ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

STUDIES ON BOVINE TRYPANOSOMOSIS AND THERAPEUTIC EFFICACY OF SELECTED TRYPANOCIDAL DRUGS IN BIRBIR VALLEYOF GAWO-DALLE DISTRICT, WEST OROMIA

By

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A thesis submitted to the school of Graduate Studies of Addis Ababa University in partial fulfillments of the requirements for the attainments of the Degree of Masters of Veterinary Science in Tropical Veterinary Parasitology

JUNE 2008

DEBRE ZEIT, ETHIOPIA

STUDIES ON BOVINE TRYPANOSOMOSIS AND THERAPEUTIC EFFICACY OF SELECTED TRYPANOCIDAL DRUGS IN BIRBIR VALLEYOF GAWO-DALLE DISTRICT, WEST OROMIA

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JUNE 2008

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ACKNOWLEDGMENT

Above all thanks to almighty GOD for he saved and protected me in all difficult situations and environment, and for he gave me health and power to accomplish my research work.

I am particularly indebted to Dr. Yacob Hailu and Dr. Dinka Ayana my advisors for their intellectual guidance and unreserved advice, motivation and courage to work on the trypanosomosis, tsetse distribution and drug sensitivity tests in experimentally infected cattle. I would like also to express great thanks from my heartfelt for devotion of their time to go through the manuscript and provision of reference materials.

My specials thank goes to Associate Dean Office for Research and Graduate Studies of Faculty of Veterinary Medicine for its financial support and overall guidance.

My sincere gratitude and great appreciation is to NTTICC for the financial, transportation and material support given to this research work, without which it could not be fulfilled. In addition to this my special word of thanks goes to Mr. Getachew Abichu and Mr.Abebe Olani members of NTTICC for their skillful technical assistance in data collection on the field and experimental works at the Centre and all staffs of NTTICC for their incredible support.

I am highly grateful to Dr, Moses N. Kyule, and Dr. Getahun Ejeta for their assistance in data management. I would like to thank Dr. Hagos Ashenafi and Dr. Bersisa Kumsa, for the encouragement and other supports they provided. I would like to thank Federal Ministry of Agriculture and Rural Development for sponsoring the post graduate course and for the support provided to me to pursue the course.

Special thanks are to my wife Bidiktu Tassisa, for her encouragement and love and my doughters Bontu and Egeree and my son Moata Waktole for their patience during my absence from home.

I would like to thank Agricultural and Rural development Office of Gawo-Dalle district and Kellem Wollega zone Agricultural and rural development desk for their cooperation.

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ABBREVIATIONS

AAT	African Animal Trypanosomosis
Ab-ELISA	Antibody Enzyme linked immunosorbent assay
Ag-ELISA	Antigen Enzyme linked immunosorbent assay
CFT	Complement fixation test
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxy nucleic acid
EDTA	Ethylene Diamine Tetracetic Acid
EEC	European Economic Commission
FAO	Food and Agriculture Organization of the United Nations
FITCA	Farming In Tsetse Controlled Areas
FTD	Fly per trap per day
GPS	Geographical positioning system
IAEA	International Atomic Energy Agency
IBAR	Inter-African Bureau for Animal Research
ILRI	International Livestock Research Institute
ICIPE	International Center for Insect physiology and Ecology
ISMM	Isomethamidium chloride
NTTICC	National Tsetse and Trypanosomosis Investigation and Control Centre
NAHRC	National Animal Health Research Center
PAAT	Programme Against African Trypanosomiasis
PCR	Polymerase chain reaction
PCV	Packed Cell Volume
SIT	Sterile Insect Technique
VSG	Variable Surface Coat Glycoprotein

ABSTRACT

A cross sectional study was carried out to determine the prevalence of bovine trypanosomiasis and tsetse apparent density using parasitological, entomological and questionnaire survey in Birbir valley Gawo Dalle district. In addition efficacy of trypanocidal drugs were evaluated in experimentally infected cattle. The study was conducted from October2007 to March 2008. The drug sensitivity experimental trial was conducted on 15 cattle divided into three groups each with five animals at National Tsetse and Trypanosomsois Investigation and Control Centre in Bedelle. Results of parasitological study revealed an overall prevalence 13.4% of bovine trypanosomosis in the study area. In the study *T. congolense* (71.8%) was identified as the dominant species followed by *T. brucei* (14.1%), *T.vivax* (10.7%) and mixed spp. (2.5%). In the study statistically significant difference in the prevalence of trypanosomosis among the study sites(X^2 = 122.112, P = P 0.000, P < 0.001) and seasons (X^2 =14.646, P=.041, P< 0.05) was observed while there was no variation between sex of animals (X^2=6.994, P=.430, P>0.05). A mean PCV of 26.26% (95%CI=25.87-26.65%) was recorded in aparasitaemic cattle while in parasitaemic animals it was 22.82% (95%CI=22.19-23.44%). The level of anemia between the trypanosome infected and non-infected animals (t = 6.566, P<0.001) found. PCV value was found to be negatively correlated with trypanosomosis prevalence (r = -0.1666). The entomological survey showed that the apparent densities of different flies across both seasons varied significantly (P<.001) among the study villages and were 2.33±SD 5.56, 7.32±SD11.8, 3.08±SD7.83, 0.37±SD1.8, and 0.11±SD0.33, 6.97±SD6.31 and 0.03±SD.12 for *Glossina m. submorsitans, G.pallidipes, G. fuscipes fuscipes, G. tachinoides, Haematopota, Stomoxys and Tabanus were recorded*, respectively. An overall mean catches of 39.3±SD 58.7 for tsetse, 0.32±SD 0.95 for Haematopota, 20.7 ±SD 18.88 for Stomoxys; and 0.09±SD 0.372 for Tabanus were recorded. The trypanocidal drug sensitivity study was used to test the efficacy of diminazene diaceturate, isometamidium chloride hydrochloride and homidium bromide against *T. congolense*, and mixed *T. congolense* and *T. brucei*. A standard technique of sensitivity evaluation was employed to assess the level of drugs resistance in the trypanosome isolates. The isolates were collected from randomly selected positive animals of Gawo Dalle district during cross sectional parasitological survey and inoculated into each experimental animal which later treated with dosages of 0.5, 1 mg/kg body weight for isometamidium chloride hydrochloride, 1, 2 mg/kg body weight for homidium bromide and 3.5, 7 and 10.5 mg/kg body weight for diminazene diaceturate. High

levels of resistance against diminazene diaceturate, isometamidium chloride hydrochloride and homidium bromide were detected in studied isolates of trypnosomes. All isolates had relapsed after treatment with different doses of trypanocides. However, one cattle was cured with high dose (1mg/kg) of isometamidium chloride hydrochloride. one cattle showed relapse of infection after 22 days and the one after 49 days to diminazene diaceturate applied to five animals to clear resistant strain at 10.5 mg/kg body weight Results of the questionnaire survey revealed that 100 %of the interviewees replied that trypanosomosis is a serious problem in the area, apart from this inexpert use of trypanocidal drugs, drug sell by farmers. very high treatment frequency which reaches about 20 times per year was revealed concerning drug use activity. Thus observed levels of drug resistance could in most cases be correlated to the drug-use practices in the district. The results of this study should be useful to define the strategy of disease control in places where resistance of trypanocides were been reported.

Key words: Cattle, Drug resistance, Diminazene diaceturate, Gawo Dalle. *Glossina*, Homidium bromide, Isometamidium Chloride hydrochloride. *Trypanosomosis*

1. INTRODUCTION

Tsetse-transmitted trypanosomosis (Nagana) is one of the most important constraints to agricultural development in the sub humid and humid zones of Africa. The rural community, which occupies 70% of the human population in Africa, has suffered severe from animal and human diseases transmitted by tsetse fly. The vector occupies nearly one third of well-watered agricultural land with suitable pastures for livestock in the continent. The genus *Glossina* occurs over some 11 million km^2 of Africa (Jordan, 1986).

The devastating effects of tsetse-transmitted trypanosomosis on the livelihoods of African communities have reached unprecedented levels across much of sub-Saharan region. Researches on the socio-economic impacts of the disease have revealed that, over 3 million heads of various livestock species in Africa are lost per year by deaths due to trypanosomosis. Furthermore, over 35 million doses of trypanocidal drugs are bought annually to treat animals against the disease and more than 70 million heads are at risk of contracting the disease so that total direct and potential losses attributable to the disease worth over 4.5 billion dollars per year (Bett *et al.*, 2004). It has been shown that in Ethiopia, at least 10 million heads of cattle are already exposed to the disease and over 14 million heads are at risk of contracting the disease at any one time such that total direct and potential annual losses from the disease exceed US$ 236 million (OAU/IBAR, 2001). Furthermore, livestock cannot be introduced into some 155,000-220,000 Km^2 of the most fertile land in the southwest and western regions (ILRI, 2002).

So far, resistance to one or more of the three-trypanocidal drugs used in cattle has been reported in at least 13 countries in sub-Saharan Africa. In addition to the 11 countries (Burkina Faso, Chad, Côte d'Ivoire, Ethiopia, Kenya, Nigeria, Somalia, the Sudan, the United Republic of Tanzania, Uganda, Zimbabwe) reported by Peregrine (1994), the Central African Republic and Zambia (Sinyangwe and Mubanga, 2004) should be included. This is probably an underestimation of the true situation, because in several countries surveys for resistance have not yet been carried out or cases of resistance have not been published. In eight of the 13 countries, multiple resistances have been reported. Recent case surveys conducted in some Sub-Saharan countries, including Ethiopia, have shown that almost all of the commercially available trypanocidal drugs are gradually losing their efficacy due to the development of multiple drug resistance to both diminazene and isometamidium (McDermott *et al.*, 2003; Tewelde *et al.*, 2004).

Furthermore, the unlikelihood of new trypanocides appearing in the foreseeable future together with the low adoption of other alternatives to the use of trypanocidal drugs create additional dilemma in the management of African animal trypanosomosis (Bett *et al.*, 2004).

Experimental studies conducted in different tsetse-infested zones of Ethiopia have revealed the occurrence of various degrees of resistance in trypanosomes to both diminazene aceturate and isometamidium chloride. Multiple drug resistance have been reported in the Abay/Didessa tsetse belt in Metekel district(Afewerk,1998)and in Didessa valley in Bedelle district (Tewelde, 2001), in Ghibe/Omo tsetse belt which is adjacent to the upper Diddessa river valley (Codjia *et al.*,1993; Rowlands *et al.*,1993; Leak *et al.*,1993; Mulugeta *et al.*,1997). In North Omo Zone, southern Ethiopia Asseffa and Abebe (2001) have also reported multiple drug resistant of *T. congolense* against diminazene aceturate and isometamidium chloride in naturally infected donkeys. Most of the studies conducted in Ethiopia to assess the therapeutic and prophylactic efficacy of trypanocidal drugs have involved experimentally infected mice where it was possible to demonstrate the general status of resistance to the drugs used in cattle after natural challenge.

As part of the BaroAkobo river system, in Gawo-Dalle district of Kellem Wollega zone tsetse-transmitted animal trypanosomosis has been incriminated as the primary cause of reduced livestock production potential in the fertile lowland and midlands. Furthermore, there are some complaints of farmers of this area on increasing death of animals due to trypanosomosis 'Gandi' which could be probably as a result of diminishing efficiency of commonly used trypanocidal drugs. Furthermore information is lacking on the dynamics of bovine trypanosomosis and tsetse challenge in Gawo-Dalle district of west Oromia.

There fore, the major objectives of this study were:

- ✓ To determine the prevalence of Bovine trypanosomosis
- ✓ To assess seasonality of the disease and associated risk factors
- ✓ To determine apparent density of tsetse species and biting flies as well as their distribution
- ✓ Assess the efficacy of selected trypanocidal drugs in cattle infected with trypanosomes isolated from the study area.

2. LITERATURE REVIEW

2.1. Significance of trypanosomosis

Trypanosomosis has for long enjoyed the dubious accolade of most important livestock disease in sub-Saharan Africa. Around nine million square kilometers are infested by tsetse flies (Budd, 1999), twice the area of the current European Union. An estimated 45 to 60 million cattle are at risk from trypanosomosis (Gilbert *et al.*, 2001) three to seven million die each year (FAO, 2003) and the productivity of the survivors in terms of draft power, milk production, growth and birth rate is lowered by 10–40% (Swallow, 2000). The direct and indirect costs have been estimated at 4.5 billion USD (Budd, 1999), which would make annual losses from trypanosomosis equal to one third the livestock G.D.P. in sub-Saharan Africa. Moreover, trypanosomosis is a zoonosis; over 60 million people living in some 250 foci are at risk of contracting the disease (WHO, 1998); there are about 300 000 cases each year (WHO, 1998), most of which go untreated, and an estimated of 1.5 million Disability Adjusted Life Years (DALY) are lost annually from sleeping sickness .

2.2. Epidemiology

African animal trypanosomosis (AAT) is a hyper-endemic, vector-transmitted disease of domestic livestock, showing a seasonal pattern where vector populations undergo seasonal fluctuations (Rogers, 2004). The epidemiology of AAT in tsetse-infested areas of Africa is determined by four biological factors operating within the physical environment, namely: trypanosomes, tsetse flies, reservoir hosts and cattle. Diverse farming systems: different cattle breeds varying in susceptibility; numerous other hosts, (wild animals and livestock) differing in their reservoir potential for trypanosomes and susceptibility to trypanosome stocks; and diverse tsetse species with varying ecological niches and host preferences and with differing vector competence for different strains of the three trypanosome species parasitic to cattle make this epidemiology a complex one.

Trypanosomosis in Africa follows the distribution and intensity of the various species of the tsetse fly. The tsetse northern limit extends across the continent from Senegal in the west to southern Somalia in the east (at about 14^0N -4^0N). The southern limit is less well defined and it varies between 10^0- 29^0S. These limits are determined by climate, often through its effect on vegetation (Bursell, 1960). Approximately 10-11 million km2 or 37% of the African continent

is infested by 23 different species of Glossina recognized to three groups (Morsitans, Palpalis and Fusca). This area includes 38 countries (Griffin, 1978).

2.2.1. Pathogen

AAT is caused by trypanosomes, unicellular protozoan parasites of the phylum Sarcomastigophora, order Kinetoplastida, family Trypanosomatidae, and genus Trypanosoma .The *Trypanosoma brucei* complex comprises three morphologically identical subspecies: *T. brucei brucei*, *T. b. rhodesiense*, and *T. b. gambiense*. Only the ancestral *T. b. brucei* is pathogenic to cattle, the other species causing acute sleeping sickness in east Africa and chronic sleeping sickness in West Africa respectively. *T. congolense* is divided into subtypes with different distributions and Pathogenecity: savannah type, forest type, Tsavo type, and Kilifi type (Majiwa *et al*., 1993, 2001). *T. congolense* is considered the most important cause of AAT in east Africa, and *T. vivax* in west Africa .But some *T. vivax* stocks from east Africa can cause hyper-acute hemorrhagic disease with high mortality *T. b. brucei* is considered less pathogenic to cattle; however cases with central nervous system involvement and high mortality have been reported (Wellde *et al.*, 1989). The 'vivax ratio' is the ratio of *T. vivax* to *T. congolense*; it is influenced by the species of tsetse, use of drugs, reservoir hosts and cattle immune responses (Ford, 1976). The vivax ratio is usually high where the overall prevalence is low; after successful vector control campaigns (perhaps reflecting greater importance of mechanical transmission); where diminazene is widely used; and where *G. Palpalis*, (a poor vector of *T. congolense*), predominates .In terms of pathology, a distinction can be made between the haematic *(T.vivax*,T *congolense*) and humoral *(brucei*) trypanosome species; the former associated with anaemia and the latter also with tissue degeneration and inflammation (Losos et al,1986). Compared to *T. congolense*, the parasitaemia is higher but anaemia less profound in *T. vivax* infections. However, it is rarely possible to clinically distinguish disease caused by different trypanosome species and mixed infections are common. Disease in cattle varies from hyper acute to chronic; the latter is more common in endemic areas. Signs are not pathognomonic, but a combination of the following are typically present: fever, anaemia, lymphadenopathy, dull and dirty coat, piloerection, change of hair colour, hair loss, weight loss, lacrimation, chancre, fatigue, anorexia, pica, abortion, salivation, nasal discharge, arched back, tucked-up abdomen, laboured respiration and irgular pulse.

An important biologic feature of pathogenic trypanosomes is the Variable Surface Glycoprotein (VSG), a protein that forms a dense coat on the trypanosome surface. With time, the host mounts an effective immune response against trypanosomes with a specific VSG coat, removing these but not other trypanosomes that have switched to a new (temporarily unrecognizable) VSG coat. These variants form the next wave of infection. Antigenic variation of the surface coat is unique to trypanosomes and the basis of epidemiological features of intermittent parasitaemia; fluctuating fever; disease chronicity; long, largely asymptomatic, incubation period; and failure to develop effective post-infection immunity.

2.2.2. Vector

Tsetse flies (genus *Glossina*) are the primary vector of trypanosomosis and the only vector capable of transmitting trypanosomes cyclically. Thirty-one species and subspecies of tsetse have been identified. Species can be divided into three subgenera, based primarily on morphological features of the adult genitalia; a classification recently confirmed by comparative gene sequence analysis and by geometric wing morphometry. The morsitans group is found mainly in savannah ecosystems and includes several important vectors of AAT including *Glossina morsitans spp.*, *G. pallidipes* and *G. austeni*. The *palpalis* group is found mainly in the riverine galleries of west and central Africa but can extend into savannah regions between river systems; less mobile than morsitans they rely on sight rather than smell to locate their hosts. Important AAT vectors in this group include *G. palpalis* and *G. tachinoides*. The fusca group are found mainly in forests and are therefore less important vectors; however the atypical *G. longipennis* and *G. brevipalpis* are found in drier areas of east Africa and are significant disease vectors.

Tsetses are unusual insects; females give birth to live offspring, both sexes feed obligatorily on blood, and mortality is low. Their longevity, mobility, and frequent feeding make tsetse highly efficient vectors, but the low rate of population growth means even small increases in mortality rate can result in population decline and even extinction (Hargrove, 2004). Vectorial capacity is the readiness to become infected while feeding on a vertebrate host and to subsequently develop an infection and transmit the trypanosome to another vertebrate host and varies from species to species. Infection rates in tsetse are determined by the parasite, the host, the vector, and the environment. They are generally low but vary greatly according to species of trypanosome, with *T. vivax* ranking the highest and *T. brucei spp.* ranking the lowest.

