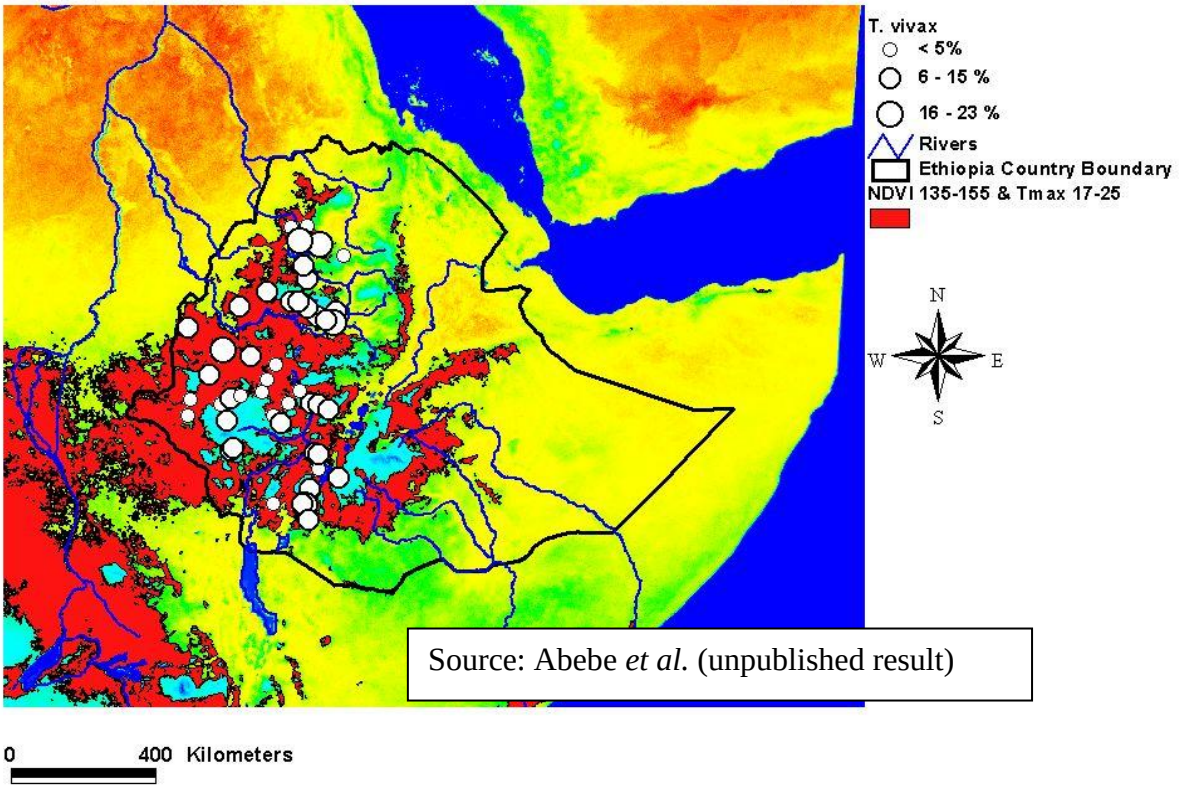
examination, close to 10% of the cattle herd was found to harbor *T. vivax* infection (Table 3 and Figure 1). The ability of *T. vivax* to adapt to ways of mechanical transmission has enabled to establish itself in the vast highland plateaus of Ethiopia (Abebe and Jobre, 1996).Following the work of Langridge (1976) and reports of Roeder *et al.* (1984), other different reports on mechanically transmitted trypanosomosis caused by *T. vivax* in the apparent absence of tsetse fly in Ethiopia came from various workers (Table 3).

TABLE 3. PREVALENCE OF *T. VIVAX* IN AREAS FAR FROM THE TSETSE BELT IN ETHIOPIA

| Location | Region | Sample size | % Prevalence | Source |
|---|---|---|---|---|
| Shoa | Amhara | 100 | 14* | Langrige, 1976 |
| Melka-Sedi | Afar | 1200 | 6* | Roeder *et al.*, 1984 |
| Gondar | Amhara | 1276 | 4.46 | Hassen, 1988 |
| Gojjam | Amhara | 1040 | 13.27 | Terefe and Abebe, 1999 |
| Gojjam | Amhara | 1148 | 9.4 | Getinet, 1994 |
| Bahir Dar | Amhara | 739 | 16.1 | Mihiret, 1995 |
| Gojjam and Gondar | Amhara | 423 | 8.71 | Abebe and Jobre, 1996 |
| Gonder | Amhara | 3194 | 12.62 | Enyew and Abebe, 1997 |
| Gojjam | Amhara | 3288 | 2 | Cherinet, 1999 |
| Gojjam | Amhara | 1040 | 13.27 | Terefe and Abebe, 1999 |
| Tigrai | Tigrai | 604 | 6.29 | Aklilu, 2002 |

*Reported in the form of outbreak

Figure 1. The Distribution of *Trypanosoma vivax* in Ethiopia




Source: Abebe *et al.* (unpublished result)

2.2.5. Status of *T. evansi* as compared to *T. vivax* in Ethiopia

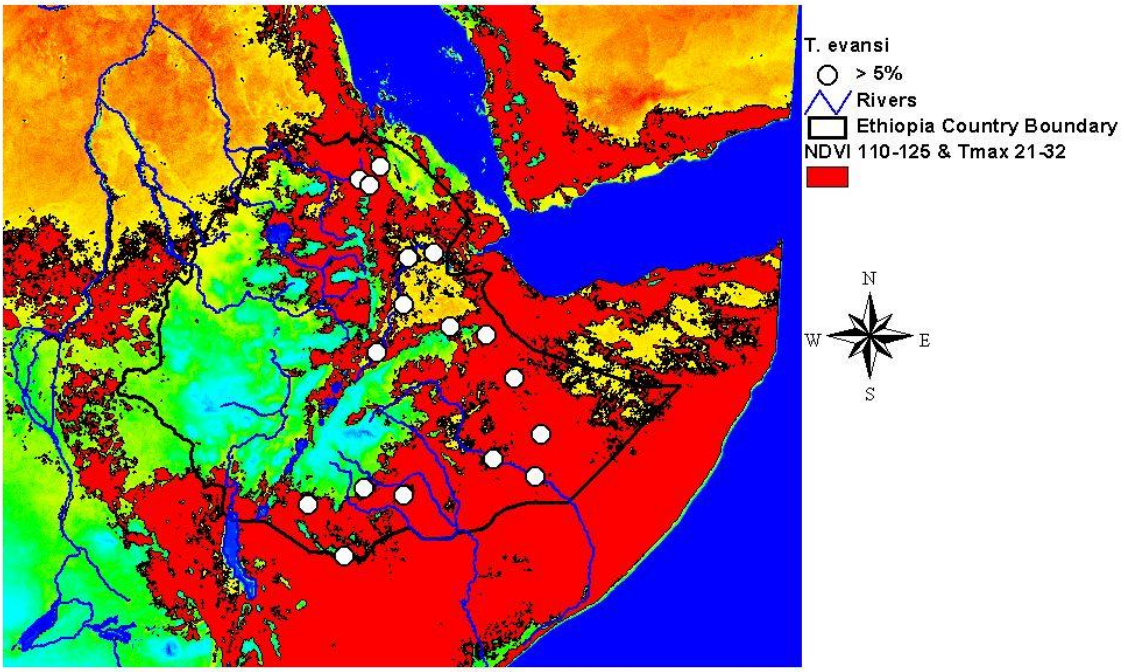
*T. evansi* causing "surra" in camels is common in the southern and eastern regions of the country (table 4). In Ethiopia, the distribution of *T. evansi* coincides with the distribution of camels in the semi-desert environment through out the eastern part of the country (figure 2). This trypanosome also occurs in the dry country of the North West near the Sudan border (Langridge, 1976). In Southern Ethiopia (Borena), the disease caused by *T. evansi* is well known to the breeders by the local name "Dhukane" and is given the first priority in its order of importance among camel diseases (Demeke, 1998; Tekle and Abebe, 2001).

TABLE 4. PREVALENCE OF *T. EVANSI* BY PARASITOLOGICAL METHOD IN ETHIOPIA.

| Location | Region | Sample size | Prevalence (%) | Source of information |
|---|---|---|---|---|
| Issa | Afar | 327 | 0.3 | Tefera, 1985 |
| Ogaden | Somali | 321 | 6.5 | Wossene, 1988 |
| Borena | Oromia | 1100 | 21.5 | Meskelu, 1990 |
| Yavello | Oromia | 294 | 31.9 | Lakew, 1993 |
| Somali | Somali | 336 | 7.7 | Issa, 1998 |
| Borena | Oromia | 609<br>324 | 6.7<br>56.5* | Demeke, 1998 |
| DireDawa | Dire Dawa | 228 | 12 | Mohammed, 1999 |
| Tigrai | Tigrai | 280 | 5 | Hailu, 2000 |
| Borena | Oromia | 391 | 10.9 | Tekle and Abebe, 2001 |

* Ab-ELISA

Figure 2. The Distribution of *Trypanosoma evansi* in Ethiopia




Source: Abebe *et al.* (unpublished result)

### 2.3. Diagnosis of trypanosomosis due to *T. vivax* and *T. evansi*

The development of anemia is the most reliable indicator of the progress of a trypanosome infection and it is simply and reliably estimated by measuring the packed cell volume percent (Murray *et al.,* 1983). The incidence, prevalence, species and sequence of appearance of different species of trypanosomes can be evaluated by examination of blood by a variety of parasitological techniques, which include blood films (thin, thick and wet), dark ground or phase contrast buffy coat method, hematocrit centrifugation technique (HCT), inoculation of laboratory animals and hemocytometer for quantification of parasites (Murray *et al.,* 1983).

Now a day, other serological and molecular tests are developed such as the CATT/ *T. evansi* test, Ag-ELISA, Ab-ELISA, IFAT and PCR diagnostic tests (Gardiner, 1989; Camus, 1996; PLITM, 2001). CATT/*T. evansi* is an experimental card agglutination test developed by Prince Leopold Institute for Tropical Medicine that is used for the detection of anti trypanosome antibodies in blood, serum or plasma of infected animals. The test is performed on a plastic card with 10 reaction zones. The reconstituted antigen suspension is mixed with blood or diluted serum/plasma and rotated for five minutes at 60 to 70 revolutions per minute on a card test rotator (PLITM, 2001). Blue clumping indicates the presence of antibodies in the test sample. The test is not strictly species specific. This may complicate the interpretation of positive results in areas where other species of Salivarian trypanosomes occur (PLITM, 2001). Till now the CATT/*T. evansi* has been evaluated for sero diagnosis of surra in dromedary camels in Mali, Mauritania, Niger, and the Canary Islands and for water buffaloes in Vietnam. For screening serum or plasma samples of dromedary camels, a sample dilution of 1:4 to 1:8 is indicated while for water buffaloes 1:8 dilution is used (PLITM, 2001).

After results of experimental infection Olaho-Mukani *et al.* (1996), suggested that levels of IgM and IgG class-specific antibodies may be a suitable indicator of the exposure status of goats to *T. evansi* because their findings indicate that, the former fall rapidly after treatment or cure, while the latter may persist for long periods.

Since many animals harboring *T. evansi* may have parasites in their peripheral blood only sporadically and in relatively small numbers, a single or even several examinations may fail to reveal the parasite in thin or wet blood films. Therefore, no single technique meets all

requirements and that where possible a combination of methods including mouse inoculation should be used. Nevertheless, tick and thin blood films should always be made (Murray *et al.*, 1983; Stephen, 1986).

Similarly, a failure to detect *T. vivax* infections in blood films, particularly in the long-standing cases where there is a low parasitemia has been known for a long time. If infections are diagnosed by the blood film technique alone, about 27% of the cases will be missed (Langridge, 1976). Parasitological methods (blood film, buffy coat technique) and serological methods (IFAT and ELISA) are the commonly employed diagnostic techniques (Murray *et al.* 1983, Gardiner, 1989). The PCR diagnostic tool is also in application recently (Solano *et. al.*, 2001). The Latin American isolate of *T. vivax* is diagnosed using indirect immunoflorescence antibody test, and ELISA, diagnostic methods (Camus, 1996).

Efficient diagnostic methods should be used to determine the prevalence of *T. vivax* in Africa, in South America and the Caribbean where DNA probes could be employed to evaluate better the contribution, if any, of ticks and other biting insects to the transmission of *T. vivax*. It is probable that new DNA probes will have to be prepared to ensure the identification of all the members of this geographically dispersed species of protozoan parasite (Gardiner, 1989).

TABLE 5. PREVALENCE OF *T. VIVAX* BY SEROLOGICAL AND PARASITOLOGICAL METHODS.

| Tests applied | Animals Positive for *T. vivax* | | | Source |
|---|---|---|---|---|
| | Cattle | Sheep | Goats | |
| Parasitological | 2.3% | 1.2% | 0.7% | Anosa *et al*. (1995) |
| Antigen-ELISA | 13.1% | 8.9% | 9.0% | |
| Parasitological | 3.34% | | | Aaron *et al.* (1996) |
| ELISA | 34.3% | | | |
| | | | - | |
| Parasitological | 10% | 5% | | Desquesnes (1997) |
| Indirect-ELISA | 50% | 19% | - | |
| Parasitological | 7.6% | - | - | |
| Indirect-ELISA | 22% | - | - | |
| Parasitological | 4.2% | | | Solano *et al*. (2001) |
| PCR | 11.5% | | | |

A similar different result for *T. evansi* in camels has been reported in Ethiopia that 56.5 % and 6.5% of the samples were positive using Ab-ELISA and parasitological techniques respectively (Demeke, 1998). Dia *et al.* (1997) has applied the CATT, IFAT and Ag-ELISA tests and seropositivity rates were 16.5% with CATT, 24.3% with IFAT and 14.0% with Ag-ELISA. Anosa *et al.*(1995) reported a ratio of 7.5:1, 6.0:1 and 12.5:1 in diagnosis of trypanosomes using Ag-ELISA and parasitological methods in cattle, sheep, and goats respectively. Similarly in the Central African Republic, Amics *et. al.* (1996), reported that the frequency of *T. vivax* ranged from 0 to 14.5% using parasitological methods and from 8.2 to 19.4% using ELISA

### 2.4. Treatment and Control of Mechanically Transmitted Trypanosomes.

In case of mechanically transmitted trypanosomes, which are maintained under natural conditions by the presence of the parasite in the blood of susceptible animals, and by the presence of hematophagous arthropods in the vicinity of infected animals, control should be directed towards elimination of trypanosomes from the blood of animals or elimination of the vectors from the environment (Luckins, 2000). In case of *T. evansi*, treatment with trypanocidal drugs is the usual method of control (Luckins, 2000) and most of the usual trypanocides have been used for treating this parasite. However, the type of drug to be used is dependent on the species to be cured and the levels of drug resistance in the region (Molyneux and Ashford, 1983).

Either of the four compounds namely Suramin, Diminazine aceturate, Isometamidium chloride and Quinapyramine have been used to treat camels, cattle, buffalo, horses and pigs for many years (Luckins, 2000). Particularly, Quinapyramine and Diminazine aceturate (Berenil) in bovines and Isometamidium chloride (Samorin) in dogs are effective against *T. evansi* (Molyneux and Ashford, 1983). Drug resistance is known to occur amongst *T. evansi* isolates and there have been reports of its occurrence in several different countries in Africa and Asia (Luckins, 2000). In Sudan, from a place named Kassala (near the west border of Ethiopia), an isolate of *T. evansi Kassala/4* stock was found to be resistant to the curative action of Suramin even at the maximum tolerated dose of Suramin for mice (Abebe *et al.*, 1983), which was attributed to an extensive and repeated use of Suramin in that area. A similar problem may be expected in the adjacent Metema areas of Ethiopia.

In treating animals positive for *T. vivax,* Homidium chloride (Novidium), Homidium bromide (Ethidium), Isomethamidium chloride (Samorin or Trypamidium), Quinapyramine dimethylsulphate (Trypacide sulphate), Quinapyramine dimethylsulphate:chloride 3:2 w/w (Trypacide prosalt) , and Diminazine aceturate (Berenil) are the various drugs so far used in different countries (Gardiner, 1989). Drug resistant *T. vivax* that were transmitted mechanically was reported by Desquesnes *et al*. (1995).

In the control of both *T. vivax* and *T. evansi*, that are transmitted acyclically, elimination of the source of infection; that is the infected animal that are in contact with the uninfected ones and between which transmission is made directly. It is not practicable to control the

transmitters as the population of biting flies is extremely numerous, widely distributed, and difficult to deal with (Langridge, 1976). In case of *T. evansi*, control is limited to treating those animals that are considered infected on the basis of clinical signs, or treatment of animals showing patent infection (Luckins, 2000). The need to control trypanosomosis in tsetse free zones is already emphasized (Abebe and Jobre, 1996) stressing the disease as a potential treat to the huge highland livestock population of Ethiopia. The authors presumed that a regular blood examination and systematic treatment of infected animals on the highland Ethiopia would undoubtedly minimize the risk of trypanosomosis due to *T. vivax*.

Control of biting flies is also an alternate and the options proposed by various people to limit the impact of biting flies are application of chemicals like malathion or Sumithion [fenitrothion] to stable walls or if necessary on the animals and managing the grazing periods for stock at peak period of biting activity of the flies (Bhasker, and Joseph, 1986; Heine; 1987) listed sprays, ear tags and pour-on formulations as the methods and permethrin, cyhalothrin, cypermethrin, fenvalerate, tetrachlorvinphos, flucythrinate, cyfluthrin and deltamethrin as the insecticides.

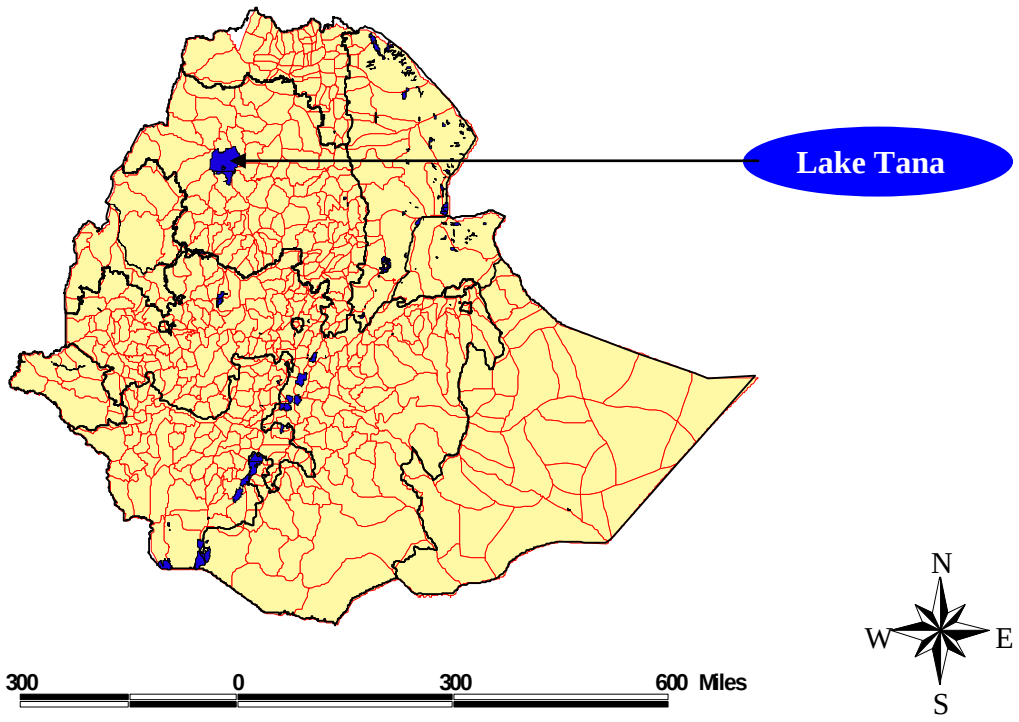
Legner, (1995) has also indicated that the manipulation of natural enemies through introduction and/or augmentation has in some cases provided satisfactory control. Management of larval habitats by sanitation is the key to stable fly control. Treatment of animals with residual insects can aid in control and thorough application to the lower body parts is important. Modified traps, either as treated targets or with solar powered electrocution grids, can be effective in reducing stable fly populations with proper use (Foil, 1996).

# 3. MATERIALS AND METHODS

## 3.1. Study Area

The study was undertaken in three discrete districts bordering lake Tana, namely Bahir Dar Zuria, Fogera, and Dembia; located in West Gojjam and South Gonder and North Gonder administrative Zones of the ANRS respectively, Ethiopia. The study districts are densely populated and most of the land is intensively cultivated and in the rainy season, particularly in the so-called Fogera plain, most of the cultivable land will be water logged.

Figure 3. Map of Ethiopia.



The Amhara National Regional State (ANRS) is found in the northwest part of Ethiopia. Geographically the region is located between north latitude 9°20' and 14°20' and East longitude 30°20' and 40°20'.

