ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

A STUDY ON BOVINE TRYPANOSOMOSIS, TSETSE CHALLENGE AND EFFICACY OF ISOMETAMIDIUM CHLORIDE IN AMARO SPECIAL DISTRICT, SOUTHERN ETHIOPIA

BY
TESHOME ASSEFA

JUNE, 2008
DEBRE ZEIT, ETHIOPIA

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

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DEDICATION

This manuscript is dedicated to my mother Mrs Ejigayehu G/mariam who is she caressed her child in freedom and still holds close to me as if I am a child.

DEDICATED

TO

MY MOTHER

EJIGAYEHU G/MARIAM

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LIST OF ABBREVIATIONS

AAT	African Animal Trypanosomosis
Ab	Antibody
AD	Apparent density
Ag	Antigen
ANOVA	Analysis of Variance
bw	body weight
CI	Confidence Interval
DNA	Deoxyribo Nucleic Acid
ELISA	Enzyme Linked Immuno Sorbent Assay
FAO	Food and Agriculture Organization
G.m	*Glossina morsitans*
IAEA	International Atomic Energy Agency
IBAR	Inter-African Bureau for Animal Resource
IFAT	Indirect Fluorescent Antibodies Technique
ILRA	International Livestock Research and Agricultural Development
IRLI	International Livestock Research Institute
ISMM	Isometamidium chloride
ISTRC	International Scientific Council for Trypanosomosis Research and Control
m.a.s.l.	meter above sea level
MoARD	Ministry of Agriculture and Rural Development
NGO	Non-Governmental Organization
NLDP	National Livestock Development Programme
NTTICC	National Tsetse and Trypanosomosis Investigation and Control Center
OIE	Office International des Epizooties
PAs	Peasant Associations
PAAT	Program against Africa Trypanosomosis
PCV	Packed Cell Volume
POP	Population
RBC	Red Blood Cell
RRA	Rapid Rural Appraisal
SNNPR	Southern Nation Nationalities People Region

SIT	Sterile Insect Technique
spp	species
SPSS	Statistical Package for Social Science
SRVL	Sodo Regional Veterinary Laboratory
STEP	Southern Rift valley Ethiopia Tsetse Eradication Programme
Tb	*Trypanosoma brucei*
Tc	*Trypanosoma congolense*
Tv	*Trypanosoma vivax*

ABSTRACT

Among livestock diseases tsetse-transmitted trypanosomosis, have been incriminated as the predominant elements in the extreme deterioration of animal resources in sub-Saharan region; Ethiopia that holds the greatest potential for expanding livestock production. The part of Amaro special district particularly mid and low land is infested with tsetse fly where animal trypanosomosis is a serious threat to livestock production. Thus the objectives of the present study were: to determine the prevalence and impact of bovine trypanosomosis and seasonal density of tsetse flies; the efficacy of Isometamidium chloride on bovine trypanosomosis. The study was conducted from October 2007 to May 2008 in Amaro special district of SNNPR. The study design involved questionnaire survey, seasonal cross sectional studies of tsetse and trypanosomosis and assessment of Isometamidium chloride therapeutic efficacy. The questionnaire survey indicated that trypanosomosis is an important problem following erratic rain fall affecting the animals and impeding agricultural activity of the area. The entomological survey revealed that *Glossina pallidipes* species was the only prevalent species along with other biting flies (Tabanids and Stomoxys*).* The apparent density of flies was significantly higher in the late rainy season 1.62 fly/trap/day,2.37 and 1.6 for *G. pallidipes*, Tabanid*s* and Stomoxys, respectively than the dry season where the density was 0.66, 1.12 and 1.03, respectively. The tsetse fly account 27 %, of the total fly catch Tabanid*s* 42.6 %, and Stomoxys 29 % during late rainy season and 24.7 %, 35 %, 39.9 % during dry season. In the low lands (<1500 m.a.s.l) the apparent density for *G. pallidipes* was statistically significant higher ($p<0.05$) than the mid land areas (>1500 m.a.s.l) in both seasons. The *G. pallidipes* caught was higher in the bush lands vegetation type followed by grass wood land and altitudinal distribution limit was up to 1550.m.a.s.l. The apparent density of *G. pallidipes* fly was positively correlated ($r=0.147$) with prevalence of trypanosomes infection. The parasitological survey of 1136 animals (585 in the late rainy and 551 in the dry seasons) indicated trypanosomosis prevalence rate to be 27.35 % and 13.79 % in rainy and dry seasons, respectively which showed statistically significant difference ($p<0.05$) between seasons .The higher infection rate found in the low land areas 31.77 % and 15.45 % than the mid land areas 17.32 % and 12.68 % in the late rainy and dry season respectively indicated a statistically significant difference ($p<0.05$). *T. congolense* was the dominant species among the trypanosomes and account 61.02 % infection as compared to *T. vivax* 24.15 %, in the over all infections. The mean PCV value of parasitaemic and aparasitaemic animal was 21.92 % (CI=21.38-22.46) and 27.31 % (CI = 27.00-27.62) where as the over all mean values of PCV

was 26.23 % (CI=25.89 -26.45). The regression analysis of herd average indicated that PCV decreased with increasing prevalence of trypanosomes infections with a negative regression coefficient of values (r= -0.31) in both seasons. A statistically significant difference ($p<0.05$) in trypanosomes infections between different age groups of cattle was also observed. A total of 69 parasitaemic cattle were selected from 3 PAs for therapeutic efficacy of Isometamidium chloride at the dose rate of 1mg/kg bw. Parasitaemia was demonstrated in 8 out of 69 cattle (11.60 %) with in 15 days; 11out of 61 animals (18.03 %) with in 30 days; 9 out of 50 (18 %) with in 60 days and 6 out of 41 (14.63 %) cattle with in 90 days. With in 90 days of post Isometamidium chloride treatment out of 69 animals 34 (49.28 %) relapsed. In the post treatment, *T. congolense* contributed for 75 %, 81.82 %, 77.78 % and 66.67 % of infections with in 15, 30, 60 and 90 days, respectively. It is evidenced that trypanosomosis is one of the most important problems for agricultural and animal husbandry operations; the situation is getting worse as control and prevention of trypanosomosis is facing challenges due to limitation of vector control activities and the development of drug resistance in Amaro special district, SNNPR.

Keywords*:* Amaro district, Isometamidium chloride, Prevalence, Relapse duration, Trypanosomosis, Tsetse fly.

1. INTRODUCTION

Tsetse-transmitted trypanosomiasis is a disease unique to Africa affecting both humans and animals. This disease occurs in about 10 million km^2 in 37 sub-Saharan countries corresponding approximately to one-third of Africa's total land area, and threatens an estimated 50 million people, 48 million cattle and a countless population of other domestic animal species. FAO has identified the reinforcement of agriculture as a key element in the fight against poverty and the improvement of food security in developing countries. The need to reduce poverty is particularly felt in tsetse infested areas of sub-Saharan Africa (IAEA, 2002).

Ethiopia has the largest livestock inventories in Africa, including more than 43 million cattle, 42 million small ruminants, 2.3 million camels and 6.4 million equines and 40 million chickens with livestock ownership currently contributing to the livelihoods of an estimated 80 percent of the rural population (CSA, 2004; FAOSTAT, 2006). The economy of the country is mainly based on agricultural sector, which contributes 40-50 % to gross domestic products and over 90 % to foreign exchange earnings while the majority of agricultural output is generated from crop and livestock integrated production system (NLDP, 1997).

Among livestock diseases tsetse-transmitted trypanosomosis, have been incriminated as the predominant elements in the extreme deterioration of animal resources in sub-Saharan region that holds the continent's greatest potential for expanded livestock production (Maser, 2005). The devastating effects of tsetse-transmitted trypanosomosis on the livelihoods of African communities have reached unprecedented levels across much of sub-Saharan region. Accordingly, 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 (Bett *et al.*, 2004).

Tsetse transmitted animal trypanosomosis is a disease complex caused by *Trypanosoma congolense, T. vivax,* and *T. brucei brucei* or mixed infection with one or more of these trypanosomes. The disease is most important in cattle but can cause serious losses in pigs, camels, goats, and sheep.

Infection of cattle by one or more of the three trypanosome species results in subacute, acute, or chronic disease characterized by intermittent fever, anemia, occasionally diarrhea and rapid loss of condition often terminating in death (ILRAD, 1990). In Ethiopia, there are five economically important animal trypanosome species. These are *T. congolense*, *T. vivax, T. brucei, T. evansi* of livestock and *T. equiperdum* in horses (Abebe, 2005).

Ethiopian tsetse and trypanosomosis situation shares many characteristics with the rest of African countries occupied by the different species of tsetse flies. Until 1976, a total of 98,000-km^2 area of Ethiopia was infested by five species of tsetse flies. In more recent years, tsetse flies have progressively invaded productive agricultural areas in the west, south and southwest parts of Ethiopia. In Ethiopia the vector fly occupies some 220,000 km^2 of areas of fertile land and about 23.15 million livestock populations are at risk to contract the disease (Abebe, 2005).

There are five species of tsetse flies distributed along the lowlands of western, southern and southwestern part of Ethiopia. *Glossina m. submorsitans, G. pallidipes, G. fuscipes fuscipes and G. tachinoides* are the most important tsetse flies while *G. longipennis* has of a minor economic importance (Langridge, 1976; Abebe, 2005).

Eventhough, Amaro special district one of the Southern Nation Nationalities and People Regional State of Ethiopia, is known to have a large cattle population reared and managed under traditional system studies were not so far conducted on the prevalence of trypanosomosis and distribution as well as seasonal apparent density of tsetse flies.

The main objectives of this present study were therefore:

- To determine the prevalence and impact of bovine trypanosomosis and seasonal density of tsetse flies and

- To determine the efficacy of isometamedium chloride on bovine trypanosomosis

2. LITERATURE REVIEW

2.1. Brief Account on Trypanosomosis

Trypanosomosis is the name given to a group of disease, which is caused by the protozoan of the genus *Trypanosoma,* which affect all domestic animals and man (Hall, 1998). Trypanosomes are belonging the genus *Trypanosoma,* the name was given in 1843 by Gruby a Hungarian doctor who worked in Paris to a blood parasite of the frogs, which he called *Trypanosoma rotatorium* (Itard, 1989). Trypanosomosis locally called "Gendi" is one of the major diseases of livestock in Ethiopia. It is found throughout the country with the exception of the highlands where it is rare or absent. The problem of trypanosomosis has increased extremely and still it is on increase due to a number of factors, which include mainly over population and over stocking of high lands, which brings people and their livestock in to direct contact with tsetse flies. The advance of tsetse flies into previously an infested areas and the wide spread of drugs resistance have shown the magnitude of the problem (Langridge, 1976; NLDP, 1997).

Trypanosomosis is a parasitic disease caused by species of flagellate protozoa belonging to the genus *Trypanosoma* which inhibit the blood plasma and body tissue (Hoare, 1970; Jordan, 1986). The genus is divided in two sections: Stercoraria (posterior station group) and Salivarian (anterior station group) and the latter one is further divided into Duttonella, Nannomonas, Pycromonas and Trypanozoon which are of veterinary and medical importance. Six species of trypanosomes are recorded in Ethiopia and the most important trypanosomes, in terms of economic loss in livestock are the tsetse-transmitted species: *T. congolense, T. vivax and T. brucei.* The closely related *T. brucei* subspecies, *T.b. rhodesiense* causes human sleeping sickness

The other trypanosome species of economic importance are *T. evansi* of camels and *T. equiperdum* of horses. Surveys conducted in 1970; revealed that *T.congolense* and *T.v*ivax were very common trypanosomes but *T.brucei.* is not a common trypanosome in cattle, sheep and goats. Its importance being that it is likely to be implicated when pack animals such as horses and mules come into contact with tsetse on the coffee trade routes. *T. evansi* causing "Surra" in camels is common in the southern and eastern regions.

It is not transmitted by tsetse flies (Langridge, 1976). The ratio of *T. congolense, T. vivax, T. brucei* and mixed infection in Gamu Gofa were 48.8 %, 20.3 %, 5.9 % and 8.5 %, respectively (Argaw and Abebe, 1988).

2.2. Prevalence and host range

In the tsetse infested areas of Ethiopia, 20-30 % of cattle are affected by trypanosomosis and in some high tsetse challenge areas the prevalence of the diseases reaches up to 50 %. *T. congolense* and *T. vivax* are the most prevalent trypanosomes that infect cattle in the tsetse infested and tsetse free areas of Ethiopia (Abebe and Jobre, 1996). In the tsetse infested areas of the country, though, the prevalence of *T. congolense* was found to be high (58.5 %) a considerable number of examined animals were also harbouring *T.vivax* infection (31.28 %). Similar findings were reported by Langridge (1976) and Leak *et al*. (1993) in which they indicated 60 % *T. congolonse* and 31 % *T.vivax* 84 % *T.congolense* and 14 % *T.vivax* infections in southwest part of Ethiopia, respectively. Higher proportions of *T. congolense* infection were detected in areas such as Gamo Gofa, Illubabor, Sidamo and Ghibe valley. The predominance of *T. congolense* infection in tsetse-infested areas of Ethiopia indicates the existence of increased contact of cattle with tsetse vectors (Table 2).

Economically, the tsetse-transmitted trypanosomes are of most importance in cattle, with 14 million heads at risk in Ethiopia. However, in addition to infection of domesticated livestock, trypanosomes are found in many species of wild mammals. All species of domestic animals are susceptible to infection with one or more species of trypanosomes, but trypanosome infections are economically important in cattle, considering its major impact in the agricultural economy of Ethiopia (NTTICC, 1996).