Infection rates are influenced by endogenous factors (including tsetse species, strain, sex, age, nutritional status and interactions with micro-organisms within tsetse); by host factors (including tolerance of tsetse, immune state and attractiveness to tsetse); ecological factors (including climate, light, wind and biomass) and parasite factors (including species and infectivity) (Molyneux, 1980; Leak, 1999). Biting insects may transmit typanosomes mechanically (and this is how *T. vivax* is transmitted in South America and Mauritius). The most important mechanical vectors are flies of the genus *Tabanus*, but *Haematopota*, *Liperosia*, *Stomoxys*, and *Chrysops* flies have also been implicated. According to Jordan (1986) and Leak (1999) who extensively reviewed the subject, there is little good evidence that mechanical transmission is of importance in Africa under natural conditions; reports of field occurrence may be due to insensitive sampling that does not detect low populations, and experimental studies that support mechanical transmission do not replicate natural conditions. Congenital transmission of trypanosomosis can take place and carnivores can be infected (with *T. brucei*) by consuming infected meat; the importance of these transmission routes is not known, but is not likely to be high. On the other hand, iatrogenic transmission is also possible and may be important when poor needle hygiene is practiced.

2.2.3. Host

Cattle-infective trypanosomes circulate in a variety of wildlife hosts, which generally tolerate infections or have a state of pre-immunity. The wildlife surveys have shown that some favored hosts of tsetse such as bushbuck, buffalo, warthog and waterbuck are major reservoirs of trypanosomes, with many animals infected. Many savannah species such as wildebeest and Zebra also harbor trypanosomes despite little overlap with tsetse distributions feeding habits (Olubayo *et al.*, 1991). Altogether, these concerted efforts have revealed a continuum of host immune mechanisms that result in tolerance or resistance. Buffalo transmits trypanosomes effectively to various species of *Glossina* (Moloo *et al.*, 1993).Small ruminants, equines, pigs, dogs and cats are also susceptible to some species of cattle-infective trypanosomes. The existence of reservoir and alternative hosts complicates the epidemiology of AAT, making it difficult to manage and perhaps infeasible to eliminate the disease. Susceptibility of cattle to trypanosomosis depends on their breed, age, behaviour, previous exposure and health status, of these the most important factor is breed Studies have shown that African cattle stem from the *in situ* domestication of a taurine (*Bos taurus*) wild ox that inhabited northern Africa around 9 000 years ago. In contrast, Zebus were mainly introduced from south Asia around 760 C.E. West

African *B. Taurus* breeds are trypanotolerant; that is, they can survive and be productive under trypanosomosis risk. This capacity is highly heritable and involves the ability to control parasitaemia, maintain weight and resist anemia .Some east African breeds are also, to a lesser degree, trypanotolerant. Although Trypanotolerance is intrinsic, previous exposure to trypanosomosis is an important determinant of disease susceptibility field studies have shown that N'Dama(Olubayo et al., 1990)have a marked capacity to acquire resistance to both *T. vivax*, and to a lesser extent, *T. congolense* (Murray *et al.*, 2004). Moreover, there is evidence of a (lesser) ability to acquire resistance in *B. indicus* types in East Africa (Murray *et al.*, 1982).There appears to be reverse age immunity to AAT (i.e. calves least susceptible).

Transmission is host-specific, with some hosts good transmitters and others poor transmitters, simply as a result of the biochemical characteristics of their blood (Mihok *et al.*, 1993). Herd management, daily activity patterns of tsetse species involved and the grazing patterns of the herds are of great influence on the transmission of the disease between tsetse flies and domestic ruminants (Uilenberg, 1998).

2.3. Tsetse and trypanosomosis distribution in Ethiopia

Above 15% of the land believed to be suitable for livestock production is affected by one or more of the following species of tsetse flies; *Glossina morsitans submorsitans, Glossina pallidipes, Glossina tachinoides, Glossina fuscipes fuscipes and Glossina longippennis,* and are confined to southern and south-western region of the country. *Glossina morsitans submorsitans* are usually found in deciduous woodland and wooded grassland, often interspersed with evergreen vegetation. *Glossina pallidipes* is almost invariably associated with extensive and fragmented thickets, including evergreen species. *Glossina longipennis* is found in dry Acacia thorn-bush and is very active after sunset and before nightfall. *Glossina fuscipes fuscipes* and *Glossina tachinoides* inhabit gallery forest, thickets and fringing vegetation on streams, rivers and lake shores (Ford *et al.*, 1976; Langridge, 1976).

Tsetse-transmitted trypanosomosis occurs in all areas infested with the fly, affecting livestock, and thereby hindering rural development. Trypanosomes causing severe losses in Ethiopia are *Trypanosoma congolense, Trypanosoma vivax* and *Trypanosoma brucei*, which are tsetse-borne. Non-tsetse-borne trypanosomosis also occur in the country due to *Trypanosoma equiperdum* and *Trypanosoma evansi*, the agent of surra mainly in camels (Dagnachew and Shafo, 1981).

2.4. Diagnosis of trypanosomosis

Accurate diagnosis of trypanosome infection in livestock is required for a proper understanding of the epidemiology of the disease in any geographical locality. Besides clinical diagnosis, direct (parasitological) and indirect (serological) diagnostic methods with varying degrees of sensitivity and specificity are available for trypanosomosis

2.4.1. Clinical diagnosis.

In general, diagnosis of trypanosome infection based on clinical signs alone is rather difficult but hematological parameters like PCV value could be reliable indicators of the progress of the disease. In regions where the disease is known to occur; fever, anemia and loss of body condition are important parameters used routinely for tentative diagnosis of trypanosomosis in areas where the disease is endemic and laboratory services are not available (Uilinberg, 1998). Definitive diagnosis of the disease is ultimately dependent on the detection of the trypanosome in blood samples from infected animals.

2.4.2. Parasitological diagnosis

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

Dark ground /Phase contrast/Buffy coat technique

The Buffy coat Zone prepared in a microhaematocrit capillary filled up to ¾ part with blood and centrifuged for 5 minutes at 12000rpm is examined for trypanosomes by cutting the capillary tube to include 1 mm of erythrocytes and 1 cm of the plasma. The Buffy coat is poured on a slide and covered with a 22x22mm cover slip. The preparation is examined using a microscope with a phase contrast and dark ground illumination. The use of 10x eyepiece in combination with a 25x objective gives optimal viewing by allowing large visual fields and sufficient magnification for ready identification of trypanosomes.

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This technique is the most sensitive of the parasitological tests for the detection of T.congolense and T.vivax detecting trypanosomes to an estimated level of just over 10^2 parasites per ml (Murray *et al.*, 1977).

In addition, species identification based on size and movement is easier to asses (Paris et al., 1982). Trypanosomes can be identified and the level of parasitaemia is estimated using a scoring system. The PCV is measured before examination of the blood for parasitaemic detection.

Other diagnostic methods including Haematocrit centrifugation technique, capillary concentration technique, biochemical tests, serological tests to detect specific humoral antibody and circulating antigens (Ab-ELISA, Ag-ELISA, CFT, Passive Haemaglutination test, etc), and the molecular tests DNA-probes and PCR techniques could be used.

2.5. Impacts of tsetse-transmitted trypanosomosis in Africa

It is variously estimated that some 45-50 million cattle live under trypanosomosis risk, in a tsetse -infested area of some 8-11 million km^2 of Africa (Budd, 1999; Gilbert *et al.*, 2001). Attempts to quantify the impact of the disease have been undertaken firstly by trying to measure its direct impact on key productivity parameters -mortality, fertility and milk production, animal traction output and weight gain- then to translate these into monetary terms, often using demographic herd or livestock population models. The indirect effects thus reflect the 'lost potential', that is the 'total loses' due to a disease, rather than the more immediately 'avoidable loses' or direct effects (Perry and Randolph, 1999). The total losses to agricultural production alone due to trypanosomosis in Africa were estimated to be US $ 4.5 Billion per year. According to Tikubet (1993) about 50 million cattle, approximately 30% of the total population in Africa are exposed to the risk of infection with trypanosomes and 3 million die every year.

2.6. Control methods of trypanosomosis

Control of vector-borne diseases, (virtually all forms of trypanosomosis are in this category except dourine), can be based on control of the causal agent, control of the vector and use of innate resistance of the host to the effect of the infection. A dense coat of variant surface glycoprotein that stimulates antibody production in the host covers trypanosomes.

There is no vaccine against the disease and, in spite of intensive research, because of the ability of trypanosomes to readily change their glycoprotein surface coat through a process called antigenic variation (Radostits and Blood, 1994).

2.6.1. Trypanotolerance

Trypanotolerance has been defined as that property of an animal that enables it to remain productive under tsetse-trypanosomosis challenge. The mechanisms that underlie this ability include control of parasite proliferation, limitation of the pathological effects of the parasites, and an acquired ability for better control of trypanosomosis (Murray *et. al.*, 1982; Murray and Dexter, 1988; D'Ietera *et.al.*, 2000). At present, trypanotolerant livestock are only found in certain areas of West and Central Africa and although they retain a certain level of productivity under tsetse challenge conditions they are considered less productive in terms of meat and milk produced. They are also known to be tolerant to streptothricosis, ticks and tick-borne diseases and to some extent, helminthiasis (D'Ietera *et.al.*, 2000). In areas that are heavily infested with tsetse flies, or when a lower level of tsetse challenge is combined with other stress factors such as malnutrition, trypanotolerant breeds of livestock will need protection of trypanocidal drugs in order to be productive (FAO 2002).

Ethiopia's 18 cattle breeds have recognizable features, similar to that of the West African N'dama, which would suggest that they may have some trypanotolerant qualities (Alberro and Haile-Mariam, 1982). This possibility was investigated in Ethiopia on four different breeds by ILRI and NAHRC. A comparative study on the response of four indigenous cattle breeds of Ethiopia, namely Abigar, Horro, Sheko and Gurage to natural challenge of trypanosomosis in the Tolley-Gullele area of the Ghibe valley has been undertaken from August 2000 until August 2004. Fifty female yearlings each of Horro, Sheko and Abigar and 31 of the Gurage were purchased from their natural habitats and introduced into medium to high tsetse-trypanosomosis challenge area of the Ghibe valley. While the natural habitats of first three breeds are naturally infested with tsetse flies and trypanosomosis, that of the Gurage is known to be very minimal, if any, and hence the Gurage breeds was used in this study as the known susceptible breed. During the study animal health, production performance and tsetse fly situation were monitored monthly.

The Sheko breed has manifested very significantly ($p<0.001$) high overall average packed cell volume (PCV) values (25%) compared to that of Abigar (24%),Horro (23%)and

Gurage(22%).It also had the lowest mean trypanosome prevalence rate of 9% against 23%of Horro,26% of Abigar and 27% of Gurage,and the least number of Berenil treatment (1.36)compared to Abigar (4.0), Horro(4.6) and Gurage(6.7).While the Abigar manifested high sensitivity and frequent death to PCV depression, the Horro showed strong resilience to PCV depression and better response to Berenil treatment assistance. This study showed that the only surviving indigenous taurine type indigenous cattle breed in Ethiopia-the Sheko-exhibited better trypanotolerant attributes than the other three breeds Abigar, Horro, Gurage), as measured by lower trypanosome prevalence rate, less severe anemia after infection, and fewer trypanocidal treatments per annum than the other breeds. These results need to be substantiated with further in-depth investigation including immune response, animal behavior and environmental influences (Lemecha *et al.*, 2006).

2.6.2. Tsetse control techniques and their principles

Tsetse vector control methods relying on large scale bush clearing and aerial spraying methods are no longer used due to environmental concerns. Tsetse control currently relies on two bait systems: insecticide-treated traps and targets and insecticide treated livestock. Sterile Insect Technique (SIT) has also been used in efforts to eradicate tsetse flies in some areas.

Parallel to the application of trypanocidal drugs on infected animals, continuous effort has been put towards controlling tsetse flies as part of an integrated approach to keep the size of vector population down to a level where the trypanosomosis problem is tolerable (Donelson, 2003). Some of the conventional methods employed to control the vectors are: selective spraying of the vegetation support of the flies, use of artificial bait devices such as insecticide impregnated traps and targets, application of small quantities of persistent powerful pyrethroids insecticides on animals, and the use of the so-called sterile insect technique (SIT) after rearing and releasing male flies (Eisler *et al.*, 2003). Although vector control techniques have some environmental adversity, banning the more primitive methods and adopting the modern strategies has proved to be very efficient.

Because of the stability of tsetse populations and their low reproductive rate, little sustained mortality pressure (additional to natural mortality) needs to be exerted on a population to cause its extinction (Weidhaas and Haile 1978).

That makes them good candidates for traps and target control methods. Not to be forgotten though are the risks of reinvasion or immigration into an area already cleared of tsetse flies. The theory behind this control method is simple: the flies are visibly attracted to a trap or target; this attraction may be further helped by the use of olfactory attractants. When the tsetse lands on a trap or target they either receive a lethal dose of insecticide, or are caught in the trap and subsequently die (Leak 1999). The effectiveness of traps and targets will depend on when the flies are active, hether they will move into the vicinity of a trap or target and finally, whether they are trapped or killed.

Insecticide-treated livestock was developed as a method of tsetse control from the concept of baited traps and targets. It is widely accepted by a majority of stockowners in Africa. Most commonly used are the synthetic pyrethroids, and of these Deltamethrin appears to be the most potent and it is also low in mammalian toxicity and has minimal environmental impact Extensive use of insecticides on cattle for tsetse control appears to have the potential to interfere with endemic stability/immunity of cattle to several tick borne diseases. Thus the long-term use of these products may jeopardize control of tick borne diseases (FAO 2002,).

Insecticide delivery methods

Chemical control depends upon sufficient contact between the tsetse fly and the insecticide for the fly to pick up a lethal dose. One stage in the tsetse lifecycle, a period of pupal development lasting from 1 to 3 months, takes place underground and protects this juvenile proportion of the population from direct chemical attack. Delivery methods therefore had to be designed to ensure that the entire target population contacts the insecticide at some point and this requires either long lasting carefully placed residual deposits or repeated space sprays spanning the full tsetse lifecycle. Based on ecological and behavioral knowledge, this requirement was engineered in a number of ways, such as :by depositing residual insecticide, either selectively on to places such as resting or breeding sites that (ground spraying), or by treating the entire habitat with residual insecticide from the air; by repeatedly spraying non-residual insecticide aerosols on to tsetse flies, either over large areas using aircraft ,or in more localized areas using handheld machines; and by attracting tsetse to artifacts that are impregnated with persistent insecticides (Maudlin, 2004).

Both ground spraying techniques and aerial spraying techniques has been widely used in the more recent past, to control or eradicate tsetse flies. Aerial spraying can be used to treat large areas rapidly .It is also used where ground access is difficult, dangerous or undesirable. How ever the method is costly and harmful to the environment and cannot be implemented in areas with ragged topography and also most insecticides are harmful to aquatic life, while the earlier compounds such as DDT, also affect terrestrial animals, including birds (Allosop, 1991). The method of choice in most countries is stationary baits, supported where possible by insecticide-treated cattle. In the future, other factors may determine that different methods, or a combination of different methods, are used. All tsetse control methods currently in use involve the application of insecticides.

Tsetse control using insecticide treated targets

In the 1970's behavioral research demonstrated the importance of host Odour in host-location by tsetse flies (Vale, 1974). Subsequent experiments isolated some of the attractant Odour from oxen, and advances in trap design suggested that if the trap was treated with insecticide, its design could be greatly simplified. The result of this research was a simple target that flies only have to land on or bump into. On contact with the target they pick up a lethal dose of insecticide, fly away and die.

Currently used targets are baited with butanone. Octenol. 4-methyl phenol, and 3-n-propyl phenol to maximize fly contact. The combination of blue and black cloth maximizes the number of tsetse that contact the insecticide as well as reducing the amount of insecticide used. The flies are attracted by the blue Colour, but prefer to alight on the black portion of the target. Therefore, only the black portion is treated with insecticide. Savannah tsetse flies are generally more attracted by the Odour than riverine group.

Sterile insect technique

Sterile Insect Technique (SIT) involves sustained and systemic release of sterile insects among the indigenous target population (FAO 2002). Males are sterilized by irradiation and then taken to a target area and released. The high numbers of sterile males released into the population makes the chance of a fertile mating very small, since females mate only once. After repeated releases of sterile males the population should die out (Williamson *et al.*, 1983; Politzar and Cuisance, 1984).

Following mating with sterile males the females become infertile for the remainder of their life spans. By continually releasing sterile males in quantities over a time span that is sufficient to cover several generations of target populations, the fertile population is progressively reduced. Eventually, so few fertile insects remain that they cannot sustain the population. For maximum effectiveness, the sterile males released must outnumber the fertile native males by a considerable margin. One way this could be achieved is by suppressing the native population through other means before SIT (Bett 2004; FAO, 2003, 2002)

2.6.3. Current chemotherapy of animal trypanosomosis

Over most of sub-Saharan Africa, bovine trypanosomosis continues to be controlled primarily by trypanocides. Chemotherapy remains the principal means of controlling the African trypanosomosis, but current drugs are not satisfactory. Chemotherapy of animal trypanosomosis depends on isometamidium, homidium and diminazene, whilst cymelarsan is used against *Trypanosoma evansi* in camels. Suramin remains in use for some animal trypanosomosis but quinapyramine was discontinued due to its capacity to induce multi-drug resistance. Resistance to the animal trypanocides, isometamidium and homidium, is widespread (Geerts and Holmes, 1998; Geerts *et al.*, 2001). Suramin resistance has also been reported in many loci. All of these drugs have been on the market for over 40 years. A full account of the history and properties of these drugs was provided by Mulligan (1970) and later reviewed by Leach and Roberts (1981). Over the past 40 years a few large European pharmaceutics companies have primarily provided these three drugs but recently several generic forms of these compounds from a wider range of companies have become available on the African market.

Although there is a consistent demand for trypanocides by African farmers, the total value of the market (about US$ 30 million) is not considered sufficient to justify investment by large pharmaceutical companies in the development and licensing of few animal trypanocides, the costs of which may exceed US$250 million for a single compound The challenge, therefore, remains to make optimal use of the three relatively old compounds until new methods of treatment cross-reactivity with new broad-spectrum anti-protozoan compounds such as those currently being developed for the treatment of malaria and cryptosporidiosis.