Figure 4. Map of the Amhara National Regional State

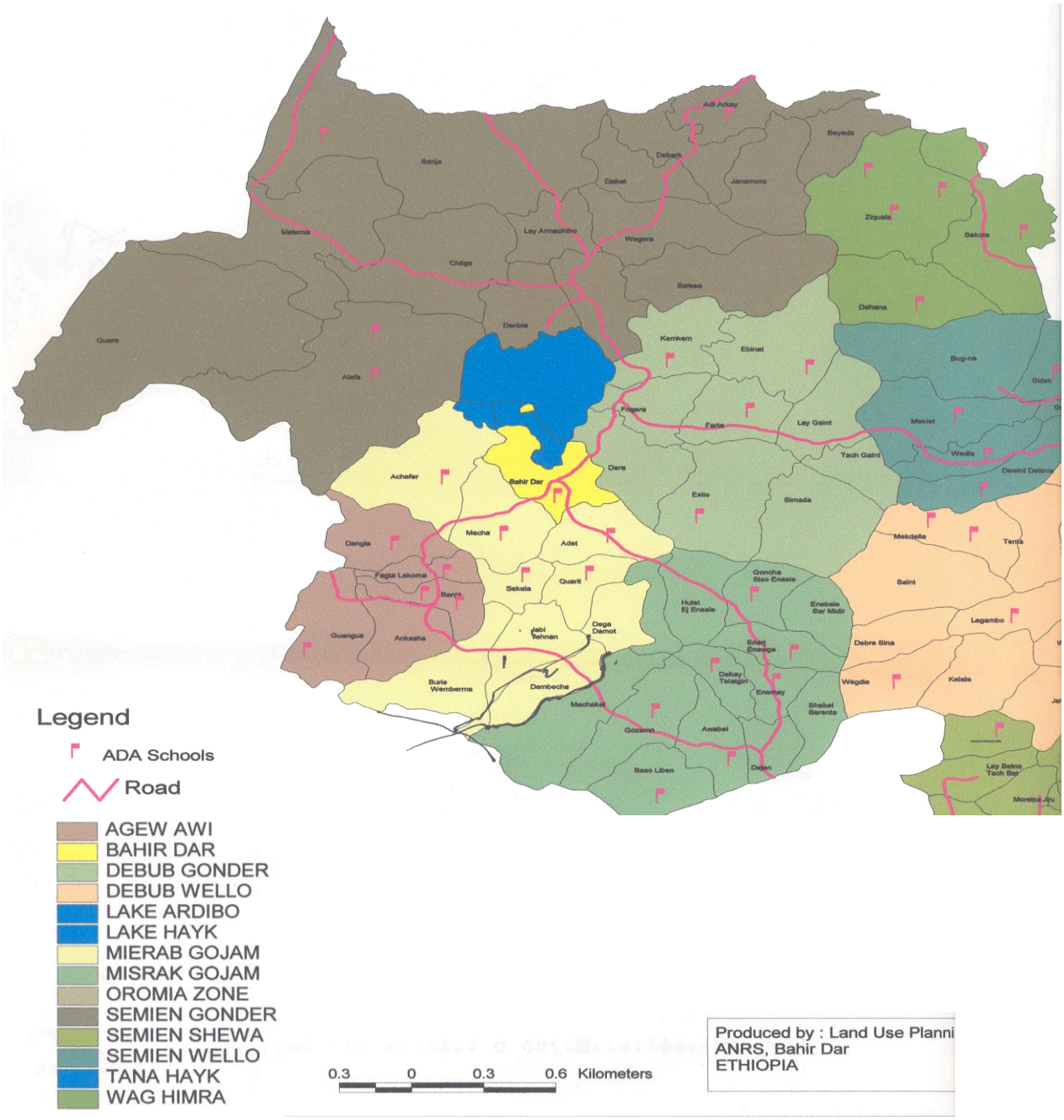
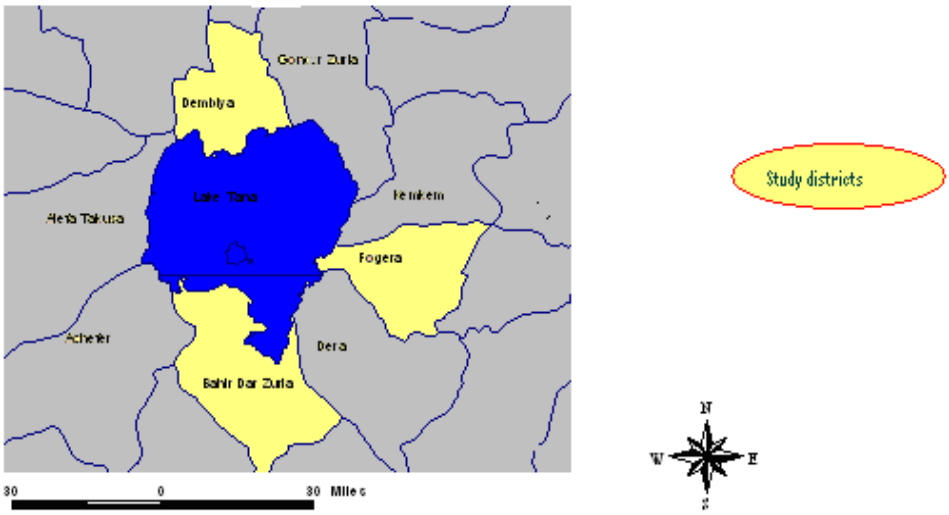


Figure 5. Map of the study districts bordering lake Tana




The region shares 25% of the total area of the country which is estimated to be 170052 sq km. Amhrara Region is bounded with Tigray Region to the north, Afar Region to the east, Oromiya Region to the south, Benishangual Gumiz Region and Sudan to the west.

ANRS is the land of diverse topography with altitude ranging form 500 at Metebia to 4620 meters above sea level at Ras Deshen. Based on altitude it is divided in to three traditional agro-ecologies zones ‘Kolla’, ‘Woinadega’ and ‘Dega’ representing 31%, 44% and 25% respectively

The livestock population of the Amhara region comprises about 10.6 million cattle, 5.7 million sheep, 4 million goats, 2.1 million equines and 17.4 thousand camels. Smallholder mixed farming dominates (80%) and livestock is an integral part of the farming system.

Out of the total area about 28.16% is under cultivation, 29.98% under grazing and browsing, 2.12% covered with forest, 12.58% covered by bush and shrubs, 16.11% not utilized, 7.26% settlement and construction site, and 3.82% belong to water bodies (BOA, 2000).

The specific study districts are located along the side of lake Tana with an altitude ranging from 1600-2200 meters above sea level. Most parts of the study districts are plain. The area has poor drainages and there occurs an annual over flooding during the rainy season leaving pockets of water bodies and swampy ditches that would stay up to the early dry period. The mean annual rainfall of the study areas ranges from 1000-1600mm and the mean annual ambient temperature varies from 15-20 $^{0}$C.

The soil type in most parts of the low plain is a heavy dark brown clay type that has a high capacity of water retention. At Fogera district, irrigated agriculture using big rivers that are flowing to join lake Tana, (namely, Rib and Gumara) is increasing recently and most of the waterlogged areas have recently shifted to rice production, especially in the Fogera plain lands that were formerly left uncultivated during most of the wet seasons.

Cattle are the major livestock kept and one of Ethiopia's cattle breed/type known by the name of Fogera cattle is by far raised in this districts. Small ruminants, especially goats are raised in drier parts of the districts that are relatively far from the plain. Donkeys together with few mules in towns are the dominant equine populations that are present around the lake where as horses are concentrated far away to the highland districts.

### 3.2. Study animals

Cattle, small ruminants and equines were used as study animals. Among the domestic animals, cattle are the dominant species raised and the Fogera cattle breed/type is concentrated in the study districts. Though small ruminants are present within the districts boundary, their population is minimal in those localities that are very adjacent to the lake (Table 6). Among the equines, donkeys are relatively abundant as we approach the lake, mules are concentrated in towns and horses are localized away from lake in areas with higher altitudes.

TABLE 6. LIVESTOCK POPULATION OF THE STUDY DISTRICTS.

| District | Cattle | Sheep | Goats | Equine | Poultry | Total |
|---|---|---|---|---|---|---|
| Fogera | 137,030 | 2,939 | 18,856 | 9,557 | 95,373 | 263,755 |
| Dembia | 152,567 | 16,792 | 38,461 | 13,597 | 361,562 | 582,979 |
| Bahir Dar Zuria | 158,564 | 8,078 | 11,749 | 7,118 | 366,606 | 552,115 |
| Total | 448,161 | 27,809 | 69,066 | 30,272 | 823,541 | 139,8849 |

*Source:BOA(2003)

### 3.3. Study design, sample collection and identification

The study was based on a questioner, cross-sectional and longitudinal type of investigations:

#### 3.3.1. Questioner survey

Field investigations were carried out by initially interviewing farmers in groups in a village and individually with the aid of a questioner about disease status, herd structure, usage of trypanocidal drugs, and physiological status. Both open-ended and closed type questionnaires were applied to interview individual farmers or in groups. Both the questioner types were tested in the field before administering to the study population. Then, the questioner was administered to group of farmers and individual cattle owners. A questioner survey format was used to collect information related to trypanosomosis and those relevant to data analysis. Data has included the physiological status of the animal, body condition score, and other relevant information. (Annexes 2, 3, 4, 5, 6 and 7).

#### 3.3.2. Cross-sectional study

##### 3.3.2.1. Site selection and sample size determination

A combination of stratified, multistage and purposive sampling methods were applied according to Toma *et al*. (1999) and Putt *et al* (1988). First the three discrete study districts were selected from three zones of the ANRS (first stage) to represent areas bordering lake Tana. Then a list of PA's within districts were compiled from a data obtained in the district's agricultural office (second stage) and sampling PA's were selected based on representation of the respective districts and accessibility. Villages were selected in collaboration with the respective district's animal health personnel, selected by purposive sampling on the basis of prior information on the problem, farmer's co-operation, logistics, share of communal grazing land and accessibility (third stage). Selected villages and herds grazing within the same grazing land were considered as strata. With in each stratum, sampling was performed irrespective of the other strata.

Then representative numbers of animals (considering sex, and age) were sampled. Animal sampling was performed initially based on the willingness of the owners. Therefore, the system of herding all the animals in one communal grazing area, the sceptic attitude of farmer's towards blood sampling and the low small ruminant population in adjacent areas of the lake were some of the reasons that had interfered with the full application of probability sampling methods.

Representative numbers of animals were sampled from each village. Parameters like age, sex, body conditions score and reproductive status (parity, lactation, pregnancy, and abortion) were recorded for each individual animal. Individual animals having an age of greater than six months were considered for blood sampling to categorize the data in a defined age group as those below one year (<1 year) from those1-4 years and from those above four years of age (> 4 years).

To determine the sample size, a trypanosomosis prevalence rate of 10% (the average prevalence for cattle in West Gojjam and South and North Gonder regions) was taken into consideration. Hence, for cattle, 900 samples (10% expected prevalence, 95% confidence level and 2% precision) were taken. However in case of small ruminants and equines since there was no information available about prevalence estimates for these species, 20% prevalence rate was used to estimate the sample size. Therefore, a sample size of 711 small ruminants, (20% expected prevalence, 95% confidence level and 3% precision) and 711 equines (20% expected prevalence, 95% confidence level and 3% precision) were assumed respectively (Putt *et al.,* 1987 and Toma *et al.,* 1999).

Therefore, a total number of 2322 domestic animals were required for the study. How ever, a total of 1509 cattle (at Fogera 592 samples in the late rainy season and 609 in early wet season, 125 at Dembia and 133 at Bahir Dar Zuria), 798 small ruminants and 749 equines were practically considered. In case of cattle the comparison of two seasons was considered (late rainy and early dry) at the Fogera district that makes the total sample size 1509 in cattle. Hence, a total of 3,056 were sampled for this study.

3.3.2.2. Parasitological and hematological examinations.

Cattle, sheep and equines were bled from the peripheral ear vain after cleaning and disinfecting the area using microhematocrit capillary tubes for the purpose of parasitological examination of trypanosomes.

Buffy coat technique (BCT) in the field: A microhematocrit capillary tube containing 70 µl of blood will be centrifuged for 5 minutes using the Hawksley micro hematocrit centrifuge, the PCV was read and the buffy coat examination done as described in Murray *et al.* (1983), the capillary tubes were cut with a diamond pointed pen 1mm below the buffy coat to include the upper most layer of RBC and 3cm to include the plasma, the contents of the capillary tube will be expressed onto a slide, mixed and covered with a cover slip (22X22 nm) and examine the slide under a microscope using X20 and X40 objective.

Thin blood stained smears (TSS): Polish clean slides with a dry, clean cloth apply a small drop of blood from a microhematocrit capillary tube to the slide, approximately 20 mm from one end; place a spreader (another slide) at an angle of $30^0$ ahead of the drop of blood. Draw it back to make contact with blood, allow the blood to run to each end of the spreader. Spread the blood along the slide in a fairly rapid but smooth motion. If the correct amount of blood is used, the slide should be covered to about 2 cm from each end, dry the slide by waving it in the air, fix for 2-5 minutes in methyl alcohol., flood with Giemsa stain (1:10 solution) for 30-60 minutes, drain and wash of excess stain, wash the backside of the slide and drip dry and examine under a microscope using the oil immersion x100 objective and perform species identification (Murray *et al*., 1983). Using the procedure of this technique, thin buffy-coat smears were prepared from those animals found positive in the buffy coat technique for the purpose of species identification

Estimation of anemia: Anemia is simply and reliably estimated by measuring packed cell volume (Murray *et al.* 1983). Collect blood from the ear vain using a set of two heparinized capillaries (3/4 full), seal one end with cristaseal, place the tubes in a microhematocrit centrifuge with sealed end outer most, load the tubes symmetrically to ensure good balance, screw on the rotary cover, close the centrifuge lid, centrifuge at 12,000 revolutions per minute for five minutes, and read the PCV% using a reader

3.3.2.3. Survey of flies

Trap deployment: From October, 2003 to February, 2004, a total of 86 (63 during late rainy season and 23 during early dry season) standard traps developed for tsetse fly trapping (66 NGU, and 20 Monoconical) (Drees and Jackman, 1998) were deployed in the three districts. All the traps were baited uniformly with octenol (1-oct-3-nel) and acetone. The pole of traps were greased to prevent fly predators mainly ants. Traps were allowed to stay at the site of deployment for a period of 72 hours before collection.

Trap deployment sites were selected to represent all habitats that could be related to fly multiplication, behavior, feeding, and other related aspects. Hence grazing lands, cattle barns, swampy areas, bushy areas, riverbanks, watering points and animal congregation sites were purposely included and extent of such habitats were recorded represented by numbers to be transformed in to percentage values expressing the level of the selected site of deployement later for analysis.

Fly preservation and identification: After 72 hours of deployment, the catchments of each trap was sorted by fly's tribe, and then counted. The cover of a new matchbox will be labeled by district, PA, trap type, date, site description, name of fly tribe, and other information that may be relevant from the aspect of data analysis was included. Then fly samples were put in the matchbox for a further species identification (Murray *et al.*, 1983).

Flies collected from traps and stored in a clean matchbox were identified at a species level at the FVM, AAU and representative samples were sent to South Africa and CIRED (Burkina Faso) for simultaneous identification by entomologists and experts in veterinary parasitology/entomology as recommended by Drees and Jackman (1998). Flies were mounted on a stereomicroscope in the department of parasitology (FVM, AAU) and species identification was done according to Oldroyid (1952, 1954, and 1957). After identification of the samples at a species level, flies were permanently preserved in a 70% ethyl alcohol (Drees and Jackman, 1998).

### 3.3.3. Longitudinal study

#### 3.3.3.1. Selection of cattle

In order to carry out a preliminary study the prophylactic activity of Isomethamidium chloride and therapeutic activity of Diminazine aceturate, 50 and 21 naturally trypanosome-infected cattle respectively were selected from villages during the cross-sectional study in the late rainy season and early dry season respectively.

Though the report of questioner did not gave a clue about the presence of drug resistance in the area and farmers are not aware of the drug resistance problems in trypanosomiasis, retrospective case book assessment and discussion with the animal health personnel has revealed that both ISMM and DIM has been in use with in the last 10 years.

Infection status of animals to be subjected for treatment was selected using the parasitological methods explained and PCV value for each cattle was determined before treatment. Each of the fifty and twenty-one animals was identified by their respective name from the owner because the farmers were not interested in ear tagging method using plastic tags. Parameters like body weight, sex, age, date of treatment and dosages used in each case were recorded.

#### 3.3.3.2. Treatment of cattle

For calculating the treatment dose, the body weights of each of the animals were estimated before treatment in a group (with farmers and animal health personnel) and using body condition scoring (Nicholson and Butterworth, 1986) methods.

Cattle selected for a field trial of drug sensitivity test were injected with Isomethamidium chloride (TRYPAMIDIUM-SAMORIN®, Manufactured by MERIAL-17, rue Bourgelat 69002 LYON-FRANCE, Manuf.date: 25/06/2003, Batch: w391971, Exp: 06/2008,) at a dose rate of 1 mg/kg body weight of deep intramuscular injection as a prophylactic activity and Diminazine aceturate (Norotryp, Norbrook Laboratories Limited, Station works, NEWRY, Co., Down, N.Ireland, U.K., BN: 3443-41, DOM: 10-2003, Exp: Oct-2007) at a dose rate of 3.5 mg/kg body weight of deep intramuscular injection as a curative drug.

3.3.3.3. Monitoring of cattle

Only 48 of the cattle selected for ISMM and 19 for DIM were presented at day zero for treatment. Animals were monitored every 30 days for a period of 90 days post Isomethamidium block treatment and every six day post treatment of Diminazine aceturate for a period of twenty-four days. Except that 1 cow was dead for unknown reasons before our arrival for sampling at day 90 post treatment 47 and 19 of ISMM and DIM injected cattle were present respectively through out the follow-up period. In each sampling date, blood samples were collected from the ear vein and consequently, the PCV of each sample was determined using a Hawksley microhematocrit reader and then parasitemia examinations were performed with the buffy coat method (Murray *et al*. 1983). An in vivo test of trypanocidal drugs in cattle has been previously recommended by Eisler *et al.*(2001)

## 3.4. Data analysis

3.4.1. Data management

Data obtained by means of the questioner, data on individual animals and parasitological examination, and data on entomology was inserted in to MS Excel Spread Sheets Program (Microsoft Corp.) to create a database and transferred to the STATA and SPSS software programs of the computer before analysis.

3.4.2. Statistical analysis:

Descriptive statistics, confidence interval, Student-t test, Pearson's correlation, and ANOVA were used to express results and compare variables. The Intercooled STATA 7 (STATA, 2001) and SPSS (SPSS, 2002) software of the Computer Program were applied for the statistical analysis. The prevalence rate of trypanosome infection was calculated as the number of parasitologically positive animals as examined by the buffy coat method (Murray *et al*. 1983) divided by the total number of animals investigated at that particular time.

Confidence intervals (95%) for the PCV of trypanosome infected and non-infected and among different physiological parameters (lactation, sex, pregnancy) were calculated. ANOVA was used to compare the prevalence rates of trypanosome infections in different districts, peasant associations, and seasons (Intercooled STATA, 2001 and SPSS, 2002). Student t-test was utilized to compare the mean PCV of the parasitic animals with that of the aparasitemic animals and to compare the PCV difference observed at the beginning and end of ISMM and DIM intervention (Intercooled STATA, 2001 and SPSS, 2002).

For the data on fly survey since the number of flies caught varied widely, the data was transformed to a logarithmic scale using the transformation $y=\ln(x+1)$ before the statistical analysis. Then student t-test was used to compare the difference of fly catch between the NGU and Monoconical traps and between the two seasons. ANOVA was applied to compare the mean fly catch difference among the different trap deployment sites.

# 4. RESULTS

## 4.1. Results of questionnaire survey

Results on group interview about trypanosomosis, vectors, drug usage, grazing systems, watering point distance, herd structure, and other relevant information has indicated that the disease trypanosomosis is the first priority in the list that has limited cattle productivity in all the three study districts. All the respondents' in-group has agreed that the disease problem is expanding from time to time. In all the districts cattle rearing is the most important among domestic animals. In all the area cattle are raised for the purpose of plowing, manure production, sale of live animal, as a gift for dowry, milk and butter sale and as a social prestige.

Pertaining to the livestock management, free grazing and stall feeding (especially during seasons of least feed availability) are mostly practiced and the grazing system is communal. Cattle are kept in herd. In seasons of least feed availability, farmers will save their crop by-products like tef straw, maize straw, rice straw, and hay to practice stall-feeding. Feed is least available in the dry season when most of the grazing land is overgrazed and mid-rainy season where the grazing land is watery and muddy. Better feed is available during the late rainy season and early rainy season when the pasture is green and the feed biomass relatively high. Animals are allowed to drink; whichever the water source may be and varied from river, stream, pond, and well. The distance of watering point varies from adjacent to the house to 5 kilometers away from the house.

The most common livestock diseases mentioned by farmers in the three study districts are trypanosomosis (*Gendi, Gechita*), fascioliasis (*berer, yegubet til*), shcistosomiasis (*yewiha til*), septicemia (*Gerefita, mich*), helmenthiasis (*yehod til*), anthrax (*abba senga, haile mot, kuriba*), pasteurellosis (*enk*), blackleg (*abba gorba*), lumpy skin disease (*yezihon beshita*), foot and mouth disease (*maz*), bloat (*hod nifat*), colic (*hod kurtet*) and diarrhea (*gechita*).

All the group interviews have agreed that trypanosomosis is present in their village. Among the seven group interviews performed 4 (57.1 %), 1 (14.3%) and 2 (28.6%) ranked

trypanosomosis to be the first, second and third disease affecting cattle productivity of the study districts respectively. Among the domestic livestock species only cattle are affected by trypanosomosis. However 1/7 (14.3%) group respond that donkeys are also affected.The symptoms of trypanosomosis in animals mentioned by farmers are lachrymation, diarrhea, listlessness of the hair, inappetance, emaciation, coughing, depression, constipation, grinding of the tooth, and normal appetite. But recalling symptoms varied among the groups. The season that animals were seen sick of trypanosomosis is at the late rainy and early dry seasons (August to January) and 5/7 (71.4 %) of the groups claimed the months of September to December as the peak disease time.

Five out of seven (71.4 %) of the groups knew that flies are transmitters of trypanosomosis and 2/7 (28.6%) of the groups do not know the role of flies as a vector. Flies specifically claimed as a vector includes the horse flies (*awira zimb, ewir zimb*), *Stomoxysis* (*woyina zimb*) and *Hippobosca* (*litif zimb, lefata zimb, chara zimb*) that are said to be abundant in the months of September and October. End of August and November are also included in 2/7 (28.6%) of the groups. The groups respond that fly population is high around ponds 6/7(85.7%), close to rivers 4/7 (57.1%), in the grazing scheme 3/7 (42.9%), and around the barn 2/7 (28.6%).

Veterinary clinics are the only treatment sources for trypanosomosis and only veterinary personnel's perform treatment of sick animals. Diminazine aceturate (Berenil) and Homidium chloride (Novidium) are the only drugs mentioned by respondents as a trypanocidal, expressed as oil and blood colored respectively, and all the groups think that Homidium chloride is more effective than Diminazine aceturate. However, the respondents were unable to tell the usual dosage of both drugs and they do not practice any traditional treatment against trypanosomosis. Five out of seven (71.4%) of the respondents claimed that the problem of trypanosomosis is expanding from time to time and 2/7 (28.6%) of the groups agreed that there is no difference of expansion in time. In all the villages, the respondents mentioned that only sick animals are taken to veterinary clinics.

Questionnaire result of farmers conducted with 90 individuals has revealed that the livestock management; grazing, watering and herding practices and veterinary practices (clinical services, drug types and quantity used to treat trypanosomosis) are similar to the results of the group interview. But, 60/90 (66.7%) of farmers respond that they follow both free grazing and stall-feeding and 30/90 (33.3%)stick only to the free grazing system.The average cattle

possessed by a single household are 6.4 that vary from 1 (minimum) to 22 (maximum). Only 38.9% (35/90) possessed 1 donkey, 22.2% (20/90), 2 donkeys, 15.5% (14/90) or 3 donkeys 1.1% (1/90). Only 9/90 (10%) of the respondents are raising small ruminants 8.9%(8/90) sheep, 1.1% (1/90) goats).However this data on interview of farmers at an individual level was restricted to the Fogera district where the drug trail was conducted.