Table 1: The prevalence of trypanosomosis in different area in Ethiopia

Study area	Species	No. of animals examined	Prevalence (%)	Authors
Upper Dedissa Valley	Cattle	992	24.38	Yimenu (1993)
Upper Dedissa Valley	Cattle	484	24.40	Nuru (1993)
Arbaminch and the surrounding	Cattle	813	12.79	Sertse (1994)
Bahar Dar and the surrounding	Cattle	739	16.10	Mihret (1995)
Areas boardering Lake Tana	Cattle	1509	6.10	Sinshaw (2004)
Areas boardering lake Tana	Small ruminants	798	0.20	Sinshaw (2004)
North West Ethiopia	Cattle	2462	12.35 – 17.07	Shimelis (2004)
Southern Rift Valley	Cattle	328	23	Bekele (2004)
Southern Nation	Cattle	1509	15.77	Daya (2004)

2.3. Biology of Trypanosomes

2.3.1. Morphology of trypanosomes

Trypanosomes are unicellular microscopic elongated spindle shaped protozoa ranging from 8.0 to 39.0µm long. All trypanosomes have flagellum which arises at the posterior end of the trypanosome from a basal body at the foot of the flagellar pocket. The flagellum runs to the anterior end of the *Trypanosome* membrane and is attached along its length to the pellivle to form andulating membrane. In stained specimen, a single centerally placed nucleus can been seen, and adjacent to the flagellar pocket. Small structure the kinetoplast which contains the DNA of the single mitochondria is observed (Mulligan, 1970). Based on difference in the genus *Trypanosoma* divided in to: Stercoraria and Salivaria species of the first group complete development in the terminal gut and are transmitted in the flies of vector.

Stercoraria which are with a few exceptions are non pathogenic and of no great economic importance to livestock Species belonging to the salivary group complete their development in the anterior part of the digestive tract and are transmitted via the vector salivary The main pathogens in salivary group categorized in four sub genus: Duttonlle (species: *T.vivax; T. uniforme*; Nanomonas (species:*T. congolense;T. simae*): Pycnomonas (*T. suis*) and Trypanozoon (species: *T. brucei*: *T. rhodesiense*; *T. gambiense*: *T. evansi and T. equiperdum*) (Mulligan,1970).

2.3.2. The life cycle in the mammalian host

The infective metatrypanaosomes undergo development and multiplication at the site of infection where a swelling or chancre may be detected in the skin; and finally the mature blood trypanosomes (trypeomastigotes) are released via lymph vessels and lymph nodes into blood circulation. Reproduction in the mammalian host occurs through a process of binary division. Trypanosomes feed by absorbing nutrients through their outer membrane, from the body fluids of the host (FAO, 1998b).

Trypanosoma congolense has been classified into three different types; savannah, forest and Kilifi (Young and Godfrey, 1983; Knowles *et al.*, 1988). The pathogencity appears to vary depending on which type or strain of *Trypanosoma congolense* is involved (Bengaly *et al.*, 2002).Strains within the savannah type are regarded as the most pathogenic to mice and cattle (Gow *et al.*, 2007). However, certain breeds of African cattle have been shown to exhibit a level of tolerance to trypanosome infection (Naessens, 2006).

2.3.3. The life cycle in the tsetse fly

The development of different trypanosome spp in site of the fly is also different. Blood stream forms (trypomastigates) ingested by the fly undergo considerable changes, in morphology as well as in their metabolism. They change in to long slender forms called epimastigotes, which multiply and finally give rise to the infective metatrypanaosme (FAO, 1998b). The life cycle of trypanosome is complex in both the tsetse fly vector and the mammalian host. Trypanosomes undergo a series of transformation in to different forms (Seifert, 1996).

The cyclic development of *T. vivax* in *Glossina* at a time of feeding on infected animal, the fly sucks up blood and trypanosomes, some trypanosomes may attach themselves to the proboscis, but others are swept along the food canal to the gut were they perish. In the labrum the anchored trypanosomes transform in to the epimastigote form, which measures 16-35 micrometers in length. These epimastigotes multiply and attach to the inner wall of labium and labrum by the tip of their flagella. The epimastigotes are detached from the location at certain stage and migrate to the hypopharynx where a morphological change occurs and these forms have been referred to as the pre-infective metacyclic trypanosomes. A further transformation then follows and the final infective stage, the metatrypanosome (metacyclic from) is produced (Lorne, 1986, Seifert, 1996).

Table 2: Trypanosomes species reported in Ethiopia

Trypanosomes	Vector	Host affected	Regional distribution
T. congolense	Tsetse	Cattle	Amhara
T. vivax and *T. brucei*			Benshangul-Gumuz
			Gambella
			Oromiya
			SNNPR
T. vivax	Biting flies	Cattle	All over Ethiopia
T. evansi	Biting flies	Camel	Afar
			Amhara
			Oromiya
			Somali
			Tigray
T. equiperdum	Via coitus	Horses and donkeys	Oromiya
T. rhodesiense	Tsetse	Human	Gambella ,Oromiya
			SNNPR

Source: Abebe, 2005.

2. 4. The vector

2.4.1. Morphology and biology of tsetse fly

Genus *Glossina* comprises of 23 species and 8 subspecies of *Glossina* identified so far (Leak, 1999). From morphological point of view, tsetse flies are elongated and robust, of various shades of brown ranging from yellowish to grayish to dark or blackish brown but never metallic. The male are usually smaller than the female (Itard, 1989). Useful features for identification include: wing being held closed over the abdomen fully over lapping one another; a piercing propocis which sticks out horizontally from the front of head; widely separated compound eyes; the distal medial cell of the wing is shaped like a butchers' cleaver and is some time referred to as the 'hatchet cell' and the hairs on the arista of antenna have further hairs branching of them (Robertson, 2004).

In temperature below 15^{o} C tsetse flies are in active and above 35^{o} C they seek refuge in rot – holes in the trees and animal burrows and deep tissues in the barks, where they remain inactive. Humidity is also important factor both for pupal and adult fly development (The highest catches of *Glossina pallidipes* were in bushes and wooden grass land in the Southern Rift Valley of Ethiopia (Vreysen *et al*., 1999). The most distinctive features of the life history of tsetse flies, shared with only a few other small families of diptera, is retention of the single egg in the uterus of the female, where it hatches to a larva and nourished by the products of a pair of modified accessory glands. This method of reproduction is referred to as adenotrophic vivparity (Leak, 1999).

Tsetse reproduce by adenotrophic viviparity i.e. the egg contains sufficient yolk to sustain the entire embryonic development and the larva is nourished in the female by special maternal organs (Vreysen, 2001). The successful burrowing in the soil by the deposited larva depends on various factors, for instance, soil particle size, moisture content of the soil and possibly the soil temperature are the most important ones. Under favorable environmental conditions (temperature and moisture of the soil) newly deposited larva is transformed, within a few hours, in to a hard almost black larva and moults to form the pre pupa, but remains within the third cuticle, which then harden to form the puparium within an hour of larviposition. Thirty days later adult fly emerges from the puparium with the sex ratio1:1.The puparial is highly dependent on temperature (Jordan, 1993).

The optimum temperature for the puparium development is about 25^{o}c (Leak, 1993). It has been noted that female fly live longer than males .As a result of this, there are always more female than males in any tsetse population. A female fly may produce about 8-10 offsprings in her life time. Consequently the rate of reproduction is much lower than in any oviparous insects and in fact resembles that of small mammals that is why the sterile insect technique (SIT) control method is facilitated (Leak, 1999).

2.4.2. Distribution of tsetse flies in Ethiopia

According to survey result conducted by Langridge (1976) five species of tsetse flies were identified in different parts of the country. The fusca group: *G. longipennis;* the Morsitans group: *G.m submorsitans* and *G. pallidipes and* the Palpalis group: *G. fuscipes fuscipes* and *G. tachinoides.* The fly belt in Ethiopia extends from the southern part of the rift valley, around the southwestern part extending along the western lowland and then escarpment to Abay valley being confined to the southern and western regions between longitude 33^{0} and 38^{0}E and latitude 5^{0}and 12^{0}N (Langridge, 1976) (Table 3). The largest belt is Omo belt, which include Ghibe, Gojeb and Omo river systems. The second belt in the eastern part of the region is the Rift valley belt, which includes Bilate river system, Abaya and Chamo Lakes and Segan river system up to Woitto and Chew Bahir (Langridge; 1976; SRVL, 2000).

During the course of consecutive surveys (Amare, 1995; NTTICC, 1996; SRVL, 2000), the distribution of tsetse and trypanosomosis were recorded in two main belts, Omo and rift valley belts. Tsetse species identified from these two belts were also *G. m. submorsitans, G. fuscipes, G. pallidipes* and *G. longipennis.* Different species of tsetse flies require particular vegetation type that would provide an optimal condition for growth and survival and vegetation is also important that provides shelter their host (Leak, 1999). The highest catches of *G.pallidipes* were in bushes and wooden grass land in the Southern Rift Valley of Ethiopia (Veryesen *et al.,* 1999).

Table 3: Tsetse infested regions and river basins of Ethiopia

Region	Major River Basin	Tsetse fly
Amhara	Abay (Blue Nile)	G.*m. submorsitans, G. tachinoides*
Beneshangul-Gumuz	Abay (Blue Nile)	*G. m. submorsitans, G. tachinoides*
Gambella	Baro/Akobo	*G. m. submorsitans, G. tachnioides*
		G. pallidipes, G. f. fuscipes
Oromiya	Abay/Didessa	*G. m. submorsitans, G. tachinoides*
	Upper Ghibe/Omo	
	Baro/Akobo	*G. pallidipes, G. fuscipes*
SNNPR	Ghibe/Omo	*G. pallidipes, G. f. fuscipes, G. longipennis,*
	Rift valley	*G. pallidipes*

Source: Abebe (2005)

2.5. Epidemiology

The epidemiology of African animal trypanosomosis is highly dependent of the parasite, vector and host factors. *Trypanosoma* species occur in a remarkable variety of genotypes with differing strains of virulence, immunogenicity and response to chemotherapeutic agents. The severity of the disease also depends on the species and strain of trypanosomes involved. For instance, *T. vivax* and *T.congolense* are known to have high virulence in cattle. The fact that the parasite infects not only cattle but also wild animals, which constitute the reservoirs of the disease, makes the epidemiology of animal trypanosomosis extremely complicated. The animal hosts differ in their response to trypanosome infection depending on the species, breed and individual animals. The level of animal husbandry practices, nutritional status, workload and physiological states (exhaustion, lactation and parturition) also play a role in the severity of the disease (Eisler *et al*., 2004).

2.5.1. Transmission

The transmission of the disease is either cyclically by tsetse flies or mechanically by hematophagous flies. Transmission by tsetse fly is a complex mechanism in which the fly remains life long carrier.

In the vector trypanosome changes through several morphological distinct stages (amastigote, promastigote and epimastigote) until it reaches trypomastigote (metacyclic stage), which is infective for mammals (Stephen, 1986, Urquhart *et al.*, 1996).

Trypanosomosis is a disease, which is cyclically transmitted by different species of tsetse flies. The tsetse fly becomes infected with trypanosomes when feeding on an infected animal. Once the trypanosomes are ingested they lose the surface coat, develop a mitochondrion and undergo a number of developmental stages before they become infected, once more, for the mammalian host these developmental stages are known as trypomastigote, epimastigote and metacyclic forms (ILRAD, 1989). Mechanical vectors cause mechanical transmission of trypanosomosis (*T. vivax*) that is accomplished through interrupted blood meals and can occur through tsetse flies and other biting flies (Tabanids and Stomoxys). Mechanical transmission requires only the blood that contains infectious trypanosomes be transmitted mechanically and occasionally be found outside tsetse infested areas (Langridge, 1976).

2.5.2. Pathogenesis of trypanosomosis

The pathogenesis of tsetse-transmitted trypanosomosis can be categorized into the following groups according to the site of host-parasite interaction. The first interaction between trypanosomes and host occur in the skin following a successful feed by an infected tsetse fly. Within a few days of bite, cattle develop a raised coetaneous swelling called a chancre, which is caused by the reaction to multiplying trypanosomes (Murray, 1983). Following enlargement of the lymph node (Lymphadenopathy) draining the chancre, generalized enlargement of lymph nodes and splenomegaly develop. This is associated with marked proliferation of lymphoid cells in the organs. In the medullar cords of lymph nodes and splenic red pulp there are increases in plasma cells and numerous large active germinal centers are also present (Leak *et al*., 1999). In addition, the red pulp of the spleen, there is an increase in the number of activated macrophages, some of which are engaged in erythrophagocytosis. Trypansomosis, like other infectious disease starts with in increase of the body temperature, a hyperthermia.

This is a result of the contact between the trypanosomes multiplying in the host and the defense system of the host (FAO, 1998b). The pathogenesis of trypanosomosis depends on the pathogen city of the strain, the animal hosts, breed, genotype, age, sex, skin type etc, and most importantly, on the method by which the infection was induced i.e. natural or artificial (Leak *et al*., 1999).

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

T. vivax and *T. congolense* species cause severe anemia and mild to moderate organ damage. Trypanosomes can also pass through the placenta and into the fetus in pregnant animals. As the result some cows abort and some calves are born before birth time. A cerebral form of the disease occurs with *T. brucei* alone or in mixed infections with the other spp (Stephen, 1986). The onset and severity of the anemia is directly related to the appearance of the parasite in the blood and to the level of the parasitaemia. The rapid decline in the hemoglobin concentration, red blood cell numbers and PCV and the clear clinical sign of pallor of the mucus membrane in infected animal leave as no doubt that anemia is a very important part of the pathogenesis of trypanosomosis.

When an animal is infected with trypanosomes, antibodies against the surface coat are produced. The problem is that these trypanosomes have multiple genes, which code for different surface proteins; this allows organisms with a new coat glycoprotein to elude the immune response.

This process is called antigenic variation and results in the persistence of the organism and this prevents the development of vaccine and permits re-infection when animals are bitten by tsetse flies carrying trypanosomes with surface coat glycoproteins of new antigenic type. Genetic resistance to animal trypanosomosis has been attributed to certain breeds of livestock, e.g. West African N'Dama.