Perhaps the greatest risk to the future use of the existing three trypanocides is the development and spread of drug resistance to the point where they become ineffective over large areas of Africa. However, other risks exist one is that, because of drug resistance (real or perceived), the market will shrink and manufacture will become unprofitable.

Drugs currently recommended for chemotherapy of animal trypanosomosis come from only three closely related groups. These are the phenanthridines, isometamidium and homidium, and the aromatic diamidine, diaminazone. Only isometamidium and homidium are recommended for prophylaxis. The incidence of resistance to these drugs is apparently increasing and the main means of controlling the disease is therefore under threat (Peregrin1994).

The control of trypanosomosis in domestic livestock depends mainly upon the use of drugs, either curatively or as a prophylactic. Resistance to the available drugs is on the increase and their continued use is expensive for livestock owners, it has been estimated that at least US$ 20 million (approximately 50 million doses) is spent annually to treat or protect animals exposed to trypanosomes in Africa. The actual amount of trypanocides used is difficult to estimate, particularly in recent years, since the distribution of trypanocides has become more decentralized, with a number of generic brands being sold, increasingly through traders and shopkeepers and less through official veterinary channels. Drug resistant trypanosomes develop through (i) under dosing, which may occur for a variety of reasons such as underestimation of animal body weight, over diluted solutions or incorrectly calculated dose volume.(ii) incorrect (and therefore ineffective) injection or (ii) an incorrect strategy of drug use Leak (1999). Measures that may delay the development of drug resistance are to reduce the selection pressure on trypanosome populations by avoiding exclusive reliance on drugs for trypanosomosis control and avoiding mass treatment of livestock at short intervals.

2.7. Current situation of resistance against trypanocidal drugs

So far, resistance to one or more of the three trypanocidal drugs used in cattle has been reported in at least 13 countries in sub-Saharan Africa. In addition to the 11 countries (Burkina Faso, Chad, Côte d'Ivoire, Ethiopia, Kenya, Nigeria, Somalia, the Sudan, the United Republic of Tanzania, Uganda, Zimbabwe) reported by Peregrine (1994), the Central African Republic (Finelle and Yvore, 1962) and Zambia (Sinyangwe and Mubanga, 2004) should be included.

This is probably an underestimation of the true situation, because in several countries surveys for resistance have not yet been carried out or cases of resistance have not been published. In eight of the 13 countries, multiple resistances have been reported. Most of the currently available information on drug resistance, however, is derived from limited numbers of case reports and does not give any indication of the prevalence of resistance in a region or a country as systematic surveys have not been conducted. There is also considerable variation in the criteria that have been used to diagnose drug resistance. The table summarizes the published reports in which a number of trypanosome isolates have been examined. Very few authors provide information on the method of sampling (randomized or not). There is an urgent need for surveys in which representative numbers of trypanosome isolates are examined for drug resistance. Such surveys should be taken at random and use agreed methods of diagnosis.

This type of survey should provide more reliable data on the true prevalence of drug resistance in regions and countries. In addition, risk analysis should help to identify the factors that influence sensitivity or resistance to trypanocidal drugs.

It is also important to stress that drug resistance is not an "all or nothing" phenomenon and the degree of drug sensitivity and resistance varies considerably between individual trypanosomes. A further factor that can influence drug effectiveness is identified in the interesting observations of Burudi *et al.* (1994) and Mamman *et al.* (1995), who reported differences in drug sensitivity according to the timing of treatment after infection and the concentration of trypanosomes in the blood.

2.7.1. Development of resistance to trypanocidal drugs

In most endemic areas, the control of trypanosomosis principally relies on the use of antitrypanosomal compounds. However; these compounds are limited in number and have been under extensive use with little/no regular monitoring. Thus, case surveys conducted in some sub-Saharan countries have shown that almost all of the commercially available trypanocides are gradually losing their efficacy due to the development of multiple drug resistance by trypanosomes (Mc Dermott *et al.*, 2003; Taylor and Authie, 2004). As the result, this scenario is currently recognized as a major impediment to livestock production in Sub-Saharan Africa.

Selection by drugs essentially takes place during asexual multiplication in the animal or human host, though there is some evidence that, during passage through the tsetse fly, genetic exchange (sexual recombination) may occur at least in Trypanosoma brucei .In the past the development of drug resistance in trypanosomes was mainly ascribed to their exposure to sub therapeutic concentrations of trypanocidal drugs. Although this is an important aspect, the intensity of drug pressure (i.e. the treatment frequency and the degree of exposure of the parasite population) is probably even more important. The immunocomptence of the host also appears to play an important role. Finally, the phenomenon of cross-resistance has been well established for instance quinapyramine usage has been shown to induce resistance to isometamidium and diminazene (Ndoutamia *et.al*, 1993; Mc Dermott *et al.*, 2003).

2.7.2. Mechanisms and genetics of resistance to trypanocides

Isometamidium

Wilkes and Peregrine (1995) showed that the trypanosome kinetoplast is the primary site of ISMM accumulation. The mechanism of resistance to ISMM, however, is less clear. Decreased levels of drug accumulation have been observed in drug-resistant populations of *T. congolense* and later work found indirect evidence of an increased efflux of drug from resistant trypanosomes (Sutherland and Holmes, 1993). Mulugeta *et al.* (1997) showed that the maximal uptake rates (Vmax) of ISMM in resistant *T. congolense* were significantly lower than in sensitive populations. It remains to be shown whether this is caused by a decreased number of protein transporters of ISMM in the plasma membrane and/or by changes in the balance between influx and efflux. The role of nucleoside transporters in resistance to ISMM by *T. congolense* remains to be examined, although changes in these transporters have been

associated with resistance to arsenical drugs in *T. brucei* (Carter and Fairlamb, 1993; Carter, Berger and Fairlamb, 1995). More recently, changes in mitochondrial electrical potential have been demonstrated in ISMM-resistant *T. congolense* by Wilkes *et al.* (1997). Although contradictory observations have been reported on the genetic stability of ISMM resistance, recent field observations in Ethiopia, based on cloned populations, showed that the drug-resistant phenotype of *T. congolense* had not altered over a period of four years (Mulugeta *et al.*, 1997).

Homidium salts

Although their mutagenic activity has been known for a long time homidium chloride and especially homidium bromide or ethidium are still widely used as trypanocidal drugs.

The mechanism of their antitrypanosomal action is not well understood. However, it has been shown that the drugs interfere with glycosomal functions, the function of an unusual adenosine monophosphate-(AMP) binding protein, trypanothione metabolism and the replication of kinetoplast minicircles. The mechanism of resistance by trypanosomes to these drugs is unknown. There are indications, however, that it is similar to that described for ISMM (Peregrine, Gray and Moloo, 1997).

Diminazene

Although diminazene probably exerts its action at the level of the kinetoplast DNA, this has not been proven *in vivo*, and other mechanisms of action cannot be excluded (Peregrine and Mamman, 1993). Similarly the molecular basis of resistance to diminazene in trypanosomes is not clear. Carter, Berger and Fair lamb (1995) showed that the accumulation of diminazene was markedly reduced in arsenical-resistant *T. brucei brucei* owing to alterations in the nucleoside transporter system (P2). However, there might be other resistance mechanisms. Similarly to ISMM, contradictory reports have also been published on the stability of resistance to diminazene. Mulugeta *et al.* (1997), however, showed that the phenotype of multiple drug-resistant (including diminazene) *T. congolense* remained stable over a period of four years. In conclusion, it is clear that much more work is required in order to elucidate the mechanism of resistance to the three currently available trypanocidal drugs. Such studies, as well as being of great value in their own right, may also provide novel methods for the detection of drug-resistant trypanosomes in the future. The same is true for the genetics of drug resistance in trypanosomes. There are three major types of genetic change that are responsible for acquired

drug resistance: mutations or amplifications of specific genes directly involved in a protective pathway; mutations in genes that regulate stress-response processes and lead to altered expression of large numbers of proteins; and gene transfer.

2.8. Detection of drug resistance

Several methods have been described to identify drug resistance in trypanosomes (reviewed by Peregrine, 1994). At present, three types of technique are commonly used to identify drug resistance: tests in ruminants; tests in mice; and *in vitro* assays (Eisler et al., 2001). None of these is, however, an ideal test and other tests are still in the phase of development or validation. The advantages and disadvantages of each of the different techniques are briefly summarized in the following sections.

2.8.1 Tests in ruminants

Tests in ruminants provide direct information from studies in ruminants using recommended doses of trypanocides. The tests commonly consist of infecting a group of cattle or small ruminants with the isolate under investigation and later, when the animals are parasitaemic, treating them with various levels of trypanocides. The animals are then regularly monitored over a prolonged period (up to 100 days) to determine the effective dose (ED), i.e. the dose that clears the parasites from the circulation, and the curative dose (CD), i.e. the dose that provides a permanent cure. For these studies, the cattle or small ruminants must be kept in fly-proof accommodation or in a non-tsetse area in order to eliminate the risk of reinfection during the study. A variation on this technique is to inoculate blood from a group of infected cattle was inoculated into a single recipient calf, which was monitored, and later, when it was parasitaemic, treated with trypanocides at the recommended dose. A breakthrough infection, indicative that one of the inoculated trypanosome populations was drug-resistant, was inoculated into groups of calves and mice to determine the level of drug resistance. This technique is useful in situations where laboratory facilities are very limited but it only allows a qualitative assessment and does not indicate how many of the isolates inoculated into a single calf were resistant. Further constraints to this technique are that not all populations might grow equally well and that sensitive isolates might overgrow resistant ones when inoculated together However this is not a consistent observation (Burudi *et al.*, 1994). A useful indication of the level of resistance can be obtained from studies in ruminants (and mice) by recording the length

of time between treatment and the detection of breakthrough populations of trypanosomes. The advantages of studies in ruminants are that most trypanosome isolates of cattle are able to grow in these hosts and that the data obtained are directly applicable to the field. The disadvantages are the long duration (a follow-up of 100 days is necessary to allow the detection of relapses) and the cost (purchase and maintenance of the animals are expensive).

Furthermore, if only one isolate per animal is tested, it is usually impractical and too expensive to examine a large number of isolates (Maudlin, Holmes, Miles, 2004).

2.8.2. Tests in mice

After expansion of an isolate in a donor mouse, groups of five or six mice are inoculated with trypanosomes. Twenty-four hours later, or at the first peak of parasitaemia, each group except the control group is treated with a range of drug doses. Thereafter, the mice should be monitored three times a week for 60 days.

The ED50 or ED95 (the effective dose that gives temporary clearance of the parasites in 50 or 95 percent of the animals, respectively) can be calculated, as can the CD50 or CD95 (the curative dose that gives complete cure in 50 or 95 percent of the animals, respectively). Groups of five mice, which allowed an easy calculation of ED80 and CD80 values are used (one out of five mice was not cleared or cured). These figures should be compared with those obtained using reference-sensitive trypanosome strains. The advantage of the mouse assay is that it is cheaper than the test in cattle. There are several disadvantages, however: i) most *T. vivax* isolates, and also some *T. congolense* isolates, do not grow in mice; ii) although there is reasonable correlation between drug sensitivity data in mice and in cattle, higher doses of drug must be used in mice (normally ten times higher) in order to obtain comparable results to those obtained in cattle because of the vast difference in metabolic size iii) precise assessment of the degree of resistance needs a large number of mice per isolate, which makes it a labour-intensive test -identification of a discriminatory dose, above which an isolate should be considered as resistant, could drastically reduce the number of mice and the amount of work to be carried out; and iv) it takes as long as 60 days to evaluate the drug sensitivity of an isolate(Eisler et al.,2001).

2.8.3. In vitro assays

The advantage of this technique is that large numbers of isolates can be examined; tests with metacyclic trypanosomes correlate well with field observations. However there are several disadvantages. *In vitro* cultivation of bloodstream forms is only possible using pre adapted lines and not using isolates directly from naturally infected animals (Peregrine, 1993). These authors have developed a simplified axenic culture system, but further research is still necessary to study the correlation with field data.

A potential problem associated with this lengthy time of adaptation is the possible selection against trypanosomes that have the phenotype of the original population. *In vitro* assays are expensive to perform and require good laboratory facilities and well-trained staff. If better techniques can be developed in order to adapt isolates more rapidly to grow *in vitro*, these assays may become more popular, especially in those laboratories where culture facilities are already established.

2.8.4. Trypanocidal drug ELISAs

As an alternative to the tests mentioned above, the use of trypanocidal drug enzyme-linked immunosorbent assays (ELISAs) in combination with parasite detection tests has given promising results for the detection of resistant trypanosomes. A competitive ELISA which allowed the detection of small amounts of isometamidium in serum of cattle was further improved by and has been validated in cattle under experimental and field conditions (Eisler *et al.*, 1996). The test is both sensitive, detecting subnanogramme concentrations, and specific. It allows the monitoring of drug levels over extended periods and the evaluation of factors influencing drug disappearance rates from the plasma. The available data indicate that there is a considerable individual variation after intramuscular injection of ISMM in cattle (Eisler, 1996). One interesting finding has been that the drug disappears more rapidly in animals challenged and becoming infected with drug-resistant trypanosome isolates than in those challenged but protected against infection with sensitive trypanosomes. Observations showed that the presence of trypanosomes in animals with an ISMM concentration of > 0.4 ng/ml suggests resistance: the higher the drug level detected the greater the degree of resistance that could be inferred (Eisler *et al.*, 1996. Similar drug ELISAs have been developed for the detection of sub nanogramme amounts of homidium bromide. The advantage of the ISMM ELISA is that large numbers of

sera can be tested within a relatively short time. The ELISA may also provide information on drug usage in an area of investigation. The disadvantage is that further studies are required to confirm the correlation between protection against tsetse challenge with various trypanosome populations and the ISMM concentration in the serum. It is not yet possible to draw firm conclusions on the sensitivity or resistance of a trypanosome population at the level of the individual animal. The ELISA should, however, give some indication of the resistance situation at the level of the herd. A further disadvantage is that, while the ELISA may indicate the level of drug withstood by a trypanosome population, it does not provide information about the level required for protection.

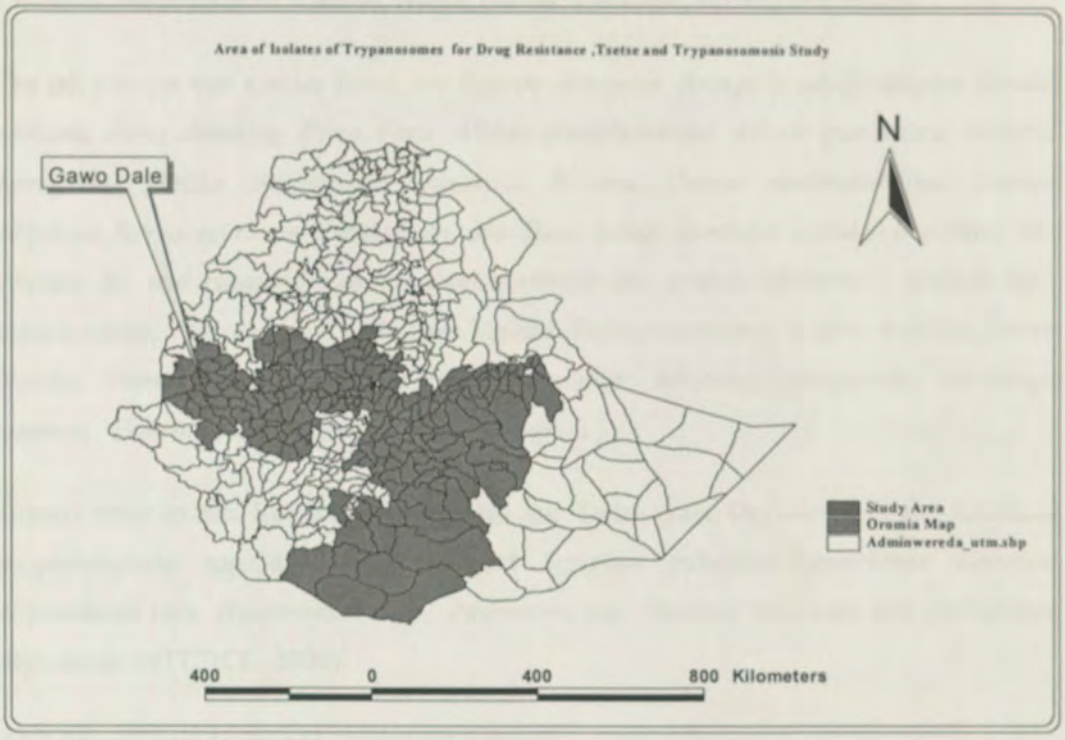
3. MATERIALS AND METHODS

3.1. Description of study area

The study was carried out in Gawo Dalle district, which is found in Kellem Wollega zone of Oromia region, west Ethiopia; in collaboration with National Tsetse Fly and Trypanosomosis Investigation and Control Center (NTTICC). This study area was selected by convenience sampling method based on previous information on tsetse and trypanosomosis challenge. Gawo Dale is located about 600 kms westwards from the capital, Addis Ababa. The district has an area of about 113,000hectar, with 65% being midland and 35% lowland. Gawo Dalle is situated in Birbir watershed, which is one of Baro Akobo river system.

The zone in which this study area is situated is bordered by: Gambella regional state to the south, Benishangul Gumuz regional state to the west, west Wollega zone to the north and Illubabor to the east. According to the survey report of NTTICC,(2006) seven districts of Kellem Wollega (namely Anfillo,Sayo, Hawa Gelan, Yemalegi walal,GawoDalle,Dalle Sadie and Lalo kille) which lie between 08^0N 25' 56" to 08^0N 58'05" and 034^0E 33'41" to 035^0E 28'48" geographical positions are infested by tsetse flies. The topography of areas around Birbir River is plain and gentle slops in most places and the areas to the west of Birbir watershed gets high elevation and with steeps slopes and chained mountainous areas like Gerjeda, Walal, Guma Guda, Guma Tina, and the western parts are usually high to mid high land and is boundary for Birbir and Dabus River systems (tributary of Abay). The perennial rivers flowing through the district and drain in to the Birbir are Hindina, Chebel, Keto, Kunni, Kile The Birbir continues into the Baro which is a tributary of the White Nile.