The mean cost of treatment per individual animal, treatment frequency of an animal sick of trypanosomosis per year, the mean annual expense incurred for trypanocidal drugs at a house hold level, taking animals for treatment of trypanocidal drug in the year and cattle dead due to trypanosomosis since last year are presented (Table 7). None of the respondents have a trypanocidal drug in their stock.There was a positive correlation ($r=0.4$, $p<0.01$) between the number of cattle at a household level and expense of trypanocidals per year.

TABLE 7. INTERVIEW RESULTS OF INDIVI DUAL FARMERS

| Interview (points of focus) | No.of Respondents | Mean | 95% CI |
|---|---|---|---|
| No of cattle per house hold | 90 | 6.7 | 5.8-7.5 |
| No of sheep per house hold | 90 | 0.4 | 0.1-0.8 |
| No of equine per house hold | 90 | 0.6 | 0.4-0.7 |
| Birr per animal per dose of trypanocidals | 90 | 10.1 | 9.4-10.8 |
| Cost of trypanocidals in birr per year per house hold | 90 | 32.3 | 27.1-37.4 |
| Frequency of treatment of cattle per year per house hold | 90 | 1.5 | 1.3-1.6 |
| Months between treatments of single cattle | 32 | 5.1 | 4.7-5.6 |
| Death of cattle due to trypanosomosis per household | 90 | 0.6 | 0.4-0.9 |

Though their was no indication about the existence of drug resistance to trypanosomes by the farmers, a retrospective case book investigation has revealed that Isomethamidium chloride, Diminazine aceturate, and Homidium chloride were treated in different years for one decade at Fogera district, simultaneously or alone. Hence, from 1993-94, only Homidium chloride was available, from 1995-96 both Homidium chloride and Isomethamidium chloride were present almost equally, from 1997-99 the only drug available was Isomethamidium chloride, then from 2000 till now, Diminazine aceturate is the only trypanocidal present in the clinic.

Therefore, Isomethamidium chloride and Diminazine aceturate were available for 5 and 4.5 years respectively. In this decade a giemsa-stained blood smear result indicates that *T. vivax* is the only species documented in the case books and it was only recorded in cattle. However, retrospective information about trypanosomosis and usage of trypanocidal drug before 10 years is not available in support of suspecting the presence of any drug resistance in the area.

### 4.2. Results of the cross sectional study

#### 4.2.1. Results of the prevalence study

Out of a total of 3056 animals (1509 cattle, 798 small ruminants and 749 equines) sampled results indicated that the overall prevalence in cattle was 6.1% (92/1509). Prevalence was significantly (t =-3.5, P<0.001.) higher during the late rainy season 9.6% (57/592) than the early dry season3.6% (22/609) at Fogera district where the two seasons were compared. Prevalence at district level has significantly (P<0.005) varied from 9.6% (57/592) at Fogera district to 4.5%(6/133) at Bahir Dar and 4% (7/175) at Dembia (Table 8) and prevalence at a PA level has varied significantly (P<0.01) from 0% (0/54) (Sebatamit, Bahir Dar) to 15.5% (37/239) (Shina, Fogera) (Table, 9).

TABLE 8. PREVALENCE OF TRYPANOSOMOSIS (*T. VIVAX*) IN CATTLE OF THE THREE DISTRICTS.

| Districts | Season | Number of cattle | |
|---|---|---|---|
| | | Examined | Infected |
| Fogera | Early wet season | 592 | 57 (9.6%) |
| | Late dry season | 609 | 22 (3.6%) |
| Dembia | Early wet season | 175 | 7 (4%) |
| Bahir Dar Zuria | Early wet season | 133 | 6 (4.5%) |
| Total | Both seasons | 1509 | 92 (6.1%) |

In cattle all the trypanosomes encountered belong to a single species of *Trypanosoma vivax.* However the trypanosoma species in sheep and goats though it seemed *T. vivax* from the buffy-coat movements, it was not possible to get in giemsa-stained buffy-coat smear due to the very low number of parasites in the blood (single parasites per field). In cattle, the variation in prevalence of *T. vivax* with regard to age and sex was not statistically significant ($p>0.05$).

TABLE 9. PREVALENCE OF TRYPANOSOMOSIS IN CATTLE OF THE PEASANT ASSOCIATIONS

| District | PA | No Sampled | Prevalence | 95%CI | Species |
|---|---|---|---|---|---|
| Fogera | Abuana kokit | 209[a] | 15 (7.2%) | 3.6-10 | *T.vivax* |
| | | 205[b] | 5 (2.4%) | 0.3-4.5 | *T.vivax* |
| | | 414[*] | 20 (4.8%) | | *T.vivax* |
| | Quahar | 144 [a] | 5 (3.5%) | 0.4-6.4 | *T.vivax* |
| | | 190 [b] | 8 (4.2%) | 1.3-7.0 | *T.vivax* |
| | | 334[*] | 14 (4.2%) | | *T.vivax* |
| | Shina | 239 [a] | 37 (15.5%) | 1.2-20.1 | *T.vivax* |
| | | 214 [b] | 9 (4.2%) | 1.5-6.9 | *T.vivax* |
| | | 453[*] | 46 (10.2) | | *T.vivax* |
| Dembia | Sankisa | 50 | 1 (2%) | -.0.2- 6 | *T.vivax* |
| | Tezeba | 50 | 1 (2%) | -.02-6 | *T.vivax* |
| | Guramba | 75 | 5 (6.7%) | 0.8-12.4 | *T.vivax* |
| Bahir Dar Zuria | Sebatamit | 54 | 0 (0%) | 0 | *T.vivax* |
| | Woramit | 73 | 6 (7.6%) | 1.6-13.5 | *T.vivax* |
| Total | | 1509 | 92 (6.1%) | 4.9-7.3 | |

There was a significant variation in prevalence among PA's ($P<0.01$)

[a] late rainy season, [b] early dry season, [*] Total for the PA

Of 798 small ruminants (122 sheep and 676 goats), only one sheep 1/122(0.82%) and 1 goat, 1/676(0.15%), were found positive for trypanosoma. However, due to the very low parasitemia (only one trypanosoma per preparation of the BCT), it was not able to identify at a species level due to negative result of the giemsa stained blood smear preparation.

None of the equines (608 donkeys and 141 mules) were positive for trypanosoma.

4.2.2. Results of the PCV values and productive parameters

The PCV of cattle was significantly ($t = 8.7781$, $P<0.001$) affected by *T.vivax* infection and it was 21.6 % (95% CI=20.9-22.3) and 25.4% (95% CI=20.9-22.3) in *T.vivax* positive and negative animals respectively. The difference between the two groups is 3.8%. In small ruminants, the PCV of one goat positive for trypanosoma was 23 and that of the sheep was 14

ANOVA has indicated that the BCS of aparasitemic animals (2.1, 95% CI= 2.0-2.2, n= 1417) was significantly ($p<0.0001$) higher than the parasitemic group (1.5, 95% CI= 1.4-1.7, n=92)

ANOVA performed for the districts has indicated that there was a significant difference ($p<0.001$) in mean PCV of the three districts (Figure 7) and peasant association ($p<0.01$) within districts (Table 10). How ever, PCV of cattle was with in the normal range for the species (Figure 6).

In one heifer at Abuanakokit, Fogera district, the PCV of the heifer was 26 on first date of sampling and was positive for *T.vivax*, but the owner refused to treat his heifer expecting our return and with out treatment the PCV became 23 after two weeks and 17 after a month and parasitemia increases as time goes on which was at a swarming rate when PCV became 17 and then the PCV profile changed to 17, 23, 24, and 26 after subjected to DIM treatment group with in three weeks. In another bull-calf at Shina, Fogera district PCV was 24 on first time of sampling and was positive for *T.vivax*, but the owner refused to treat his animal. Next time, after two weeks % PCV became 22 and the infection was observed and then after a month PCV stayed constant at 22, but the parasites were absent and after 1.5 months PCV was 23 and the parasites were still absent.

TABLE 10. PCV OF CATTLE IN THE STUDY DISTRICTS AND PA'S

| Districts | PA | Animals sampled | Mean PCV* | 95% CI |
|---|---|---|---|---|
| Dembia | Tezeba | 50 | 30.9 | 29.6-32.1 |
| | Sankisa | 50 | 28.7 | 27.5-29.9 |
| | Guramba | 75 | 25.4 | 24.4-26.4 |
| Fogera | Abuanakokit | 209 | 24.3 | 23.8-24.8 |
| | Quahar | 144 | 25.7 | 25.1-26.3 |
| | Shina | 239 | 25.7 | 25.2-26.2 |
| Bahir Dar Zuria | Sebatamit | 54 | 25.5 | 24.4-26.5 |
| | Woramit | 79 | 25.1 | 24.2-26.0 |

*There is a significant difference in PCV among PA's (Anova $p<0.001$)

Association of PCV (n=1509) with body condition score, and presence of *T. vivax* infection using Pearson's Correlation indicated that PCV was positively related with BCS ($r = 0.123$, $p<0.05$), and negatively related with presence of *T. vivax* infection ($r = -0.221$, $p<0.05$). A negative relationship was also observed between BCS and *T. vivax* infection ($r = -0.138$, $p<0.05$) (Figure 8).

In cattle, PCV has not differed significantly ($p>0.05$) among the different age groups (0.5-2, 2-4 and > 4 years of age category). How ever there was a highly significant ($p<0.0001$) variation in PCV between the late rainy season and early dry season (Figure 9)

Analysis of variance and Pearson's correlation statistics for productive and reproductive indices in female cattle (n=762) of age ≥4 (females at reproductive age) were performed to predict and relate PCV with other parameters respectively (Tables 11 and Table 12). By taking PCV as an outcome variable using ANOVA has revealed that PCV was significantly negatively affected by parity ($P<0.01$), and lactation ($p<0.05$). However, body condition score ($P<0.001$) and pregnancy ($P<0.01$) had a positive impact on PCV. Though abortion had lowered the PCV values, there was no statistical difference ($p>0.05$) with those with out abortion.

TABLE 11. PCV OF FEMALE CATTLE (≥4 YEARS) CATEGORIZED BY REPRODUCTIVE STATUS.

| Reproductive parameters | Reproductive status | No of animals | Mean PCV | 95% CI |
|---|---|---|---|---|
| Lactation | Non-lactating | 347 | 26.5 | 26.0-27.0 |
| | Lactating | 415 | 25.8 | 25.5-26.2 |
| Pregnancy | Non-pregnant | 661 | 25.9 | 25.6-26.2 |
| | Pregnant | 101 | 27.9 | 27.1-28.7 |
| Abortion | No abortion | 729 | 26.2 | 25.9-26.5 |
| | Aborted | 33 | 25.7 | 24.4-27.0 |

TABLE 12. RELATIONSHIP AMONG THE REPRODUCTIVE AND PRODUCTIVE INDICES IN FEMALE CATTLE ≥ 4 YEARS OF AGE

| | BCS | Parity | Pregnancy | Lactation | PCV | Infection (*T. vivax*) |
|---|---|---|---|---|---|---|
| BCS | | | | -0.162(**) | 0.238(**) | -0.072(*) |
| Parity | | | | | -0.123(**) | |
| Pregnancy | | | | | 0.163(**) | |
| Lactation | -0.162(**) | | | | -0.079(*) | |
| PCV | 0.238(**) | -0.123(**) | 0.163(**) | -0.079(*) | | -0.186(**) |
| Infection (*T. vivax*) | -0.072(*) | | | | -0.186(**) | |

** Correlation is significant at the 0.01 level * Correlation is significant at the 0.05 level. Only significant values are presented (n=762, Pearson's Correlations)

Figure 6. PCV profiles in cattle of the three study districts

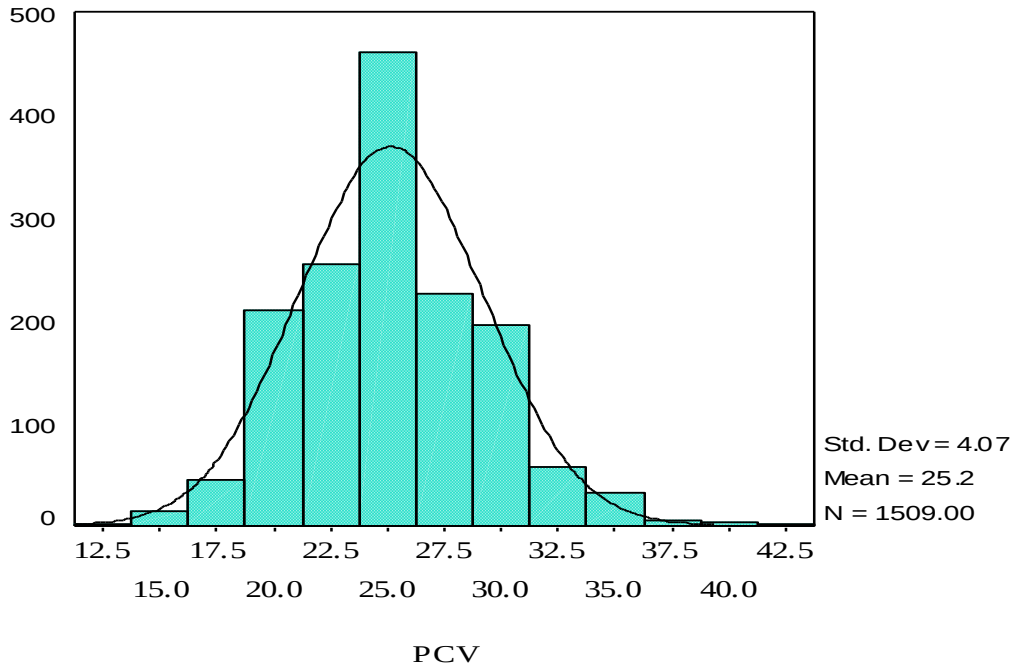
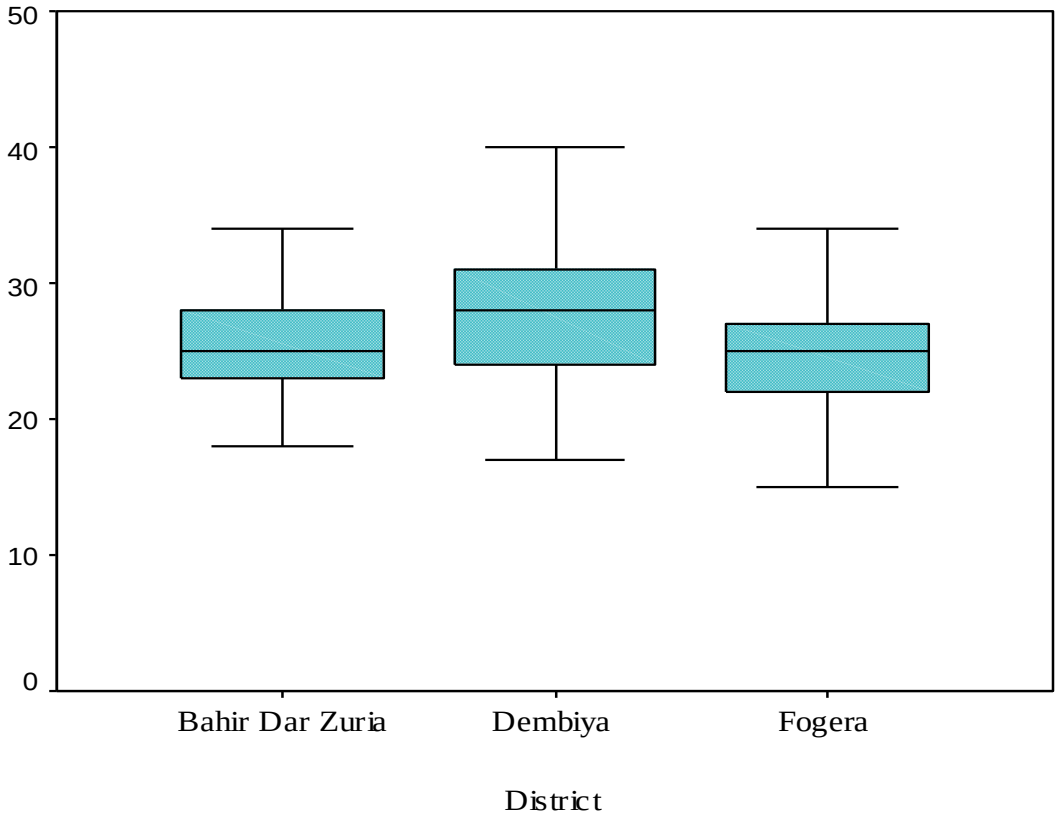



Figure 7. PCV of cattle per study districts



There was no a statistically significant (p>0.05) variation in % PCV of male (22.8, 95% CI= 22.2 -23.5, n=93) and female (22.54, 95% CI=22.3-22.8, n=583) small ruminants. Pearson's correlation analysis of PCV with other productive and reproductive variables (n=546) in female goats at reproductive age has indicated that age (r=-0.149**), BCS (r=0.172**), Parity (r=-0.184**), Pregnancy(r=0.150**), Lactation(r=-0.146**), and abortion (r=-0.097*) were significantly associated with PCV values (** Correlation is significant at the 0.01 level *Correlation is significant at the 0.05 level).

Analysis of variance and student t-test test of independence for female goats at reproductive age has revealed that BCS ($p<0.05$), parity ($p<0.05$), pregnancy ($p<0.01$), lactation ($p<0.05$) and abortion ($p<0.05$) were found to be sources of variation for the % PCV of these animals (Annex 10).

Figure 8. Body condition score in relation to *T. vivax* infection and PCV in cattle

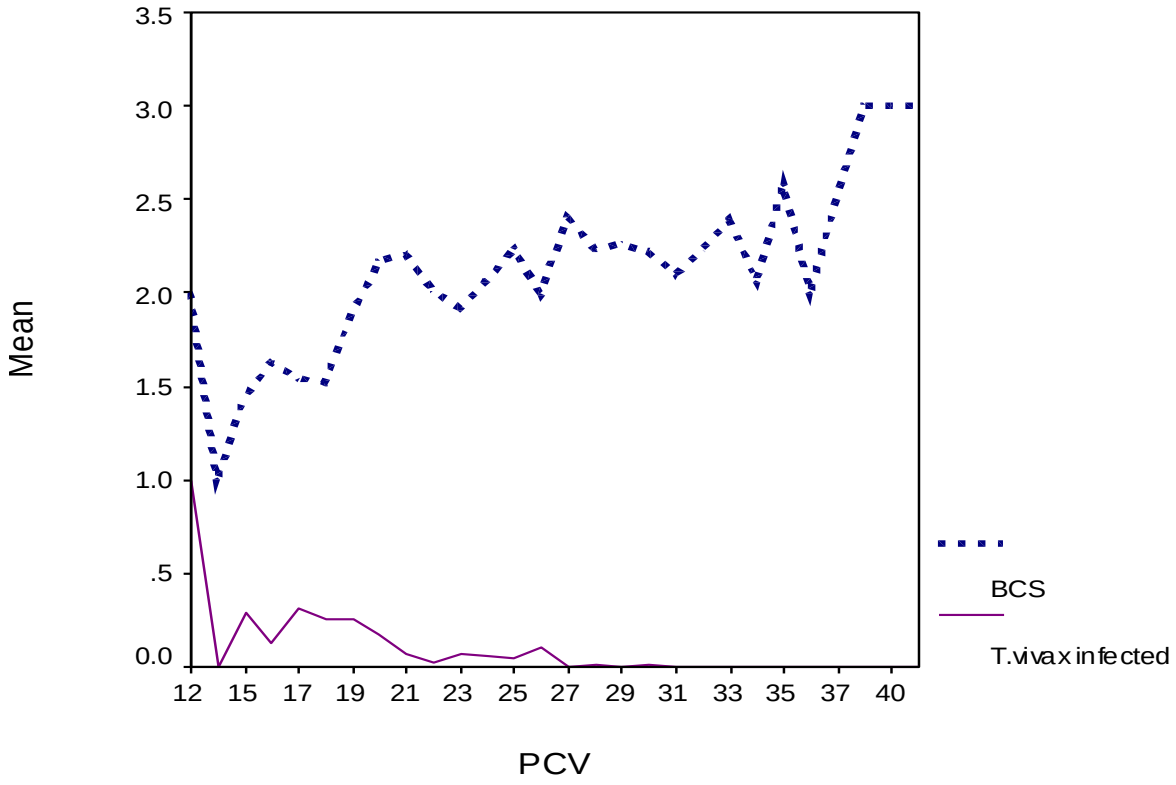
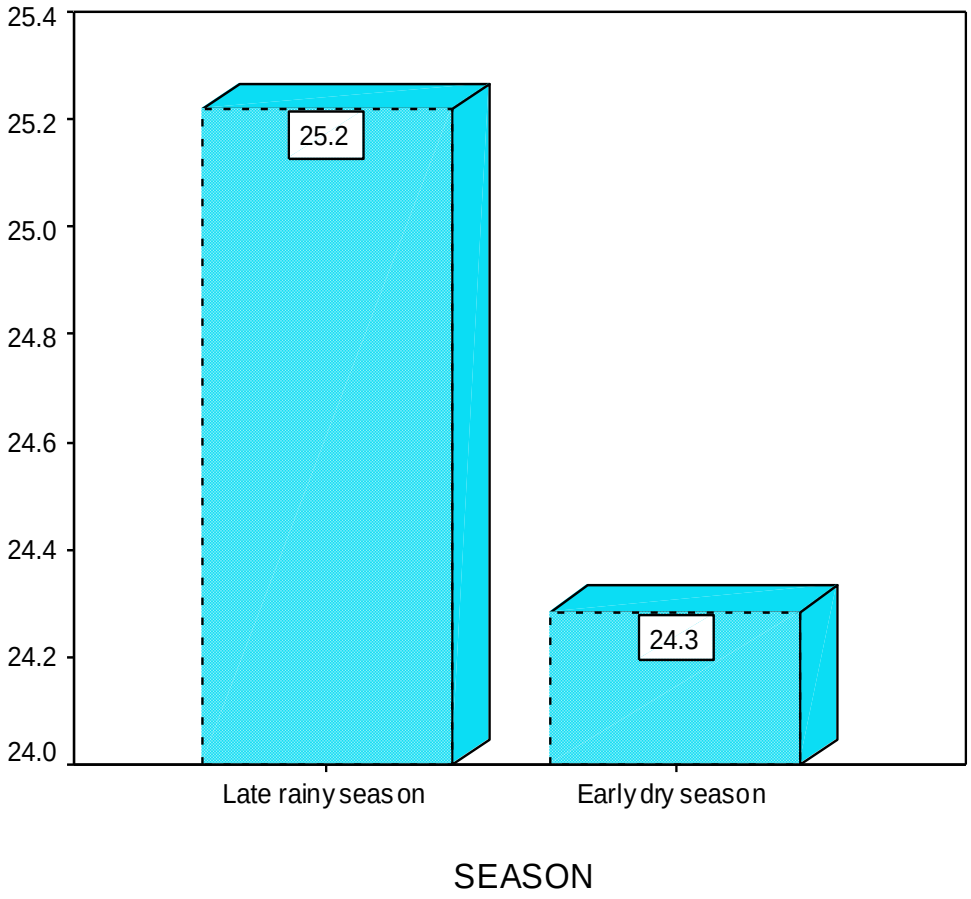


Figure.9. PCV of cattle in the two seasons at Fogera district.