This resistance is manifested by ability to withstand the adverse effects of trypanosomes by regulating parasite growth and their ability to prevent or reduce the rate and degree of development of anemia (Seifert; 1996; Murray, 1998). Metabolic disorders are observed in the host due to a trypanosome induced hypothyroid status and pituitary dysfunction during trypanosomosis (Abebe *et al*, 1993). Pathology in tissue is associated with the relative ability of the trypanosomes to invade extravascular space and organs (Taylor and Authiė, 2004). *T. congolense* is mainly confined to the blood, while *T. vivax* and *T. brucei* also invade the tissue. There is remarkable intraspecies variation in the pathogenicity of different parasite stock, especially stock isolated from distinct geographical regions (Taylor and Authiė, 2004).

2.6. Diagnostic methods

2.6.1. Clinical diagnosis

In general diagnosis of trypanosomes infection based on clinical signs alone is rather difficult, but hematological parameters like PCV could be reliable indicators of the progress of the disease. Intermittent fever can be observed due to the variation in parasitaemia and if the animal survives, the disease becomes chronic and there is development of anemia and emaciation. Therefore, fever, anemia and loss of body condition are important parameters used for tentative diagnosis of trypanosomosis in areas where this disease is endemic and laboratory service is not available (FAO, 1998a). The skin often loses its suppleness ("turgor") because of dehydration, the eyes are sunken and at this stage the classical signs of anemia are obvious, the visible mucous membranes are pale and the blood is watery in appearance. The emaciation is associated with weakness and in the final stages results in inability to stand and in pressure sores and ulceration of the skin over the bony prominences. There is very often an increased secretion of tears (lachrymation) (Uilenberg, 1998).

2.6.2. Parasitological diagnosis

Parasitological diagnosis is the direct demonstration of the parasite in blood or less frequently in other body fluids. The security of the parasites and the fluctuating nature of the parasitaemia limit the use of laboratory tests based on demonstration trypanosomes in accessible body tissues such as the peripheral blood (Doyle, 1977).

Direct examination techniques, (Wet blood film)

Wet films of fresh blood, usually obtained from the ear vein, jugular vein or the tail constitute the simple, inexpensive and rapid method. Trypanosomes can be recognized by their movement among the red blood cells. Depending on the size and movement of the trypanosome a presumptive diagnosis can be made of the trypanosome species. The diagnostic sensitivity of the method is generally low but depends on the examiner's experience and the level of parasitaemia. Sensitivity can be improved significantly by lysing the RBCs before examination using a haemolytic agent such as sodium dodecyl sulfate (OIE, 2004; FAO, 1998a).

Dark ground or phase contrast buffy coat technique

The buffy coat zone prepared in a microhaematocrit capillary tube filled with 70 µl of blood and centrifuged for 5 minute at 12,000 revolutions is examined for trypanosomes by cutting the capillary tube to include 1mm of erythrocyte and 1cm of the plasma. The Buffy coat is poured on a slide and covered with a 22x22 mm 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. This technique is the most sensitive of the parasitological test for detection of *T.c* and *T.v* detecting trypanosomes to an estimated level of just over 10^2 parasites per ml. (Murray *et al*., 1977). In addition, species identification based on size and movement is easier (Paris *et al*., 1983). Trypanosomes can be identified and the level of parasitaemia estimated using a scoring system (Murray *et al*., 1983). The PCV is measured before examination of the blood for parasitaemic detection.

Capillary concentration

Because of the tendency of *T. congolense* to be retained amongst red blood cell (RBC) these technique was designed to create a large differential density between the RBC and the parasite This was achieved by mixing infected blood with a strongly hypertonic non-toxic medium (Walker solution). On centrifugation the denser red cells separate from the trypanosomes, which display normal motility. Equal volume of diluents and blood are mixed on a micro titer titration plate. After being allowed to stand for a minimum of 15 minutes, plain capillary tubes are three quarter filled from the wells, sealed and spin for 2 minutes in a micro haematocrit centrifuge.

The capillary tubes are placed on a clean microscope slide and the Buffy coat zone covered with a few drops of diluents beneath a cover slip. This technique is more sensitive than haematocrit centrifugation test in the detection of *T. congolense*. However, it needs more time to prepare the samples and PCV cannot be measured at the sometime (Walker, 1972 ;).

Thin blood smear

Thick smears contain more blood than thin smears and hence, have a higher diagnostic sensitivity. Thin smears on the other hand allow trypanosome species identification. Trypanosome species can be identified by their morphological characteristics (OIE, 2000):

2.6.3. Use of experimental animals

The sub inoculation of blood into rodents, usually mice or rats, is particularly useful in revealing sub patent infections. The laboratory animals are injected intraperitoneally with 0.2-5 ml (depending on the size) of freshly collected blood. They are bled three times a week for at least 2-7 months. Collected blood is examined using the wet film method. Nevertheless, the method is not practical; it is expensive and diagnosis is not immediate. The method is highly sensitive indetecting *T.brucei* infections. However, some *T. congolense* strains are not easily transmitted and *T. vivax* rarely infects laboratory rodents. Also animal inoculation should be avoided as it raises serious animal welfare concerns (Schlater and Bossche, 2004).

2.6.4. Serological diagnosis

A PCR method has been developed as a tool for the diagnosis of infections with African animal trypanosomes. Specific repetitive nuclear DNA sequences can be amplified for the three types of *T. congolense* (Masiga *et al.,* 1992; Desquesnes, 1997; Desquesnes and Davila, 2002). Unlike their prohibitive cost for routine use, PCR restriction fragment length polymorphism (RFLP) assays have been recently developed that allow the identification of all *Trypanosoma* species as single or mixed infections using one single test (Desquesnes *et al.*, 2001; Delespaux *et al.,* 2003). Species-specific monoclonal anti bodies produced against procyclic forms of *T. congolense, T. brucei* and *T. vivax* were used to develop antigen-captured enzyme linked immunosorbent assay (Ag-ELISA) for the diagnosis of bovine trypanosomosis (Nantulya *et al.,* 1989).

Several antibody detection techniques have also been developed to detect trypanosomal antibodies for the diagnosis of animal trypanosomosis. The methods of choice are the Indirect Fluorescent Antibody Test (IFAT) (Katende *e`t al*., 1987; Greiner *et al.*, 1997) and the trypanosomal antibody-detection, ELISA (Luckins, 1977; Hopkins *et al.*, 1998). ELIS As using *T. congolense* precoated microtitre plates have been developed (Rebeski *et al.,* 2000). They detect immune responses to current and past infections and can, therefore, only provide a presumptive diagnosis of active infection. Sample collection and storage is made easy through the use of filter papers. All of these factors make the antibody ELISA a very useful test for large-scale surveys to determine the distribution of tsetse-transmitted trypanosomosis (Schlater and Bossche, 2004). The present serological tests are not sufficiently specific to reveal conclusively the identity of the infecting trypanosomal species (Nantulya *et al*., 1987).

2.7. Treatment and control of trypanosomosis

Historically sodium antimony tart rate has been the only relatively successful remedy for African Cattle Trypanosomosis between the two world wars. It was, however, difficult to use, the tissue irritation attendant upon its injection required that it be administered intravenously and a complete cure could, at best, be assured only by repeated treatments. Control became widespread with the arrival of synthetic insecticides in the 1940s.

Selective spraying of the vegetation support of the flies and later the application of insecticides by aircraft opened the way for large scale tsetse eradication (Uilenberg, 1998). Currently, there are three principal control strategies for tsetse-transmitted trypanosomosis in Africa: selection and breeding of trypanotolerant cattle; chemotherapy and chemoprophylaxis using trypanocidal drugs and vector (mainly of tsetse fly) control/eradication (insecticidal spraying, insecticidal targets, traps, and the sterile insect technique) (McDermott and Coleman, 2001).

2.7.1. Control strategies against the parasite

Chemotherapy and chemoprophylaxis

Isometamidium chloride has been used in the field for several decades prophylactically or therapeutically for livestock suffering from trypanosomosis due to infection with *T. congolense* and others (Leach and Roberts, 1981). Treatment and prevention of African animal trypanosomosis nowadays relies essentially on three drugs namely: Homidium salts, Diminazene aceturate and Isometamidium chloride salts of these drugs have been in use for more than 45 years in Africa. However, almost all of these trypanocidals are gradually losing their efficacy due to drug resistance (Delespaux and Koning, 2007; Williamson, 1970).Isometamidium is a phenanthridinium compound and is marketed as both a therapeutic and prophylactic agent. In the dose range recommended for prophylactic purposes (0.5-1mg/kg of bw), the compound has been used successfully to maintain the productivity of Zebu cattle exposed to tsetse challenge in both village and ranch management systems in East Africa (Moloo *et al*., 1987). However, considerable variation in prophylactic activity has been observed in that a dose of 1mg/kg bw has been shown to confer prophylaxis to cattle for 2-22 weeks. Variation in drug susceptibility between different trypanosome populations appears to be the major factor determining the duration of prophylaxis (Peregrine *et al*., 1997). The trypanosome kinetoplast is the primary site of Isometamidium accumulation. The main mode of action of the drug is the cleavage of kDNA-topoisomerase complexes. The mechanism of resistance to Isometamidium is less clear. Several workers have shown that accumulation of Isometamidium is significantly lower in resistant populations than in sensitive ones (Holmes *et al*., 2004).

2.7.2. Vector Control

Use of insecticide

Each of the available options for tsetse control or eradication has its own advantages and specific limitations. The strategies for using these options may vary considerably depending on the specific objective, technical and logistical feasibilities and cost requirements. However in most circumstances, viable agricultural systems can be established effectively only when several methods are combined (FAO, 2001).

Live bait technology, Pour-on, an efficient technology in tsetse infested areas within a high density of cattle, but disadvantage are the high frequency of treatment and high cost of insecticides (Oloo *et al.*, 2000). Tsetse flies are highly susceptible to the action of insecticide, and many different products starting with DDT and dieldrin up to the more recently introduced and less harmful pyrethroids, have been used over the past 50 years to control and eradicate tsetse. So far, the use of insecticide has not produced insecticide resistance in tsetse flies. This can presumably be attributed to the low selection pressure for resistance and the fact each female tsetse fly only produces few descendants during her life span (FAO, 1998b) apply on domestic animals, so that flies settling on such animals are killed.
Synthetic pyrethroid formulations are applied by spraying, dipping or used as a "pour-on" formulation, which is more expensive but does not need any pump, spray-race or dip (Uilenberg, 1998).

Use of Traps and targets

The development of insecticide impregnated, odour-baited traps (Dransfield *et al.*, 1990) and targets (Vale, 1993) and insecticide treated cattle as pour-on (Shereni, 1990) which attract and kills tsetse offer the prospect of cheaper alternative with less damage to the environment (Jordan, 1988). On the other hand, baits (traps, targets or animals) are now-a- days used widely to replace ground broad casting of the insecticides (Vale, 1993). In Ethiopia, these techniques have been tried and are still in use in the different tsetse infested areas. The use of insecticide impregnated target and application of pour-on on cattle in the area has suppressed the tsetse population from 4.1 to 0.9 fly/ trap/day.

As the result the prevalence of bovine trypanosomosis has dropped from 27 to 6 % in two years time (Abebe *et al*., 2004). Clausen *et al.,* (1992) stressed that efficient tsetse control will lead to a reduction in use of trypanocidal drugs and this will leave their role as efficient means of cunning the disease in case of an outbreak.

Sterile insect technique

The sterile insect technique (SIT) is one area-wide insect pest management method where the insect pest is controlled or eradicated by affecting its reproductive capacity. It relies on the production of sterile males (target insect) in mass-rearing facilities and release in sustained numbers in the natural habitat large enough to outnumber the wild pest population (Vreysen, 2001). Males are sterilized by radiation at the appropriate stage and then taken to the selected area and released. Eventually, so few fertile insects remain that fertile matings do not occur and the population is eliminated (Feldmann and Hendrichs, 2001).

2.7.3. Use of Trypanotolerant Cattle

Certain local breeds have developed a tolerance to trypanosome infections during the centuries spent in areas strongly infested by Glossina. This ability, named trypanotolerance, results from several biological mechanisms under multigenic control (Hanotte *et al.*, 2003). Indeed, some breeds present the remarkable capacity to control their level of parasitemia, to resist the development of severe anemia during the infection, and to remain productive in a zone strongly infested by tsetse flies.These two characteristics (limited parasitemia and anemia) are known to be highly heritable and genetically linked to cattle productivity (Hill *et al.*, 2005).

More than one single gene, it seems probable that two pools of genes, are involved but all the techniques used up to now have failed to identify them (Berthier *et al.,* 2006) such as the “normal” Boran Zebu, Indian breeds of zebu and other “exotic” breeds such as European taurine breeds. However, the resistance of West African taurine breeds appears to be considerably more pronounced (FAO, 1998b).

The only surviving indigenous taurine type indigenous cattle breed in Ethiopia the Sheko exhibited better trypanotolerant attributes than the other three breeds (Abigar, Horro and Gurage), as measured lower trypanosome prevalence, less severe anemia after infection, and fewer trypanocidal treatments annum than the other breeds. Moreover, the Sheko breed maintained its physiological functions under prevailing trypanosomosis challenge and compared favorably with the other breeds in its reproductive performance. While the Abigar manifested high sensitivity and frequent death to PCV depression, Horro had strong resilience to PCV depression better response to Berenil 1 treatment assistance (Lemecha *et al*., 2006).

2.8. Drug resistance

Drug résistance is defined as a loss of sensitivity by a certain of an organism to a compound to which it had been previously susceptible because of misuse of trpanocidal drugs and lack of use of trypanocidal drugs and lack of essential information dissemination at all levels., the effectiveness of trpanocidal drugs is often limited and this is mainly due to the development of drug resistance (Conner, 1992). Eisler *et al*. (2000) have developed a method for the assessment of trypanosomosis risk and the level and prevalence of resistance to ISMM, utilizing cattle population under natural challenge in the field. The first reports of Isometamidium use date from 1963 and the first case of resistance to Homidium and cross-resistance between Homidium bromide/chloride and Isometamidium chloride was reported in 1967 (Delespaux and Koning, 2007).