Figure 1: Map of study area



3.1.1. The wild life and natural vegetation

Wild animals which have been seen and recorded in this district include Baboon, Colobus-monkey ,Vervet-monkey, African Buffalo (Syncerus caffer), Bush pig (Potomacheorus porcus), Warthog, (Phacoeheorus aethiopicus), Bushbuck (Tragelaphus Scriptus), Lion, Crocodile, Hippopotamus, Gazelle, Hyena, Dik-dik, Porcupine, Pythons and Snakes.

The tall riverine tree species found *are Pygeum africanum, Aningeria adolfiriederici, Cordia africana, Ficus thonning, Ficus Vasta, Albizia grandibracteata, Albizia gummifera, Millettia*

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Ground cover include Rubus spp., Agerratum Spp., Sudan grass, Oxytenanthera sp., Solanacea sp.,pteridophyte spp.*Garecina glutea* and *Syzygium guineense.Hyparrhenia elongate, Hyparrhenia rufa, Hyaparrhenia spp., Pennisetum spp., Panicum maximum* and *Andropogen abyssinicus* (NTTTICC, 2006).

3.1.2. Human population and agricultural activities

In the mid-altitude region, the majority of human population resides and a simultaneous overcrowding, overgrazing, soil erosion, land degradation and overall resource deterioration prevail. The human population of the district is estimated to be 111784. The total livestock population of the Gawo Dalle district is about 29722 cattle, 5700 sheep, 4895 goat and 1840 equines. On the other hand, the lowlands are scarcely populated mainly due to animal trypanosomosis. Mixed livestock and crop farming is the dominant form of production in the area. In the area the main crops produced are maize and sorghum and coffee is also widely used as the main cash crop (CSA, 1998).

The area is closely situated to settlements, forest and woodland next to Ketto river, a tributary of the Birbir River which is a conducive environment for tsetse distribution. *

3.2. Study design and sample size determination

3.2.1. Questionnaire survey

A structured questionnaire format was developed and administered to randomly selected house hold heads to obtain information on herd composition; the major livestock health problems; animal management (grazing areas, watering points); main roles of animals; socioeconomic activities; source and usage of trypanocidal drugs; suspected trypanocidal drugs failure and loss due to trypanosomosis (Annex 10). Accordingly, 80 household heads were randomly selected from the population of livestock owners of ten sites.

3.2.2. Cross sectional study

Repeated cross sectional studies were conducted to determine the seasonal dynamic of tsetse population and other biting flies and the prevalence of bovine trypanosomosis in late rainy and dry seasons, which were from October to November 2007, and in March 2008 respectively.

Parasitological survey and methodology

The main aim of the study was to determine the prevalence of trypanosomosis infection at different sites and season as well as to isolate the strains that were used to infect the cattle to conduct experimental trial on trypanocidal drug sensitivity. The study was carried out by simple random sampling technique based on the selection of house-hold heads by lottery system (as it was possible to get sample frame based on records of peasant association) during late rainy and dry season on all animals of selected house hold heads found in different peasant association of the Gawo-Dalle district. To determine the sample size required to conduct the cross sectional study; the following formula was used (Thrusfield, 2005):

$$n = \frac{T^2 x Pexp x [1-Pexp]}{L^2}$$, where;

n – The required sample size; to get the total number we multiply n by 2.

T- Student's T-value at a given confidence level (1.96 at 95% confidence level);

P- Expected prevalence of trypanosomosis infection in the study area, and

L- Accepted absolute error/level of precision (5%)

Expected P = 25%, Then n= 252 households (All cattle were sampled from each house holds). One household has an average of 3 cattle, hence our total sample size was = 252 * 2 (both seasons) *3 = 1512. However 1525 animals were sampled in both seasons for this study.

To determine the seasonal prevalence of bovine trypanosomosis and estimate the potential risk factors associated with the disease, cross sectional parasitological surveys was conducted. Sample collection and parasitological examination was performed according to the following working procedures:

Blood samples were collected after properly securing the animal and aseptically preparing around the veins. In this study a small quantity of paired blood samples were obtained from the marginal ear vein after pricking the vein with the tip of a lancet.

The Buffy coat /phase contrast/ dark ground technique had been used for parasitological examination. This technique is recommended for diagnosing low parasitaemia, helps to identify trypanosome species and its quantification. Paired blood samples were collected from auricular vein of each animal using two heparinized Haematocrit capillary tubes centrifuged at 12000 rpm for 5 minutes and then measured by Haematocrit reader to know PCV values for determination of anemia.

The capillary tube was cut by diamond pencil 1mm below the Buffy coat RBC junction to include the top layer cells. The content of the capillary tube was expressed onto a clean microscope slide, covered with a 22x22 mm cover slip. Then the slide was examined for trypanosomes based on the type of movement in the microscope field. Confirmations of trypanosome species by morphological characteristics were done after staining the blood smear with Giemsa and examination with oil immersion microscopy with 100x power of magnifications (Murray et al., 1977). Then all the relevant data (species of trypanosome, number and identification of the animals by location, peasant association, village, owner, cattle's name given by owner, parasitaemic and non parasitaemic animals, PCV values, sex) were recorded for analysis.

Entomological survey and its methodology

Monopyramidal traps, which are mostly used and recommended by NTTICC, were used to catch the flies in both late rainy and dry season. NGU traps are efficient for savannah species (Vale, 1982).

However, in the study conducted in around Sor River that is a tributary of Birbir River in Baro Akobo River system for odour bait trial monopyramidal was highly efficient to sample *G.pallidipes*, *G.fuscipes fuscipes* and *G.morsitans submorsitans* (NTTICC, 2004). Monopyramidal was found also very efficient to sample *G.m.submorsitans* and *G. tachinoides* in the survey conducted in Limu shay area in Didessa district in upper Didessa valley (Feyisa, 2004).

To assess the apparent densities, distribution and species of tsetse flies and other biting flies in different seasons and vegetation pattern the survey was carried out according to the following protocol. Entomological data was collected twice in late rainy and dry seasons of the year 2007/2008. During the survey 110 Monopyramidal traps were deployed in selected sites where it seems was a suitable habitat for tsetse flies. Every trap was Odour-baited with Acetone, Octenol and Cow urine, which are Odour attractants for tsetse flies. The underneath of each trap pole was smeared with grease in order to prevent the ants climbing up the pole towards the collecting cage that could damage the tsetse flies. The deployment time was 72 hours. After a specified period of deployment the flies captured in the collecting cage were sorted by sex, species, and sites and recorded. A hand lens or stereomicroscope was used for the identification of tsetse fly species. Other biting flies were also recorded. The species of tsetse flies were identified based on the characteristic morphology (Ford *et al.*, 1976; Langridge, 1976; Leak *et al.*, 1999). Other biting flies were also separated according to their morphological characteristics such as size, color proboscis and wing venation structures at the genus level.

3.3. Trypanocidal drug sensitivity trial

3.3.1. Experimental animals

Drug resistance test of trypanosomes on Diminazene diaceturate, Isometamidium chloride hydrochloride and Homidium bromide was carried. For the drugs to be tested, a total of 15 indigenous zebu (Horro breed) aged 15 to 18 months were purchased from a market in the tsetse free area of Illubabor zone one month prior to the experimental infection and grouped into three treatment groups (Five animal per group). Prior to the experiment, the animals were dewormed

using a broad spectrum anthelmintic (Albendazol 2500 mg), injected with 20 % oxytetracycline, 7 % solution of Diminazene and sprayed with acaricide.

The animals were ear tagged, housed at night in animal house constructed previously for other study purpose in the National Tsetse Fly and Trypanosomosis Investigation and Control Center (NTTICC) which is located in Beddelle at an altitude of 2000 m.a.s.l. and is thus free of tsetse fly. The animals were maintained on grass land reserved for experimental animals on isolated grazing area in the campus of NTTICC and in addition they were given the by products of barely from Bedelle Brewery factory. Potable water was also provided to the animals.

3.3.2. Experimental design and field isolates of trypanosomes

Blood was collected from cattle and examined by Buffy coat methods and positive samples were examined using Giemsa staining for species identification. Blood from parasitaemic cattle were collected into EDTA- treated Vacutainer tubes, placed in Cold chain with ice and carried to the site of the experiment (NTTICC) which is found at a distance of about 400 km from Gawo-Dalle district, the study area. Subsequently, immediately after arrival at NTTICC the isolates were placed in incubator at 37^{o}C for about 30 to 40 minutes and by confirming the viability of the trypanosome. The Parasite isolates were from village 5, village -6 and Igu koffale peasant association or sites. These sites are found in the same area or adjacent to each other and therefore the stabilates can be considered as Gawo-Dalle isolates. The isolates were selected and inoculated into each cattle of the treatment groups. For diminazene group T.congolense 2-isolates from village -5, 2 isolates from Igu-koffale and one from village -6 were used. For Isometamidium chloride hydrochloride treatment group the mixed T.congolense and T.brucei trypanosome isolates selected were 2 from Igu-kofale, 2 from village -6 and one from village -5 were injected into cattle. For the homidium bromide group the T.congolense trypanosomes 2-isolates from village -6, 2-isolates from village-5 and one isolate from Igu-kofale were used. We brought above 50 samples from the field and selected the above mentioned isolates based on their site and species.

The isolates were injected into the jugular vein of the cattle. Three days after inoculation, and continuing until the end of the experiment, the animals were monitored on regular basis for PCV and parasitaemia (Annex 4 to9). Summery of the protocol is indicated as below:

Table 1: Summery of a protocol for drug resistance testing in cattle according to Eisler *et al.*, 2001

1	Number of groups	3
2	Treatment groups per drug.	1
3	Total number of Cattle	15
4	Number of animals per group	5
5	Inoculum:	
	➢ Number	$1x10^5$
	➢ Route	IV
6	Drug dosage (mg/kg BW):	
	➢ Diminazene aceturate7% Group I.	3.5, 7.0 and10.5
	➢ Isometamidium1% Group II.	0.5and1.0
	➢ Homidium2.5% Group III	1.0and 2.0
7	Drug administration	
	➢ Time	First peak of Parasitaemia
	➢ Route	IM
8	Parasitological examination	
	➢ Method	Buffy coat
	➢ Frequency	3 times per week
9	Duration of follow-up	100days
10	Interpretation of results	
	➢ Isolate sensitive	5/5 cured
	➢ Isolate resistant	Less than 5/5cured

3.3.3. Treatment and monitoring

Animals inoculated with the trypanosome isolates under investigation were treated with one of the commonly used trypanocidal drugs diminazene diaceturate, isometamidium chloride hydrochloride and homidium bromide at recommended doses, when the animals show the first peak of parasitaemia and clinical signs. Inoculated animals were monitored and followed for 100 days. The parasitaemia were monitored according to Paris et .al. (1982) in that estimation of trypanosomes per field at 250 magnification is scored (score is 6+= if >100 per field, 5+= if >10, 4+=1-10, 3+ =1per fields-1 per 10 fields, 2+=1-10 per slide and 1+ if 1 Trypanosoma per field is obtained).

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3.3.4. Trypanocidal drugs

Diminazene diaceturate (Veriben®, Lot No 437A1; Ceva Sante Animale, France), Isometamidium chloride hydrochloride125mg (Veridium ™, Lot No144A1; Ceva Sante Animale, France) and Homidium Bromide (Ethidium®,Lot No 48A1; Laprovet, France) were used for drug sensitivity study. Each drug was administered intramuscularly to cattle.

3.3.5. Drug sensitivity test in cattle

The experimental animals were divided into three treatment groups of five animals each. Each trypanosome isolate of the three peasant association was inoculated into the experimental cattle. The samples with higher than 10 trypanosomes per field at 250 magnification power of microscope were used as a peak parasitaemia for the isolates. On the first days of high parasitaemia post infection all animals were treated with 7% diminazene diaceturate, 2% isometamidium chloride hydrochloride and 2.5% solution homidium bromide at a dose rate of 3.5, 0.5 mg/kg and 1mg/kg body weight, respectively. The second treatment, at the double dose of the initial treatment, was given to all the same animals immediately after showing relapse and peak parasitaemia. The third dose was given to diminazene group only and was at dose rate of 10.5 mg/kg body weight in order to clear the resistant strain.

3.4. Data management and statistical analysis

Data collected from questionnaire survey, vector and trypanosome infection survey were entered into MS excel spreadsheet programme to create database. Data on all aspects of the above objectives were collected properly, handled carefully and analyzed systematically. For the analysis of data, statistical software programme: SPSS 15 for windows versions was used.

Information that were generated through questionnaire survey to compute frequency of responses and percentage of summarized data on basic livestock health problems were presented, and analyzed for frequency distribution and percentage expression in tabular and diagrammatic forms. Vector survey data were analyzed using two-sample t-test to compare seasonal mean catches and ANOVA to compare the mean catches in different study areas. In cross-sectional study, the late rainy and dry season prevalence of bovine trypanosomosis, hematological values and entomological results were presented and analyzed. Prevalence of bovine trypanosomosis can be expressed as the number of parasitaemic animals through Buffy coat microscopic study to the total number of animals examined (%). Hematological findings were expressed as percentage of the RBC to the total blood content (%).

In all cases, a 95% CI were employed to extrapolate sample results to the target population in the study area so as to assess seasonal dynamics of the parasite and the vector. In order to compare trypanosomosis prevalence and the pooled data of mean PCV between aparasitaemic and parasitaemic animals in both seasons, a combination of frequency distribution and student's t-test values and correlation were done to compare the relationship of PCV value with trypanosome infection rate. For data obtained from drug sensitivity testing in experimentally infected animals, information on the occurrence of relapse infections were summarized and analyzed. Hence, interpretations of the results were made based on the conditions described in annex 11 Eisler *et al* (2001).

4. RESULT

According to the information gathered from district agricultural office the surveyed areas in Birbir valley particularly in the bottom valley of Chanka was settled about twenty years ago by people from Northern Ethiopia due to drought problem and at the beginning the government purchased oxen and gave them which became victims of tsetse and trypanosomes and due to this devastating problem it supported them by tractor to prepare the land and ploughed for few years. The people later penetrated into the land by clearing the bush and started to keep livestock in the environment where the animals could live for up to few months to years only with the help of drug .The veterinary drugs were given freely by organization like O.R, A. as a mass treatment for about four years in this area and all the high trypanosome challenge districts in this zone.

4.1. Questionnaire survey

A total of 80 farmers who live in ten areas located in Birbir river Valley of Gawo Dalle District were interviewed. About 47.5%of respondents were settled in the areas twenty years ago because of drought problems in the Northern Ethiopia. They were mainly questioned on herd composition, major animal health problems, livestock management, socioeconomic activities, sources and usage of trypanocidal drugs and their efficacies.

All of the 80 respondents judged that bovine trypanosomiasis ranks first as the major animal health problem impairing agricultural development in their areas. The interviewees characterized the trypanosomiasis ("Gandi" or "Qoqsa") by signs like emaciation, rough hair coat, milk loss, cough and geophagia .All the respondents ranked cattle as the most susceptible class of livestock to trypanosomiasis followed by equines and small ruminants. 87.5% of the respondents complained that the wet (early and late rainy) seasons of the year are the most peak periods for trypanosomosis, while 12.5%of them replied that trypanosomiasis occurs all the time. About 96.2 % of the respondents know that flies are the transmitters of the disease while 3.8% do not know any thing about the fly. Apart from this, none of them were able to differentiate tsetse flies from other biting flies.

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4.1.1. Livestock management

All respondents use free grazing in large herds' .Out of the 80 respondents 57.5%keep cattle in valley bottom in the bush and savannah, and 47.5% at the shoulder of the valley and grassland. Most of them 95%responded that feed shortage is common in dry season from February to April.

4.1.2. Socio-economic activities

The main source of the income is ranked to be first from coffee production by 32.5%of the respondents and 67.5% reported mixed crop and livestock production as the main source of income how ever 100%of them responded they keep livestock

4.1.3. Livestock disease occurrence

The most common disease-affecting cattle is trypanosomosis (100%of respondents), followed by CBPP, ticks, Pasteurellossis and blackleg. 100% of the respondents reported trypanosomosis mostly affects cattle. About 35 %of the respondents reported that trypanosomosis started about 40 years ago (old native dwellers) and 65% of the respondents replied that they know trypanosomosis above 20 years in the area and all of the settlers which arrived 23 years ago said that the disease is there when they arrived. Accordingly the information gained from the 35% old native dwellers is more reliable to estimate the first historical occurrence of the outbreak of trypanosomosis in the area. Regarding the trend of the disease in the past five years 21.3% responded that it has increased, 52.5%replied it has decreased and 26.3% reported no change.

4.1.4. Usage of trypanocidal drugs

100%of the farmers interviewed used trypanocidal drugs out of which about 61.3%used the drug for the last 15years and 27.5% of the respondents used for about 40yearsand about 11.2%of them replied that they used for the last 10 years. 55%of the respondents reported that they treat any sick animal and 45% replied that they treat all animals monthly either by isometamidium or diminazene. The animals are treated for trypanosomosis with high frequency or at short interval according to the response of majority of interviewees and 12.5%; 67.5%; 15%and 5% of the treatment interval at about less than 20 days, every month, and 1to 2month and 3 to 6 month treatment interval were reported by the respondents respectively (Figure 2)and 52.6% of respondents reported trypanocidal drugs are administered by farmer themselves or non-professionals at home (Fig.-3).