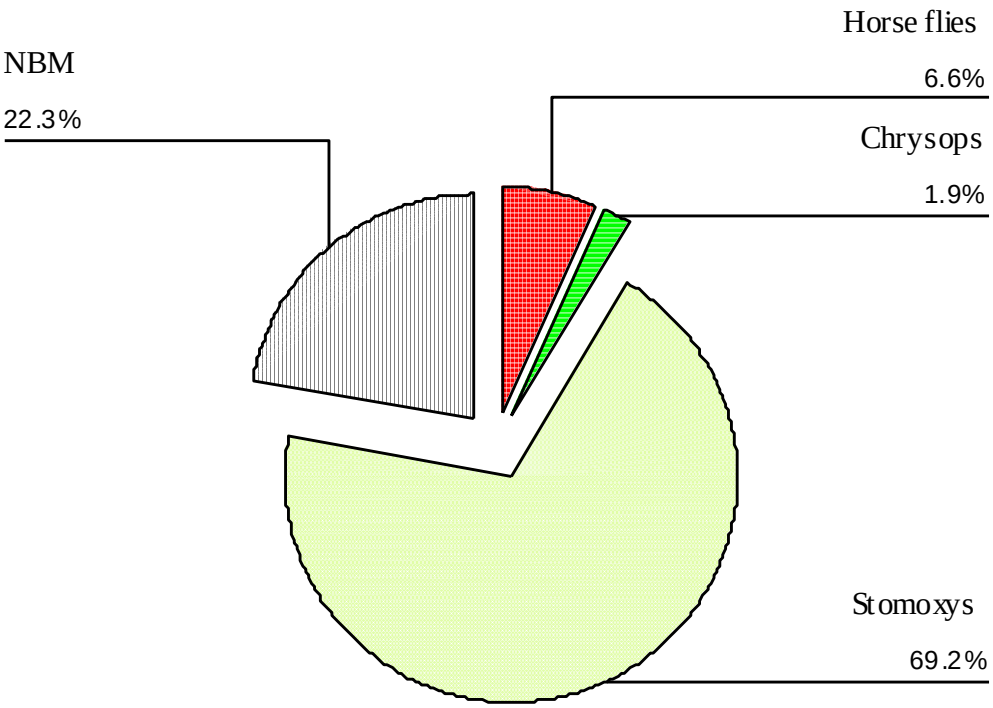



4.2.3. Results of the fly survey

From 86 traps deployed at the three study districts, a total of 71,273 flies were caught. Of which 49,353 (69.2%) belong to the family *Stomoxys*, 15,875(22.3%) to non-biting muscidae, 4,715 horse flies (6.6%) and 1,330 (1.9%) *Chrysops* (Figure 10). The overall apparent density of flies was 276.3 flies/trap/day. The number of fly tribes collected was significantly different ($p<0.001$) for each fly category, being in the order of *Stomoxys* (69.2%), non-biting muscidae (22.3%), horse flies (6.6%), and *Chrysops* (1.9%).

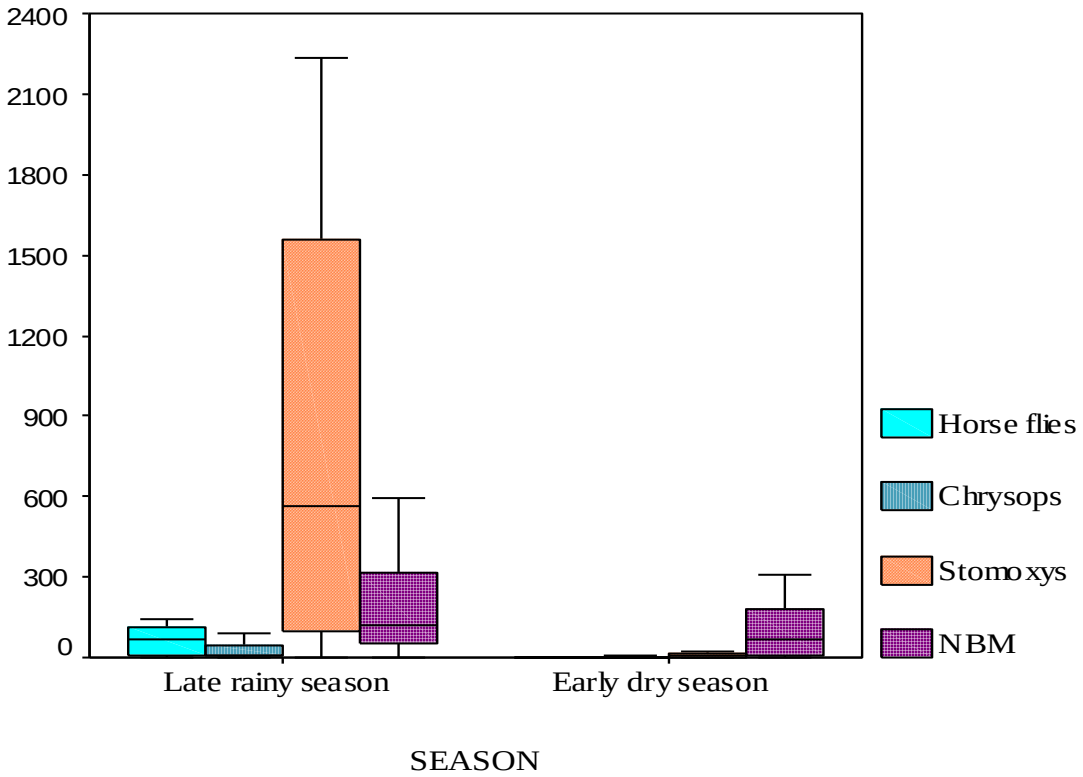
Results on fly survey in this study has revealed the presence of various fly tribes and genera including horse flies, *Stomoxysis*, *Chrysops*, *Hematopota* and non-biting Muscidae. A *Hippobosca* was also collected by hand from the body of cattle at Dembia district and this fly genus was not observed to inter in any one of the two trap types.

Figure 10. The relative % distribution of total fly catch



Of the genera's collected *Atylotus, Chrysops, Hematopota Hippobosca, Stomoxys, Tabanus* and genera of non-biting muscidae were present. From the representative samples subjected for species identification, 3,920 *Atylotus agrestis,* 591 *Chrysops streptobalia*, 15 *Stomoxys calcitrans*, 27 *Stomoxys nigra*, and 5 *Hippobosca variegata* were confirmed to be present in the three study districts. One single sample that belong to the genus *Hematopota* and another single specimen of the genus *Tabanus* were present, however, species identification of was not possible since their wings and legs were partially broken No tsetse fly was captured in any of the study districts. Among the horse flies, *A. agrestis* was the most abundant and all the *Chrysops* belong the species of *Chrysops streptobalia.* Samples in the *Stomoxys* part identified at CIRDES, Burkina Faso were *Stomoxys calcitrans, Stomoxys nigra, S. pulla, S. pallida, S. sataiens, S. taieniata*.

Figure. 11. Fly catchments between the late rainy and early dry seasons



Seasonal comparison of fly catches using 23 traps per season at Fogera district (17 NGU and 6 Monoconical traps per season) indicated that there is a remarkably significant variation ($p < 0.0001$) in overall fly genera number between the two seasons for horse flies, *Chrysops* and *Stomoxys*. Exception was observed for the NBM in that there was no seasonal difference for the group. This seasonal variation was consistent when each trap type (either NGU or Monoconical) was compared for each season (Figure 11, Figure 12, and Figure 13).

Figure 12. Seasonal comparison of catchments of the NGU trap.

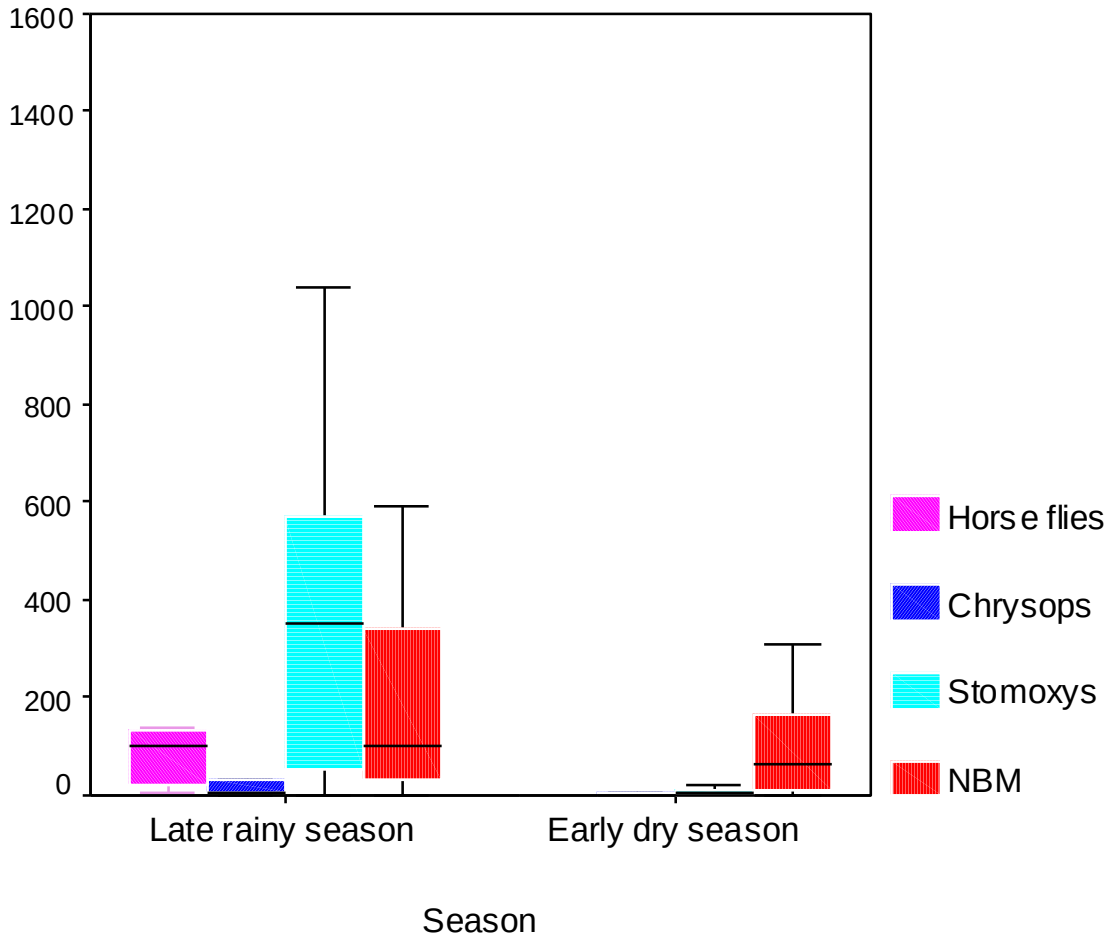
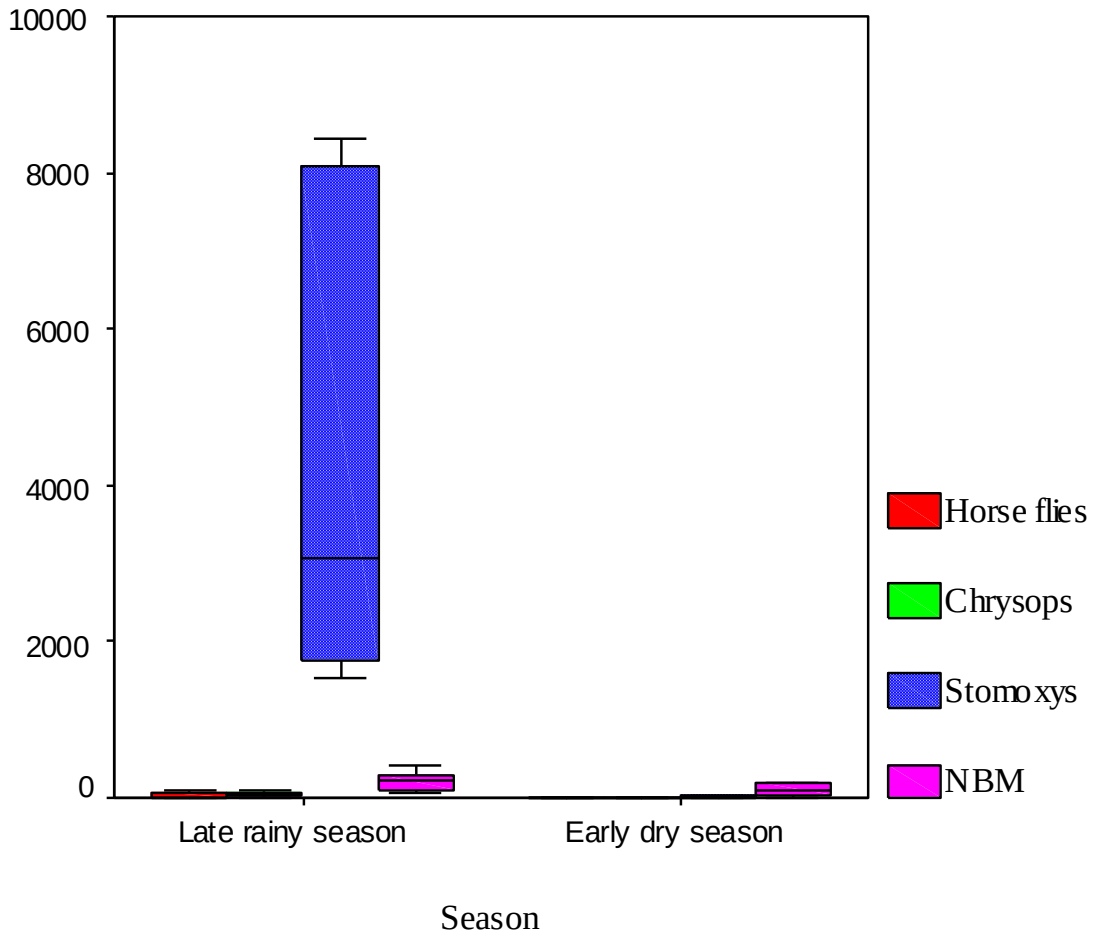
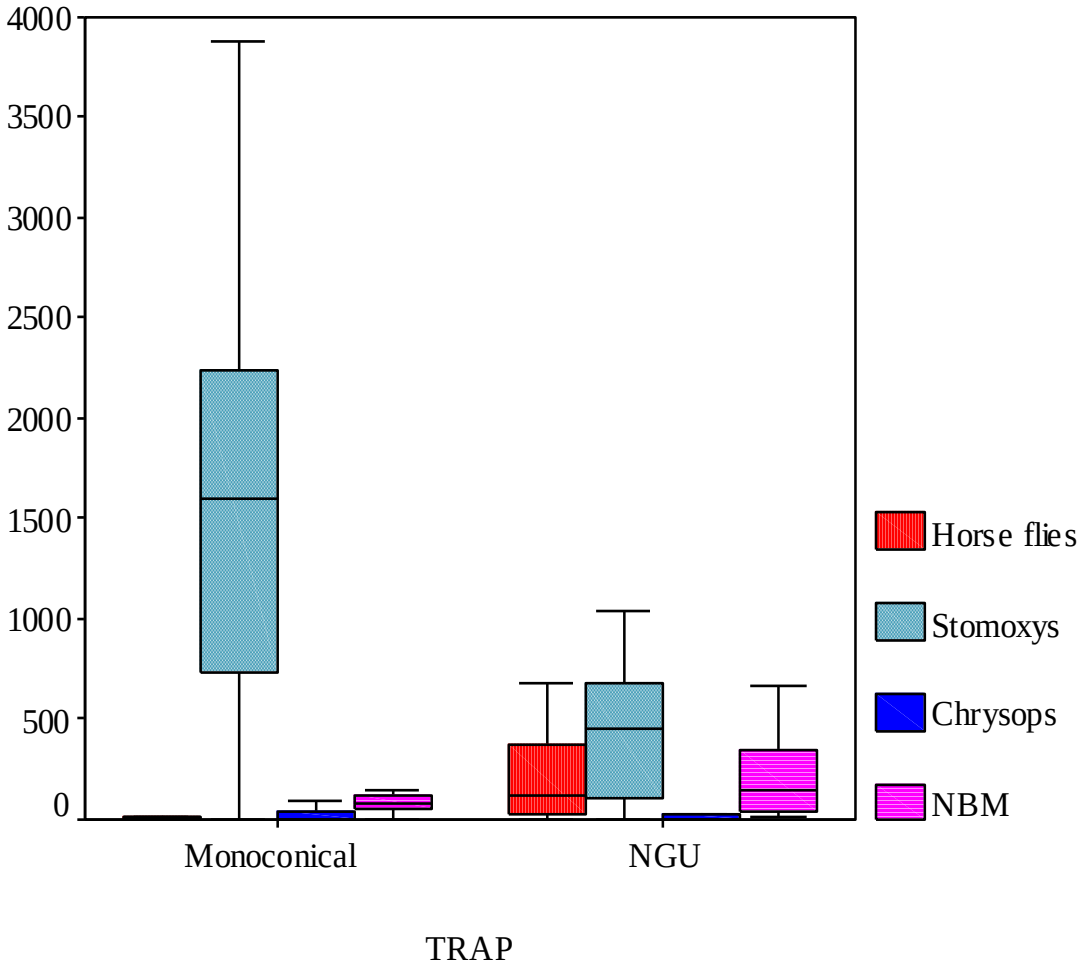


Figure 13. Seasonal comparison of catchments of the Monoconical trap.



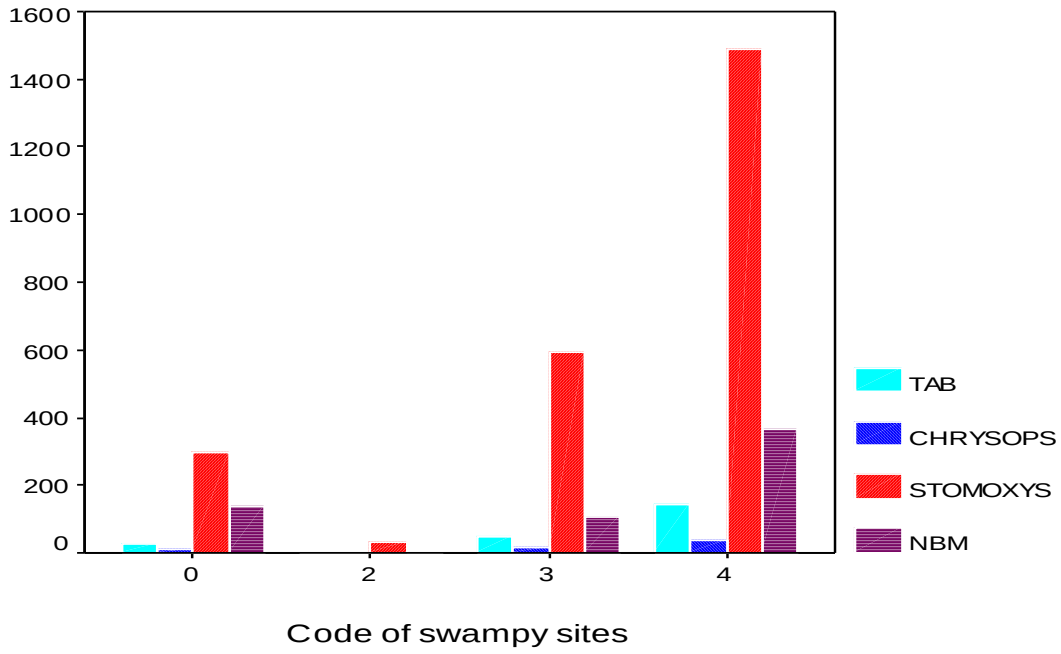

Comparison of the NGU and monoconical trap has indicated that the NGU trap had a significantly ($P<0.05$) high catch of horse flies than the Monoconical where as the Monoconical trap had a significantly high catch of *Stomoxys* ($p<0.0001$) over the NGU trap. But there was no significant difference in catchments between the two traps for non-biting muscidae (NBM) and *Chrysops* species (Figure 14).

Figure 14. Comparison of the Monoconical and NGU trap



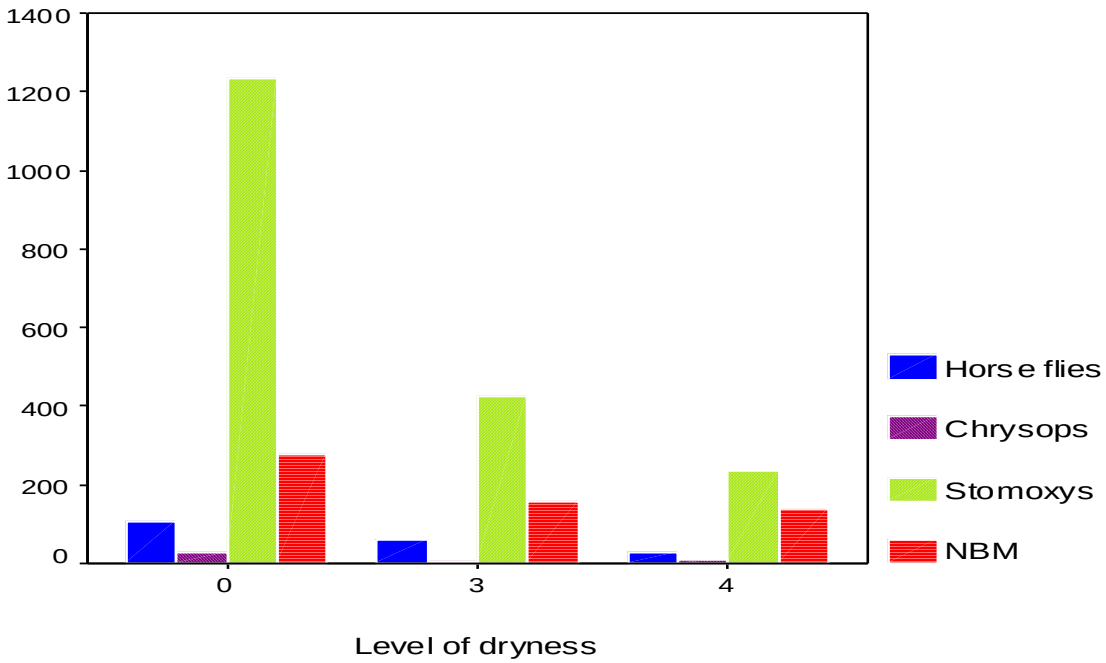

Traps deployment sites were categorized and compared for farmers village, grazing land, swampy locations, cattle barns, dry sites, cattle congregation areas, river banks, watering points, high way, and areas covered with vegetation. Comparison of each fly type by trap deployment sites using ANOVA has indicated that the catch of horse flies, catch of *Chrysops*, and *Stomoxys* ($p<0.001$) and non-biting muscidae ($p<0.05$) was significantly high in and around swampy areas and low at dry sites ($p<0.001$). All the other site variables included in the ANOVA model did not show any significant catch difference (Figure 15 and Figures 16).