The main mode of action of Isometamidium chloride was suggested to be the cleavage of kDNA-topoisomerase complexes, causing the desegregation of the mini circle network within the kinetoplast (Shapiro and Englund, 1990), though findings later shown dyskinetoplastic trypanosomes observed at least as sensitive to Isometamidium chloride as kinetoplastic lines (Kaminsky *et al.,* 1997).This explanation was supported by observations from later studies who showed that the trypanosome kinetoplast as the primary site of Isometamidium accumulation in *T.congolense* (Wilkes *et al.,* 1995) which was later confirmed for the hemoflagellate *Cryptobia salmositica* (*Kinetoplastida, bodonina*) (Ardelli and Woo, 2001; Woo, 2003) and for *T. brucei* (Boibessot *et al.,* 2002), using more sophisticated chromatographical and microscopical techniques.and later work found indirect evidence of an increased efflux of drug from resistant trypanosomes (Sutherland and Holmes, 1993).

Under field conditions, some of the factors resulting in resistance in cattle are repeated use of drugs (Leak, 1999), to small doses, under estimation of animal weights, an abscess at the site of injection (Itard, 1981) and under estimation of local trypanosomiasis challenge leads to drug resistance (Jordan, 1986).

Moreover, irregular treatment with chemoprophlactic or termination of the treatment while the animal is still exposed to the injection and if prophylactic drugs are given to an infected animal instead of curative drugs (Itard, 1981; Jordan, 1986). In Ghibe valley, south west Ethiopia where *G.pallidipes*, *G. fuscipes* and *G. m. sub morsitans* are prevalent species, all 12 cases produced infection with T. congolense showed resistance to treatment with Diminazene aceturate at dose of 7.0mg/kg bw, 92 % of infection were also resistant to Isometamedium chloride at a dose of 0.5mg/kg bw and Homidium at a dose of 1.0mg/kg bw (Peregrine *et al*., 1994b). In southern region drug efficacy studies with 1mg/kg, 3.5 mg/kg and 0.5 mg/kg bw, Ethidium, Diminazene and Isometamidium showed that 18.4 % and 35.4 % of cattle were infected with recurrent parasiteaemia by ten and twenty days respectively (Habtewold, 1993).

2.8.1 Mechanism genetics of resistance to trypanocides

The mechanism of resistance to Isometamidium chloride (ISMM), however, is less clear despite, certain suggested mechanisms from experimental finding results .Decreased levels of drug accumulation have been observed in drug resistant populations of *T. congolense* (Sutherland *e al.,* 1991) and later work found indirect evidence of an increased efflux of drug from resistant trypanosomes (Sutherland and Holmes, 1993).

Isomethamedium

Peregrine (1994a) showed that the trypanosome kintoplast is the primary site of Isometamidum (ISMM) accumulation. The mechanism of resistant to ISMM, however, is less clear. Decreased levels of drug accumulation have been observed in drug resistant populations of *T. congolense*. Recently, Mulugeta *et al*. (1997) showed that the maximal uptake rate (V max) of ISMM in resistant *T. congolense* were significantly lower than in sensitive populations.

Although contradictory obs ervations have been reported on the genetic stability of ISMM resistance, recent field observations in Ethiopia, based on cloned populations, showed that the drug-resistance 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 along time, Homidium chloride and especially Homidium bromide or Ethidium are still widely used as trypanocidal drugs. The mechanism of there anti trypanosomal action is not well under stood. The mechanism of resistance by trypanosomes to this drug is unknown. There are indications; however that is similar to that described for ISMM (Leak, 1999).

Diminazene

Although Diminazene probably exerts its action at the level of the kinetoplast DNA, this has not been proven in vivo, and other mechanism of action can not be excluded. The molecular basis of resistance to Diminazene in trypanosomes is not clear. 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 conclusions, it is clear that much more work is required in order to elucidate the mechanism of resistance to three currently used drugs (Geerts *et al*., 1999).

3. MATERIALS AND METHODS

3.1. Study area.

The study was conducted in Amaro special district of the Southern Nation Nationalities and People Regional State of Ethiopia. Amaro special district is located in the Southern part of the country, which is bounded on the north and east by Gelana district (Ormoiya) and on the south by Burji special district (SNNPR) and by Bulle Horra district (Oromiya) and to the West Gamu Gofa Zone (SNNPR) (Chamo and Abaya lakes).

The capital city of the district, Kelle town, is located 478 km south of Addis Ababa and 203 km south of Awassa, the regional capital city. The district is characterized by plain, mountainous and undulating terrain. The altitude ranges from 1200 to 3600 m.a.s.l. The Amaro mountain chain, Dello Mountain being the highest peak drops sharply to south east and gently northwest till it reaches the lowest elevation at Gelana River and Chamo Lake respectively. The extremes of the elevation characterize the diversity of agro- climate and vegetation cover of the area.

3.1.1. Climate

On the average, the area used to receive a minimum of 735 mm and a maximum 1200 mm of rainfall per annum. Currently the area is characterized by in adequate and erratic rainfall pattern. The rainfall pattern is bimodal. The community indicates that the normal rain seasons are during the month of mid march to mid July and mid September to mid November. There are three distinct agro climatic zones, namely highland, midland and lowland covering 30 %, 38 % and 32 % of the district total land are (1534.07 Km^2) respectively. The annual average temperature is between 15^0 C and 28^0 C (CSA and Amaro, 2006).

3.1.2. Human population.

The total human population of the district in year 2006 was estimated at 139,727 (CSA,2006) Rural population was 96.7 % while the urban population was only 3.3 %.The total household in the district is 28,084 average family sizes of about 5 persons (Amaro,2006).

3.1.3. Livestock population

Table 4: Livestock populations in Amaro special district

Livestock	Population
Bovine	61,380
Caprine	42.021
Ovine	36,202
Equine	3,693
Avian	177,050

Source: Amaro (2006)

3.1.4. Socioeconomic situation and farming systems of the study area

Agriculture was the major livelihood of people with a mixed farming system and livestock played on integral role for agricultural activity. Livestock also provided meat, milk, cash income and manure. The livestock species reared were cattle, sheep, goat and equine. Communal grazing was the traditional way of feeding animals and crop residue used extensively during the dry period in the study area. The major crops produced in the district were maize, enset, teff, barley, wheat, cassava, sweet potato etc. Small-scale irrigation was also traditionally practiced for a long period by a large number of farmers. Most of the PAs have potential source of water for irrigation, which were used by farmers. Twelve permanent rivers were used to irrigate 191 hectares of land area out of potentially irrigable 10,000 hectare. Animals used to drink water from springs, irrigation ditches and rivers (Amaro, 2006).

3.1.5 .Constraints of livestock Production and efforts done to control tsetse and Trypanosomes

In the Amaro special district, infectious disease like black leg, pasteurellosis, contagious bovine pleuropneumonia (CBPP), contagious caprine pleuro pneumonia (CCPP), lumpy skin disease (LSD), Newcastle disease (NCD) and others (Amaro, 2006).

Parasitic diseases such as dictyocaullosis, fasciollosis, trichostrongylosis, cysticercosis and protozoan diseases of which tryponosomosis is the primarily constraints of livestock production. Lack of grazing land in the high lands was due to over stocking and expansion of cultivated land. Tsetse and non-tsetse transmitted trypanosomosis is now becoming critical problem in the lowland and midland of the area and *Glossina pallidipes* was the main vector of trypanosomosis in the area (Amaro, 2006).

Gamule PA area was recorded to have the highest disease prevalence by Agri-service Ethiopia Amaro Integrated Food Security Program (Agriservice, 2002) and thus has been included as part of the study area

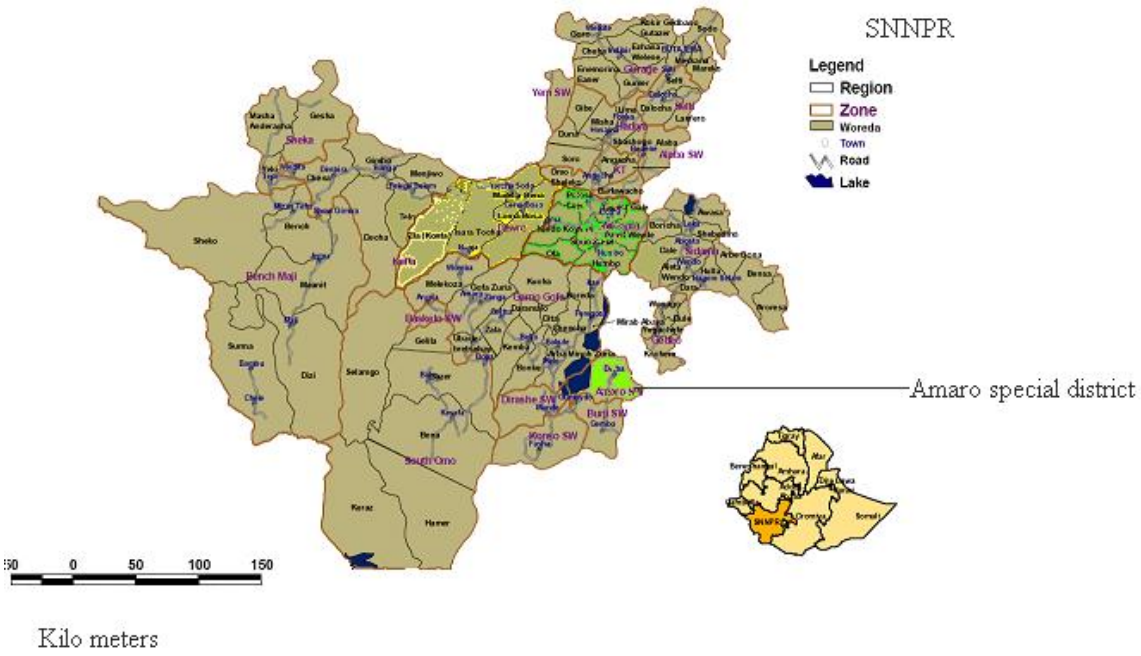

Figure1 Map of study areas
Source: CSA (2004)

3.2. Study period

The present study was conducted from October, 2007 to May, 2008 in Amaro special district of SNNPR involving both seasons of the year.

3.3. Study population

A total of 15,000 cattle from the 5 PAs (Gamule, Jello, Korebiko Golbe and Jijolla) and bordering PAs of the study area were the study population. PAs were randomly selected from low lands and mid lands of Amaro special district.

3.4. Study design

3.4.1. Study methodology

The study was based on questionnaire, vector (tsetse fly) and parasitological survey and longitudinal study.

Questionnaire survey

To assess the perception of farmers on the occurrence of tsetse and trypanosomosis, herd composition, production income source, use and source of trypanocidal drugs, livestock constraints, socioeconomic status, delivery of the drug for treatment of their animals and other control methods of trypanosomosis and tsetse flies, a questionnaire survey was undertaken. A total of 80 farmers were selected randomly in the study area for this purpose. The questionnaire used for interviewing the farmers have been indicated in Annex-1

Vector studies (tsetse fly survey)

For the study of apparent density, species, age, sex and distribution variation of tsetse fly, and its relation to seasonal, altitudinal and vegetation type, tsetse fly sampling was made in the study sites. The vegetation types and altitude as well as watering points were recorded during sampling. The vegetation types were categorized as bushland, wood, grassland and cultivated land.

Data collection for tsetse fly

Data was collected in two seasons during the study period: In the late rainy season (October-November, 2007) and during the dry period (January -February, 2008).

Tsetse fly was trapped using NGU traps baited with acetone and three week fermented cow urine. The NGU trap, developed by Brightwell *et al*. (1987) at Nguruman in Kenya for tsetse control. In selected sites of each PAs in the study area 12 traps were deployed preceding sun rise in the morning and remained in position for 72 hours.

Before they were set, they were checked carefully to make sure that there were no holes or tears in the material especially in the net cone or in the cage. Prior to setting traps area for trap deployment was cleared in set radios around the trap to standardize visibility of the trap (FAO, 1992).

All traps were set at the same height above ground level. The caught flies were adequately protected from ants by coating part of trap poles with grease. About 120 traps were deployed in the study period. During trapping acetone was dispensed from open vials through 2-6 mm diameter in order to get release rate of more than 150 mg/hr and the hole of dispenser for cow urine was with a diameter of about 45 mm in order to get release rate of about 100 mg /hr (FAO, 1992) All odour releasing containers were placed on the ground about 30 cm above the ground. The different fly catches in each trap were counted, identified and analyzed according to their sex, age, and apparent density.

Age assessment was determined using the method described by Challier (1965) , where flies with a perfect wing were recorded as wing fray category 1 and those with a heavily damages wing category 6. The relative apparent density of tsetse fly was calculated and expressed as the number of tsetse fly/ trap/day (Leak *et al.*, 1987).

Parasitological studies

To determine the prevalence of trypanosomosis a cross sectional study was carried out twice during study period, after rainy season and during the dry period in 5 PAs (Gamule, Globe, Jello, Jijolla and Kore Biko PAs).

3.4.2. Sampling method and sample size determination

Cluster sampling method was the sampling strategy (Martin, 1987). The sample size was determined based on the expected prevalence of 20 % (Abebe and Jobre, 1996) and absolute desired precision of 5 % and 95 % level of confidence. Accordingly a total 245 animals were needed to be sampled. But in case of cluster sampling used the sample size, by rule of thumb, is to be inflated twice (Thrusfield, 2005). This would have result in a sample size of 490 units, but to make the study more precise, 585 and 551 (total 1136) units were sampled in late rainy and dry seasons, respectively.

The sample size determination formula:

$$n = \frac{1.96^2 \, p_{exp}(1-pexp)}{d^2}$$

Where,

n= required sample size
pexp= expected prevalence
d= desired absolute precision

The clustering based on herds (locally Monna) is a group of animals from one village that share the barn at night and graze together. The average animals per hard were estimated about 45-60 animals. Total 272 herds (Monna) are found in the study area. From these herds 12 herds were selected at random to get 590 animals needed for the study.

Sample collection and parasitological examination

Blood sample was collected form auricular vein of sampled animals. During sampling sex and age of the animals were recorded. Blood sample was collected twice in the study period, in late rainy season and during the dry period. Dark ground/ phase contrast buffy coat method (Murray *et al.,* 1977) was employed to study the prevalence of trypanosomes. Blood sample from each animal was collected in two heparinized capillary tubes upto ¾ th of the height of the tubes and sealed at one end with crystaseal. These were centrifuged at 12000 rpm for 5 minutes (Murray *et al*., 1977).