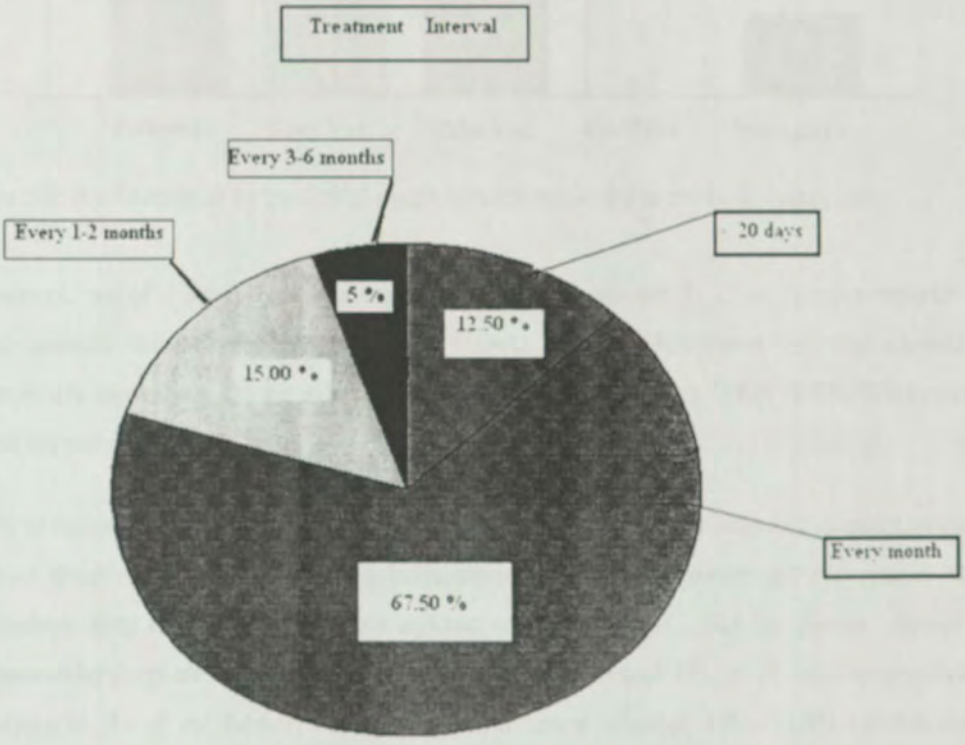

Figure 2: Treatments Interval of Cattle in Gawo-Dalle district against trypanosomosis

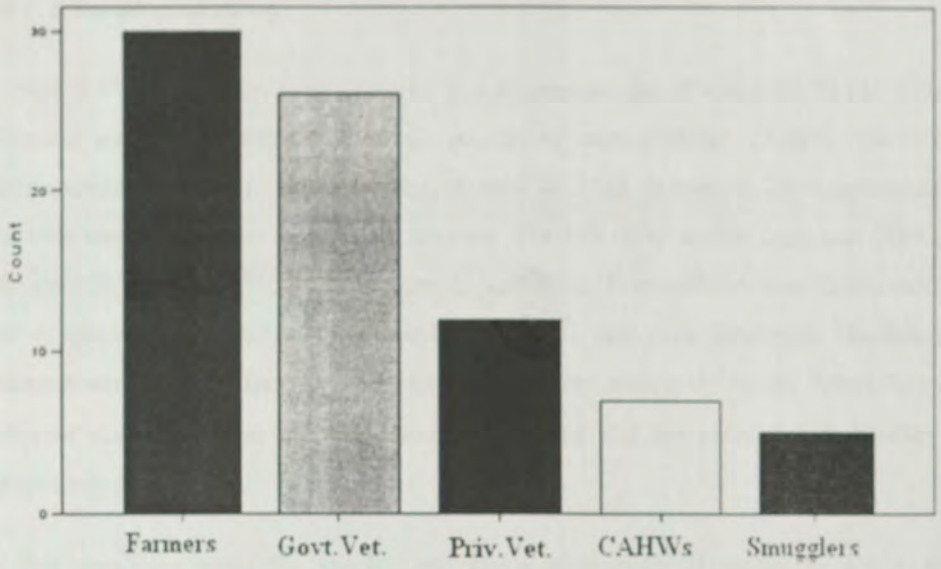

Figure 3: Application of trypanocidal drugs carried out in the areas by different body

However, out of the total animals treated for trypanosomosis 81.2%of farmers reported that their animals did not recover from the disease and 18.8%reported that the animals are completely recovering. On the average each farmer spends about 8.71Birr (0.93USD) to treat an adult animal once.

28% of the respondents replied that they use diminazene, 11%used isometamidium chloride and 55%of them reported they use both isometamidium and diminazene and only about 4%use homidium salts to treat their animals against trypanosomiasis. 55of the farmers replied that trypanocidal drugs treatments were applied to sick animals and 45% to all cattle as prophylactic measure.36.3% of the farmers replied that diminazene whereas 25%, 21.3% isometamidium said both diminazene and isometamidium and 17.5%said don't know which is most effective. About 50% of the farmers reported that they use the correct dose of trypanocidal drugs and about 50% has no idea of drug dose.

4.2. Cross sectional study

4.2.1. Entomological survey

A total of 4088 tsetse flies were captured in both seasons out of which 59.1% (2416) belong to *Glossina pallidipes*, 18.8%(770) *to G. morsitans submorsitans*, 24.88% (1017) *to G. f. fuscipesand2.96%*(121) to *G.tachinoides*. A total of 2128 Stomoxys, 25 Haematopota and 10 Tabanus were caught also during both seasons .The late rainy season catch was 2519 of which 54.55%, 21.32%, 21.24% and2.9% were *G.pallidipes, G.m.submorsitans. G.fuscipes fuscipes* and *G.tachinoides* respectively. In addition 1167, 1 and zero Stomoxys, Haematopota and Tabanus were caught respectively during the late rainy season fly survey. Mean fly catches in different study areas and late rainy and dry seasons and are presented in Annexes 1and 2 respectively.

In this area four tsetse fly species are found abundantly (*G.pallidipes* and *G.morsitans submorsitans)* which belong to savannah species are predominating over the other two species G.fuscipes fuscipes and G.tachinoides, the riverine group. The apparent densities of 7.32, 3.08, 2.33 and 0.37for G.pallidipes, G.f.fuscipes, G.m.submorsitans and G.tachinoides were recorded respectively; whereas the apparent density of 6.97, .11 and .03 were obtained for Stomoxys, Haematopota and Tabanus respectively in the study area (Annex 3).Tsetse overall mean apparent density (fly/trap/day) of tsetse species is 13.10± SD 19.58.and the apparent densities across both seasons were summarized in Annex 3.

Higher mean catch was obtained during late rainy season than dry season (t = 31.321, P<.001); and (t =17.42 P<.001) for total Glossina in areas .Statistically, significant differences were also observed for other biting flies between seasons (t =11.57, P<0.001); (t=3.494, p=0.001) and (t=2.562, P<0.05) for Stomoxys, Haematopota and Tabanus respectively (Table 2). The apparent densities recorded in altitudes less than 1400 m.a.s.l. was higher than that obtained in higher altitudes in the areas (Table 4).

Table 2: One sample T test analysis of fly catch in different areas and seasons

	t	df	P	Mean Difference	95 % CI
Area	17.415	109	.000	3.818	3.38 - 4.256
Season	31.321	109	.000	1.500	1.41 - 1.59
G.m.submorsitans	4.397	109	.000	2.333	1.282 - 3.385
G.pallidipes	6.505	109	.000	7.321	5.091 - 9.552
Total G.f.fuscipes	4.129	109	.000	9.245	4.81 - 13.68
G.tachinoides	2.436	109	.016	.367	0.67 - 0. 66
Total Glossina	7.019	109	.000	13.103	9.403 - 16.803
Haematopota	3.494	109	.001	.111	0.05 - 0.17
Stomoxys	11.578	109	000	6.965	5.773 - 8.157
Tabanus	2.562	109	.01	.030	01 - 0 .05

Table 3: The fly apparent densities (fly/trap/day) of Late rainy and dry season in Gawo-Dalle district of Birbir valley 2007/2008(95% CI= 1.41_1.59)

Season		Flies							
		Glosina Species					Biting Flies		
		G.m.s.morsitanse	*G.palli dipes*	*G.f.fusc ipes*	*G.tach inoides*	Total *Glossina*	*Haemat opota*	*Stomo xys*	*Tabanus*
Late	Mean	3.94	10.36	3.31	.44	18.05	0.01	8.53	0.00
rainy	S.D.	7.16	14.59	9.24	1.65	24.14	0.045	8.16	0.000
Dry	Mean	.73	4.28	2.85	.29	8.16	0.22	5.41	0.06
	S.D.	2.47	7.05	6.19	1.52	11.88	0.445	2.97	0.171
Total	Mean	2.33	7.32	3.08	.37	13.10	0.11	6.97	0.03
	S.D.	5.57	11.80	7.83	1.58	19.58	0.332	6.31	0.124

There were statistically significant differences in mean catches between areas being (F=8.102,df =7, P< 0.001),(F=0.984,df =7,P< 0.05),(F=0.926,df=7, P<0.05) (F= 0.806. 7df, P< 0.05) for tsetse, Stomoxys, Haematopota and Tabanus respectively. The different fly densities were assessed per season where (18.1and 8.2) for tsetse ;(8.53 and 5.4) for Stomoxys (.01and.22) for Haematopota and (.00and.06) for Tabanus flies relative densities (fly/trap/day were recorded in late rainy and dry seasons, respectively.

Table 4: Apparent densities of Glossina species at different altitude

Altitude m.a.s.l		Flies							
		Glossina Species					Biting Flies		
		G. m.s morsitanse	G. pallidipes	G.f. fuscipes	G. tachi noides	Total Glossina	Haema topota	Stomoxys	Tabanus
<1400	Mean	3.058	9.416	4.19	.50	17.156	.11	6.790	.03
	S.D	6.311	13.057	8.879	1.824	21.331	.315	6.829	.125
>1400.	Mean	.310	1.472	.00	.00	1.782	.12	7.454	.02
	SD	1.019	2.69	.000	.000	3.475	.380	4.624	.124
Total	Mean	2.333	7.321	3.08	.37	13.103	.11	6.965	.03
	SD	5.565	11.804	7.829	1.578	19.58	.332	6.309	.124

4.3. Parasitological Survey

4.3.1. Trypanosome infection

In ten peasant associations of Gawo-Dalle district repeated cross-sectional surveys of trypanosome infections were conducted in two seasons. A total of 1525 cattle (759 in late rainy and 766 in dry season) were sampled to determine the prevalence of bovine trypanosomosis and to assess the associated risk factors.

Table 5: Overall Prevalence of trypanosome infection in the cattle subpopulation by area, season and sex in ten peasant associations

Group (variables)		No.of animals examined	Trypanosome species identified							No. infected and Preval (%)	95 % CI (.17-23)
			T.c.	T.b	T.v.	T.th.	T.c. + T.b.	T.b. + T.v.	Tc. + Tv.		
Area	Village-7	128	18.8	3.1	1.6	0.0	0.8	0.0	0.0	31(24.23)	.21- .46
	Village-6	138	14.5	1.4	2.2	0.0	0.0	0.0	0.0	25(18.11)	.14- .34
	Village-5	151	13.2	2.6	1.3	0.7	0.7	0.0	0.0	28(18.55)	.17- .40
	Igu-kofale	178	10.7	4.5	1.7	0.0	0.0	0.0	0.0	30(16.9)	.16- .34
	Cherecha	140	4.3	0.7	1.4	0.0	0.0	0.0	0.0	9(6.4)	.03- .17
	Chankbururi	136	9.6	1.5	3.7	0.0	0.0	0.0	0.7	20(14.8)	.14- .42
	Village-11	138	16.7	3.6	0.0	0.0	0.7	0.0	0.7	30(21.8)	.18- .46
	Dog-Adami	174	2.9	0.6	0.6	0.0	0.0	0.6	0.0	8(4.7)	.01- .17
	Village-1	150	10.7	1.3	2.0	0.0	0.0	0.0	0.0	21(14.0)	.10- .28
	Dogo-ruri	192	1.0	0.0	0.5	0.0	0.0	0.0	0.0	3(1.56)	- 01-.06
Spp.prevalence		206	71.8	14.1	10.7	1.0	1.0	1.0	1.0	-	.17-.23
Season	Lrainy	759	10.8	1.7	2.4	0.1	0.1	0.0	0.0	115(15.1)	.13-.18
	Dry	766	8.6	2.1	0.5	0.1	0.1	0.3	0.1	91(11.80	.095-.141
Sex	Feme	546	9.3	1.1	0.9	0.2	0.2	0.0	0.0	64(11.7)	.090-.144
	Male	979	9.9	2.4	1.7	0.1	0.1	0.2	0.1	142(14.5)	.123-.167
Over.prevalence		1525	9.7	1.9	1.4	0.1	0.1	0.1	0.1	206(13.44)	.12-.15

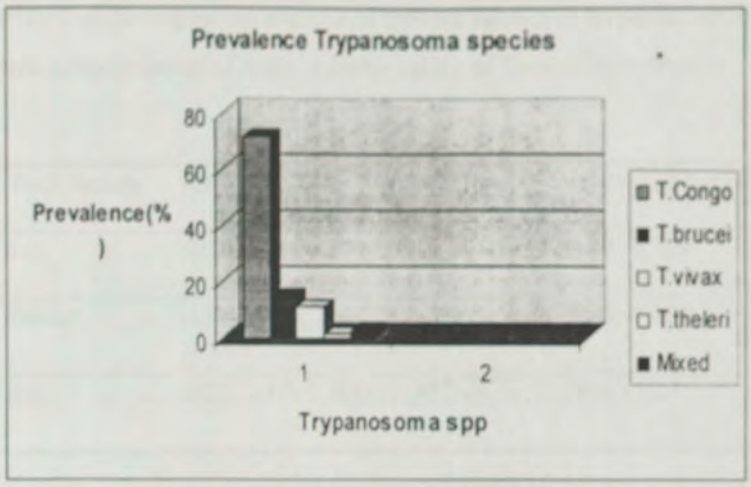

Figure 4: Prevalence of different Trypanosomes species

There was a statistically significant difference in the prevalence of trypanosomosis among the study sites(X^2 = 122.112, P = P 0.000, P < 0.001) and seasons (X^2 =14.646, P=.041, P< 0.05) whereas, (X^2=6.994, P.430, P>0.05) with in sex of animals which reveals there is statistically no significant differences in trypanosomosis infection between sex.

The overall prevalence for the bovine trypanosomosis in the area was found to be 13.44% across both seasons. Among the prevailing trypanosome species, T.congolense was the dominant one accounting for 71.8% followed by T.brucei14.1%, T.vivax 10.7% and mixed infections constituted 2.5% .In the present finding we have recorded 1% T.theillerii which is a non pathogenic species to cattle. The seasonal prevalence of trypanosomiasis was found to be 15.1% and 11.8% in the late rainy and dry months, respectively (Table.5). The multivariate analysis performed using logistic regression model adjusted for assumed risk factors (Season, area and sex) for trypanosome infection is presented in (Table.6). The risk of trypanosomosis was higher in the late rainy season as compared to the dry season (OR =1.332, 95%CI=. 988-1.704) .The high risk is by far attributable due to site or area of animal (OR=.855, 95%CI=.810-.902).

Table 6: Multivariate logistic regression analysis of the risk factors in trypanosome infection in the cattle subpopulation of areas in Birbir valley of Gawo-Dalle district

Risk factors	S.E.	df	P	Odds.ratio	95.0% C.I.for OD
Sex	.163	1	.191	1.238	.899 -1.704
Season	.153	1	.060	1.332	.988 -1.796
Site	.027	1	.000	.855	.810 -.902

4.4. Haematological findings

In the present study the mean PCV in aparasitaemic cattle was 26.26% (95%CI=25.87-26.65%) while that of the parasitaemic animals was observed to be 22.82% (95%CI=22.19-23.44%).The mean PCV values for parasitaemic and aparasitaemic animals in late rainy season were 23.28% (95%CI=22.43-24.12%) and 26.37% (95%CI=25.64-27.10%) respectively. While the mean PCV values for parasitaemic and aparasitaemic animals in the dry season were 22.23 % (95%CI=21.29-23.17%) and 26.15 %(95%CI=25.82-26.49%). There was a statistically significant difference in the level of anemia between the trypanosome infected and non-infected animals (t = 6.566, P<0.001).The mean PCV values in the late rainy season were 25.90%(95%CI =25.27-26.54%) and the dry season values 25.69%(95% CI=25.36-26.54%). respectively (Table .8).The mean PCV value was found negatively correlated with the trypanosome prevalence (r = -.1666).

Table 7: Mean PCV values of aparasitaemic and parasitaemic animals

	N	Mean PCV	SD	Std. Error	95% Mean	C I	Minimum	Maximum
Aparasitaemic	1319	26.26	7.308	.201	25.87	26.65	10	233
Parasitaemic	206	22.82	4.570	.318	22.19	23.44	11	37
Total	1525	25.79	7.098	.182	25.44	26.15	10	233

Table 8: Mean PCV values by season

Season	N	Mean	Std. Deviation	95% Confidence for Mean	Interval	Minimum	Maximum
Late rainy	759	25.90	8.917	25.27	26.54	10	233
Dry	766	25.69	4.644	25.36	26.02	12	41
Total	1525	25.79	7.098	25.44	26.15	10	233

r = - 0.166

Table 9: T- test analysis of PCV values of infected and non infected cattle by seasons

PCV	Test of significance level of PCV by season					
	t	df	P	Mean Difference	95% Interval of the Difference	Confidence
Late rainy	80.034	758	.000	25.904	25.27	26.54
Late rainy aparasitemic	71.110	643	.000	26.373	25.64	27.10
Laterainy parasitaemic	54.667	114	.000	23.278	22.43	24.12
Dryseason aparasitaemic	152.299	674	.000	26.153	25.82	26.49
Dryseason parasitaemic	46.798	90	.000	22.231	21.29	23.17
Total	6.566	1523	.000	3.445	2.416	4.47

4.5. Results of drug sensitivity tests

The parasitological results of the sensitivity testing of each of the experimental cattle or Isolates from the three Peasant associations for diminazene 3.5 mg/kg, 7mg/kg and 10.5mg/kg; isometamidium chloride hydrochloride 0.5, 1mg/kg and homidium bromide 1 and 2mg/kg body weight are presented in Annexes.4.to.6, where as the summarized result of drug sensitivity test is indicated on table 10 and 11 bellow.