Figure 15. Fly catchments in relation to the level of swamp.




0-0%, 2-50%, 3-75% and 4-100%

Figure 16. Fly catchments in relation to the level of dryness.



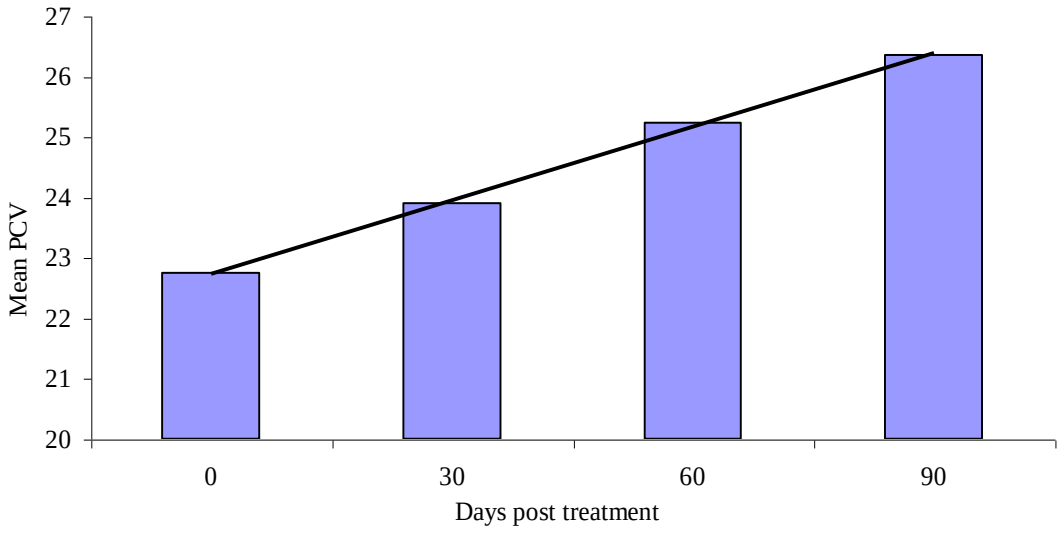

0-0%, 2-50%, 3-75% and 4-100%

### 4.3. Results of the longitudinal study

4.3.1. Results of drug sensitivity trial on Isomethamidium chloride

None of the ISMM treated cattle were found positive through out the post treatment period of our follow-up. The mean PCV of the study animals has improved during the post treatment period. There was a mean increase in PCV by 3.6% from the beginning at day zero of the experiment (22.8, 95%CI=21.8-23.6) to end of the experiment of day 90 (26.4, 95%CI=25.4-27.3) (Figure 17). A statistically significant increase in PCV was observed starting from the day 30 ($p<0.01$) and a highly significant variation ($p<0.0001$) was observed at the end of the experiment (day 90 post treatment) and PCV has significantly differed between each month of the post treatment period (Annex 21).

Figure 17. PCV profiles of cattle treated with Isomethamidium chloride




$y = 1.2146x + 21.521$

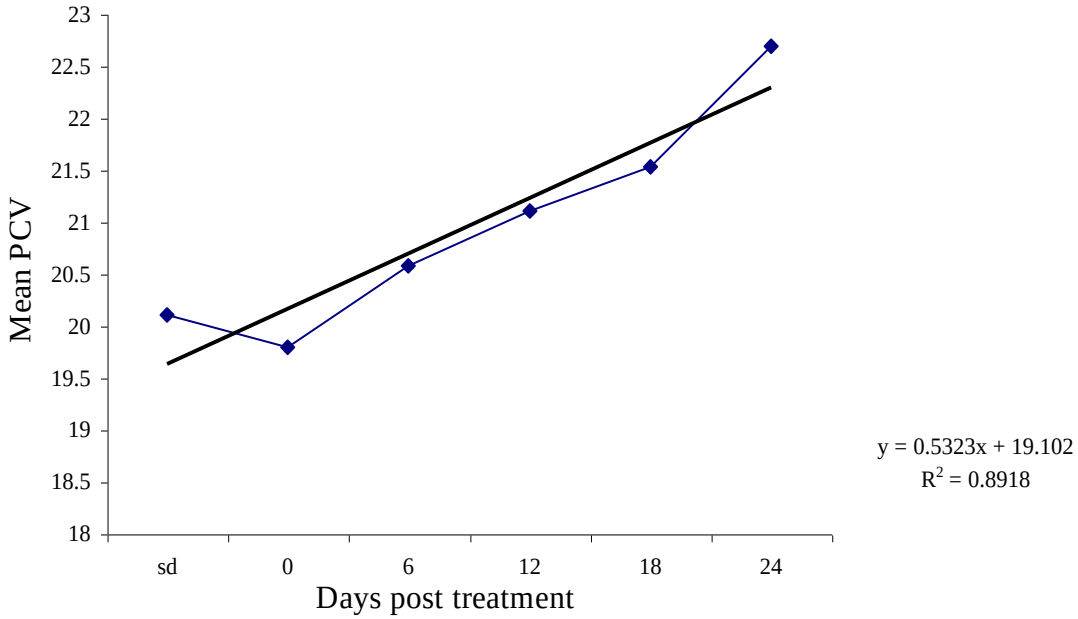
$R^2 = 0.9989$

4.3.2. Results of drug sensitivity trial on Diminazine aceturate

All cattle treated with DIM were negative for trypanosoma through out the study period.

There was also an improvement of 2.9 % of PCV in cattle after treatment of DIM with in 24 days of the experiment from a mean of 19.8 (95%CI=8.7-20.9) at day of treatment to a mean of 22.7(95%CI=21.7-23.7) at day 24 of the end of the experiment (Figure, 18). Though there was no a statistically significant variation in PCV between the day of screening and 12 days post treatment, a significant variation was observed with in six days between day zero of treatment and six day after (Annex 21).

Figure 18. PCV profiles of cattle treated with Diminazine aceturate



## 5. DISCUSSION

Questioner survey in this study indicated that the problem of trypanosomosis in cattle is expanding from time to time. This is supported by the awarness and increased use of trypanocidal drugs in the study areas and recent reports on prevalence of trypanosomosis. The period of fly abundance mentioned by farmers has well correlated with our results that a high fly population is found in the late rainy season (October and November) than the early dry season (December and January). The sites of fly abundance from the respondents and the trapping results of this study are not far apart in that most flies' genera are caught near swampy sites, and grazing scheme.

Veterinary clinics being the only source of veterinary drugs reported by farmers and the alternate application of trypanocidal drugs at the veterinary clinic has prevented the development of drug resistance as partially confirmed by the results of drug resistance trial in the present investigation. The symptoms of trypanosomosis described by group respondents are not different from what is available in literature (Stephen, 1986, Nyindo, 1992, Mare, 1998). This indicates that the farmers are well aware of the disease. in alienation from other diseases.

The unique importance of mechanically transmitted trypanosomosis in cattle apart from other domestic animals as reported by villagers become more pronounced by the fact that infection of small ruminants and equines sampled in the present study were negligible and negative respectively. The livestock management system; grazing, watering and herding practices and veterinary services reported by the farmers is a common trend in a mixed farming system in Ethiopia. The small number of farm animals possessed by a single household is also common in the medium and highlands of Ethiopia where livestock are mainly kept for the purpose of plowing, transport, and source of income. The small ruminant population in this study PA's is low which might be attributed to the swampy nature of the study areas and absence of browsing plants in PA's around lake Tana.

The cost of trypanocidal drug treatment for a single animal, the annual cost per house hold , the frequency of treatment and loss of 0.6 adult cattle per year per house hold reported by the

farmers indicates that apart from loss of productivity, mechanically transmitted trypanosomosis is affecting the economics of farmers through the mentioned costs incurred and direct loss from mortality. In Zambia, the interview results of Bossche *et. al.*(2001) has indicated that more than 75% of the farmers were willing to treat only clinically sick animals. In general from the farmers side, the factors enhancing the development of resistance to trypanocides in trypanosomes are not yet present.

The percentage prevalence of trypanosomosis observed in this study is with in the range of other previous reports of studies conducted in neighboring and similar districts of this study and has varied from 2 % to 16% (Getinet 1994; Mihiret 1995; Eneyew and Abebe; 1997, Cherinet; 1999, Terefe and Abebe, 1999).

The presence of variation in prevalence of *T. vivax* among the districts and the different PA's in districts, zones and regions of northwestern Ethiopia where there are no tsetse fly reports is already documented (Mihiret 1995; Getinet 1994; Terefe and Abebe, 1999; Aklilu, 2002). A prevalence study conducted in a similar and adjacent survey sites of this study by Hassen (1988), reported zero prevalence at Gayint, Wogera, Estie, Armachiho, Simada, and Debark to a 3/60 (5%) at Kemkem, 5/87 (5.75%) at Dera, 6/80 (7.5%) at Dembia and 22/261 (8.43%) at Fogera. Similarly, in a wide area survey conducted in Zambia, Sinyangwe *et al*. (2001), reported that prevalence in individual villages varied between 0 and 64% and this prevalence has varied widely not only between villages but also between visits. A prevalence variation that laid between zero and 43% has also been reported by Mwambo *et. al.*(2001) in Tanzania.

Except a single report by Mihiret (1995) at Bahir Dar Zuria district that documented a prevalence of up to 21% in one village, other reports are below the 15.5% of the present study. This variation among districts and PA's of prevalence in the present study could be attributed to the biting fly population and type present in each locality, which is dependant on microclimate, animal herd density, distance between herds and other various factors (Foil, 1996).

A significantly high rate of infection following the months with high rainfall reported in our study is due to the emergence of biting flies at a high rate as it has matched with the high number of biting fly collected. However the decrease in prevalence in the early dry season would be complimented by the fact that owners take their animals for treatment and as the infected animal number decreases, the source of infection decreases. Hence, in concomitant

with the decrease in fly population during the dry season, the prevalence of *T. vivax* is expected to decrease. The self-cure phenomenon following *T. vivax* infection would also contribute to the decrease in prevalence. In French Guiana, Desquesnes *et al*., (1996) reported that self-cure in cattle was observed at a mean rate of 15-20% per year. This self-cure phenomenon is ascribed to the smaller number of variable surface glycoprotien in *T. vivax* (Gardiner, 1989).

Such seasonal difference in the prevalence of *T. vivax* infection rates was reported by adjacent areas of the present study districts (Getinet, 1994; Mihiret, 1995; Eneyew and Abebe, 1997; Cherinet, 1999 Terefe and Abebe, 1999). In Nigeria, a study conducted on zebu cattle has revealed a higher infection rates during rains (9.3%) than in the dry season months (1.5%). In this area tsetse were encountered at low density and *T. vivax* was the predominant species accounting 81% infections in the rainy season and 100% in the dry season (Kalu, 1996). This suggests that biting flies would mediate *T. vivax* infections when the tsetse fly density is either low or absent.

Similarly, Tamasaukas *et al*., (1996) indicated that bovine trypanosomosis caused by *T. vivax* was low during part of the dry season and suggested that it is due to major changes in the environmental and agro-ecological conditions of the farms during this season in comparison with those observed in the rainy season. D' Ieteren *et. al.* (1988), found that prevalence for cattle and sheep was 2-4 times lower in the dry season than the months with highest rain fall.and Kalu and Lawani (1996), reported that infection rates doubled during the rainy season (7.6%) as compared with an average of 3.8% during the dry season

The very low prevalence in sheep and goats and zero in equines might be due to that biting flies prefer cattle than other domestic animals (Kniepert, 1981) and in a mixed farming system in the study areas where different species of animals are kept together in a communal grazing area, biting flies would preferably attack cattle, leaving most of the small ruminants and equines uninfected. However, the presence of single infections in one sheep and one goat indicates that these species would get the infection and other diagnostic methods apart from the parasitological techniques might reveal the extent of the infection rate in these small stocks. Kniepert, (1981) showed that the rate of attack by species of Tabanidae on cattle depended on the position, size and coloration of the host. Tabanid species studied preferred a particular site for feeding caused by variations in the length of the hair, thickness and tensile

strength of the skin of the animal. The tabanids preferred sites in which these characters correlated with the absolute and relative length of their proboscis.

A high prevalence rate in cattle and low in small ruminants has been reported by different workers previously. Coulibaly *et al*., (1988) and Defly *et al*. (1988,) indicated that livestock species had a major effect on trypanosome prevalence. They found that prevalence in trypanotolerant cattle was 3 times than in sheep kept in the same area, but there was no difference between the trypanosome prevalence of sheep and goats. This difference in cattle and sheep was greatest where *T. vivax* causes about 90% of parasitemia in cattle and about 95% in sheep. Kalu and Lawani (1996), observed a prevalence of 5.3, 1.2 and 0.7% in cattle, sheep, and goats respectively. In southwest Ethiopia, in a tsetse-infested area, Dinka and Abebe (in press) has reported a 5.1 % (7.6% in sheep and 3.6% in goats) prevalence of trypanosomosis in small ruminants and the prevalence varied from village to village.

Similarly, Hendy, (1988), in east African short horn zebu found a prevalence of 16%. However, no parasites were found in goats (0/280) and only 4/172 sheep were found parasitemic. He proposed that there might be a problem in the diagnosis of disease. This may be a particular problem in goats and sheep, especially if some degree of trypanotolerance exists. Detection of trypanosomes in the blood may then be difficult. However, more sensitive/specific tests such as the PCR technique (Solano *et al*., 2001) would be applied to reaffirm our findings. Desquesnes *et al*., (1996) reported in sheep that depending on the management of the farm, clinical signs might vary from nil to significant loss of weight and condition and even abortion in the ewes. If the management level of a farm is satisfactory, clinical signs may be absent and the infection inapparent even if the trypanosome is circulating.

A variable result with regard to *T. vivax* infection in sheep was reported by Desquesnes *et al*., (1996) that in one site 163 sheep samples collected, all were negative and at other times, of 164 samples collected several showed sub clinical infection by *T. vivax* and others showed drastic clinical signs including abortion. Similarly, Anosa *et al*.(1995), reported a parasitological prevalence of 4.5, 2.7 and 2.2% in cattle, sheep and goats and Nawathe, *et. al*.(1995) found a 0.9% prevalence in small ruminants in Nigeria. Consideration of the presence of infection in small ruminants in the present study is important since they would act as a reservoir of infection for cattle.

Desquesnes (1997) has skeptically put equines susceptibility to the Latin American *T. vivax* strain. Perhaps, though equines are preferred for attack by biting flies next to cattle, they might not be susceptible to the mechanically transmitted strain of *T. vivax* present around lake Tana.

Results on hematological values reported in the present study where *T. vivax* was the only species of *Trypanosoma* encountered in cattle of the three districts, the degree of anemia as measured by the PCV was profound. Such significant difference in PCV of cattle due to trypanosomosis in ruminants is available in various works done so far and that of *T. vivax* infections is tabulated in the literature review part of this paper (Defly *et al*. 1988, D' Ieteren *et. al.* 1988, Malo *et. al.* 1988, Mulatu *et. al.* 1988 Ordner *et. al.* 1988, Getinet, 1994, Mihiret, 1995, Abebe and Jobre, 1996, Kalu, 1996; Enyew and Abebe, 1997, Terefe and Abebe, 1999, Aklilu, 2002).

Desqesnes and Dia, (2003, 2004), demonstrated that hematocrit values of infected cattle (after experimental mechanical transmission of *T. vivax* with *Atylotus agrestis* and *Atylotus fuscipes* respectively) has decreased during the infection period indicating the notable pathogenic effect of mechanically transmitted *T. vivax* and this transmission of *T. vivax* proved to be very efficient with an incidence observed of 75%, from an initial prevalence of 20 % infected animals (donor animals).

In this study, the decrease in PCV of *T. vivax* infected heifer at Abuanakokit, Fogera district from 26 to 17 in one month and an increase in parasitemia and the return to normal of 26 after DIM treatment indicates that mechanically transmitted *T. vivax* causes a dramatic drop in PCV in cattle. The absence of *T. vivax* in the bull-calf at consecutive sampling times at Shina, Fogera district and the relatively constant PCV that stayed at 22-23, indicates that this bull-calf might have developed an intrinsic self-cure or the parasites may be not be present in sufficient number in the peripheral blood stream to warrant diagnosis. However, more information on PCV profiles is required to verify the present clues.

PCV depression observed in the single sheep and goat in this study suggests that in the future, trypanosomosis in small ruminants in the study districts if expanded (or if other wise prevalence is already high by applying more sensitive tests) is a warning that these species are at a high risk of the disease. Previously, Van Dam *et al*. (1997), had experimentally infected goats with *T. vivax* and the goats has developed anemia and their PCV decreased gradually in

all infected animals with time after infection to an average 17 % in week 4 post infection (control animals had a mean PCV of 38%). The infected sheep in our finding has a similar PCV value and the goat had a history of abortion. Similarly, as observed in the single sheep infected at Dembia district of this study, Defly *et al*. (1988) already indicated that *T. vivax* infection in sheep had depressed PCV. In southwest Ethiopia, in a tsetse-infested area, the PCV of parasitemic small ruminants was significantly lower than the aparasitemic group (Dinka and Abebe, in press).

Taylor (1998) indicated that anemia persists during the chronic stages of infection when parasitemia is generally quite low, probably because different mechanisms are involved in its genesis during the acute and chronic stages of infection. This suggests that control of parasitemia and control of anemia is unrelated in the chronic phase when immune infections are depressed and anemia is sustained through dyserythropoiesis.

In the present study, in female cattle (infected or non infected) and goats at reproductive age, PCV was negatively associated with lactation and parity. Formerly, Ordner *et. al.* (1988), found that one or more trypanosome parasitemic months detected in the cow during the breeding year depressed the average PCV in the same period by 4% units. They stated that, lactation status of the cow has significantly affected the average PCV level during the breeding year, which was 1.5% lower in lactating as compared to dry cows. Similarly, in sheep, the mean PCV of lactating ewes was lower than that of gestating ewes (D' Ieteren *et. al.* 1988).

PCV was positively associated with the body condition score of cattle and there was a low body condition score in *T. vivax* infected animals than non-infected groups. This variation in PCV in relation to reproductive and productive indices would be important if considered with the aspect of predisposing factors to *T. vivax* infection and taking PCV as one major criteria of assessing *Trypanosoma* infection. *T. vivax* was found in different peasant associations in the three districts, and it seems that species (hosts) are important factors for the development of infection and the disease as mostly detected in cattle, negligible in small ruminants and nil in equines.

In general, the present findings indicated that trypanosomosis due to *T. vivax* has established in the three districts in affecting cattle productivity and small ruminants are at a higher risk of

infection and development of the disease in the three districts bordering lake Tana, ANRS, Ethiopia.

Findings of the fly survey in this study could be considered as a preliminary work. In Ethiopia, though there has not been a specific study on biting flies in alienation from the usual tsetse fly studies, few authors has reported the name of some of the biting flies as *Tabanidae* and *Stomoxynae* at a family level (Eniyew and Abebe, 1997; Kidane-Mariam, 2000). How ever, Kigaye, and Jiffar (1991) in a survey of ectoparasites of cattle in Harar and Dire Dawa districts, south eastern part of Ethiopia has reported the presence of 10 species of "stable flies" *Stomoxys calcitrans, S. nigra (S. niger), S. sitiens, Lyperosia spinigera (Haematobia spinigera), S. varipes, S. bilineata [S. niger bilineatus], S. brunnipes, Lyperosia minuta [H. minuta], L. thirouxi [H. hirouxi]* and *H. hirtifrons.* 7 "housefly" species, one *H. variegata*, and 2 tabanids (*Chrysops obliquefasciata* and *Hematopota atellicorne*).

How ever, various workers in different African countries have reported the presence of different biting and non-biting fly tribes/ genera found in the present study. The most abundant *Atylotus agrestis* and *Stomoxys* species in our collection were previously reported from Sudan, Saudi Arabia, Nigeria, Burkina Faso, Mauritania and elsewhere (Bowden, 1976; Adeyefa, and Dipeolu, 1986; Leclercq, M. 1986; Amoudi and Leclercq 1988; Amoudi, 1989; Burg, *et al*., 1991; Amoudi, and Leclercq, 1992;Amoudi and LeClercq, 1993; Amoudi and Leclercq, 1996; Dia, *et al.*, 1997, Dia, *et al.*, 1998).

Acapori *et al.* (2001), reported that out of 2471 *Stomoxyinae* captured; *Stomoxys niger* (70.7%) and *S.calcitrans* (29.3%) were identified. The *Stomoxynae* represnted by two species only, made up about 45% of the biting flies captured. They will have to be reckoned when evaluating the impact of biting insects on cattle. This finding is similar to our reports that *Stomoxynae* are present at a high population. In another study, D'Amicus *et al.* (1996) had encountered 5 species or sub-species of *Stomoxynae* namely *Stomoxys nigra, S. taeniata, S. tiens,* and *S. omega omega*.

In a study conducted in Mauritania, Dia *et al.* (1998) has reported a similar composition of flies as has been found in this study (*Tabanus, Atylotus, Stomoxyn*ae, and *Hippobosca*) and they have collected *Hippobosca* from the body of the animals as has been done in our study. Hussein, *et al.* (1991) has also reported *Atylotus farinosus* and *A. agrestis*. Iwualaand Onyeka, (1977) in Guinea, West Africa reported three types of biting flies as *Stomoxys*, *Chrysops* and

*Hippobosca* and several types of non-biting muscidae. From the collection of Dipeolu, (1975) in Nigeria, *Stomoxys nigra* and *S. calcitrans* were the most abundant. Kangwagye, (1977) in Uganda reported the presence of *Haematopota, Tabanus*, *Chrysops,* and *Stomoxys*. In Burkina Faso, the distribution of fly species under natural condition was *Atylotus agrestis* 20%, *A.fuscipes* 4%, *Chrysops distinctipennis* 12%, *Tabanus taeniola* 2%, *T. sufis* 16%, and *Stomoxys niger* 46% (Desqesnes and Dia, 2003).