Packed cell volume (PCV) was determined using heamatocrit reader (Woo.1970). After the PCV was read, capillary tubes were broken 1 mm below the buffy coat to include the red blood cell layers and the content was expressed on microscopic slide and covered with a 22x22 mm cover slip. The content was examined under x40 objective to identify trypanosomes by their motility (Murray *et al*., 1977). From positive samples thin blood smears were made, fixed with methanol for 5 minutes and stained with Giemsa solution for 30 minutes and examined under x 100 objective using oil immersion to detect the species of *Trypanosomes*

3.4.3. Longitudinal study

During cross sectional study in the beginning of November 2007, 69 Zebu cattle naturally infected with trypanosomes were selected from 3 PAs to assess curative and prophylactic effect of isometamidum chloride (1 mg /kg bw).The area was selected based on information gathered from the society and on the result of the trypanosomes prevalence during cross sectional study; Golbe. Gamule and Jello PAs were selected for ISMM chloride block treatment using purposive sampling method. Animals in each PA were ear tagged using yellow plastic tags which allow easy identification of animals during each visit for parasitolgical and haematological examination. The ear tag number and owner's name for each animal were registered.

Cattle were treated intramuscularly with Isometamidium chloride / Veridium (1 mg /kg bw) (LOT 142 A1 Mfd. 02/2007 Exd. 02 /20012 CEVA Saite Animale 3501 Libourne France). For calculating treatment dose, the body weight of each of the study cattle were estimated from measurements of heart girth and length of the animal (Arora e*t al.,* 1981).Treated animals were monitored on 15, 30, 60, and 90 days post Isometamidium chloride block treatment. The blood sample were microscopically examined and positive parasitaemic animals (the relapsed) were recorded and treated with diminazine aceturate with 7 mg /kg bw and excluded from the study.

3.5. Data analysis

For the management, analysis and interpretation of data statistical analysis was employed with Microsoft Excel and SPSS (Version 15, 2006) for Microsoft windows was used.
The following parameters were analyzed. The mean prevalence of trypanosomosis in different sites and seasons was compared by chi square test, the abundance of tsetse between the two seasons (the seasonal distribution and apparent density) was compared by Independent sample t-test.

The total fly catches in relation to variables measured (season, vegetation type altitude level and climate) were analyzed using One Way ANOVA. Independent sample t-test was employed to compare the mean PCV of the parasitic and non parasitic animals. The prevalence of trypanosomes infection in different seasons was subjected to Logistic Regression analysis.The effects of area and seasons of sampling on the herd average PCV was investigated by analysis of variance. The relationship between the parasitological prevalence of trypanosomal infections and herd average PCV was examined by Correlate Bivaritae Analysis. Data on the questionnaires was summarized using frequency distribution and percentages. Significant level was determined at $p< 0.05$ for all statistical results

4. RESULTS

4.1 Questionnaire survey

History of farmer's settlements

A total of 80 farmers were interviewed from 5 PAs, all indicated that they practice mixed farming system. The interviewerd individual farmers were selected randomly from the study area. All the interviewed farmers responded that they started to live in the study area 38 years ago 50 % more than 25 years and 15 % 17-23 years, from starting of settlement. Trypanosomosis was reported to exist since the beginning of their settlement. The farmers still settlle in the low land areas from high land PAs of Amaro special district by adjusting their own indigenous social and cultural management system.

Livestock management

Livestock was reared primarily for draught power, milk, meat, manure and as gift for dowry. The composition of livestock species in the area was such that cattle account for 59 %, small ruminants 40.67 % and equine 0.33 %. The number of animals per respondents in the study area was found to vary between 1 and 48 with an average of 9.43 cattle.

Majority of the respondents (94.6 %) indicated that free grazing during rainy and dry seasons was a common practice where as 5.6 % of respondent's practiced stall feeding for milking cows and fattening animals. In dry season all respondents used to feed their animals with crop residues mainly from 'teff', maize, enset and cassava.

Livestock watering points were close to grazing areas (0.5-3 Kms) except watering point in Jello PA (Gellana River) where, in dry season (January-march) 92 % respondents responded that their animals got water from irrigation cannels and perennial rivers during rainy and dry seasons whereas, 8 % of respondents animals got water from the streams.

Seasonal livestock feed abundance is indicated by rapid rural appraisal (RRA) technique (Snow and Rawlings, 1999) (Figure 2).

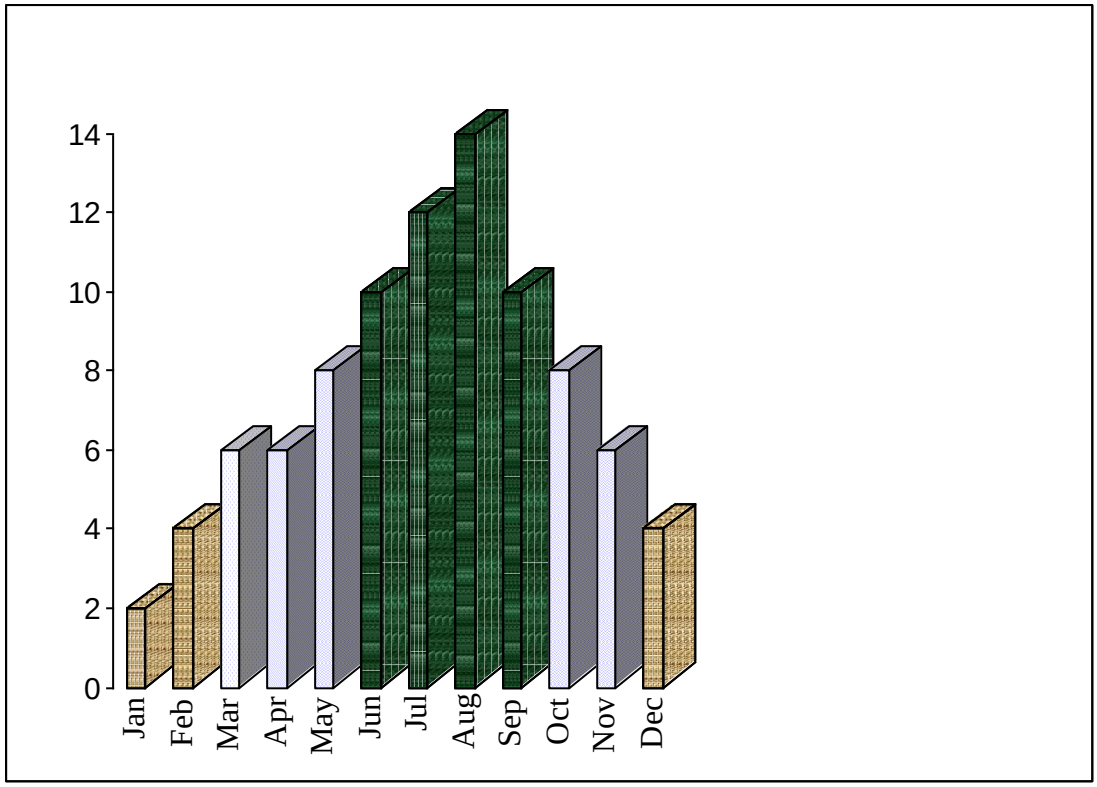

Figure 2: Seasonal livestock feed abundance as indicated by respondents (RRA method) in Amaro special district, SNNPR

Key: Score 1-4 low abundance, 5-8 medium abundance and 9-14 high abundance of feed

Constraints to Agricultural activities in the study area

According to the 95 % of respondents, erratic rain was the main constraint for their agricultural activity and 100 % of respondent ranked animal disease (trypanosomosis) as second constraint, arable land and veterinary service as third. Based on the interview result the main livestock disease in order of importance are trypanosomosis, black leg, CBPP, CCPP, pasturellosis, external (ticks) and internal parasites as shown in Figure 3.

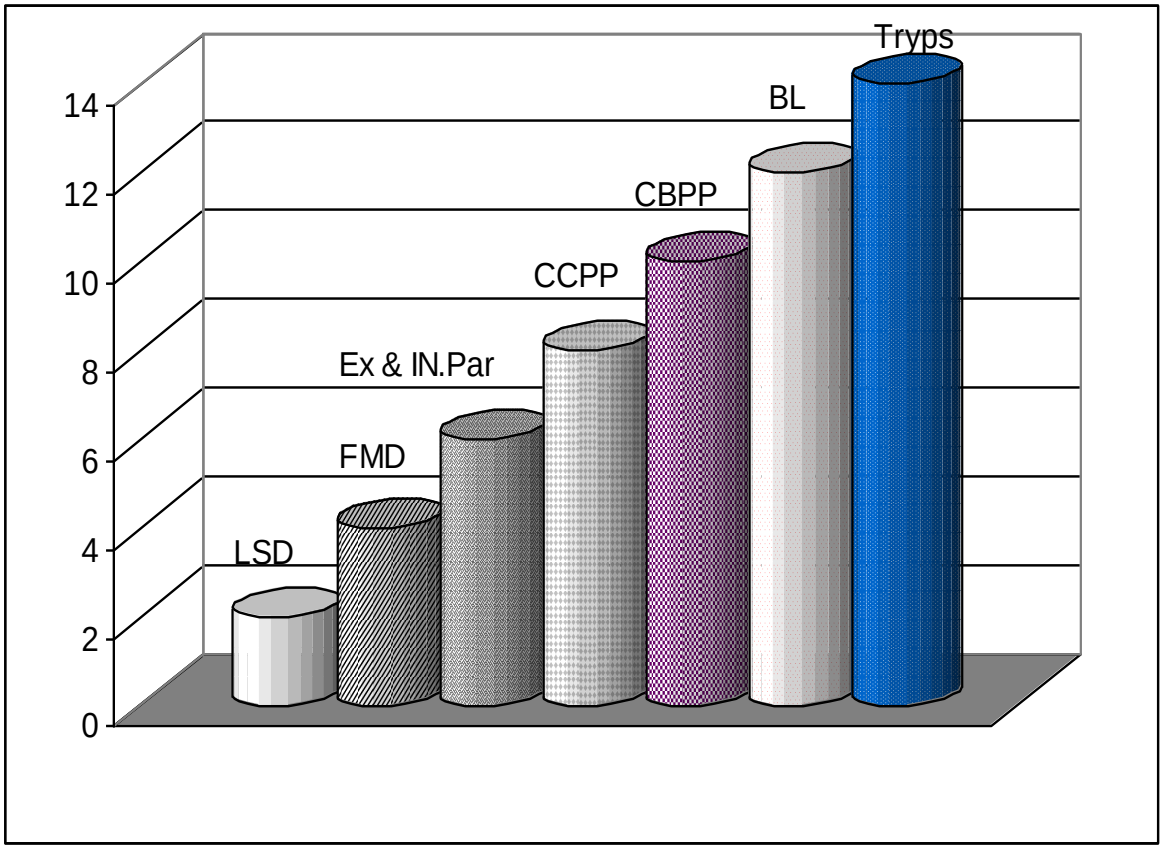

Figure 3: Major livestock disease in the study area Amaro special district, SNNPR

Key:

LSD: Lumpy skin disease; FMD: Foot and Mouth Disease

Ext. and In. Parst: External and internal parasite

CCPP: Contagious Caprine Pleuro Pneumonia

CBPP: Contagious Bovine Pleuro Pneumonia

Tryps: Trypanosomosis

According to respondents, 70.5 %, 24.5 % and 5 % sick animals of the study area were treated by owners, veterinary personnel and both by veterinary personnel and owners respectively as shown in Figure 4.

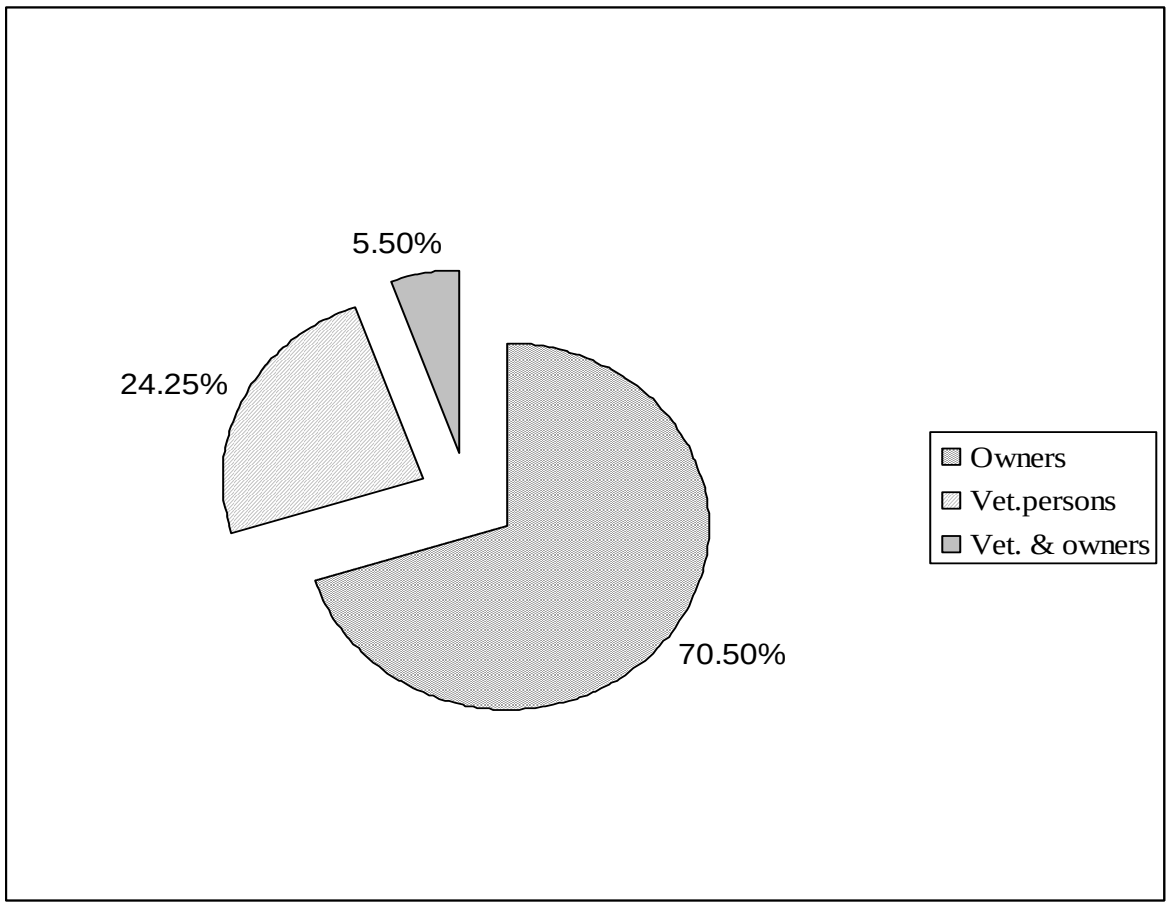

Figure 4: Persons involved in the treatment of animal trypanosomosis in Amaro special, dfistrict, SNNPR

The common trypanocidal drug used in the area by farmers were Diminazene aceturate which they identified by its color, and is locally called as "Bulla, Bale Meskel" followed by Isometamidium chloride locally known as "Kennini Kersi". Majority (95 %) of interviewed community members responded that they used one sachet of Diminazene aceturate dose per draught animal (oxen) and milking cow, 5 % used 1 sachet for two animals. For others (bull, heifers) 85 % of respondents used one sachet for 2-3 animals, 15 % used 1 sachet for each animal.