Table 10: Sensitivity to Diminazene Diaceturate, Isometamidium Chloride and Homidium bromide of *Trypanosoma congolense* and mixed *T.congolense* and *T.brucei* isolates from Gawo-Dalle district in Cattle

Isolate	ID.No	Group	First Rx	Rd	Second RX	Rd	Third Rx	Rd
Village-5	V500/02	Diminazene	3.5mg/kg	8	7mg/kg	14	10.5mg/kg	
Village-5	V501/02	Diminazene	3.5mg/kg	8	7mg/kg	14	10.5mg/kg	
Village-6	V604/01	Diminazene	3.5mg/kg	8	7mg/kg	10	10.5mg/kg	49
Igukofale	IK06/02	Diminazene	3.5mg/kg	8	7mg/kg	25	10.5mg/kg	
IguKofale	IK09/03	Diminazene	3.5mg/kg	8	7mg/kg	13	10.5mg/kg	22
Resistant			5/5		5/5		2/5	
Village-6	V600/01	Isometamidium	.5mg/kg	3	1mg/kg	15		
Village-5	V500/03	Isometamidium	.5mg/kg	18	1mg/kg	90		
Village-6	V605/02	Isometamidium	.5mg/kg	4	1mg/kg	18		
Igukofale	IK07/02	Isometamidium	.5mg/kg	4	1mg/kg	10		
Igukofale	IK08/02	Isometamidium	.5mg/kg	4	1mg/kg	10		
Resistant			5/5		4/5			
Igukofale	IK00/04	Homidium	1mg/kg	0	2mg/kg	0		
village-5	V503/01	Homidium	1mg/kg	10	2mg/kg	5		
village-5	V510/03	Homidium	1mg/kg	0	2mg/kg	x		
village-6	V611/03	Homidium	1mg/kg	4	2mg/kg	4		
village-6	V612/03	Homidium	1mg/kg	0	2mg/kg	5		
Resistant			5/5		4/4			

Table 11: Summary of Sensitivity of Gawo Dalle isolates of *T.congolense* to Diminazene diaceturate, and Homidium bromide and mixed *T.congolense* and *T.brucei* field isolates to Isometamidium chloride hydrochloride

Treatment group	Drug/dose (mg/kg)	Number of cattle relapsed	Mean relapse in days
Diminazene	3.5	5/5	8.4
	7.0	5/5	15.2
	10.5	2/5	35.5
Isometamidium	0.5	5/5	6.6
	1.0	4/5one cured	13.25
Homidium bromide	1	5/5	2.8
	2	4/4 one died	3.5

4.5.1. Development of infection and drug sensitivity (response) in cattle

After experimental infection the animals showed first parasitaemia at 6.2, 7.8, and 6.6 days. In diminazene group four animals developed parasitaemia in seven days, except one animal (IK0602), which showed parasitaemia in 3days post-infection. Three animals in isometamidium and two animals in homidium groups also became parasitaemic in 3 days post infection. The animal tags V600/01, IK0004 were parasitaemic at 19 and 17 days respectively.

The parasitological results for various doses of diminazene diaceturate, isometamidium chloride hydrochloride and homidium bromide when the same animals were given showed that the relapse occurred in 8.4 days and 15.2 days to the dose levels of 3.5mg/kg and 7mg/kg body weight respectively as a group in10to25 days after the second treatment in diminazene group. whereas 6.6 and 13.25 relapse days recorded in0.5 and 1mg/kg of isometamidium group and trypanosomes reappeared in animals within 0to10 days(2.8days) in homidium bromide group at both dose level of 1mg/kg and 2mg/kg body weight when the same animals were given the trypanocidal drugs at different dose level(Table.10).The relapse to 10.5mg/kg body weight of diminazene occurred in only two animals with tag IK 09/03 at 22 days and V604/01 at 49 days post treatment ,and no relapse in the rest out of the five treated animals in about 50 days post treatment .

Three cattle of homidium group tag Ik00/04,V51003 and V61203failed to respond completely to homidium bromide at 1mg/kg while the other two responded and relapsed in very short time in 4days (tag. V611/03) and 8 days (tag noV50003. The animal tag V510/03 of homidium group died at 14 days post treatment or a month after challenge by infection. The animal tag IK00/04 again failed to respond to the second dose of homidium bromide (2mg/kgbodyweight) while the other three animals responded but relapse of parasitaemia occurred in about 4.3 days. The animals of this group died within the interval of two months post infection due to high parasitaemia except one with tag No V61203 .In isometamidium treatment group the five cattle responded but relapsed in 3-18days range (6.6days mean) to 0.5mg/kg body weight. Then 10-18 days range to the second (1mg/kg body weight). The animal tag V50003which showed relapse in 18 days to the first dose0.5mg/kg body weight of isometamidium chloride hydrochloride was cured by the second dose (1mg/kg) of isometamidium. This animal which became sensitive to 1mg/kg of isometamidium was with PCV of 25%on the day of treatment and increased progressively up to the pre infection period PCV level in the 100 days follow up period. It has in creased in body weight from 79-109kg also. The animalsV60502 and IK0702 of isometamidium group responded to second treatment and relapsed at 14and 17 days respectively. During the protected period there were no improvements of PCV as it was similar to PCV that recorded in infection period. The animals tag no. V600/01 and IK0802 died after treatment with second dose (1 mg/kg) isometamidium due to relapse and failure of the drug to completely clear the parasite from the body.

The mean PCV of all animals were high before infection and it was 35.93 days (33-44). Post treatment the animals showed parasitaemia at different time interval. The level of PCV increased progressively in one of the cattle (V50003), which became sensitive to the isometamidium 1mg/kg (Table.10). The infection caused a comparable decrease in PCV in all groups and it decreased to mean PCV 22.06 %(95%CI=21.45-22.68%), 19.982 %(95%CI=19.22-20.74%) and 17.878 %(95%CI=17.05-18.71) in diminazene diaceturate, isometamidium chloride hydrochloride and homidium bromide groups respectively post infection and post relapse periods (Table 12).

In diminazene group the mean days the animals protected or remained free of parasite post treatment in the peripheral blood was 8 days and 15.2 days by the dose rate of 3.5mg/kg and 7mg/kg body weight respectively.

The subsequent parasitaemic relapse prevented the recovery of PCV in the uncured cases, but PCV fully recovered in cured cases. In general PCV value is higher compared to the other groups after the relapse.

After the trypanocidal treatment the PCV began to recover immediately in most of the cattle however, there was a relapse of infection in short interval of time *in* all groups post-treatment specially by first dose and PCV decreased as soon as the parasite broke to blood .In the effectively cured cattle, PCV continued to recover until day 91 pt, when the mean PCV value of the cattle (V500/03) was almost comparable with the pre-infection mean PCV .The relapsed cases had a mean PCV of 22.07 ±3.996%,compared to 36.2%,19.98±4.025 vs. 36.2% and 17.88±4.486%Vs 35.4%pre-infection mean PCV of diminazene diaceturate, isometamidium chloride hydrochloride and homidium bromide group animals.

Table 12: One-Sample Test of CV for parasitemic and aparasitemic states in the treatment groups

PCV	t	df	P	Mean Difference	95 %CI
PCVDDA6	71.153	165	.000	22.066	21.45 -22.68
PCVDD0	33.998	46	.000	23.213	21.84 -24.59
PCVISM6	52.066	109	.000	19.982	19.22 -20.74
PCVism0	36.822	67	.000	27.206	25.73 - 28.68
PCVHM.0	27.097	8	.000	17.556	16.06 -19.05
PCVHM6	42.737	114	.000	17.878	17.05 -18.71

NB : pcvdd6 = pcv values of parasitaemic animals in diminazene group
pcvdd0 = pcv values of aparasitaemic animals in diminazene group
pcvism6 = pcv values of parasitaemic animals in isometamidium group
pcvism0 = pcv values of aparasitaemic animals in isometamidium group
pcvhm 0 = pcv values of aparasitaemic animals in homidium group
pcvhm.6 = pcv values of parasitaemic animals in homidium group

5. DISCUSSION

5.1. Questionnaire survey

Results of the questionnaire survey conducted among 80 family heads of the community members indicated the presence of eight veterinary clinics (four private and four public) in the study area but still alarming loss due to trypanosomosis exists in the area. All of the respondents agreed that the animal trypanosomosis ranks first and they are using isometamidium and (or) diminazene groups to treat their animals.

Overall results of the questionnaire survey showed that all (100%) of the interviewees reported that trypanosomosis was a serious problem to keep livestock in this district. In a questionnaire survey conducted at Bedelle, west Ethiopia, by Tewelde (2001) indicated also that trypanosomosis was the most important livestock disease (95.5%of the respondents replied in the area). In the present finding, all the farmers interviewed reported that trypanosomosis cases occur either in the late or at the start of rainy season or dry season or during both seasons. Afework (1998), Tewelde (2001) also reported similar findings. During the questionnaire survey the interviewee responded that about 52.65% treat their animals at home by themselves or by non-professionals. Afework (1998) and Tewelde (2001) also found 43% and 57% drug application by farmers or uncertified individuals, which agrees with the present finding. In the present finding 80% of the interviewed farmers responded that they treat their cattle at up to a month interval and the respondents complain that if they do not treat at about a 15 days to a month interval the animals would die. This shows that there is either high pressure of trypanosomosis Uilenberg (1998) or the treatments were not effective in curing sick animals. The frequency of treatment in the present result was, however, higher than the one reported by Afework (1998) and Tewelde (2001) who reported 3 to 4 times treatment per year. The old indigenous inhabitants interviewed or 27.5%of the respondents recall the date of the first trypanosomosis out breaks and use of trypanocidal drug was being in 1960s (above 45years) and that time they used to treat their animals by one of homidium salts ("Red tablet"), while 61.3% and 11.2 of the respondents replied that they used trypanocidal drugs for the last15 and 10years respectively. Moreover, 55%, 28.8%, 11.5%, and 3.8% of the farmers responded that they currently use isometamidium chloride, diminazene aceturate, isometamidium and homidium respectively to treat their animals against trypanosomosis. About 58% of farmers

responded that they are using the correct dose of trypanocidal drug and about 40% have no idea of the quantity or dose of drug used.

In the survey conducted in Zambia, for example (Van den Bossche *et al.*, 2000), have shown that despite farmers were administering most of the trypanocidal treatments, there was little evidence of under dosing, though there was a strong tendency to use curative (diminazene) rather than prophylactic drug (isometamidium). On the other hand we observed during our survey that there is uncontrolled use of trypanocidal drugs as we observed different shops engaged in selling of different generic trypanocidal drugs particularly in village-6of the study area even though the majority of the interviewee responded to purchase the drug from private veterinary clinics. In the present survey 55% of the interviewees responded that trypanocidal drugs are applied to any sick animal and 45% replied that they treat all their animals (Mass treatment) irregularly. In this study above 80% of the respondents complained that the treated animals did not recover. This is in agreement with the result of the present experimental study.

5.2. Cross sectional study

Vector survey was undertaken to identify tsetse species and other biting flies, assess distribution and apparent densities in late rainy and dry season. During the present survey, 4 species namely *G.m.submorsitans, G.pallidipes, G.fuscipes fuscipes* and *G.tachinoides* were identified.

The entomological survey showed that the apparent densities of different flies across both seasons varied significantly (P<. 001) among the study sites or villages and were 2.33±SD 5.56,7.32±SD11.8, 3.08±SD7.83, 0.37±SD1.8, and0.11±SD0.33, 6.97±SD6.31and0.03±SD.12 for *Glossina m. submorsitans, G.pallidipes. G. fuscipes fuscipes, G.tachinoides,* Haematopota, Stomoxy*s* and Tabanus *were recorded* respectively. Totally39.3±SD 58.7for tsetse and 0.32±SD 0.95, 20.7 ±SD 18.88; and 0.09±SD 0.372 mean catches were recorded for Haematopota, Stomoxys, and Tabanus respectively. Apparent density ranging from 0.6to93.44fly/trap/day of *G.m.submorsitans, G.pallidipes, G.fuscipes fuscipes* and G.tachinoides was also reported in the same area, Gawo Dalle district (NTTICC, 2004).

However low apparent densities for G.pallidipes (2.4 and 0.6) in wet and dry seasons; for G.fuscipes fuscipes (0 .1 and 0.06) in wet and dry seasons respectively were reported in southern rift valleys of Ethiopia Similarly the mean fly catches of G.pallidipes was1.42 and for

G.fuscipes 0.29 in Ghibe valley (Leak et al., 1993). When we compare the present finding with the report of Leak (1993) it is found to be higher.

Regarding the seasonal distribution of flies, the mean catch of 54.15and 24.47($P<0.001$) for tsetse, 0.0182and0.6182 ($p<0.001$) for Haematopota, 25.58and15.87 ($P<0.001$) for Stomoxys and 0.00and.18 ($P<0.05$) for Tabanus fly were recorded during late rainy and dry season, respectively. This could be explained by favorable environmental factors such as enough moisture, vegetation growth and suitable habitat during the wet season

In all these sites tsetse flies have highly infested and it is difficult to keep livestock in the area even being under regular treatment. In the present distribution records *G.pallidipes* has covered a vast area with high apparent densities in the studied sites. This could be due to the fact that this species inhabits wide range of vegetation types, which are available in the area. While *G.m.submorsitans* was found to be restricted almost to the savannah vegetation and thus its distribution is highly dependent on the season and vegetation cover.

G.f.fuscipes is distributed along the large rivers of Birbir and its tributaries like Ketto, Chabal, Hindina, Kile and others, which cross the study area, and thus provides conducive environment (Tall gallery forests and l dense thickets along the river bank) for the distribution of G.f.fuscipes. When we compare the savannah and riverine species, the riverine species of tsetse flies were found to be less affected by seasonal variation, which could be explained, by the prevailing riverine vegetation and fringes of riverine forests, which are used as shades for coffee plantation in almost all areas.

During the study period, the sex ratio for the collected tsetse flies was assessed. Higher number of females was recorded and it is in agreement with the results reported by other worker Sori (2006) in East Wallaga west Ethiopia respectively. Leak (1999) also reported that in unbiased sample female would comprise 70-80%of the mean population.

The relation ship observed between disease prevalence and tsetse apparent densities was in agreement with some established facts. The density of tsetse flies in the area and the level of animal fly contact will determine the level of infection. This is farther influenced by the vectorial capacity of the fly, an availability of its preferred host, which is not necessarily domestic livestock (Radostitis et al., 1994).

In the present finding, the highest prevalence of trypanosome infection was recorded in village-7 (24.4%) and then followed by Village-5and village-6 with 18.5and 18.2%respectively. In the present study, over all prevalence of 15.1%e was recorded.

However, relatively higher prevalence (25%) of trypanosomosis was reported in the same district, Gawo-Dale district by (NTTICC, 2004). This disparity emanates from the fact that the previous survey was conducted only during the wet season. *T. congolense* with the prevalence of72.3% was the predominant species identified during this study. This is because tsetse flies are the main vectors of *T. congolense* infection in the area. This is completely in agreement with the reports of Tewelde (2001) in west Ethiopia (75%), and higher than that reported by Rowlands *et al* (1993) in southern Ethiopia (37%) and Abebe and Jobre (1996) for tsetse infested areas of the country (58.5). In the present finding the prevalence of trypanosomosis has varied significantly ($P< 0.05$) between the two seasons with higher prevalence in the late rainy season. This situation could be due to the presence of high population of tse tse during the wet season.

During the present work the mean PCV of parasitaemic animals for both seasons was 22.82 for parastemic and for aparasitaemic animals it was 26.26 % indicating difference between trypanosome infected and non-infected animals. This is in agreement with the work of Rowlands *et al.*, 2001 who indicated Cattle with mean PCV values less than 26%were considered as anaemic, condition considered to be the main sign of trypanosomiasis. A narrow difference was recorded in PCV values for parasitaemic animals in late rainy (23.28%) and dry seasons (22.23%). In the present findings, the mean PCV value was found negatively correlated with trypanosomiasis prevalence ($r = -0.1666$). This is in agreement with the result reported by Feyisa (2004) in south west Ethiopia 21.65%and 25.54%in parasitaemic and aparasitaemic animals respectively; Sori (2006) who reported 20.22%and 27.23%for parasitaemic and aparasitaemic cattle respectively in west Ethiopia.

5.3. Drug sensitivity tests in cattle experimentally infected with isolates of Gawo-Dalle

Each infected cattle in all groups received 2ml of blood with high parasitaemia or the same number of trypanosomes in the infective inoculums. This was to eliminate the possible influence of infective dose on the Prepatent period and subsequent parasitaemia (Murray and Dexter, 1988). It appeared that *T.congolense* single infection as well as *T.congolense and T.brucei* mixed strains were resistant to prescribed curative doses of diminazene diaceturate

(3.5and7 mg/kg) and to curative as well as prophylactic and higher doses of isometamidium chloride hydrochloride (0.5and 1 mg/kg) and to homidium bromide at 1and 2 mg/kg body weight.

In the present work with 15 trypanosome isolates collected from cattle in Birbir valley Gawo-Dalle district, the out come of the trypanocidal drug resistance tests in cattle clearly showed the presence of trypanosome strains that have developed resistance to all currently available trypanocides. Except for one isolate sensitive to 1 mg/kg bodyweight isometamidium chloride, all were found to be resistant to 3.5, 7.0 mg/kg, 0.5, 1mg/kg and 1,2mg/kg body weight of diminazene, isometamidium and homidium bromide, respectively.

In homidium bromide treatment group 3/5 animals completely insensitive to 1mg/kg while the other two responded but the relapse occurred at the 4th and 10 th day of treatment. The isolates of *T. congolense* and mixed *T.congolense and T.brucei* from Gawo Dalle district of Birbir valley were found to be resistant to the curative doses of diminazene diaceturate at a dose rate of 3.5 and 7mg/kg body weight; again the isolates were found to be resistant to curative and prophylactic doses of isometamidium chloride hydrochloride at doe rates of 0.5 and 1 mg/kg body weight respectively; and to homidium bromide at dose rates of 1mg/kg and 2mg/kg body weight in cattle. The results showed that diminazene diaceturate at both dose of 3.5 and 7 mg/kg, 1.0and 2.0mg/kg homidium bromide failed to clear the parasite and it was found that five out of five animals in each group of both diminazene diaceturate and homidium bromide were resistant with the isolates of *T. congolense*. Similarly the isolates (*T.congolense* and *T.brucei*) were resistant to isometamidium chloride hydrochloride at a dose0.5mg/kg body weight (five out of five; 100% were relapsed) and to 1.0 mg/kg body weight (four out of five) (80%) failed to clear the infection except one cattle with tag number00/03which was cured by 1mg/kg of isometamidium chloride hydrochloride. The five animals in diminazene treatment group when further treated by 10.5 mg/kg body weight of diminazene for the purpose of clearing of drug resistant strains, relapse of infection has occurred again at this higher dose used in one animal with tag number 09/03 on the 22nd day post treatment.

When we compare the higher doses of diminazene (3.5, 7 mg/kg first and second dose respectively) and isometamidium (0.5, 1 mg/kg first and second dose) difference in relapse interval of first and the second dose about two for Diminazene and about six times for isometamidium was observed.