In Turkey, Kilic, (1999), reported 156 species and 12 subspecies. Of which, 19 species and 2 subspecies are from the subfamily *Chrysopsinae* and 125 species and 10 subspecies are from the subfamily Tabanine. The composition of genera was *Tabanus* (67.9%), *Chrysops* (19.2%), *Atylotus* (11.2%), and *Ancala* (1.6%). In Cote d'Ivoire, Acapori *et al.* (2001) reported that of 3104 caught specimens, 4 genera and 16 tabanid species were identified. The most abundant of caught species were *Tabanus taeniola* (26.4%), *T. par* (15.6%), *T. laverani* (14.9%), and *Chrysops distinctipennis* (12.3%). The least abundant species were *Atylotus albipalpus* (6.9%), *Chrysops lonicornis* (6.9%), *T. brumpti* (4.8%), *T. gratus* (3.7%), *At. agrestis* (2.5%), *At. fuscipes* (1.8%), *T. biguttatus* (1.4%), *T. recardia* (0.5%), *T. boueti* (0.4%), *T. pluto* (0.3%), and *An. fasciata* (0.2%).

The large count of biting flies during the rainy season collected using traps developed for tsetse fly trapping was complimented by the use of 1-octen-3-ol and acetone. Different authors confirmed that the use of attractants namely, 1-octen-3-ol, acetone, $CO_2$, ammonia, phenols and cow urine baited traps alone or in combination had improved the catch of *Tabanidae* than the non-baited traps (French and Kline, 1989; Jaenson, *et al.,* 1991; Leprince *et. al*., 1991; Hribar, *et al.* 1992; Hayes, *et al*. 1993; Foil and Hribar, 1995; McElligott, and Lewis, 1996; Djiteye, *et al.* 1998; McElligott, and Lewis, 1998; Kristensen and Sommer, 2000; Ngare and Mwendia, 2001). In one experiment, Vale (1982) demonstrated that the catch of *Stomoxynae* and non-biting *Muscidae* in the presence and absence of odor was 83.1 and 38.3 % respectively.

The efficiency of the NGU trap over the Monoconical in trapping the horse flies and the Monoconical over the NGU for *Stomoxys* observed in this study was due to that trap design, and color are factors of attraction and larger flies prefer larger tarps and vise versa. This efficiency of the NGU trap over the Monoconical in trapping the horse flies has been previously observed by different workers (Amsler, *et al.* 1994; Amsler. and Filledier, 1994a and 1994b; Foil. and Hribar, 1995; Djiteye, *et al*. 1998). The NG-2G and F3 traps and the

screen-trap were significantly more effective (X1.7 to 8.7) than the Biconical and Monoconical ones in having a high catch of *Tabanidae*.The large screen produced relatively small catches of *Stomoxyinae* and non-biting muscidae, where as the small screen produced relatively large catches of these flies (Vale, 1982).

The population peak of most species, during the late rainy season including those with higher vector potential, suggests that the rainy season can be considered as the period of potentially higher risk of mechanical transmission of pathogens by biting flies. This high population density of biting flies recorded in this study at the end of the rainy season is due to that biting flies requires a wet habitat for multiplication and larval growth is also dependent up on wet soil/mud. High population density of various biting flies following the rains is reported by various workers in different countries (Kangwagye, 1974; Dipeolu, 1975; Bowden; 1976; Bowden 1977; Kangwagye, 1977; Davis and Sanders, 1981; McElligott. and Galloway, 1991; Gorayeb, 1993; Cilek *et al* . 1994; Dia,, *et al*. 1997. McElligott, *et al*. 1998).

Dia, *et al*. (1997) found that species of *Atylotus agrestis, Tabanus taeniola, T. sufis, Haematobia minuta* and the hippoboscids *(Hippobosca camelina* and *H. variegata)* were particularly abundant during the end of the rainy season, but could be found throughout the year at a very low density. Similarly, Dia, *et al*. (1998) in Mauritania, found that most of the *Tabanidae* were caught between October and November at the end of the rainy season. Bowden, (1976) suggested that the most important single factor affecting the phenology of *Tabanidae* in Nigeria was the incidence of rainfall and Bowden; (1977), observed that *Chrysopsinae* emerge after heavy falls of rain. Dipeolu, (1975) indicated that flies were more numerous during the rainy season and the intensity of the early rains influenced the subsequent abundance of *Stomoxys*. Gorayeb. (1993) indicated that the correlation of certain climatic factors with the seasonal abundances of common tabanid species was investigated and significant relations were found for some species with air temperature, relative humidity of the air, rainfall, insulation, and evaporation potentiality or light intensity.

Different ecological habitats where flies are numerous or scarce investigated so far indicated that each fly genera has adapted to a certain locality for breeding, feeding, resting and host seeking. Dia, *et al.,* (1998) reported that high number of *Tabanidae* was collected from an area with ample water and traps placed in the pasture near this watery area caught 80% of the population. Janzen and Hunter, (1998) collected most of the *Chrysops* from a bog habitat. Baribeau and Maire (1983) collected *Chrysops, Tabanus* and *Atylotus* from a fen habitat.

Burger, *et al*., (1981) and Lewis, (1987) reported that the most consistently favorable collecting sites for *Chrysops* were natural or artificial ponds which is agreement with what has been observed in our study. Dale and Axtell (1976) has trapped highest numbers of *Tabanus* in the marsh while the lowest were obtained from inside nearby woods. Lago and Testa (1990) reported that in an area surrounding tidal marshes *Chrysops* and *Tabanus* were the common horseflies. Matthysse, *et al.*, (1974) collected a greater number of Tabanids in a pasture containing livestock than traps isolated from animals. The largest numbers of tabanids were caught in the gallery and the fewest in the forest (Acapori, *et al.*2001). Dia, *et al*., (1997), found that in most of the time, *T. taeniola* and *A. agrestis* were caught in pastures, while *T. sufis* was caught by traps placed near water.

Collection of adult flies is also dependent on larval habitat. Foil, (1996) reported that the larva of tabanids feed on organic debris and small invertebrates in a variety of aquatic to semi aquatic habitats. However, stable fly larvae develop in manure-spilled feed and decaying vegetation. Cattle manure on cattle feed lots is an important medium for stable fly larval development and Hafez, *et al.*, (1970) in Egypt reported that larvae of *Tabanus taeniola* were found mainly in the mud of the banks of irrigation canals

In general, the fly survey in this study demonstrated that there is a high fly density during the late rainy season and different biting fly genera/species. Therefore, apart from transmission of Trypanosma, these biting pests are important in transmitting many other livestock diseases so far studied (Krinsky, 1976; Foil, 1989) and their economic importance in livestock productivity through loss of weight and condition (loss of blood, annoyance, predisposing to infection) should be considered together. Hollander and Wright (1980), estimated the blood loss in cattle caused by tabanids to be more than 200 ml/animal/day and *Stomoxynae* and tabanids cause weight loss due to blood loss and annoyance as well as create feeding lesion sites which may promote contaminative transmission of agents or myiasis, (Foil, 1996). The most abundant fly species *Atylotus agrestis* found in our study has been recently demonstrated as an effective mechanical vector of *T. vivax* at high rate of 63% (Desqesnes and Dia, 2003) and the authors concluded that in Africa, the epidemiology of trypanosomosis in cattle is also tabanid dependent and the eradication of tsetse flies will not necessarily lead to eradication of *T. vivax.*

In the present study using ISMM as a prophylactic and DIM as a curative drug it is demonstrated that *T. vivax* present in the study district is sensitive to both drugs. Previously,

an in vivo test of trypanocidal drugs in cattle was recommended by Eisler *et. al.*(2001) for the investigation of drug resistance in *T. vivax*, which is not usually infective for mice and the in vivo tests in cattle are also helpful in predicting the effectiveness of curative trypanocidal drugs at recommended dose for cattle infected with a particular trypanosome isolate than the conventional multiple dose tests in mice.

The findings in this study suggest that the use of DIM to treat infected cattle could be used to reduce infection rates and loss of productivity in cattle. Ilemobade, (1988) indicated that chemotherapy is very effective in the control of sporadic trypanosomosis due to mechanical transmission, seasonal fly dispersal and scattered tsetse foci. In an experiment conducted in sheep and goats in Nigeria, Kalu, (1995) reported that ISMM at 1mg/kg dose rate has effected in parasitological cure of *T. vivax* infections, but stocks 1/21 were consistently resistant to 7 mg/kg of DA. In another experiment conducted in Nigerian sheep, Anosa *et al.* (1995) demonstrated that all *T. vivax* infections were cured with in two weeks of post treatment with DA.

Previously it was demonstrated that prophylaxis treatment using ISMM has marginally improved mean PCV, reduced the number of detectable parasitemia and increased daily live weight gain (Malo *et al.,* 1988). In Ethiopia, Mulatu *et al.* (1988) indicated that, of the parasitemic animals receiving a Berenil treatment, 23 % had a parasitemia detected at the next monthly sampling. Despite a high trypanosome prevalence, about 20%, East African zebu cattle, given systematic therapeutic treatment, had low mortality rates, reasonable live weights and provided milk and draught power. This indicates that DIM is important to improve cattle productivity in *T. vivax* infected herds. Ilemobade, (1988) indicated that in Nigeria *T. vivax* is proved to be more susceptible to DIM than *T. congolense*.

In Ethiopia, Afework *et. al*. (2000) reported that PCV in local zebu cattle has significantly improved with in 30 days of ISMM treatment and out of ten cattle positive for *T. vivax*, only one cattle had a relapse at day 90 of post treatment. Similarly in Boran breed cattle of Kenya, Münstermann *et. al.* (1992) has indicated that following ISMM or DIM treatment, animals has regained their individual mean PCV after two weeks. This was confirmed in the present study where cattle regained their normal PCV after three weeks of curative treatment with DIM. The significant PCV variation observed between each month post treatment of ISMM in the present was not observed in Afework *et. al*.(2000) that did not vary between days 30, 60, and 90 post treatment. This might be due to the presence of relapsing trypanosomes and mixed

infections in their study as compared to the absence of relapsing trypanosomes and a single species infection of the present study.

Previously, mechanically transmitted drug resistant *T. vivax* to Diminazene aceturate was observed in experimental infections in both cattle and sheep in South America (Desquesnes *et al*, 1995) and in an experiment in calves, parasites disappear after treatment with Diminazine aceturate at day 14 and 17 and parasitological examinations were negative just after the treatment and for a period of more than two weeks to relapse 18 days and 19 days later (Desquesnes, 1997). Luckins, (2000) also reported that drug resistance is known to occur among mechanically transmitted *T. evansi* isolates and there have been reports from several different countries in Africa and Asia.

However, in the present field trail, alternate and infrequent uses of trypanocidal drugs, absence of drug in the farmers house (available only in veterinary clinics and applied by professionals), and the recent history of the disease in the study districts would have contributed for the absence of relapsing infections.

## 6. CONCLUSION

In general, the present study indicated that trypanosomosis due to *T. vivax* is an important disease limiting cattle productivity and small ruminants are infected that would act as a source of infection for cattle. Infection with *T. vivax* has negatively affected the PCV and BCS of cattle. This indicates that *T. vivax* infection of cattle in the study area causes loss of body weight and production.

The presence of various biting flies and the absence of tsetse flies in this investigation indicates that *T. vivax* infection in the study area is caused by mechanical transmission mediated by biting flies. The presence of biting flies at a higher density during the late rainy season and the concomitant higher prevalence of *T. vivax* in this same season supports that biting flies are the main epidemiological factors for *T. vivax* infection. The presence of biting flies in the early dry season at a small number would help to assure the continuity of *T. vivax* circulations in cattle herds at a low rate and when the next rainy season favors vector multiplication those circulating infections would flare up in a major proportion of the herd.

*T. vivax* infection in cattle at Fogera district is sensitive to both ISMM and DIM at a prophylactic and normal curative doses respectively. This suggests that chemotherapy is important in controlling infections and limiting loss of cattle productivity in the study areas.

Therefore, a particular attention towards *T. vivax* infection in cattle is essential to control the impact of the disease on cattle productivity. Development of control options that could minimize biting flies especially in seasons of high vector population is another task. Treatment of *T. vivax* positive cattle with DIM would be effective and economical for the time being till drug resistant problems might arise due to DIM and applications of ISMM as a prophylactic drug would not be economical so long as DIM works. It is also of particular importance to exercise a prudent use of the available trypanocidal drugs not only to effect disease control, but also to holdup the development of drug resistance to trypanocides.

After the eradication of tsetse flies, mechanically transmitted trypanosomosis (*T. vivax* and *T. evansi*) will remain as major problems unless control program is devised along with the current tsetse control activities. To this effect control of *T. vivax* and *T. evansi* need to be included in the PATTEC initiatives.

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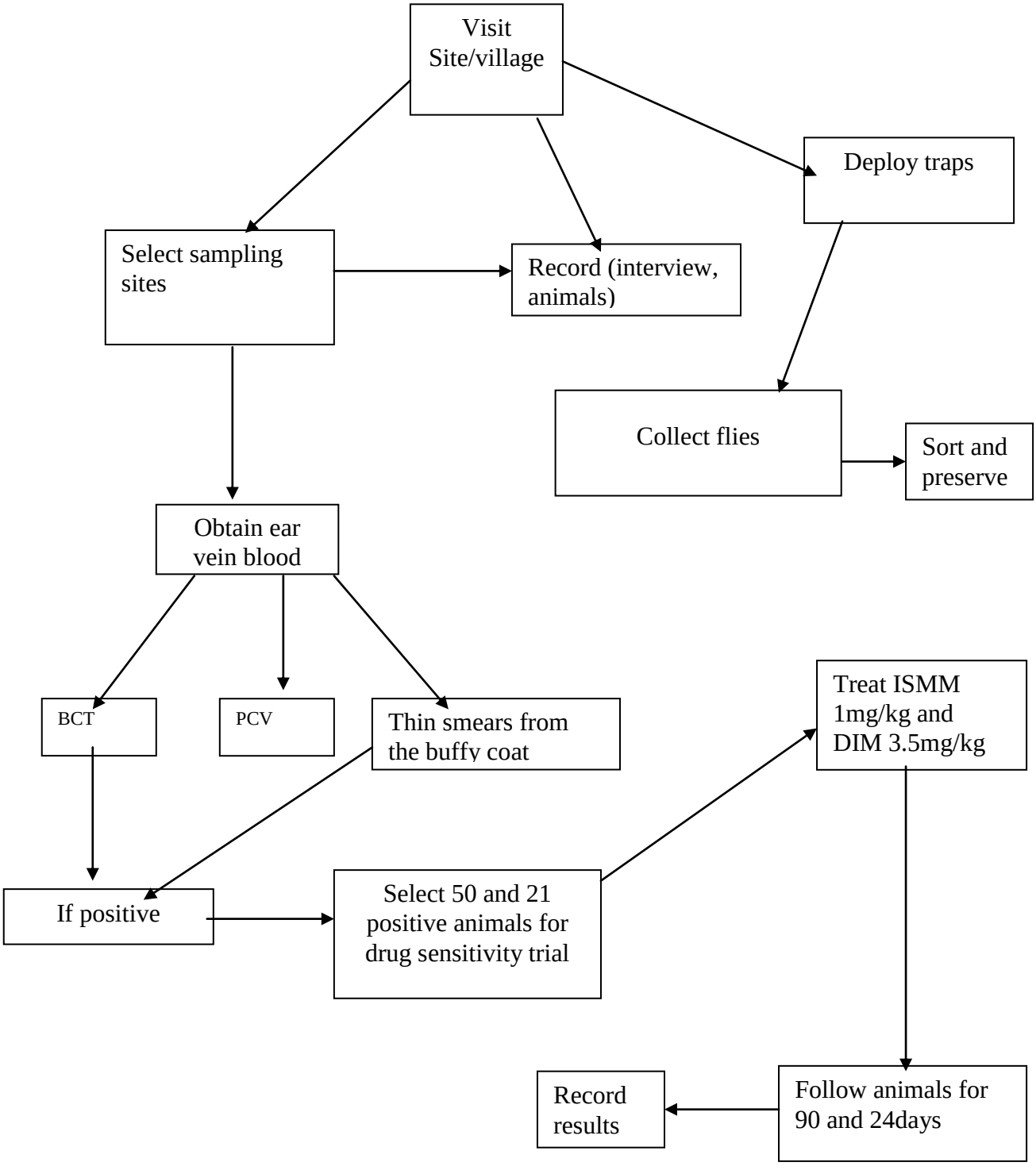
## 8. ANNEXES

Annex 1. Field flow chart of the study period



Annex 2. Questionnaire set to interview group of farmers.

Peasant Association…… Village……Date……Code……

**A. Livestock management**

1. What is the grazing management of your animals?

Free grazing
Tether
Stall feeding

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

……
…….

3. Where do animals graze? Is grazing communal or private?

……
…….

Where is the location of livestock watering point? (Stream, lake, pond, river, wheel)

……
……

Does the stream or river flow all around the year or it dries up in long dry season?

Season of stream availability ……..
Season it dries up…….

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

……
……...

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

……
……

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

……
…….

In which season is it least available?

……..
……..

10. What are the feed types available in different season?

| Season | Feed types |
|---|---|
| …… | …… |
| …… | …… |
| …… | …… |
| …… | …… |
| …… | …… |
| …… | …… |

**B. Livestock Diseases**

What are the most common diseases affecting your livestock?

…….
…….
……

Does trypanosomosis occur in this area? (Yes, no, other)?

If yes, what is the rank of trypanosomosis with regard to animal losses compared to other diseases?

……...
……...

Which livestock does trypanosomosis most affect?

Cattle (yes, no, other)
Sheep (yes, no, other)
Goat (yes, no, other)
Equine (yes, no, other)
Others (specify)

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

……...
……...

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

……...

Do you know that flies transmit trypanosomosis? (Yes, no, others)
If yes, which flies do you think to transmit trypanosomosis? Specify

……...
……...

In which season or month are such flies most abundant?

……...

Where is the fly population high?

In areas close to river
In the grazing scheme
In the bush
Around the lake
Around the pond
Around the barn
Others (specify)

**C. Treatment**

1.Where are the common treatment sources?

Veterinary clinic
Local farmers
Smugglers
Others (specify)

2. Who is applying the treatment?

Local farmers
Veterinarian, assistant veterinarian, animal health technicians
Smugglers
Others (specify)

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

……
……
……

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

……
……

Which ones are less effective?

……
……

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

All your animals
Only sick animals
Only mature oxen
Only cows in milk
Others (specify)

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

……
……

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

……..

Do you think that the problem of trypanosomosis is expanding to new areas?
(Yes, no, we do not know) If yes what are the new areas affected?

……

Thank You!

Name of interviewer ……..

Date…… Signature…….

Annex 3. Questionnaire Set to Interview Individual Farmers

Peasant Association…… Village……Date…….Code……

**A. Livestock management**

1. Which livestock do you keep?

a. Cattle (yes, no, other)
b. Sheep (yes, no, other)
c. Goat (yes, no, other)
d. Equine (yes, no, other)
e. Others (specify)

2. How many animals do you privately own?

a. Cattle……
b. Sheep ……
c. Goat ……
d. Equine ……
Others (specify) ……

3. How do you manage your animals?

a. Free grazing
b. Tether
c. Stall feeding

4. If you follow a free grazing system, is it in herd or in free groups?

…….

5. Where do animals graze?

…….

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

……..

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

……..

**B. Treatment**

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

At home
In vet. Clinic
Others (specify)

2. Who is applying the treatment?

You yourself
Veterinarian or assistant veterinarian
Animal health technician
Drug smugglers
Others (specify)

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

……

4. What quantity of trypanocidals do you use to treat your animals? (in tab, sachet etc.)
……
……

5. How much money do you pay to get a single mature animal treated? (Specify the species)

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

One times only
Two times
Three times

7. Can you tell how much expense is incurred in payment for treatment against

trypanosomosis since last year?

……
……
……

8. Of the cattle treated last time

How many are healthy at present?……..
When were these animals lastly treated? ?……..
Calculate days between treatment?……..

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

……

10. Do you have any trypanocidal medicine now in stock?
(Yes, no, other)
If yes can you show please?
How many months since you acquired it? …….
How do you use it? ……

Thank You!

Name of interviewer ……..

Date…… Signature…….

Annex 4. Format for recording of drug trial on DIM

| Owners Name | Cattle's name | Sex | Age | BCS | Species | BW | Dose in ml | Date of sampling | PCV on D0 | Date of treatment | PCV D6 | PCV D12 | PCV D18 | PCV D24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | |

Annex 5. Format for recording of drug trial on ISMM

| No | Owners Name | Animal ID | Sex | Age | BW | Species | PCV on D0 | Date of treatment | Dose | Parasitemia on Days post treatment | | | PCV on days post treatment | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 30 | 60 | 90 | 30 | 60 | 90 |
| | | | | | | | | | | | | | | | |

Annex 6. Data collection format for individual animals

Annex 7. Data collection format for fly identification

| Id | Age | Sex | BCS | Source | Parity | Pregnancy | Lactation | Abortion | PCV | Result | Trypanosoma species |
|---|---|---|---|---|---|---|---|---|---|---|---|

*g-grazing area, f-farmers village, s-swampy area, rb-river bank, br-barn, dr-dry area, ct-covered with trees/bushes, cg-cattle congregation area, hw-high way, wp-watering point.

| Pa | Trap | Code of sites that traps were deployed* | | | | | | | | | Name and Count of fly |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | g | f | s | rb | br | dr | ct | cg | hw | wp | |

Annex 8. PCV of cattle in the three districts (late rainy season)

| Variable | Obs | Mean | 95% CI | Remark (ANOVA) |
|---|---|---|---|---|
| Bahir Dar Zuria | 154 | 25.2* | 24.6-25.8* | P < 0.0001 |
| Dembia | 479 | 27.4* | 26.9-27.8* | |
| Fogera | 759 | 25.8* | 25.5-26.1* | |
| Total | 1,392 | 26.3 | 26.0-26.5 | |

- *Groups differed significantly

Annex 9. PCV of small ruminants in the three districts

| Species | District | Number of animals | Mean | 95% CI |
|---|---|---|---|---|
| Goats | | | | |
| | B/dar | 60 | 23.8 | 22.9-24.7 |
| | Dembia | 55 | 22.7 | 21.7-23.8 |
| | Fogera | 561 | 22.4 | 22.2-22.7 |
| Total | | 676 | 22.6* | 22.3-22.8 |
| Sheep | B/dar | 12 | 28.7 | 26.5-30.9 |
| | Dembia | 62 | 25.8 | 24.6-27.0 |
| | Fogera | 48 | 24.8 | 23.3-26.4 |
| Total | | 122 | 25.7* | 24.8-26.6 |

*P<0.01

Annex 10. PCV of female goats≥1 year partitioned by reproductive status.

| Grouping variable | Grouping variable status | n | Mean | 95% CI | Remark |
|---|---|---|---|---|---|
| Abortion | No abortion | 481 | 22.5 | 22.2-22.8 | P< 0.05 |
| | Abortion | 65 | 21.6 | 20.9-22.3 | |
| Lactation | Non-lactating | 256 | 22.9 | 22.5-23.3 | P <0.001 |
| | Lactating | 290 | 22.0 | 21.7-22.3 | |
| Pregnancy | Non-pregnant | 410 | 22.1 | 21.9-22.5 | P < 0.001 |
| | Pregnant | 136 | 23.2 | 22.7-23.7 | |
| Total | | 546 | 22.4 | 22.2-22.7 | |

Annex 11. PCV of cattle categorized by age and season.