With regard to treatment frequency per year, they responded that they treat with trypanocidal drugs an average of 12 times per year for oxen and milking cows (Table 5). For other animals, treatment frequency per year was less than mentioned above. Among the respondents 35 % have the experience of treating their animal for 25 to 35 years and 65 % of them have the experience of 20 to 25 years.

Seventy-five percent of respondents indicated that source of trypanocidal drugs was the private pharmacies, while the 25 % used the drug from veterinary clinics. Respondents using Diminazene aceturate as trypanocidal drug were 87 % and 13 % of respondents used Isometamidium chloride.

Table 5: Interview result of individual farmers in Amaro special district, SNNPR

Interview points (No of Respondents: 80)	Mean	95 % CI
No of cattle per house hold	9.43	7.25-11.62
No of goats per house hold	8.93	7.10-10-78
Frequency of treatment of cattle per house hold	12.30	11.54-13.06
Death of cattles in one year due to trypanosomosis per house hold	1.12	0.75-1.5
Annual income from livestock per house hold in Birr.	571.81	421.45-722.18
Milk yield per cow per day	1.26	1.14-1.39

Regarding the impact of trypanosomosis, 98 % respondents mentioned death of animals, extra expenses for purchasing trypanocidal drugs and loss of farming land.

The status of trypanosomosis

Among the respondents 70 % revealed that occurrence of trypanosomosis was increasing with time, 25.5 % reported decrease, whereas 4.5 % revealed no change. According to 64 % of the respondents the disease had a history of 35 to 38 years of occurrence and 36 % respondents indicated that they knew the disease at the start of settlement in their locality. Almost 100 % of the respondents consider trypanosomosis as a disease of cattle followed by small ruminants and equine. The main clinical signs of trypanosomosis as described by the interviewed respondents indicated in Table 6.

Table 6: Clinical signs of trypanosomosis indicated by respondents in Amaro special district, SNNPR

Clinical signs of trypanosomsis	Local name indicated by respondents
Poor coat covering	Chemechenge
Emaciation	Hodi-hodi
Inappetance	Mata muwaso
Geophagia	Sama mudi
Diarrhoea	Shoti
Abortion	Gewa kessi
Discharge of blood from the body	Sussi Kessi

Majority of the respondents (94.3 %) treated their cattle after observation of clinical signs during any season and 5.7 % of respondents treated without any clinical sign as prophylactic. The question about transmission of trpanosomosis was responded as follows: 45 % of respondents indicated that the transmitter of the disease was tsetse fly (‘Wutsetse Gendi”), 55 % of respondents indicated that the transmitter of the disease was any blood sucking fly (‘Ficha”) i.e., Tabanids and tsetse flies .The agroecological occurrence of trypanosomosis was high in areas bordering Jello river and Gelana river(low land) as indicated by 85 % of respondents and 15 % of them indicated in midlands. Majority (92 %) of respondents reported that their animals contact trypanosomosis from any where but more from bushlands and wood grass land where animals move for grazing, whereas 8 % of respondents reported that their animal equally infected when they graze in any type of vegetation.

Control methods of trypanosomosis

Majority (95 %) of respondents revealed that method of trypanosomosis (“Gendi”) was controlled by using trypanocidal drugs and applying of insecticide(Deltamethrin) on the back of animal and insecticide impregnated targets which were initiated by Agriservice Ethiopia Amaro Program Office in 9 PAs, whereas, 5 % of respondents indicated only use of trypanocidal drugs.

Among the respondents 85 % did not know traditional methods to treat sick animals infected with trypanosomes and 15 % responded tried to treat sick animals with traditional methods locally they call it “Sibaka, and Tirro” but mostly not effective and animals died. Regarding the efficacies, 57.5 %, 45 % and 2.5 % of the respondents reported the trypanocidal drugs to be effective, non effective and unknown, respectively. About use of agricultural inputs 95 % of respondents did not use commercial fertilizer instead of manure.

4.2 Entomological survey

During the survey, a total of 375 tsetse flies were collected, of which 224 (59.73 %) flies were female and 151 (40.23 %) were males. Among the other flies, 576 Tabanids, 469 Stomoxys and 2102 other Muscid flies were caught. The mean density of flies were 1.62 (CI=1.51-1.72), 2.37 (CI=2.23-2.51), and 1.6 fly/trap/day (CI=1.45-1.65) for tsetse, Tabanids and Stomoxys respectively in late rainy season and 0.66 (CI=.60-0.72), 1.12 (CI=1.05-1.20) and 1.03 fly/trap/day (CI=0.94-1.11) for tsetse, Tabanids and Stomoxys, respectively in dry season. During the survey periods, a single species, of tsetse fly, *G. pallidipes,* was identified. Tabanid and biting flies of the muscid group were caught both with the tsetse flies and in the areas where tsetse flies were not caught. The tsetse fly account for 27 %, of the total fly catch, Tabanids 42.6 %, and Stomoxys 29 % during late rainy season and 24.7 %, 35 %, and 39.9 % respectively during dry season. The proportion of the mean fly catch per trap and per day (apparent density) in the late rainy season and in the dry season is shown in Figure 5.

The total mean apparent densities of flies were 1.15, 1.76 and 1.30 fly/trap/day for tsetse, Tabnids and Stomoxys respectively in the study areas.

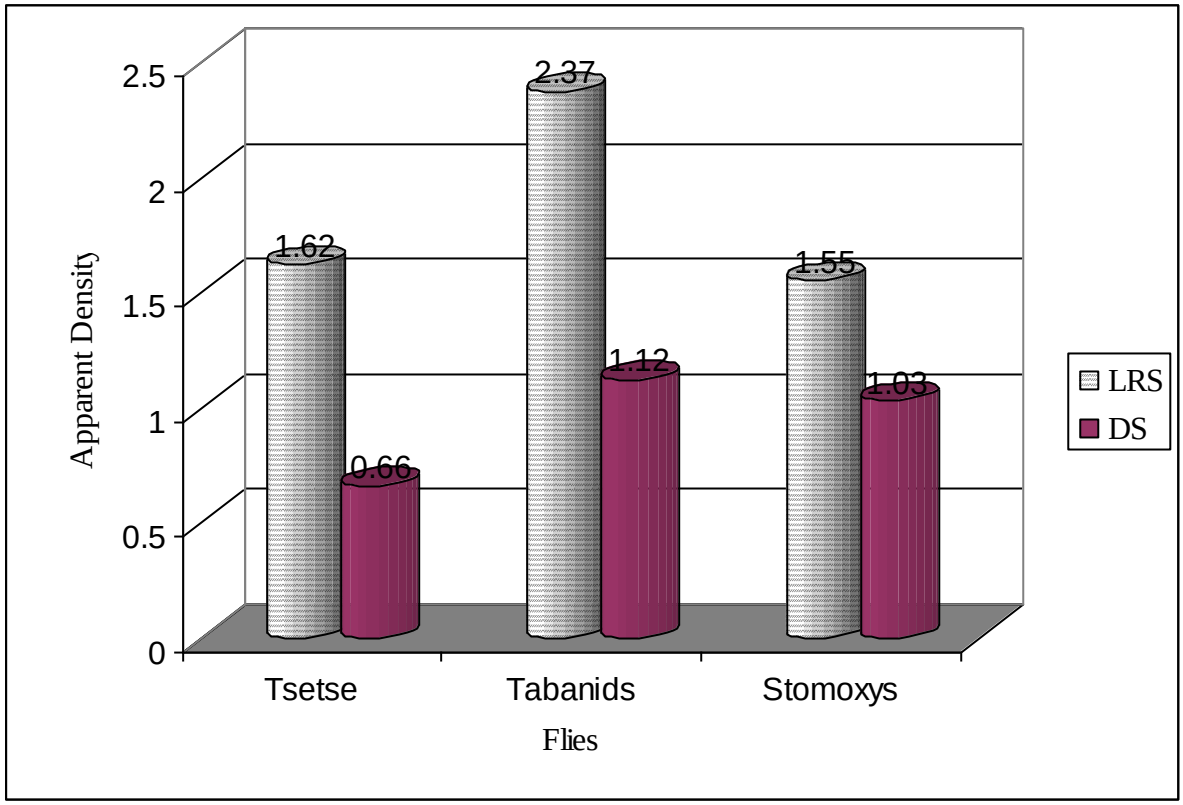

Figure 5: Apparent density of flies in late rainy (LRS) season and in the dry (DS) season in Amaro special district, SNNPR.

The mean catches of *G. pallidipes* in late rainy season at PAs of Korebiko, Jijolla, Gamule, Jello and Golbe were 1.49, O.37, 1.35, 2.42 and 2.05 flies /trap/day,respectively with statistically significant difference among them ($p < 0.05$) and during dry season 0.74, 0.27, 0.66, 0.69 and 0.76 fly / trap / day, respectively ($p < 0.05$).

The highest catch of tsetse fly was in Jello PA and the lowest was in Jijolla PA (Table 7). The mean catches of *G. pallidipes* showed statistically significant difference ($p<0.05$) between season and PAs.

Table 7: Apparent density of flies in different PAs in late rainy and dry season in Amaro special district, SNNPR

PA	Season	Flies/trap/day		
		Tsetse	Tabanids	Stomoxys
Kore biko	Late rainy	1.49	2.07	1.33
	Dry	0.74	1.17	1.00
Jijolla	Late rainy	0.37	1.07	0.78
	Dry	0.27	0.94	1.07
Gamule	Late rainy	1.35	2.86	1.39
	Dry	0.66	1.14	1.09
Jello	Late rainy	2.42	2.95	2.51
	Dry	0.69	1.07	0.55
Golbe	Late rainy	2.05	2.16	1.51
	Dry	0.76	1.12	1.39

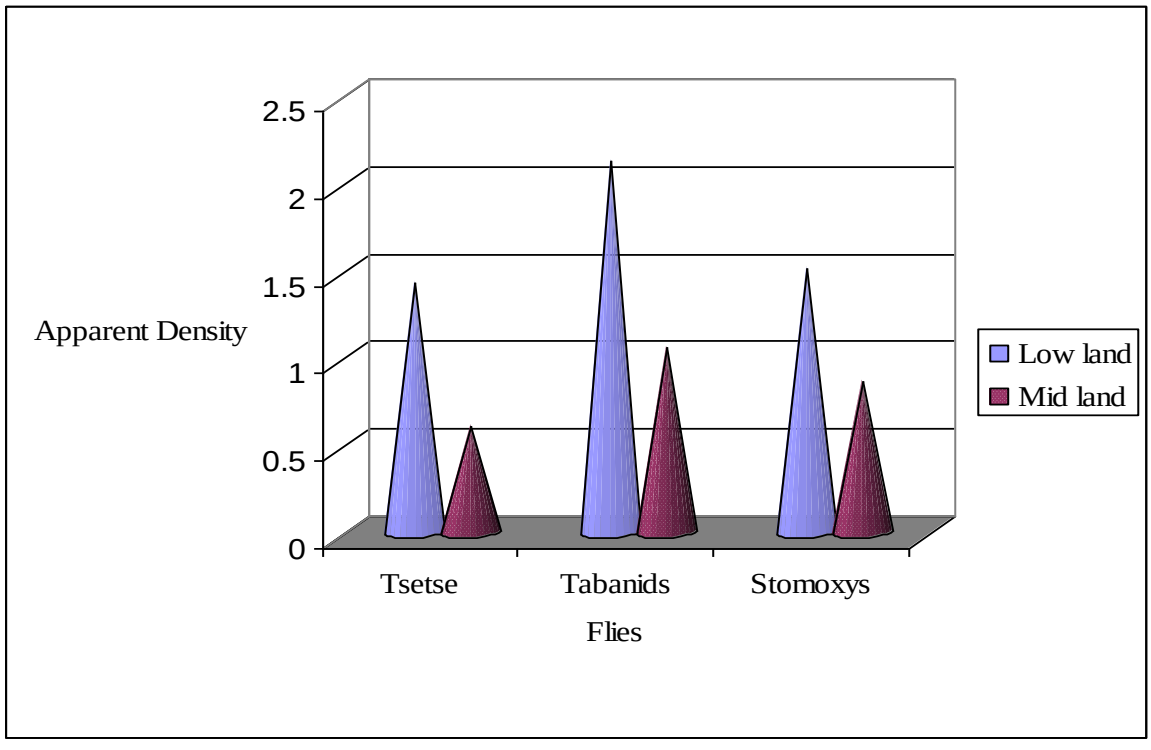

Figure 6: The mean apparent density of flies in different altitude in Amaro special district, SNNPR

In the late rainy season below 1500 m.a.s.l density of tsetse fly recorded was 1.99 fly/trap/day and above 1500 m.a.s.l 0.78 fly/trap/day, whereas, during dry season the apparent density of tsetse flies was 0.77 fly/trap/day in areas of below 1500 m.a.s.l and 0.47 fly/trap/day in areas above 1500 (Table 8) Higher ($p<.05$) apparent density for biting flies (Tabanidis and Stomoxys) also found in the areas below 1500 m.a.s.l.