The present result agrees with the report of (Geerts et al., 2001) which stated that resistance to all drugs for veterinary trypanosomosis has also reached severe levels, especially in areas with high transmission rates and high levels of drug utilizations.

These results are in accordance also with earlier reports from the southwest (Codja et al., 1993; Mulugeta et al., 1997; Rowlands *et al*., 1993 and Tewelde, et al., 2004) and northwest Afewerk et al. (2000) of Ethiopia. In a trial made to compare the therapeutic efficiency of diminazene aceturate at doses of 3.5 and 7.0 mg/kg body weight in two of the herds at Ghibe, Rowlands *et al*. (1993) indicated that although the proportion of animals that relapsed by day 20 following treatment decreased in the higher dosage (25% vs 55%), it was not able to cure all the infections. The present work was again more in accordance with experimental work that was further substantiated by Codjia et al. (1993) in which they inoculated blood samples from 12 trypanosome isolates collected from cattle in the Ghibe valley in 1989 into Boran (*Bos indicus*) calves. Twelve isolates produced infections, which were shown to be T.congolense and resistant to treatment with diminazene aceturate at a dose of 7.0mg/kg boy weight. Eleven of the infections were also resistant to isometamidium chloride at a dose of 0.5 mg/kg body weight., where, except for one isolate sensitive to 0.5 mg/kg body weight isometamidium chloride, all were found to be resistant to 7.0 mg/kg.0.5 mg/kg and 1.0 mg/kg body weight of diminazene, isometamidium and homidium chloride, respectively. This multiple-resistant phenotype was even expressed at the clonal level. Mulugeta et al. (1997) indicated a long-term occurrence of *T. congolense* resistant to diminazene, isometamidium and homidium in cattle of Ghibe, Ethiopia. Recently, Afewerk et al. (2000) showed the presence of multiple-drug-resistant *T. congolense* in the village cattle of Metekel district, northwest Ethiopia. Chaka et al., (2003), *T. congolense* isolates from Ghibe, Bedelle, Sodo and Arbaminch showed a similar level of drug resistance to treatment with 3.5 mg/kg body weight diminazene and 0.5 and 1.0 mg/kg isometamidium. The magnitude of drug resistant trypanosomes across Ethiopia is not well documented. However, the present study on a few isolates of *T. congolense* indicated the potential risk for the future in the greater part of tsetse infested areas, where the proportional infection rate of cattle by *T. congolense* is increasing Abebe G. (1996) and where dependence on regular drug treatment for trypanosomosis control, which is a common practice now in Ethiopia, may lead to the risk of major drug resistance development. This has been observed by Bourn and Scott (1978) at the Angar Guttin settlement area, Ethiopia, where during the introduction of working oxen to the area the proportion of infection rates by *T. vivax* was greater, but it was gradually overtaken by *T. congolense*. The authors also indicated that, at Anger Guttin settlement area, 36 days after

prophylactic treatment with 1 mg/kg body weight isometamidium 66% of the oxen were found infected (positive for trypanosomosis), most of the infections being due to *T. congolense*.

Rowlands et al. (1993) for instance indicated the dynamic nature of the epidemiology of drug resistant infection in the Ghibe valley, which was 6% in 1986 and increased to 14% in 1989.Chemotherapy and chemoprophylaxis are the common and practical methods available for the control of animal trypanosomosis, but their effectiveness is being eroded by the emergence of drug resistant trypanosomes. Of the drugs available for the treatment of animal trypanosomosis, diminazene aceturate and isometamidium chloride have been most used because more attention should be given to a use of their availability and relatively low toxicity to cattle. The circumstances tending to produce resistant parasite populations include under dosing with trypanocidal drugs, irregular use of prophylactics, and their discontinuation while cattle remain at risk, and the high incidence of trypanosomosis Whiteside E.F., (1960).

The present questionnaire survey result revealed that there are irregular mass(45%) treatments and very high treatment frequency (80% every month)with diminazene diaceturate and isometamidium chloride hydrochloride and homidium bromide in the area both of which are main predisposing factors for drug resistance development. The respondents have also replied that the trypanocidal drug has been in use for over fourty years which can also lead to trypanocidal drug resistance and this is in agreement with the report of Waller, (1994)that stated the repeated use of chemicals as pesticides or chemotherapeutic agents inevitably leads to the development of resistance in the target organisms and illustrated that resistance systematically occurs with in approximately ten years following the introduction of antimicrobials, insecticides, trypanocides and anthelmintic to the market .This also occurred with the trypanocidal drugs, such as isometamidium chloride (ISMM), the homidium salts and diminazene aceturate, which were introduced during the 1950s to Africa: acquired resistance was already published during the 1960s (Finelle and Yvore, 1962). Therefore use of trypanocidal drugs in this area for several decades, the irregular mass treatments the high treatment frequency, the high transmission rate existing or favored by multi-species of very high tsetse fly densities, widespread inexpert use of trypanocidal drugs (all these factors were revealed by the questionnaire and observation during the present study) altogether must have induced a high level of drug resistance to the currently available drugs in the *T. congolense* and *T. brucei* populations in this area.

6. CONCLUSION AND RECOMMENDATION

The study conducted on the distribution of Bovine trypanosomosis, the vectors and drug sensitivity trial on the isolates taken from GawoDalle district, west Oromia, gave important information on the current disease situation and the problem of drug resistance. In this district high prevalence of trypanosomiasis was found and is the major constraint to cattle production. The most widely distributed species is found to be T.congolense followed by T.vivax and T.brucei. There are four different species of tsetse flies distributed in the areas. Different conducive environment exists in the area that maintains tsetse fly distribution with high density and trypanosomosis with high transmission rate (particularly the evergreen coffee plantation with its shade make favorable environment). There is always an animal fly contact in almost all seasons favoring the consistent occurrence of the disease. The potential land existing in the area and the existing livestock will be efficiently utilized if the problem of trypanosomosis particularly drug resistance is resolved. The high degree of drug resistant populations of T.congolense to diminazene diaceturate 3.5and 7mg/kgbod weight, to Homidium bromide 1and 2mg/kg(even lack of sensitivity of T.congolense populations to homidium)as well as resistance of T.congolense and T.brucei mixed infections to 0.5 and 1mg/kg body weight of isometamidium is a serious threat to cattle production in this area.

This study has hence showed chemotherapeutic or chemoprophylaxis agents that are currently in use in Africa would not be effective to control trypanosomiasis in this area. Based on the conclusions, the following recommendations are forwarded:

- An immediate intervention is required in the study area in order to save the animal population under treat due to trypanosomosis
- More emphasis should be given to the vector control in integrated disease control strategy in the area
- Treatment should only be given to clinically sick animals in order to reduce the frequency of drug usage and occurrence of resistance
- Strict supervision on the usage of trypanocidal drugs handling and distribution should be implemented
- Mechanisms should be put in place to monitor the prevalence of resistant strains, the degree of resistance and the number of drugs towards which resistance has developed in different areas of the country

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8. ANNEXES

Annex 1: Mean catch of different Glossina species and biting flies in Gawo-Dalle district in Bir̀ir Valley in 2007/2008

Site		G.m.s. morsitanse	G.pallidipes	G.f.fuscipes	G.tachinoides	Total Glossina	Haematopota	Stomoxys	Tabanus
1	M	16.40	60.45	22.70	4.20	103.75	.1000	17.25	.05
	SD	19.629	46.029	34.334	7.696	67.045	.44721	10.572	.224
2	M	14.00	25.85	2.25	.00	42.10	.5500	22.05	.15
	SD	28.178	41.011	6.520	.000	74.090	1.39454	15.534	.489
3	M	.50	6.65	14.30	.00	21.45	.3000	26.65	.20
	SD	1.192	9.505	34.065	.000	40.076	.92338	35.564	.523
4	M	.70	7.30	.00	.00	8.00	.0000	25.20	.20
	SD	1.059	9.900	.000	.000	10.467	.00000	18.594	.632
5	M	.00	1.50	.00	.00	1.50	.5000	21.80	.00
	SD	.000	1.716	.000	.000	1.716	1.08012	12.639	.000
6	M	10.00	30.80	15.40	3.60	59.80	.2000	10.00	.00
	SD	17.250	38.807	25.479	10.373	43.034	.63246	4.619	.000
7	M	4.50	15.90	7.80	.10	28.30	.2000	17.90	.00
	SD	3.979	12.758	10.315	.316	20.532	.42164	7.031	.000
8	M	.00	.20	.00	.00	.20	.7000	21.20	.00
	SD	.000	.422	.000	.000	.422	1.49443	7.554	.000
Total	M	7.00	21.96	9.25	1.10	39.31	.3182	20.73	.09
	N	110	110	110	110	110	110	110	110
	SD	16.696	35.413	23.487	4.735	58.739	.94754	18.882	.372

Annex 2: Mean fly catch in late rainy and dry seasons in Gawo_dalle district Birbir Valley in 2007/2008

Season		G.m.s.m orsitanse	G.pallidi pes	G.f.fus cipes	G.tachi noies	Total Glossina	Haema topota	Stomo xys	Taban us
Late	M	11.82	31.07	9.93	1.33	54.15	.0182	25.58	.60
rainy	N	55	55	55	55	55	55	55	55
	SD	21.459	43.763	27.718	4.937	72.434	.13484	24.479	.600
Dry	M	2.18	12.85	8.56	.87	24.47	.6182	15.87	.18
	N	55	55	55	55	55	55	55	55
	SD	7.411	21.147	18.554	4.558	35.630	1.26916	8.512	.512
Total	M	7.00	21.96	9.25	1.10	39.31	.3182	20.73	.69
	N	110	110	110	110	110	110	110	110
	SD	16.696	35.413	23.487	4.735	58.739	.94754	18.882	.372

Annex 3: The fly apparent densities (fly/trap/day) in different areas of Gawo-Dalle district in Birbir valley 2007/2008(95%CI in areas =3.38_4.25)

PA	GmAD ±SD	GpAD ± SD	GffAD ± SD	GtAD± SD	HmAD ± SD	Sto.AD± SD	Tab.AD ± SD	TotGl AD± SD
1	5.46(6.54)	20.15(15.34)	7.57(11.44)	1.40(2.56)	.03(.15)	5.75(3.52)	.02(.07)	34.6(22.4)
2	4.67(9.39)	8.62(13.67)	.752.17)	.00(.00)	.18(.46)	7.35(5.18)	.05(.16)	14.0(24.7)
3	.167(.39)	2.21(3.17)	4.77(11.35)	.00(.00)	.10(.31)	8.88(11.85)	.07(.17)	7.2 (13.4)
4	.23 (35)	2.43(3.00)	.00(.00)	.00(.00)	.00(.00)	8.40(6.20)	.07(.21)	2.7(3.5)
5	.00 (.00)	50(.57)	.00(.00)	.00(.00)	.17(.36)	7.27(4.21)	.00(.00)	.50(.57)
6	3.33(5.75)	10.27(12.94)	5.13(8.49)	1.20(3.46)	.07(.21)	3.33(1.54)	.00(.00)	19.9(14.4)
7	1.50(1.33)	5.30(4.25)	2.60(3.44)	.03(.11)	.07(.14)	5.97(2.34)	.00(.00)	9.4(6.8)
8	.00(.00)	.07(.14)	.00(.00)	.00(.00)	.28(.60)	7.68(2.85)	.00(.00)	.067(.14)
TAD	2.34(5.56)	7.32(1 1.8)	3.08(7.83)	.37(1.80)	.11(.33)	6.97(6.31)	.03(.12)	13.1(19.58)
95% CI	1.28(3.39)	5.09(9.55)	4.81(13.68)	.07(.66)	9.4(16.8)	.05(.17)	5.77(8.1)	.01(.05)

pa= peasant association, village-7(1), village-6(2), village-5(3),Igu kofale(4),Chanka bururi(5), village-1(6), village-11(7) Doganoadami (8) ;tad=total apparent density ;ad=apparent density ; Gm=*G. m. submorsitans*,gp=*G.pallidipes*,Gff=*G. fuscipes fuscipes*,Gt=G. *tachinoides* = haematopota, sto= stomoxys, tab= tabanus, tot.Glad=total Glossina apparent density

Annex 4: Multivariate logistic regression analysis of the interaction of risk factors in trypanosome infection in the cattle subpopulation of area in Birbir valley of Gawo-Dalle district 2007/2008

Risk factors	Prevalence (%)	P	Odds ratio	95% Confidence Interval for
Village-7	24.23	.000	20.154	6.006 67.622
Village-6	18.11	.000	13.965	4.121 47.325
Village-5	18.55	.000	14.440	4.295 48.549
Igu-Kofale	16.90	.000	12.792	3.828 42.749
Cherecha	6.40	.030	4.335	1.151 16.324
Chanka-Bururi	14.80	.000	11.520	3.360 39.499
Village11	21.8	.000	17.548	5.230 58.880
Dogano-Adami	4.70	.105	3.033	.792 11.626
Village-1	14.00	.000	10.267	2.999 35.149
Dogano-Bururi	1.56	.	.	. .
Late rainyseason	15.1	.060	1.337	.988 1.809
Dry season	11.8	.	.	. .

Annex 5: Sensitivity to doses of diminazene diaceturate of T.congolense isolate from GawoDalle district and parasitaemia estimated according to Paris *et. al.* (1982)

Cattle	Dosein	Days of follow up												
	mg/kg	0	2	4	7	9	11	14	16	18				
V500/02	3.5DDA	6+	0	0	0	4+	5+	5+	6+	6+				
V501/02	3.5DDA	6+	0	0	0	4+	4+	5+	5+	6+				
V604/01	3.5DDA	6+	0	0	0	4+	5+	5+	5+	5+				
IK06/02	3.5DDA	6+	0	0	0	0	3+	3+	4+	6+				
IK09/03	3.5DDA	6+	0	0	0	5+	5+	5+	5+	6+				
DCattle	Dosein													
	mg/kg	21	23	25	28	30	32	35	37	39	42	44	46	49
V500/02	7 DDA	0	0	0	0	0	0	2+	4+	4+	4+	4+	4+	5+
V501/02	7 DDA	0	0	0	0	0	0	4+	6+	6+	6+	6+	6+	5+
V604/01	7 DDA	0	0	0	0	0	5+	5+	5+	5+	5+	5+	4+	4+
IK06/02	7 DDA	0	0	0	0	0	0	0	0	0	0	0	4+	5+
IK09/03	7 DDA	0	0	0	0	0	0	0	0	0	0	0	0	0
Cattle ID		Days of follow up												
	Dosin mg/kg	53		56	58	61	63	65	68	70	72	75		
V500/02	0.5DDA	5+		0	0	0	0	0	0	0	0	0		
V501/02	10.5DDA	5+		0	0	0	0	0	0	0	0	0		
V604/01	10.5DDA	4+		0	0	0	0	0	0	0	0	0		
IK06/02	10.5DDA	0		2+	0	0	0	0	0	0	0	0		
IK09/03	10.5DDA	0		5+	0	0	0	0	0	0	0	3+		
Cattle ID		Days of follow up												
	Dosin kg/kg	79		82	84	86	89	91	93	95	98	100	103	
V500/02	10.5DDA	0		0	0	0	0	0	0	0	0	0	0	
V501/02	10.5DDA	0		0	0	0	0	0	0	0	0	0	0	
V604/01	10.5DDA	0		0	0	0	0	0	0	0	0	3+	4	
IK06/02	10.5DDA	0		0	0	0	0	0	0	0	0	0	0	

Annex 6 : Sensitivity of T.congolense to different doses of isomethamidium chloride hydrochloride to from Gawo-Dale district and the parasitaemia estimate

CaatleID	Dosemg/kg	Day0	D2	D4	D7	D9	D11	D14	D16	D18		
V600/01	ISMM0.5	6+	5+	5+	4-	0	5+	6+				
V500/03	ISMM0.5	6+	2+	0	0	0	0	0	0	0		
V605/02	ISMM0.5	6+	4+	0	5-	5+	6+	6+	6+	6+		
IK07/02	ISMM0.5	6+	2+	0	2-	6+	6+	5+	5+	6+		
IK08/02	ISMM0.5	6+	3+	0	3-	3+	5+	6+	6+	6+		
CaatleID	Dosemg/kg	D16	D18	D21	D23	D25	D28	D35	D37	D39	D42	
V600/01	SMM 1	0	0	0	0	0	0	4+	5+	5+	5+	
V500/03	SMM 1			2+	2-	5+	6+	0	0	0	0	
V605/02	SMM 1			0	0	0	0	0	6+	6+	6+	
IK07/02	SMM 1			3+	0	0	0	4+	6+	5+	6+	
IK08/02	SMM 1			0	0	0	0	6+	6+	5+	5+	
Cattle	Dosemg/kg	D44	D46	D49	D51	D53	D56	D58	D61	D63		
V600/01	SMM 1	5+	3+	6+	6+	x	x	x	x	x	x	
V500/03	SMM 1	0	0	0	0	0	0	0	0	0		
V605/02	SMM 1	5+	5+	2+	2+	5+	4+	4+	5+	3+		
IK07/02	SMM 1	5+	5+	5+	5+	5+	5+	5+	4+	3+		
IK08/02	SMM 1	x										
Cattle D	Dosemg/kg	68	D70	D72	D75	D77	D79	D82	D84	D86	D91	D95
V600/01	SMM 1	x	x	x	x	x	x	x	x	x	x	
xV500/03	SMM 1	0	0	0	0	0	0	0	0	0	0	0
V605/02	SMM 1	5+	4+	3+	3+	4+	5+	4+	6+	3+	4+	4+
IK07/02	SMM 1	4+	4+	4+	3+	4+	4+	3+	4+	5+	4+	3+
IK08/02	SMM 1	x	x	x	x	x	x	x	x	x	x	

Annex 7: Sensitivity of T.congolense to different doses of homidium bromide to from GawoDale district and the parasitaemia estimate