Annex 12. Total fly catch, and apparent density in the three districts.

| Category Variable | Age (years) | Observation | Mean PCV | 95% CI | Remark |
|---|---|---|---|---|---|
| Age | < 2 | 106 | 24.5 | 23.6-25.4 | P>0.05 |
| | 2-4 | 329 | 25.4 | 24.9-25.8 | |
| | >4 | 1074 | 25.2. | 24.9-25.4 | |
| Season | Late rainy season | 592 | 25.2 | 24.9-25.5 | P<0.0001 |
| | Early dry season | 609 | 24.3 | 24.0-24.6 | |

*A.D. Apparent density (fly/trap/day) **Mean catch per trap/72 hrs

| Season/trap | | *Stomoxys* | NBM | Horse flies | *Chrysops* | Total |
|---|---|---|---|---|---|---|
| | Sum | 49120 | 13252 | 4699 | 1279 | 68350 |
| | Mean | 779.7 | 210.3 | 74.6 | 20.3 | |
| Late rainy season | A.D. | 259.9 | 70.1 | 24.9 | 6.8 | |
| | Sum | 233 | 2623 | 16 | 51 | 2923 |
| | Mean | 10.1 | 114.0 | 0.7 | 2.2 | |
| Early dry season | A.D. | 3.4 | 38.0 | 0.2 | 0.7 | |
| | Sum | 29019 | 13469 | 243 | 326 | 31994 |
| | Mean | 1450.9 | 204.1 | 12.15 | 16.3 | |
| Monoconical (n=20) | A.D. | 483.6 | 68.0 | 4.0 | 5.4 | |
| | Sum | 20334 | 13469 | 4472 | 1004 | 39279 |
| | Mean** | 308.1 | 204.1 | 67.7 | 15.2 | |
| NGU (n=66) | A.D*. | 102.7 | 68. | 22.6 | 5.1 | |
| | Sum | 49353 | 15875 | 4715 | 1330 | 71273 |
| | Mean | 573.9 | 184.6 | 54.8 | 15.5 | 828.8 |
| Total | A.D. | 191.3 | 61.5 | 18.3 | 5.2 | 276.3 |

Annex 13. Catchments of the Monoconical and NGU traps.

| Fly name | Trap type | No of trap | Mean [#] | 95% CI |
|---|---|---|---|---|
| Horse Flies | Monoconical+ | 14 | 2.2[**] | 1.5-2.9 |
| | NGU+ | 14 | 4.4[**] | 3.9-5.4 |
| *Stomoxys* | Monoconical | 14 | 6.8[*] | 5.6-8 |
| | NGU | 14 | 5.5[*] | 4.6-6.4 |
| *Chrysops* | Monoconical | 14 | 1.9 [a] | 0.9-2.9 |
| | NGU | 14 | 2.2[a] | 1.2-3.2 |
| NBM | Monoconical | 14 | 4.1[b] | 3.3-4.9 |
| | NGU | 14 | 4.9[b] | 4.0-5.7 |

** p<0.001 * p<0.05.a,b. Those with similar letters do not differ significantly.# Mean catch after logarithmic transformation of the data.+ Traps were deployed at uniform ecological sites and same period.

Annex 14. Fly catchments of the late rainy and early dry seasons at Fogera district.

| Fly name | Season | No of traps | Mean | 95% CI | Remark |
|---|---|---|---|---|---|
| *Stomoxys* | Late rainy season | 23 | 5.8 | 4.8-6.8 | P < 0.001 |
| | Early dry season | 23 | 1.7 | 1.1-2.3 | |
| NBM | Late rainy season | 23 | 4.5 | 3.7-5.2 | P>0.05 |
| | Early dry season | 23 | 3.91 | 3.3-4.6 | |
| Horse flies | Late rainy season | 23 | 3.7 | 3.0-4.3 | P <0.001 |
| | Early dry season | 23 | 0.3 | 0.03-0.5 | |
| *Chrysops* | Late rainy season | 23 | 2.8 | 2.1-3.5 | P <0.001 |
| | Early dry season | 23 | 0.8 | 0.4-1.2 | P <0.0005 |

*Data after logarithmic transformation.

Annex 15. Fly catchments of the NGU trap in the late rainy and early dry seasons at Fogera district.

| Fly tribe | Season | Trap | Mean | 95% CI | Remark |
|---|---|---|---|---|---|
| *Stomoxys* | Late rainy season | 17 | 420.3 | 195.8-644.7 | P<0.01 |
| | Early dry season | 17 | 8.6 | 2.0-15.1 | |
| NBM | Late rainy season | 17 | 218.4 | 49.9-386.9 | P>0.05 |
| | Early dry season | 17 | 101.9 | 48.0-155.7 | |
| Horse flies | Late rainy season | 17 | 111.5 | 44.2-178.9 | P<0.01 |
| | Early dry season | 17 | 0. 9 | 0.3-2.0 | |
| *Chrysops* | Late rainy season | 17 | 43.7 | 6.7-80.7 | P<0.05 |
| | Early dry season | 17 | 2.4 | 0.4 -4.3 | |

Annex 16. Fly catchments of the Monoconical trap in the late rainy and early dry seasons at Fogera district

| Fly tribe | Season | Trap | Mean | 95% CI | Remark (t test) |
|---|---|---|---|---|---|
| *Stomoxys* | Late rainy season | 6 | 3567.3 | 876.0-6258.7 | P<0.01 |
| | Early dry season | 6 | 14.5 | -0.8-29.8 | |
| NBM | Late rainy season | 6 | 184.8 | 42.1-327.5 | P>0.05 |
| | Early dry season | 6 | 148.5 | -34.3-31.3 | |
| Horse flies | Late rainy season | 6 | 33.5 | -8.9-75.9 | P<0.05 |
| | Early dry season | 6 | 0.2 | -0.3-0.6 | |
| *Chrysops* | Late rainy season | 6 | 36.3 | 1.8-70.8 | P<0.05 |
| | Early dry season | 6 | 1.8 | 0.02-3.6 | |

Annex 17. Fly catch records of the NGU and Monoconical traps

| Trap pairs | Flies Genera / tribe/ name | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Horseflies* | | *Stomoxys* | | *Chrysops* | | NBM | |
| | Mono | NGU | Mono | NGU | Mono | NGU | Mono | NGU |
| Pair 1 | 13 | 178 | 2154 | 280 | 0 | 0 | 73 | 168 |
| Pair 2 | 7 | 6 | 1460 | 570 | 2 | 0 | 22 | 186 |
| Pair 3 | 2 | 54 | 96 | 555 | 0 | 2 | 52 | 667 |
| Pair 4 | 4 | 447 | 404 | 440 | 0 | 5 | 20 | 100 |
| Pair 5 | 14 | 684 | 1006 | 769 | 5 | 6 | 55 | 127 |
| Pair 6 | 8 | 368 | 1671 | 696 | 10 | 80 | 66 | 1000 |
| Pair 7 | 100 | 446 | 1534 | 1042 | 93 | 23 | 74 | 591 |
| Pair 8 | 68 | 138 | 3882 | 108 | 42 | 31 | 412 | 46 |
| Pair 9 | 10 | 132 | 2238 | 676 | 50 | 213 | 86 | 348 |
| Pair 10 | 5 | 115 | 8426 | 353 | 16 | 9 | 287 | 340 |
| Pair 11 | 10 | 99 | 1757 | 474 | 7 | 3 | 149 | 124 |
| Pair 12 | 10 | 25 | 3567 | 25 | 90 | 101 | 101 | 10 |
| Pair 13 | 1 | 6 | 736 | 61 | 0 | 1 | 118 | 27 |
| Pair 14 | 0 | 2 | 1 | 3 | 0 | 0 | 0 | 8 |
| Total catch per trap | 252 | 2700 | 28932 | 6052 | 315 | 474 | 1515 | 3742 |

Horse Flies include genera's of the tribe Tabanini other than Chrysopini and *Hematopota*.

Annex 18. Fly catches between the late rainy and early dry seasons at Fogera District

| Traps*** deployed at the same site | Name of genera / tribe / family / | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *Horse Flies | | *Chrysops* | | *Stomoxys* | | Non-biting Muscidae | |
| | Season | | | | | | | |
| | Rainy+ | Dry** | Rainy | Dry | Rainy | Dry | Rainy | Dry |
| 1 | 87 | 0 | 6 | 13 | 570 | 0 | 99 | 4 |
| 2 | 416 | 0 | 209 | 4 | 486 | 1 | 1316 | 1 |
| 3 | 8 | 0 | 10 | 3 | 1534 | 10 | 74 | 200 |
| 4 | 446 | 1 | 80 | 3 | 1042 | 43 | 591 | 93 |
| 5 | 138 | 0 | 23 | 2 | 108 | 0 | 46 | 10 |
| 6 | 3 | 0 | 0 | 9 | 5 | 0 | 2 | 74 |
| 7 | 100 | 0 | 93 | 1 | 3882 | 19 | 412 | 8 |
| 8 | 68 | 1 | 42 | 6 | 2238 | 16 | 86 | 470 |
| 9 | 132 | 0 | 31 | 0 | 676 | 2 | 348 | 270 |
| 10 | 21 | 0 | 7 | 3 | 53 | 4 | 88 | 255 |
| 11 | 115 | 3 | 213 | 0 | 353 | 35 | 340 | 312 |
| 12 | 5 | 0 | 50 | 0 | 8426 | 1 | 287 | 31 |
| 13 | 140 | 9 | 135 | 0 | 1580 | 20 | 364 | 166 |
| 14 | 4 | 1 | 0 | 0 | 0 | 12 | 0 | 150 |
| 15 | 6 | 0 | 2 | 0 | 13 | 0 | 8 | 10 |
| 16 | 14 | 0 | 9 | 0 | 824 | 2 | 152 | 34 |
| 17 | 116 | 0 | 5 | 0 | 284 | 10 | 127 | 208 |
| 18 | 10 | 0 | 16 | 0 | 1757 | 1 | 149 | 38 |
| 19 | 99 | 0 | 9 | 0 | 474 | 5 | 124 | 64 |
| 20 | 110 | 1 | 8 | 0 | 566 | 0 | 65 | 9 |
| 21 | 24 | 0 | 3 | 0 | 86 | 4 | 33 | 60 |
| 22 | 10 | 0 | 7 | 0 | 3567 | 40 | 101 | 144 |
| 23 | 25 | 0 | 3 | 0 | 25 | 8 | 10 | 12 |
| Total | 2097 | 16 | 961 | 44 | 28549 | 233 | 4822 | 2623 |

* Horse Flies include genera's of the tribe Tabanini other than Chrysopini and *Hematopota*.

+Rainy- Late rainy season, **Dry- early dry season.

*** 23 traps deployed at a similar specific sites during the late rainy season were again deployed in the early dry season.

Annex 19. Records on results of ISMM drug trail

| No | Owners Name | Animal ID | Sex | Age | BW | Species | PCV D0 | Date of treatment | Dose in ml | Parasitemia On days 30 | 60 | 90 | PCV post treatment on days 30 | 60 | 90 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Shumet Getu | Gurum | f | 6 | 200 | *T.vivax* | 26 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 25 | 26 | 30 |
| 2 | Bizualem Damite | Timula | f | 12 | 150 | *T.vivax* | 17 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 22 | 23 | 21 |
| 3 | Mekuanint Zeleke | Timula | f | 1.5 | 100 | *T.vivax* | 22 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 22 | 23 | 22 |
| 4 | Asmare Fentahun | Mechal | f | 5 | 150 | *T.vivax* | 24 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 25 | 25 | 29 |
| 5 | Shumet Damite | Zeger | m | 7 | 200 | *T.vivax* | 25 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 27 | 27 | 27 |
| 6 | Hailu Zewidineh | Muday | f | 7 | 160 | *T.vivax* | 23 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 25 | 26 | 28 |
| 7 | Sendeku Wasse | Kolba | m | 8 | 160 | *T.vivax* | 23 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 22 | 23 | 21 |
| 8 | Awoke Belay | Felika | f | 5 | 150 | *T.vivax* | 25 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 24 | 24 | 26 |
| 9 | Belete Tegegne | Worka | f | 5 | 150 | *T.vivax* | 18 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 21 | 26 | 20 |
| 10 | Melese Belay | Yimer | m | 8 | 200 | *T.vivax* | 24 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 24 | 26 | 30 |
| 11 | Gebru Belay | Sale-Egizer | m | 5 | 160 | *T.vivax* | 19 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 21 | 23 | 24 |
| 12 | Sendeku Wasse | Birile | f | 7 | 200 | *T.vivax* | 26 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 27 | 28 | 28 |
| 14 | Shitawmar Zeleke | Kolba | m | 3 | 150 | *T.vivax* | 26 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 23 | 23 | 27 |
| 15 | Kassewmar Getu | Setegn | m | 6 | 125 | *T.vivax* | 17 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 18 | 21 | 23 |
| 16 | Getu Amare | Atalay | f | 9 | 150 | *T.vivax* | 19 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 21 | 21 | 22 |
| 17 | Ambaw Getu | Adem | m | 5 | 150 | *T.vivax* | 24 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 27 | 28 | 25 |
| 18 | Zigale Wube | Merkeb | m | 4 | 150 | *T.vivax* | 26 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 24 | 24 | 24 |
| 19 | Ayanaw Engidaw | Shemila | m | 7 | 125 | *T.vivax* | 20 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 24 | 26 | 26 |
| 20 | Ayanaw Engidaw | Yibir | m | 5 | 125 | *T.vivax* | 26 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 27 | 28 | 28 |
| 21 | Bizuneh Mekuanin | Damena | m | 6 | 150 | *T.vivax* | 23 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 25 | 25 | 29 |
| 22 | Nigat Engidaw | Mateb | f | 5 | 100 | *T.vivax* | 24 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 26 | 27 | 30 |
| 23 | Mogninet Bizuneh | Adey | f | 3 | 100 | *T.vivax* | 23 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 23 | 24 | 28 |
| 24 | Yeneneh Ande | Kolba | m | 1 | 75 | *T.vivax* | 19 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 23 | 23 | 24 |
| 25 | Getish Bizuneh | Birile | f | 5 | 150 | *T.vivax* | 26 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 27 | 28 | 33 |
| 26 | Melkie Melese | Tikim | f | 3 | 100 | *T.vivax* | 24 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 24 | 29 | 29 |
| 27 | Simegnew Ande | Wolela | f | 7 | 150 | *T.vivax* | 19 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 21 | 24 | 25 |
| 28 | Tafete Belete | Saso | f | 6 | 125 | *T.vivax* | 22 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 22 | 24 | 27 |
| 29 | Teka Worku | Kokeb | m | 8 | 175 | *T.vivax* | 23 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 25 | 27 | 28 |
| 30 | Andalew Deres | Shasho | m | 3 | 100 | *T.vivax* | 21 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 23 | 23 | 25 |
| 31 | Amare Muche | Mechal | f | 8 | 125 | *T.vivax* | 15 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 23 | 19 | _ |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 32 | Nigat Engidaw | Shasho | m | 3 | 75 | *T.vivax* | 23 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 26 | 27 | 26 |
| 33 | Adebabay Worku | Zimnew | f | 5 | 150 | *T.vivax* | 25 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 28 | 29 | 30 |
| 34 | Nigus Belay | Gurum | m | 4 | 150 | *T.vivax* | 26 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 25 | 27 | 28 |
| 35 | Abere Dagnew | Wasnesh | f | 10 | 175 | *T.vivax* | 25 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 24 | 28 | 28 |
| 36 | Siyum Sendek | Setegn | m | 8 | 200 | *T.vivax* | 20 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 25 | 26 | 31 |
| 37 | Nigus Belay | Mateb | f | 3 | 100 | *T.vivax* | 25 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 19 | 26 | 30 |
| 38 | Tigabu Belete | Kinmewal | m | 7 | 175 | *T.vivax* | 17 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 25 | 27 | 28 |
| 39 | Tigabu Belete | Nurilign | m | 4 | 150 | *T.vivax* | 20 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 21 | 23 | 24 |
| 40 | Tafete Belete | Marwiha | m | 7 | 150 | *T.vivax* | 26 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 28 | 30 | 27 |
| 41 | MuCheye Demis | Nega | m | 7 | 125 | *T.vivax* | 19 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 19 | 19 | 19 |
| 42 | Yeneneh Ande | Setegn | m | 5 | 150 | *T.vivax* | 26 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 24 | 22 | 23 |
| 43 | MuCheye Demis | Enjeraw | m | 7 | 150 | *T.vivax* | 26 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 26 | 29 | 28 |
| 44 | Asmamaw Nigus | Bule | m | 4 | 100 | *T.vivax* | 23 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 24 | 23 | 26 |
| 45 | Nigat Engidaw | Dinkuan | f | 9 | 125 | *T.vivax* | 26 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 27 | 31 | 32 |
| 46 | Kibiret Engidaw | Gurum | f | 8 | 125 | *T.vivax* | 24 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 24 | 28 | 30 |
| 47 | Ambaw Getu | Shibab | f | 8 | 150 | *T.vivax* | 24 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 25 | 24 | 25 |
| 48 | Ayanaw Engidaw | Kolo | m | 2.5 | 50 | *T.vivax* | 23 | 1/3/96 | 1mg/kg | 0 | 0 | 0 | 25 | 28 | 29 |

Annex 20. Records on the results of DIM drug trial.

| No | Owners Name | Cattle's Name | Sex | Age | BCS | Species | BW | Dose (ml) | PCV on Sampling | Date of Treatment | PCV On Days | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | 0 | 6 | 12 | 18 | 24 |
| 1 | Alemu Lakew | Cheru | M | 9 | 1 | *T.vivax* | 225 | 11 | 20 | 1/5/96 | 20 | 22 | 20 | 23 | 23 |
| 2 | Genet worikineh | Workie | M | 8 | 1 | *T.vivax* | 175 | 8 | 20 | 1/5/96 | 19 | 19 | 22 | 22 | 23 |
| 3 | Misiganaw Kidane | Mekendo | F | 3 | 1 | *T.vivax* | 80 | 4 | 23 | 1/5/96 | 25 | 25 | 23 | 23 | 25 |
| 4 | Adugnaw Fekade | Nega | M | 8 | 1 | *T.vivax* | 200 | 10 | 17 | 1/5/96 | 18 | 20 | 19 | 18 | 20 |
| 5 | Tseganew Abate | Adege | M | 8 | 2 | *T.vivax* | 225 | 11 | 20 | 1/5/96 | 19 | 22 | 22 | 20 | 22 |
| 6 | Sendeku Getu | Mateb | F | 4 | 1 | *T.vivax* | 125 | 6 | 19 | 1/5/96 | 18 | 22 | 20 | 20 | 21 |
| 7 | Asires Tsige | Saso | F | 6 | 2 | *T.vivax* | 175 | 8 | 24 | 1/5/96 | 23 | 23 | 22 | 22 | 24 |
| 8 | Asires Tsige | Kolba | M | 1.5 | 1 | *T.vivax* | 50 | 2.5 | 21 | 1/5/96 | 21 | 22 | 23 | 24 | 25 |
| 9 | Gizachew Widineh | Degwas | F | 7 | 2 | *T.vivax* | 180 | 8.5 | 18 | 1/5/96 | 19 | 19 | 23 | 23 | 25 |
| 10 | Takele Kassie | Teka | M | 6 | 2 | *T.vivax* | 200 | 10 | 21 | 1/5/96 | 20 | 20 | 22 | 21 | 22 |
| 11 | Takele Kassie | Mateb | F | 3 | 4 | *T.vivax* | 150 | 7.5 | 24 | 1/5/96 | 22 | 22 | 23 | 24 | 24 |
| 12 | Hailu Zewidineh | Menorget | F | 3 | 3 | *T.vivax* | 100 | 5 | 17 | 1/5/96 | 17 | 17 | 23 | 24 | 26 |
| 13 | Atalay Dejen | Midir | F | 4 | 2 | *T.vivax* | 150 | 7.5 | 24 | 1/5/96 | 24 | 24 | 24 | 25 | 25 |
| 14 | Chakilo Negirew | Mateb | F | 4 | 1 | *T.vivax* | 175 | 9 | 18 | 1/5/96 | 16 | 16 | 15 | 17 | 18 |
| 15 | Mengistu Negirew | Zenge | M | 4 | 3 | *T.vivax* | 180 | 9 | 20 | 1/5/96 | 18 | 19 | 21 | 21 | 22 |
| 16 | Melesse Agimasu | Marew | M | 5 | 2 | *T.vivax* | 200 | 10 | 19 | 1/5/96 | 20 | 22 | 22 | 22 | 23 |
| 17 | Misiganaw Bayile | Desalegn | M | 10 | 2 | *T.vivax* | 200 | 10 | 19 | 1/5/96 | 18 | 19 | 18 | 20 | 21 |
| 18 | Asrat Molla | Kolba | M | 4 | 1 | *T.vivax* | 180 | 9 | 19 | 1/5/96 | 19 | 21 | 21 | 21 | 22 |
| 19 | Asrat Molla | Nega | M | 8 | 1 | *T.vivax* | 250 | 12 | 19 | 1/5/96 | 20 | 17 | 18 | 19 | 20 |