Table 8: Apparent density of tsetse and other biting flies in different altitude in rainy and dry season in Amaro special district, SNNPR

Types of flies	Season	Altitude	fly/trap/day	95 % CI For the mean
Tsetse	Late rainy	Above 1500 m.a.s.l	0.78	0.23-1.10
		Below 1500 m.a.s.l	1.99	1.56-2.18
	Dry	Above 1500 m.a.sl	0.47	0.11—0.88
		Below 1500 m.a.s.l	0.77	0.38-1.00
Tabanids	Late rainy	Above 1500 m.a.s.l	1.17	0.77-1.92
		Below 1500 m.a.s.l	2.89	2.38-3.19
	Dry	Above 1500 m.a.s.l	0.96	0.043-1.19
		Below 1500 m.a.s.l	1.22	0.62-1.43
Stomxis	Late rainy	Above 1500 m.a.s.l	0.88	0.56-1.57
		Below 1500 m.a.s.l	1.85	1.53-2.24
	Dry	Above 1500 m.a.s.l	0.86	0.49-1.50
		Below 1500 m.a.s.l	1.12	0.62-1.34

The apparent density of flies showed statistically significant ($p<0.05$) difference between altitudes. Higher apparent density of tsetse flies caught in bush land vegetation type in late rainy season and dry season. The apparent density of flies in 3 vegetation type showed that there is statistically significant difference between vegetation types ($p<0.05$). The higest catch of tsetse flies was in bush land and the lowest in cultivated land in both seasons (Table 9).

Table 9: Apparent density of tsetse and other biting flies in different vegetation type during the late rainy and dry season in Amaro special district, SNNPR

Vegetation type	Late rainy season			Dry season		
	Types of flies			Types of flies		
	Tsetse	Tabanids	Stomoxys	Tsetse	Tabanids	Stomoxys
Bush land	2.00	2.83	1.75	1.02	1.46	1.00
Cultivated land	1.00	1.10	0.96	0.23	0.55	0.52
Wood grass land	1.37	3.06	1.92	0.40	1.13	1.00

Of the total tsetse fly caught, 59.73 % and 40.23 % were females and males respectively in two seasons. In late rainy season proportion of male and female was 61 % and 39 %, respectively and in dry season 64.4 %, and 35.6 %, respectively. Based on the wing fray analysis the average age of the population of tsetse flies were 31 days in the late rainy and 26 days during the dry season as shown in Annex 2. Flies were found up to 1550 m.a.s.l.

4.3. Parasitological survey

4.3.1. Trypanosome prevalence

A total 1136 animals (cattle) were examined in both seasons i.e. 585 during the late rainy season and 551 during the dry season. The prevalence of trypanosomosis was determined and compared with risk factors such as seasons, sex and age. The overall trypanosomes infection prevalence in cattle was 20.77 % of which 61.02 % *T. congolense,* 24.15 % T.viviax, 8.05 % *T.burcei* and 6.78 % mixed infection (*T. congolense* and *T. vivax*). There was statisticaly significant ($p<0.05$) difference in occurrence between different species of trypanosomes.

The prevalence of trypanosomosis during the late rainy season was 27.35 % of which 59.37 %, 23.12 %, 9.37 % and 8.12 % for *T congolense, T.vivax, T.burcei* and mixed infection (*T. congolense* and *T. vivax*) respectively with a statistical significant difference ($p<0.05$). Whereas during dry season the prevalence of trypanosomosis is 13.79 % of which *T.congolense* infection covered 64.47 % and *T.vivax* 26.31 %, *T.brucei,* 5.26 %, mixed (*T.congolense and T.vivax*) 3.95 %, ($p<0.05$) as indicated in Table 10. The risk of infection in the dry season was 2.38 time odds ratio lower than late rainy season (95 % CI=1.73-3.18) with a statistical significant difference ($p<0.05$).Prevalence of trypanosomes infection was positively correlated with tsetse apparent density ($r=0.147$) and correlation was satistically significant ($p<0.05$). Biting flies also had positive correlation with prevalence of trypanosomes infections, Tabanids ($r=0.01$) and Stomoxys ($r=0.087$) correlation was statistical significant ($p<0.05$) as indicated in Table 9 and 10.

Table 10: Prevalence of trypanosomosis infection in two seasons in Ammaro special district, SNNPR

Season	Infected	Non infected	Total	Trypanosomes spp. diagnosed				Prevalence (%)
				T.congolense	*T.vivax*	*T.brucei*	Mixed (*T.c* and *T.v*)	
Late rainy	160	425	585	95	37	15	13	27.35
Dry	76	475	551	49	20	4	3	13.79
Total	236	900	1136	144	57	19	16	20.77

Animals in late rainy season 2.38 odds ratio times more infected with trypanosomes than animals in the dry season.The total prevalence of trypanosomosis (Table 11) was 19.37 % for female and 21.92 % for male in two seasons but there was no statistically significant difference between sex groups within the same seasons ($p>0.05$) as indicated in Table11. There was statistically significant difference ($p<0.05$), between age groups with in the same seasons observed in the age groups in the study season (Table12). Higher infection observed in adult animals (24.39 %) and lower in young animals (9.09 %) ($X^2 = 23.54$, $p<0.05$) The relatively lower infection rate observed during the dry period in calves (8.24%) and in young animals (18.81 %).

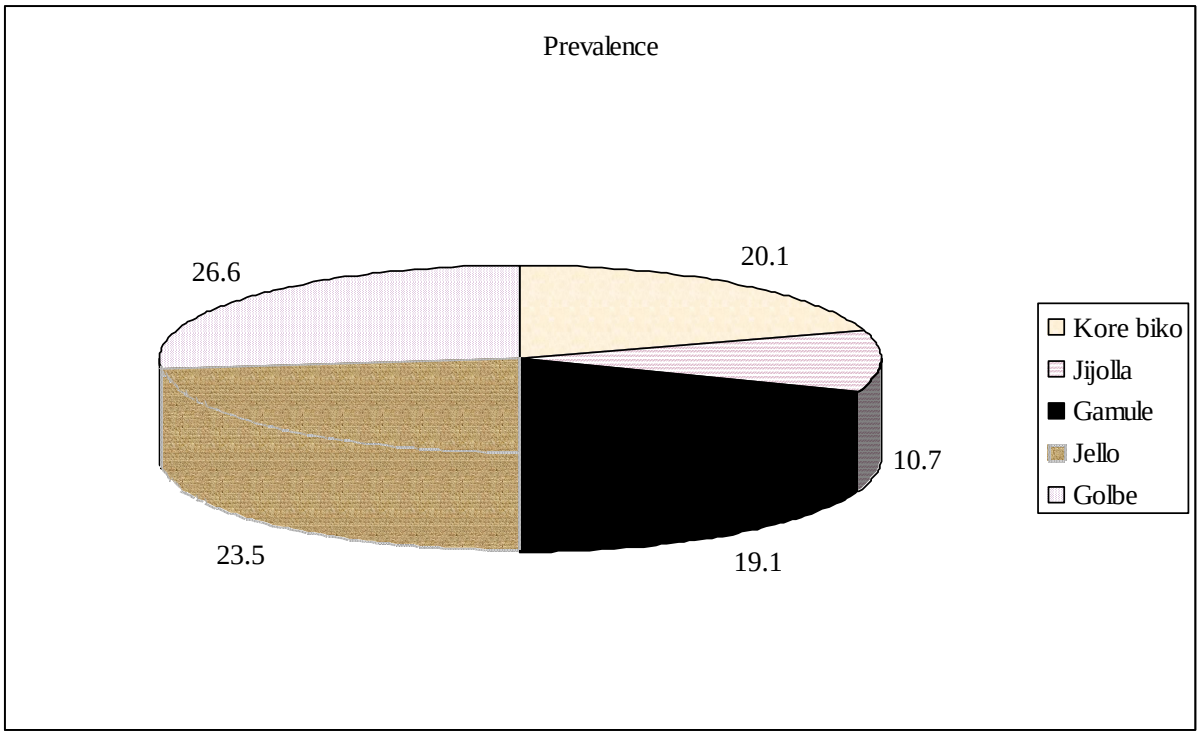

Figure 7: Prevalence of trypanosomosis in different PAs in Amaro special district, SNNPR

There is statistically significant ($p<0.05$) different in prevalence of trypanosomosis between different PAs

The highest Prevalence recorded in PA Golbe and Jello PA and the lowest was in Jijolla PA (10 %) (Figure7).

Table 11: Prevalence of trypanosomosis in female and male in Amaro special district, SNNPR

Sex	Infected	Non infected	Total	Trypanosomes spp. diagnosed				Prevalence (%)
				T.congolense	*T.vivax*	*T.burcei*	Mixed *(Tc&Tv)*	
Male	137	488	625	85	29	14	9	21.92
Female	99	412	511	59	28	5	7	19.37
Total	236	900	1136	144	57	19	16	20.77

There was no statistically significant ($p>0.05$) difference between female and male animals in trypanosomes infection (X^2 =0.317).

Table 12: Prevalence of trypanosomosis in age group in Amaro special district, SNNPR

Age	Infected	Non infected	Total	Trypanosomes spp.diagnosed				
				T.congolense	*T.vivax*	*T.burcei*	Mixed (*T.c &T.v)*	Prevalence (%)
Calf (<1 year)	20	200	220	12	6	0	2	9.09
Young (1-3 year)	56	204	260	41	9	5	1	21.53
Adult (>3 years)	160	496	656	91	42	14	13	24.39
Total	236	900	1136	144	57	19	16	20.77

There was statistical significant variation in prevalence between PAs (X^2=14.56, p<.0.05) as indicated in Table 13.

Table 13: The prevalence of trypanosomosis in different PAs in dry and late rainy season in Amaro special district SNNPR

PA	Late rainy season				Dry season			
	Infected	Non infected	Total	Prevalence (%)	infected	Non infected	Total	Prevalence (%)
Korebiko	43	84	127	25	16	87	103	15.53
Jijolla	7	63	70	10	7	54	61	11.47
Gamule	44	121	165	26.66	18	141	159	11.32
Jello	40	75	115	28.75	13	98	111	11.71
Golbe	42	82	124	33.87	22	95	117	18.80

The highest prevalence of trypanosomes infection was recorded in Jello and Golbe PAs (Table13). There is statistically significant difference ($p<0.05$) in prevalence of trypanosome infection between different PAs. The prevalence of trypansomosis in different altitudes accounts 23.8 % for low lands (below 1500 m.a.s.l) and 14.8 % for mid lands (above 1500.m.a.s.l). There was statistically significant difference in prevalence of trypanosomosis between altitudes (X^2 = 12.39, $p<0.05$). Animals in low land 1.79 (CI=1.29-2.49, $p<0.05$) odds ratio times more affected than animals in mid land. The prevalence of trypanosomosis in late rainy season and dry season in mid altitude accounts 17.32 %, 12.68 % and in low lands 31.77 % and 14.45 % respectively, as indicated in Table 14.

Table 14: Prevalence of trypanosomosis in different seasons and altitudes in Amaro special district, SNNPR

Altitude	Season	Infected	Non infected	Total	*Trypanosomes spp*				Prevalence (%)
					Tc	*Tv*	*Tb*	Mixed	
Mid altitude (>1500amsl)	Late rainy	31	148	179	18	5	4	4	17.32
	Dry	26	179	205	15	8	3	0	12.68
Low altitude (<1500 m.a.s.l)	Late rainy	129	277	406	77	32	11	9	31.77
	Dray	50	296	346	34	12	1	3	14.45
Total		236	900	1136					20.77

The prevalence of trypansome infection was high in late rainy season in low altitude than in mid altitude with statistically significant (X^2 =13.00, $p<0.05$) difference, where as in dry season there was no statistically significant (X^2 =1.42, $p>0.05$) difference between mid altitude and low altitude even though high prevalence was recorded in low altitude (Table 14).

Table 15: Prevalence of trypanosomosis in different altitudes and age groups in late rainy season in Amaro special district, SNNPR

Altitude	Age	Infected	Non infected	Total	Prevalence (%)
Mid altitude	<1year (calves)	1	37	38	2.70
	1-3 years (young)	4	29	33	12.12
	>3years (Adult)	26	82	108	24.07
Low altitude	<1year (calves)	11	66	77	14.28
	1-3 years (young)	35	68	103	33.98
	>3years (Adult)	83	143	226	36.73
Total		160	425	585	27.35

Low prevalence trypanosomes infection was recorded in late rainy season in mid (X^2 =9.79) and low (X^2 =13 .65) altitude in calves where as high prevalence recorded in Adult animals with a statistical significant difference ($p<0.05$) (Table 15).

Table16: Prevalence of trypanosomosis in different altitudes and age groups in dry season in Amaro special district, SNNPR.

Altitude	Age	Infected	Non infected	Total	Prevalence (%)
Mid altitude	<1year (calves)	3	39	42	7.14
	1-3 years (young)	4	29	33	21.21
	>3years (Adult)	19	111	130	14.61
Low altitude	<1year (calves)	5	58	63	7.94
	1-3 years (young)	13	78	91	14.29
	>3years (Adult)	32	160	192	16.67
Total		76	475	551	13.79

Low prevalence of trypanosomes infection was recorded in dry season in mid (X^2 =1.61) and low ((X^2 = 2.93) altitude in calves where as high prevalence recorded in Adult animals with none statistically significant difference ($p>0.05$) (Table 16).

The prevalence of trypanosomosis was determined from a total of 1136 sampled from 24 herds, 12 herds during the late rainy season and 12 herds during the dry season, to assess the effect of trypanosomosis on the herd average PCV values.