Cattle ID	Dosemg/kg	Day0	D4	D7	D9	D11	D14	D16	D18	D21	D23	
IK00/04	Ethidium 1	6+	6-	6-	6+	6+	6+	6+	6+	6+	6+	
V503/01	Ethidium 1	6+	0	0	0	5+	4+	4+	5+	5+		
V510/03	Ethidium 1	6+	4-	4-	2-	2+	5+	6+	6+	6+	x	
V611/03	Ethidium1	6+	5-	2-	0	2+	5+	5+	6+			
V612/03	Ethidium 1	6+	2-	2-	5+	6+	6+	6+				
Cattle ID	Dosemg/kg	D18	D21	D23	D25	D28	D30	D32	35	D37	D39	
IK00/04	Ethidium 2	6+	6-	6-	6+	6+	6+	6+				
V503/01	Ethidium 2	5+	0	0	2+	6+	2+	1+	5+	5+	4+	
V510/03	Ethidium 2	3+	x	x	x	x	x	x	x	x	x	
V611/03	Ethidium 2	0	0	2-	4+	6+	5+	5+	5+	6+	5-	
V612/03	Ethidium 2	5+	0	4-	1+	3+	4+	2+	4+	5+	6-	
Cattle ID	Dosemg/kg	D42	D44	D46	D49	D51	D53	D56	D58	D61	D63	
IK00/04	Ethidium 2	x	x	x								
V503/01	Ethidium 2	6+	2-	5-	6+	6+	6+	x				
V510/03	Ethidium 2	x	x	x	x	x	x	x				
V611/03	Ethidium 2	4+	6-	5-	5+	4+	5+	5+	x			
V612/03	Ethidium 2	4+	2-	4-	5+	6+	2+	6+	5+	4+	4-	
Cattle ID	Dosemg/kg	68	D70	D72	D75	D77	D79	D82	D84	D86	D89	D91
IK00/04	Ethidium 2	x	x	x								
V503/01	Ethidium 2	x	x	X								
V510/03	Ethidium 2	x	X	X								
V611/03	Ethidium 2	x	x	X								
V612/03	Ethidium 2	4+	4-	4-	6+	6+	4+	4+	4+	6+	6-	4-

Annex 8: PCV values of diminazene, isometamidium and homidium groups cattle after challenge and treatment by their respective drug

Day	V50002	Rx	V50101	Rx	V69401	Rx	IK0602	Rx	IK0903	Rx
PI	35	no	32	no	40	no	30	no	34	no
pp	36	no	40	no	40	no	38	no	37	no
pp	33	no	34	no	38	no	28	no	31	no
pp	28	no	25	no	23	no	33	no	29	no
pp	25	no	31	no	30	no	20	no	27	no
pp	29	3.5	27	3.5	29	3.5	18	3.5	25	3.5
day0	27		29		30		15		25	
day1	22		30		28		17		28	
day2	27		34		29		23		30	
day4	25		29		26		20		27	
day7	24		30		25		25		25	
day9	27		34		25		22		26	
day11	20		29		24		24		23	
day14	18	7	28	7	15	7	17	7	22	7
day16	16		27		15		16		18	
day18	21		26		18		20		23	
day21	20		28		22		22		25	
day23	23		26		21		23		21	
day25	22		28		28		28		26	
day28	25		24		24		20		25	
day30	25		26		24		18		22	
day32	25		25		21		19		19	
day35	23		26		20		19		16	
day37	20		23		1[illegible]		18		20	
day39	24		15		26		17		23	
day42	22		17		26		17		18	
day44	22		22		35		18		23	
day46	19		21		1[illegible]		12		20	
day49	23		25		24		13		22	
day51	17	10.5	20	10.5	22	10.5	11	10.5	24	10.5
day53	19		24		26		18		19	
day56	21		20		25		20		22	
day58	20		27		23		20		23	
day61	22		26		22		19		23	

day63	23		23		19		21		22	
day65	22		26		20		23		20	
day68	24		17		22		18		27	
day70	23		23		22		18		20	
day72	23		23		23		18		24	
day75	20		21		19		14		18	
day77	19		24		20		14		20	
day79	22		23		21		15		29	
day82	23		28		23		14		25	
day84	24		25		21		12		20	
day86	17		20		22		11		23	
day89	23		25		19		14		24	
day91	23		25		22		16		24	
day93	22		24		19		17		25	
day95	24		27		22		23		26	
day98	24		30		24		22		20	
day100	24		27		21		18		20	
day103	24		33		20		23		20	

Annex 9: PCV values of isometamidium group animals after challenge and post treatment

Days	V60001	Rx	V50003	Rx	V60502	Rx	Ik0702	Rx	IK0802	Rx
PI	32	no	45	no	36	no	33	no	35	no
pp	34	no	44	no	39	no	34	no	30	no
pp	35	no	38	no	33	no	29	no	32	no
pp	27	no	30	no	33	no	33	no	30	no
pp	28	no	25	no	33	no	23	no	25	no
pp	30	no	26	no	33	no	24	no	19	no
day0	28	0.5	27	0.5	33	1	28	1	15	1
day1	31		18		33		23		15	
day2	29		26		33		28		16	
day4	27		28		33		28		17	
day7	26		32		33		30		18	
day9	22		31		33		26		19	
day11	30		25		33		25		18	
day14	23		25		33	1	23	1	15	1
day16	22		28		33		23		17	
day18	15	1	22		33		22		16	
day21	20		22		33		22		12	
day23	15		22		33		22		13	
day25	19		22		33		18		13	
day28	23		25	1	33		20		16	
day30	23		27		33		23		15	
day32	20		26		33		23		16	
day35	17		27		33		21		15	
day37	13		30		33		16		14	
day39	17		31		33		20		Died	
day42	17		30		33		20			
day44	14		28		33		19			
day46	18		30		33		19			
day49	14		30		33		20			
day51	15		32		33		21			
day53	15		35		33		26			
day56	12	1	27		33		17			
day58	20		33		33		23			
day61	21		30		33		20			
day63	23		29		33		23			
day65	27		34		33		23			

day68	died		30		33		20			
day70			30		33		19			
day72			33		33		19			
day75			29		33		20			
day77			32		33		17			
day79			29		33		20			
day82			31		33		22			
day84			30		33		22			
day86			30		33		27			
day89			29		33		25			
day91			29		33		24			
day93			33		33		22			
day95			30		33		20			
day98			32		33		25			
day100			39		33		28			
day103			31		33		23			

Annex 10: PCV values of Homidium bromide (Ethidium) group cattle after challenge and treatment

Day	Ik0094	Rx	V50301	Rx	V51003	Rx	V61103	Rx	V61203	Rx
PI	34	no	37	no	32	no	35	no	34	no
pp	34		43	no	33	no	36	no	35	no
pp	33		32	no	31		26		30	
pp	32		30	no	32		34		33	
pp	32		24	no	23		24		27	
pp	31		25	no	25		23		23	
day0	31		25	1	19	1	20	1	32	1
day1	28		18		20		21	no	26	
day2	26		18		30		21		28	
day4	31		16		18		13		29	
day7	24		16		11		14		25	
day9	24		15		18		13		25	
day11	24		16		15		14	2	20	
day14	25		17		dead		14		20	2
day16	24		15				15		17	
day18	20	2	17				14		17	
day21	20		19				18		18	
day23	18		17				20		17	
day25	20		17				23		16	
day28	19		16				20		17	
day30	19		15				18		17	
day32	15		12				18		17	
day35	16		13				16		16	
day37	15		14				17		21	
day39	13		18				20		19	
day42	16		14				16		18	
day44	14		14				14		16	
day46	15		14				15		20	
day49	12		24				12		17	
day51	15		14				12		21	
day53	13		11				11		21	
day56	12	2	11				died		16	
day58	12		died						17	
day61	17								16	

day63	13								20	
day65	15								17	
day68	14								20	
day70	13								18	
day72	16								17	
day75	dead								19	
day77									19	
day79									16	
day82									18	
day84									15	
day86									14	
day89									12	
day91									15	
day93									20	
day95									16	
day98									24	
day100									27	
day103									25	

Annex 11: structured questionnaire to interview individual farmers.

I. General information of farmer's identity, location

A. DATE_____;

B. Village (PA) _____

C. Name of interviewee_____; sex (M/F) ____; Age _____

Ethnic group_____; first language_____;

Household size: No. Wives___; No. boys___; No. girls____; others____

Education status: A) educated B) can read C) illiterate

II. Livestock management

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

2. If yes, which species and number?

cattle____; sheep ___; goat____ equine__; others (specify)_____;

3. How do you manage your animals?

a) Free grazing b) stall feeding c) Tether

4. If cattle are allowed to free grazing, are they in large herd or small group?

a) In large herd b) In small group

5. Where do cattle graze? a) In savannah grassland b) In the bush c) On grassland

d) Valley bottom (river side) e) Valley top f) a, b and d h) c and e

6. How long is the grazing area from cattle barn? _____km

7. How long is the watering point from your homestead? _____km

8. During which season (months) is the feed abundant? _____;

9. During which season (months) is the feed shortage common? _____

II. Socio-economic data

1. What is the main source of your income? a) Livestock b) crop production

c) Other specify) _____

2. For what purpose do you keep livestock? a) Milk b) meat c) manure d) source

Of draft power e) income f) paying dowry g) wealth banking

h) Others (specify) _____

3. What is the daily milk yield of a lactating cow in liter? _____

4. IF a lactating cow gets sick due to trypanosomosis the daily milk yield) decreases by 50% b) decreases by 100% c) no change

5. What is the draught ox work out put in hours per day? _____

6. What is the problem with breeding cow? a) Abortion b) delay in age at first calving c) Long calving interval d) birth of under weight calves d) others (specify) ____

7. How many calves do a cow gives on average totally? _____

iv. Livestock disease occurrence

I What are the most common diseases affecting cattle? Please list them in decreasing order of importance in local language

1. _____2._____3._____4._____5._____6._____

2. Is trypanosomosis one of the problems to your animals? Yes No

3. If yes;

3.1. Which species does it mostly affect? A) Cattle b) goats c) sheep d) equine e) all

3.2. What is the rank of trypanosomosis among others? _____

3.3. When do you think has it started? a) 20 years ago b) 10 years ago c) 5 years d) 40 years

3.4 During which season (months) does it mostly occur? a) Wet season b) Dry season c) All the time

4. Was there an increase or decrease in its occurrence sine the last five years?

a) An increase b) a decrease c) no change d) I have no idea

5. What are the major clinical signs that an animal affected by trypanosomosis shows?

_____,_____,_____,_____.

6. Do you know that tsetse flies transmit the disease? a) Yes b) No

c) Others (specify) _____

7. In which season are tsetses most abundant? A) Wet season B) Dry season C) All time

8. How many animals have you lost since the last 12 months from trypanosomosis?

A) Cattle: Adults_____; young_____; b) sheep_____; c) goat_____; d) equine____

v .usage of trypanocidal drugs and the suspected drug failures

1. Which trypanosomosis control methods were applied in your area? a) Use of trypanocidal drugs.b) Tsetse trapping/Target c) Pour-on application d) others (specify)_____
2. Where is the common treatment place? a) Public vet. Clinic b). Private vet. Clinic c) At home d) Others (specify) _____
3. If treatment is out of vet. Clinic (public, private) please can you tell reasons?

4. How far is veterinary clinic from your home? _____Km

5. From where do you obtain the medicine?

a) Vet. Clinic (private clinic, public clinic) b) from human pharmacies c) local farmers d).Drug smugglers

6.Who applies the treatment? a) Farmer b) government vet professionals c) private Vets d) CAHWs e) smugglers f) others (specify) _____

7. Which trypanocidal drugs are you commonly using to treat your animals? (Name/type/color)_____Why you prefer to use this drug? _____

8. What quantity of each trypanocidal drugs is used?

8.1. If Berenil: a) 1 sachet for 1 adult cattle b) 1 sachet for two adult cattle c) other

8.2. If Trypamidium a) 1 sachet for 10 adult cattle b) 1 sachet for 15 adult cattle c) 1 methods (specify) _____sachet for 20 adult cattle d) no idea

8.3. If homidium salts: a) 1 tablet for 1 adult cattle b) 1 tablet for two adult cattle c) No idea

8.4. Do you use the same non sterilized syringe needle treat animals? Yes/No

9. Since when have you been using these drugs for the treatment of your cattle? a) Since the last 15 years b) since the last 10 years c) since the last 5 years

10. Which of the drugs is/are most effective? _____

11. Which of the drugs is/are less effective? _____

12. Do you have any trypanocidal drug at your home? A) Yes b) No

13. If yes:

13.1. Would you show us? _____

13.2. In what form did you obtain? (In solution, Sachet, tablet)

13.3. How long you keep prepared drug? _____ days/weeks

13.4. Where and how do you store them? _____

13.5. What time has elapsed since you acquired them? ____days/months

14. In your opinion, what should be done to the drug smugglers if they are captured? With drugs? a) They should be punished by local community b) Implementer should take his own action c) they should be released freely D) I have no idea

15. How much do you pay for treating one adult ox? _____birr

16. Can you tell how much did you pay for treating your animals against trypanosomosis since last year? ____birr

17. When you treat animals against the disease, which ones do you usually treat?

a) Treat all cattle b) treat only mature oxen c) Treat only mature cows

d) Treat only sick ones e) Treat cows in milk and oxen f) other (specify)_____

18. Was there cure after these treatments? a) Yes b) No

18.1. If yes in how many days they restart to produce a) milk _____b) draft_____

19. How many of your cattle get treatment by trypanocidal drugs? _____a) out of the treated how many were cured____b)how many died after treatment

19. If no cure; what do you think is the reason? _____

20. Could you tell us the interval between treatments of the same animal?

In days/months.a)< 20 days b) one month c) 2to3 months d) 3to 6months e) once per year

THANK YOU FOR YOUR COOPERATION!!

Annex 12: Protocol for trypanocidal drug sensitivity testing in cattle

1. About one month prior to experimental work, the animals were kept in a fly-free area. In order to remove existing parasite burden, treated with a broad-spectrum anthelmintic Albendazol 2500mg, with diminazene aceturate at 3.5 mg/KG, with long acting oxytetracycline at 20mg/ KG BW and acaricide sprayed for external parasites.
2. Beginning 2 weeks prior to inoculation of trypanosome isolate, and continuing until the end of the experiment, monitored PCV and parasitaemia at least three times a week by examination of peripheral blood from marginal ear vein using phase-contrast Buffy coat technique.
3. After confirming the viability of a trypanosome isolate microscopically, inoculated the isolate into a jugular vein of a cattle, and clinically and parasitological continued monitoring as described above. When a significant deterioration in clinical condition was observed, PCV and parasitaemia were monitored the same day.
4. At the first peak of parasitaemia, weighed the animal using weigh band and treated intramuscularly, on the same day, with one of the following: a 20% w/v solution of isometamidium chloride hydrochloride at a dose of 0.5mg/Kg BW; a 7% w/v solution of diminazene diaceturate at a dose of 3.5mg/kg BW or a 2.5% solution of homidium bromide at a dose of 1.0mg/Kg BW. When cattle relapsed (i.e. trypanosomes reappeared after treatment with the first dose trypanocidal drug), they had been monitored at least three times per week for a maximum of 50 days from the date of relapse, to obtain the peak parasitaemia level and Pathogenecity of drug-resistant trypanosomes.
5. Relapsing cattle were re-treated when the PCV failed by one-fifth of the value measured at the time relapse was first detected, or when the PCV failed below 15%, or when considered necessary on the basis of clinical examination. When treatment was necessary, the animal was weighed and treated intramuscularly, on the same day, with the second most commonly used drug, using the double dose of the same drug type given above.
6. When cattle relapsed again (i.e. when trypanosomes reappeared after treatment with the second drug),In case of diminazene group relapsing cattle were retreated when the PCV failed by one-fifth of the value measured at the time relapse was second detected or when PCV was found less than 15% by 10.5mg/kg body weight .Whereas in case of Isometamidium chloride hydrochloride and Homidium bromide groups they had been monitored at least three times per week for a maximum of 50 days from the datof relapse

in order to obtain information on the Pathogenecity of drug-resistant trypanosomes, and then the experiment terminated.

7. The experiment terminated as relapse occurred in all except one in isometamidium group which did not relapsed to the second treatment dose within 100 days following administration of the first and second doses of each trypanocidal drug for isometamidium and homidium bromide groups. For diminazene group also it terminated in 100 days.

Annex 13 Photo showing sample collection

9. CURRICULUM VITAE

Personal Information

Name:	Waktole Terfa Eteya
Nationality:	Ethiopian
Sex:	Male
Place of birth:	Dembi-Dollo, Wollega
Date of Birth:	18th April, 1966
Martial Status:	Married
Academic qualification:	Doctor of Veterinary Medicine (DVM)
Scientific membership:	Member of the Ethiopian Veterinary Association (EVA)

Proficiency:

Language skill:

- ✓ Oromiffa: Speaking, reading and writing
- ✓ Amharic: Speaking, reading and writing
- ✓ English: Speaking, reading and writing,
- ✓ Russian: Speaking, reading and writing,

.Educational Background

- 1972-1978: Grade 1-6. Addo Saint Michael Catholic Mission Elementary school, Dembi-Dollo, Wellega.
- 1978-1983: Grade 7-12. Kellem Comprehensive secondary school, Dembi-Dollo, Wollega. Award: Ethiopian School leaving certificate Examination,
- 1984 -1985 Addis Ababa University Bahir-Dar Teacher's College. Awarded: diploma in Biology.
- 1988-1994. X USSR Ukrainian state, Agricultural University, Faculty of Veterinary Medicine,
- 2005 to the present: Addis Ababa University, Faculty of Veterinary Medicine in tropical veterinary Parasitology

Work Experience

- From July 1985-Aug 1988 – Juniour secondary school teaching at Wayu in Horo Guduru of Wallega .
- From April 1/1995- January1997, Nedjo District Agricultural office veterinary team leader .in Oromia Agricultural development Bureau.
- From January 1997-October 2000 as Zonal Veterinarian in West Wollega Zone Agricultural development department. .
- From October 2000 –June2004 as a Piot Station Coordinator in NTTICC.
- From July 2004-June 2006 served as Training Coordinator in NTTICC.
- From July2006-to ate as Investigation team leader in in NTTICC (Bedddelle)
- From September 2006 –to date as acting head of NTTICC

Consultations:

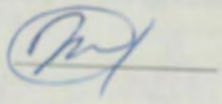
I have consulted NGOs like save the children Wolliso impact area, ACORD Ethiopia (Agent s for coordination of research in development) Gambella branch on tsetse flies and trypanosomosis in their area on different times.

Advice and technical assistance was given to undergraduate students o the Faculty of Veterinary medicine attached to the NTTICC from 2000-2008 to conduct their research

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: Waktole Terfa Eteya

Signature: 

Date of submission: June 25, 2008

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

Academic Advisors:

Dr. Yacob Hailu (D.V.M, MSc, PhD, Assistant Professor) _____

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