Annex 21. Mean PCV of cattle on days post treatment of ISMM or DIM

| Drug Type | Days post treatment | Number of animals | Mean PCV | 95% CI |
|---|---|---|---|---|
| Isomethamidium chloride | 0 | 48 | 22.8 | 21.9-23.6 |
| | 30 | 48 | 23.9 | 23.2-24.6 |
| | 60 | 48 | 25.2 | 24.4-26.1 |
| | 90 | 47 | 26.4 | 25.4-27.3 |
| Diminazine aceturate | DS* | 19 | 20.1 | 19.0-21.2 |
| | 0 | 19 | 19.8 | 18.7-20.9 |
| | 6 | 19 | 20.6 | 19.4-21.8 |
| | 12 | 19 | 21.1 | 20.0-22.2 |
| | 18 | 19 | 21.5 | 20.5-22.6 |
| | 24 | 19 | 22.7 | 21.7-23.7 |

*DS- Date of screening

Annex 22. T-test results of comparison between days post treatment of ISMM and DIM

| Drug Type | Days post treatment compared (t- test) | t value | Level of Significance |
|---|---|---|---|
| Isomethamidium chloride | day0=day30 | t =3.0912 | P < 0.01 |
| | day0=day60 | t =6.0568 | P < 0.0001 |
| | day0=day90 | t =8.4805 | P < 0.0001 |
| | day30=day60 | t =4.8477 | P < 0.0001 |
| | day30=day90 | t =6.2600 | P < 0.0001 |
| | day60=day90 | t = 3.4838 | P < 0.001 |
| Diminazine aceturate | *SD=day0 | t =1.1895 | P > 0.05 |
| | SD=day6 | t = -1.1630 | P > 0.05 |
| | SD=day12 | t = -1.9494 | P < 0.05 |
| | SD=day18 | t = -2.9266 | P < 0.01 |
| | SD=day24 | t = -4.9027 | P < 0.001 |
| | day0=day6 | t = -2.2757 | P < 0.05 |
| | day0=day12 | t = -2.7533 | P < 0.01 |
| | day0=day18 | t = -3.7418 | P < 0.001 |
| | day0=day24 | t = -6.0696 | P < 0.0001 |
| | day0=day6 | t = -2.2757 | P < 0.05 |
| | day0=day12 | t = -2.7533 | P < 0.01 |
| | day6=day18 | t = -3.7418 | P < 0.001 |
| | day6=day24 | t = -6.0696 | P < 0.0001 |
| | day12=day18 | t = -1.5689 | P > 0.05 |
| | day12=day24 | t = -7.1600 | P < 0.0001 |
| | day18=day24 | t = -7.3333 | P < 0.0001 |

*DS- Date of screening

Annex 23. Pictures of materials, study animals and parasites

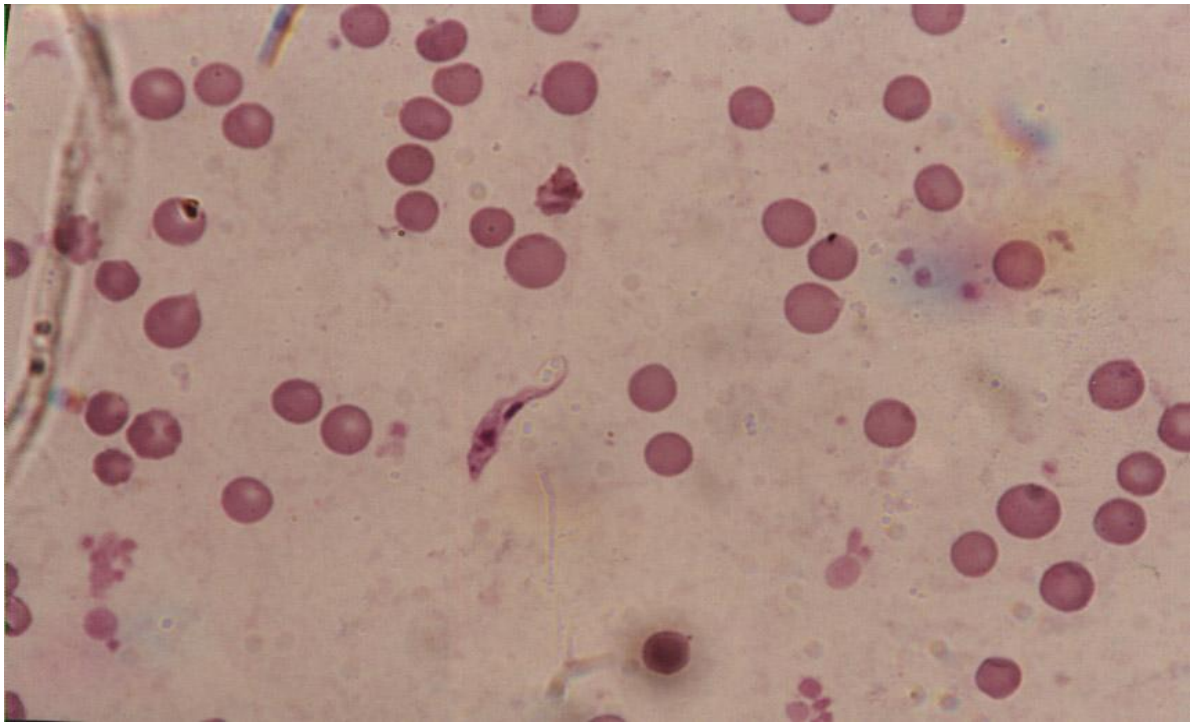


Plate 1. *T. vivax* from cattle at Abuanakokit, Fogera district

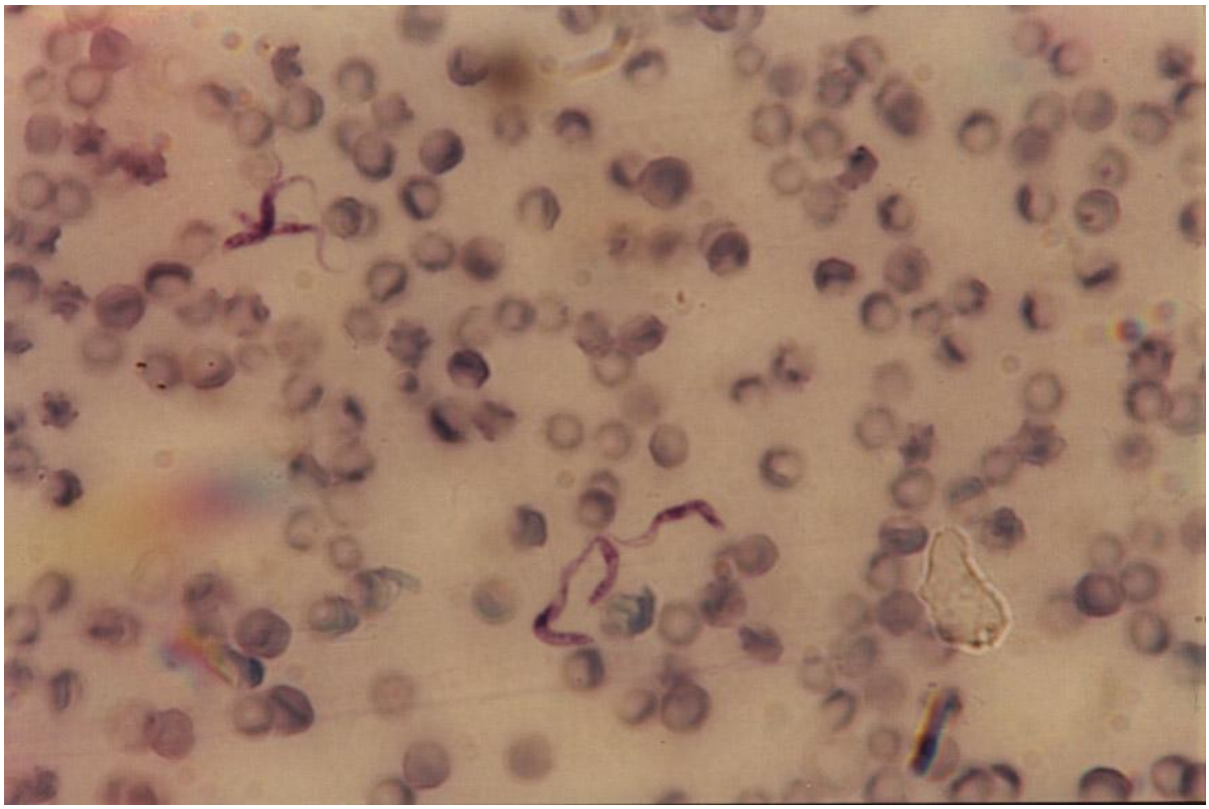


Plate 2. *T. vivax* from cattle at Quahar, Fogera district

Plate 3. An ox positive for *T. vivax* and subjected for ISMM drug trial.

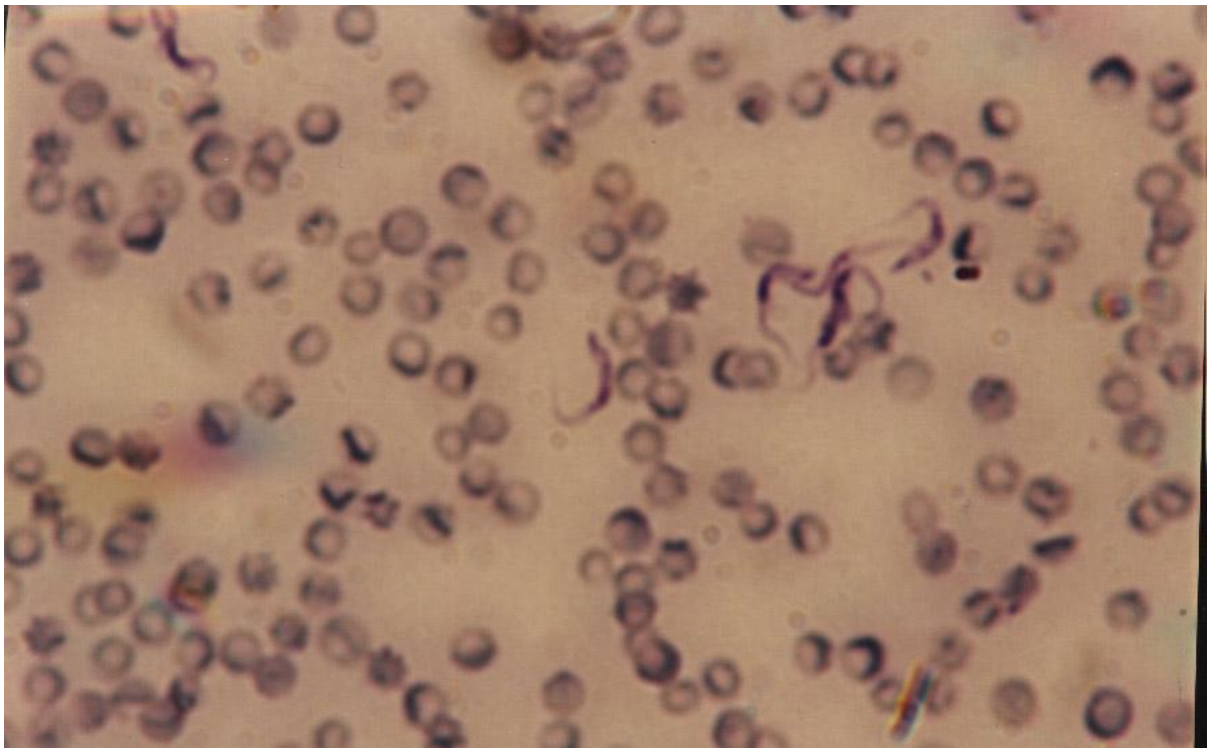


Plate 4. *T. vivax* from cattle at Shina, Fogera dustrict

Plate 5. Deployment of the NGU trap at Woramit, Bahir Dar district

Plate 6. Cattle grazing near the swampy areas of lake Tana

Plate 7. Sampling of Donkeys Abua village, Fogera district

Plate 8. A cow with *T. vivax* at Shina, Fogera, subjected to drug trial of ISMM

Plate 9. Deployment of the monoconical trap

Plate 10. Sampling of animals after ISMM injection at Shina, Fogera district

Plate 11. Sampling of sheep grazing with cattle near a swampy area, Kokit village, Fogera district

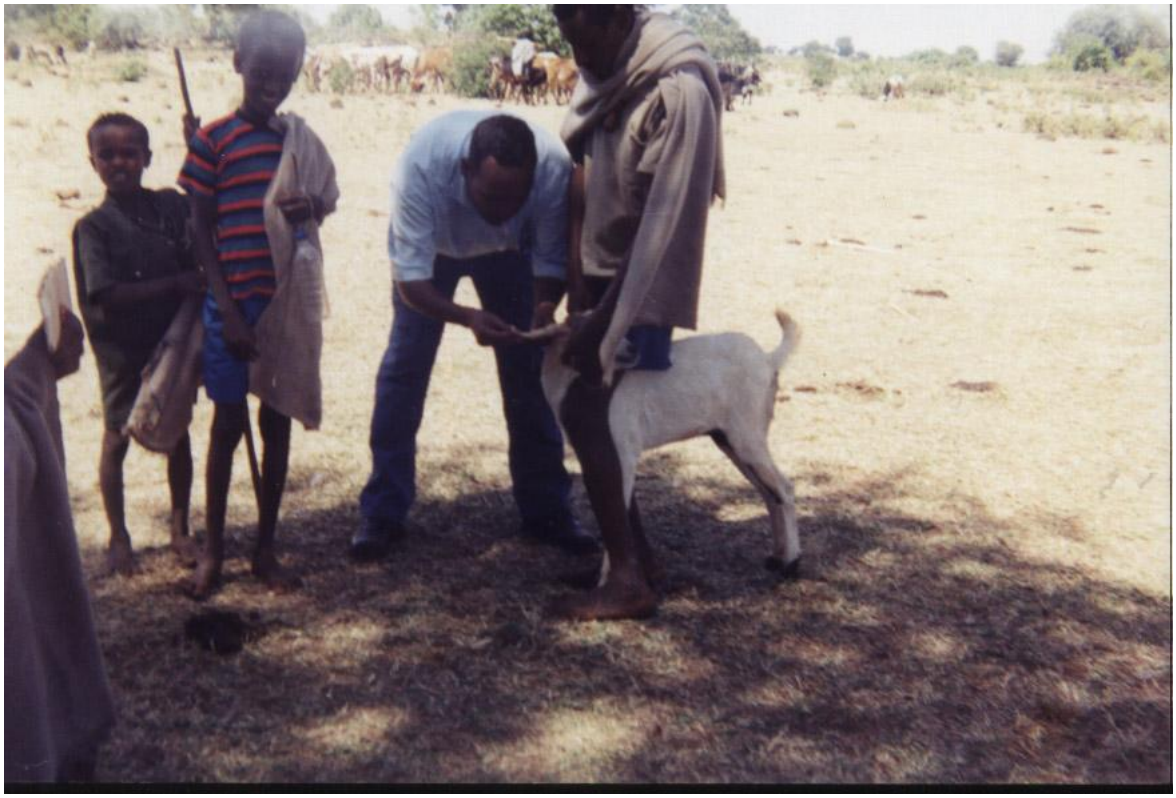


Plate 12. Sampling of goats herded with cattle, Woramit, Bahir Dar district

Plate. 13. *Hipobbosca variegata* collected from body of cattle, Tezeba, Dembia (October, 2003)

Plate14. *Chrysops streptobalia*, head and thorax, Quahar, Fogera district (November, 2003)

Plate 15. *Chrysops streptobalia*, wing patterns, Sebatamit, Bahir Dar (November, 2003)

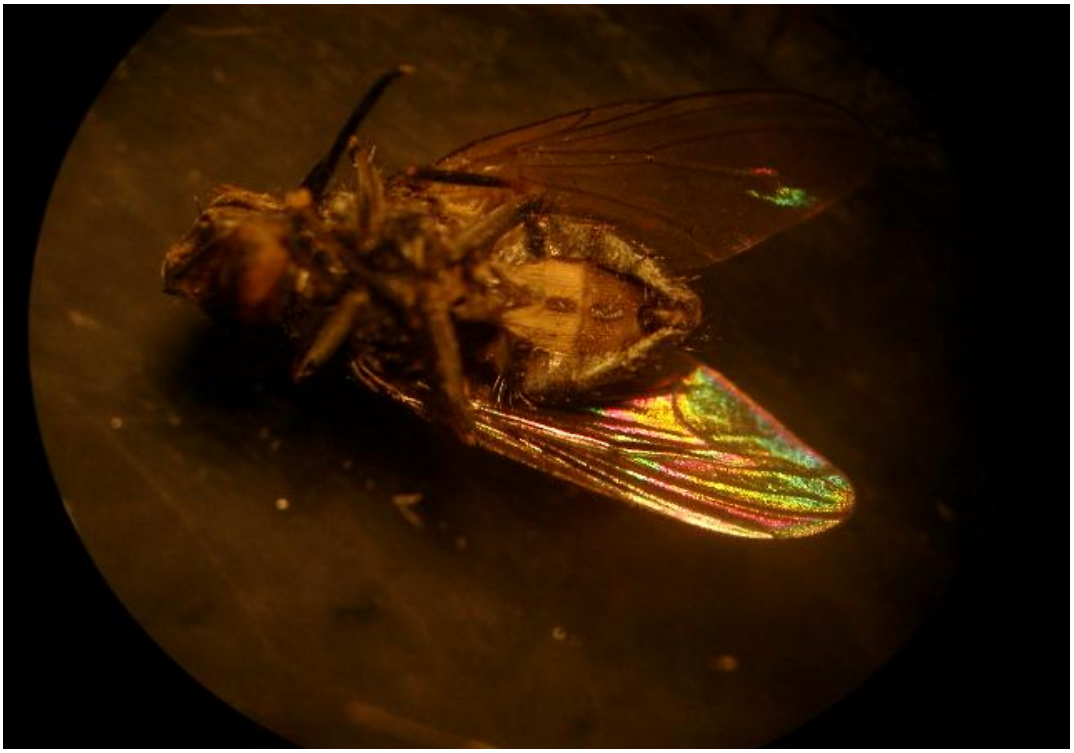


Plate 16. *Stomoxys*, ventral part, Guramba, Dembia (October, 2003).

Plate 17. *Atylotus agrestis* (frontal part of the head, and fading eye band)

Plate 18. *Atylotus agrestis*, dorsal part (wing and yellow stripes of abdomen)

## 9. CURRICULUM VITAE

**Personal Information:**

Nationality: Ethiopian

Sex: Male

Place of birth: Gojjam, Ethiopia

Date of birth: May 27, 1968

Religion: Christian of the Orthodox Church

Marital status: Single

Number of children: One

Contact address: Alekaw Sinshaw

P.O.Box: 55147

Addis Ababa, Ethiopia

E-mail: sinshaw_t @ yahoo.com

**Education:**

| Name of School | Year | Award |
|---|---|---|
| Faculty of Veterinary Medicine | 1986-90 | DVM degree |
| Faculty of Science, AAU | 1985 | Pre-veterinary transcript |
| Debremarkos High School | 1982-84 | National School leaving certificate |
| Finoteselam High School | 1981 | School Certificate |
| Dembecha Junior School | 1979-80 | National Certificate |
| Shellel Elementary School | 1973-78 | National Certificate |

**Professional experience:**

***June, 2000 to September, 2002****:* Coordinator of the National Livestock Development Project and Disease Surveillance and Economics Expert at the Agricultural Bureau of the Amhara National Regional State, Bahir Dar, Ethiopia.

***May,1999 to June, 2000****:* Research Officer at Bahir Dar Regional Veterinary Laboratory ,Amhara National Regional State, Bahir Dar, Ethiopia.

***September, 1993 to April, 1999****:* Technical Coordinator and Veterinary Officer of Metekel Cattle Breeding and Improvement Ranch and instructor of trainees on artificial insemination in collaboration with the National Artificial Insemination Center, Chagni, Ethiopia.

***November, 1991 to April , 1992*** *:* Veterinarian at the Agricultural Department of East Gojjam Zone , Amhara National Regional State, Debre Markos, Ethiopia.

***May, 1992 to August 1993****:* Veterinarian at the Agricultural Department of East Gojjam Zone, Amhara National Regional State, Dejen, Ethiopia.

**Certificates awarded:**

A certificate from ILRI-KETRI for the participation in the course of trypanosomosis and tsetse control, (August, 2002, Nairobi, Kenya).

Two certificates from the Ministry of Agriculture for instructing trainees on artificial insemination (1995 and 1997).

A certificate from Professionals United Together Computer Center for completing the courses on MsDose, Windows, Spreadsheet and Access computer programs (2001).

**Languages:**

English-Written and Spoken
Amharic-Written and Spoken

**Membership:** Member of the Ethiopian Veterinary Association

**Hobbies:** Reading, Movies and Swimming

**Research Activities and publications:**

Epidemiological investigation of trypanosomosis (*T. vivax)* in domestic animals of three districts bordering lake Tana, Ethiopia (MSc thesis FVM, AAU, June, 2004)

Study on ethnoveterinary practices in east Gojjam zone of ANRS; Ethiopia (presented at a workshop held in Kombolcha , ANRS January 2001 ).

Study on progeny history and productivity of ruminants in east Gojjam zone of ANRS; Ethiopia (presented at a workshop held in Kombolcha , ANRS January 2001 ) .

The effect of *Glinus lotoides* on fascioliasis in sheep around Bahir Dar (2000-2001).

Sero-epidemiological study of ovine brucellosis and its effect on fertility in Northwestern Amhara Region, Ethiopia (2000).

Sero-epidemiological study of caprine brucellosis and its effect on fertility in Northwestern Amhara region of Ethiopia (2000).

Distribution of ticks and tick born diseases of cattle at Metekel area, Ethiopia; Published in *Journal of the Ethiopian veterinary association* (Vol. I V No.1, 2000 PP: 40_ 60).

Reproductive performance and estrus behavior of Fogera breed cattle at Metekel ranch, Ethiopia (Published in the proceedings of the $12^{th}$ annual conference of the Ethiopian Veterinary Association, June, 1998 PP: 75_92).

The relationship between parasitosis and body condition score of sheep in the highlands of East Gojjam Zone, Ethiopia (Presented and submitted to the Bureau of Agriculture, ANRS, Bahir Dar, Ethiopia, April, 1996).

The effect of plane of nutrition on growth, puberty, estrus behavior, and subsequent fertility in Zebu X Fresian heifers of the Ethiopian highlands (DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, June, 1991, Debre Zeit, Ethiopia)

## 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 university

Name_____

Signature_____

Date of submission_____

This thesis has been submitted for examination with my approval as university advisor

Advisor:

Prof. Getachew Abebe _____