Table 17: The overall prevalence of trypanosomes infection in sex, age and altitude categories during the study period in Amaro special district, SNNPR

Variable		Infected	Non infected	Total	Trypanosomes SPP diagnosed				
					T.c	*T.v*	*T.b*	Mixed	%
Sex	Male	137	488	625	85	29	14	9	21.92
	Female	99	412	511	59	28	5	7	19.37
Age	<1Yr	20	200	220	12	6	0	2	9.09
	1-3	56	204	260	41	9	5	1	21.53
	>3	160	496	656	91	42	14	13	24.39
Altitude	<1500 m.a.s.l	179	573	752	111	44	12	12	23.80
	>1500 m.a.s.l	57	327	384	33	13	7	4	14.84
Total		236	900	1136	144	57	19	16	20.77

The prevalence of trypansome infection was high in late rainy season in low altitude than in mid altitude with statistical significant (X^2 =13.00, p<0.05) difference, where as in dry season there was no statistical significant (X^2 =1.42, p>0.05) difference between mid altitude and low altitude even though high prevalence recorded in low altitude (Table 14). The relatively higher infection rate was observed in male (21.92 %) than female animals (19.37 %), but the difference was not statistically significant (p> 0.05). There was statistically significant difference between the age groups of animals.

4.4. Hematological results

The overall mean PCV value of cattle at study area was 26.23 % (CI=25.89-26.49), there was statistically significant (p<0.05) difference in PCV value between different groups of animals. The overall PCV of parasitaemic and aparasitaemic animal was 21.92 % and 27.31 % (CI=21.38-22.46, 27.00-27.62) respectively with statistical significant (p<0.05) difference between them. The mean PCV value of parasitaemic and aparasitaemic animals during the late rainy season was 21.23 % and 26.45 % and during the dry season was 23.37 % and 28.07 % respectively.

The range of PCV values in parasitaemic animals was from 14 %-35 % and in aparisitaemic animals from 14 %-40 % in the late rainy season while in the dry season from 15 %-40 % in aparasitaemic animals and 16 %-35 % for parasitaemic animals.

The frequency distribution of PCV values of parasitaemic and aparasitaemic animals in the late rainy season and in the dry season are shown in Figures 8 and 9. The mean PCV in late rainy and dry season in low altitude was 24.75 and 27.46 respectively and in mid altitude 25.64 and 27.37, respectively.

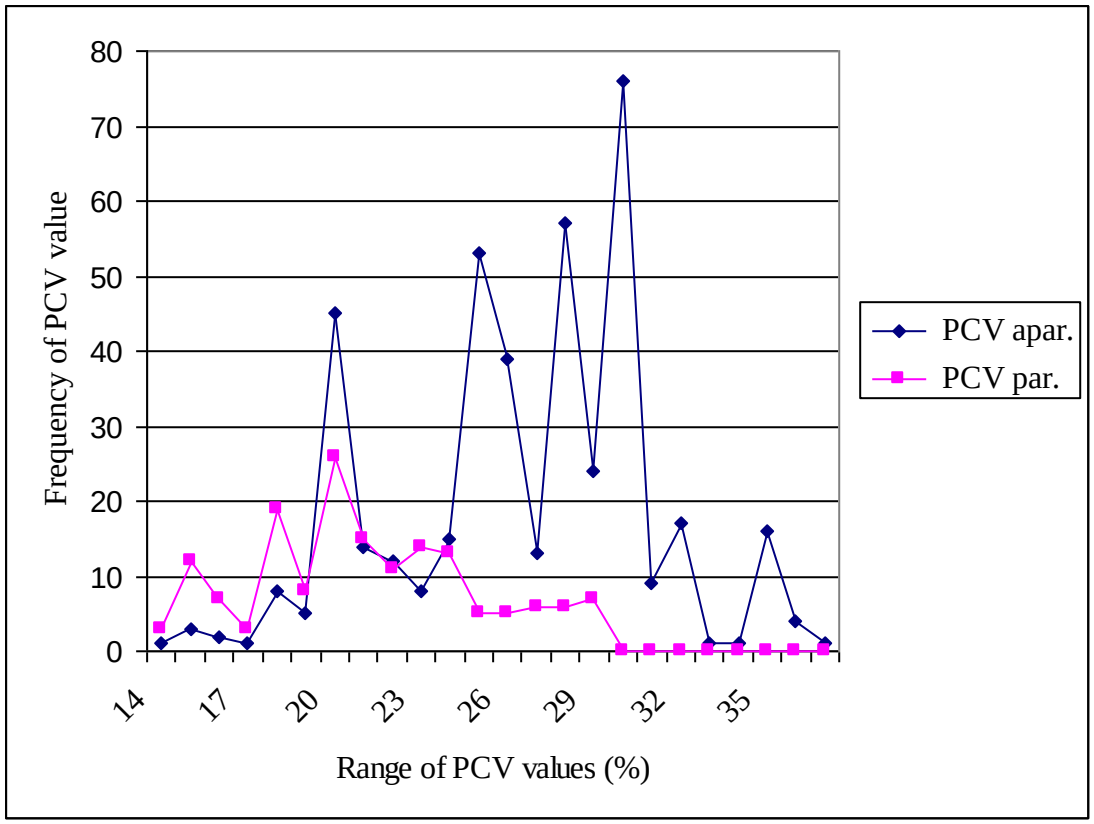

Figure 8: Frequency distribution of PCV values of parasitaemic and aparasitaemic animals during the late rainy season in Amaro special district, SNNPR

Key

PCV.apar. =PCV value of non parasitic animals

PCV.par. =PCV value of parasitic animals

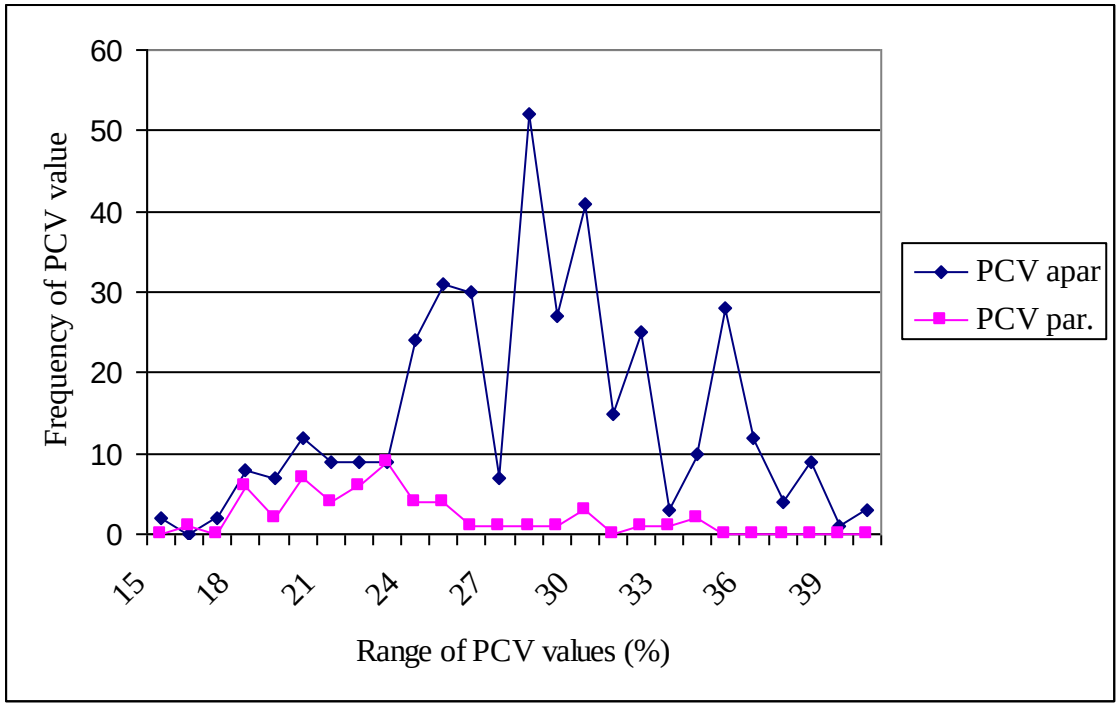

Figure 9: Frequency distribution of PCV values of parasitaemic and aparasitaemic animals during the dry season in Amaro special district, SNNPR

Key

PCV.apar. =PCV value of non parasitic animals

PCV.par. =PCV value of parasitic animals

As the herd prevalence of trypanosome infection increased, the average PCV values of the herds decreased considerably (Figures 11& 12).

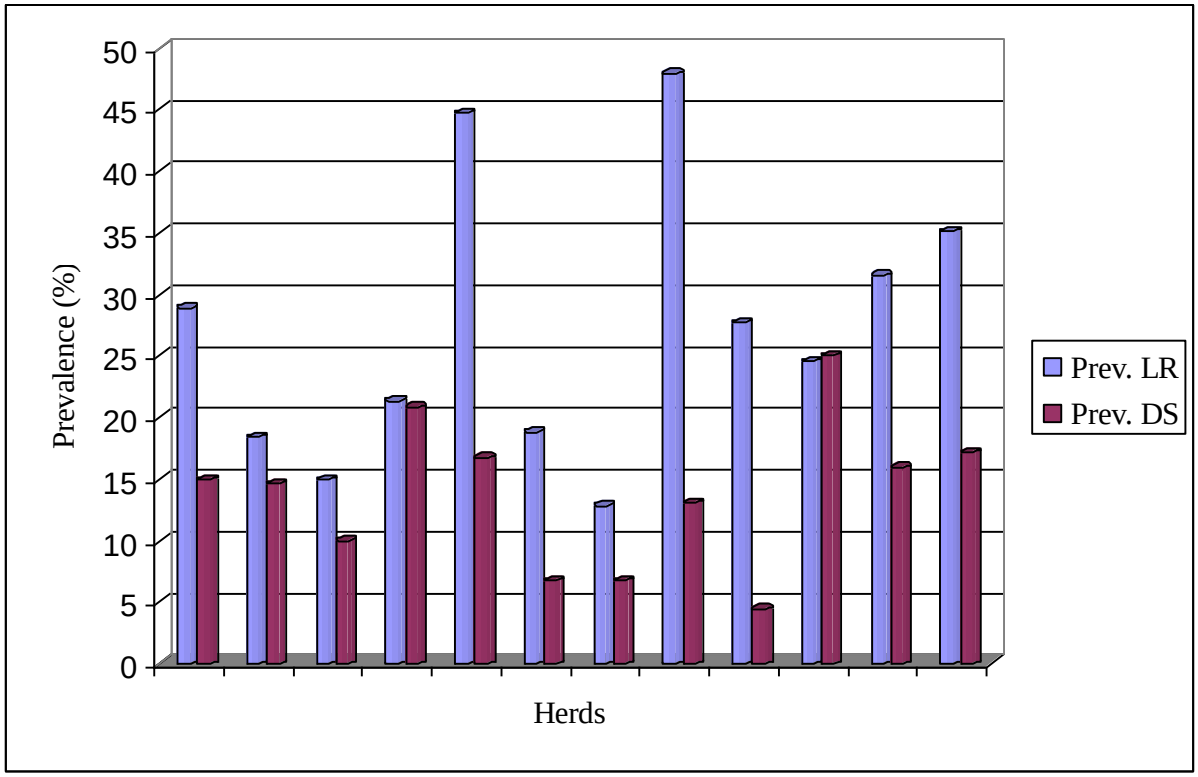

Figure 10: The comparison of herd prevalence of trypanosome infection between seasons in Amaro special district, SNNPR

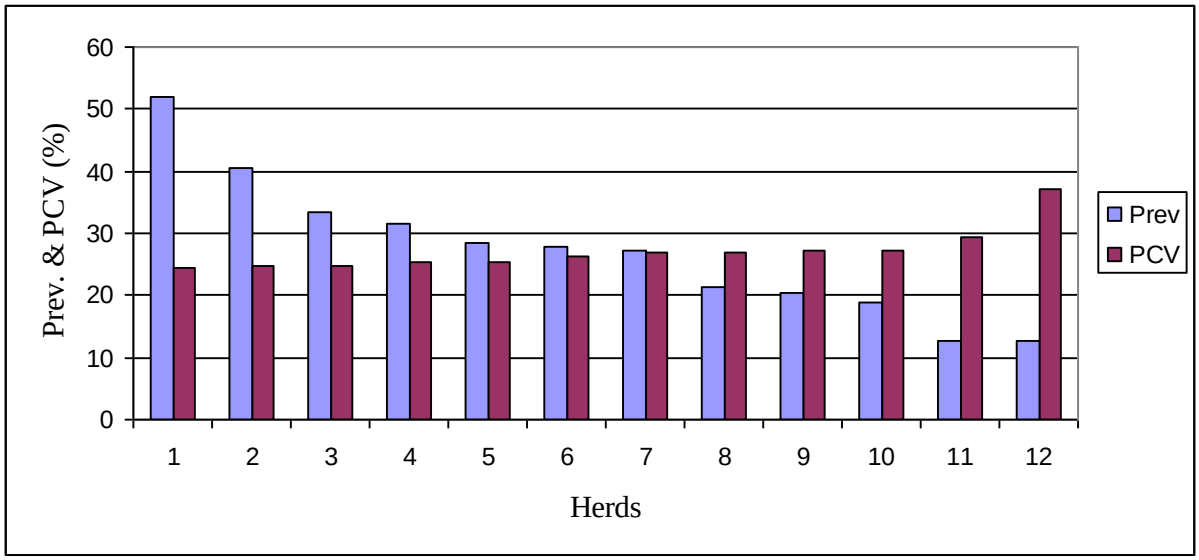

Figure 11: The mean PCV values and herd prevalence of trypanosome infection in the herds during the late rainy season in Amaro special district, SNNPR

Prev.LR=Prevalence of trypanosomosis in late rainy season

Prev DS= Prevalence of trypanosomosis in dry season.

The PCV profile in herd level analysis indicated that the herd average PCV was dependent on herd prevalence that regression coefficient were (-0.245) negatively correlated.

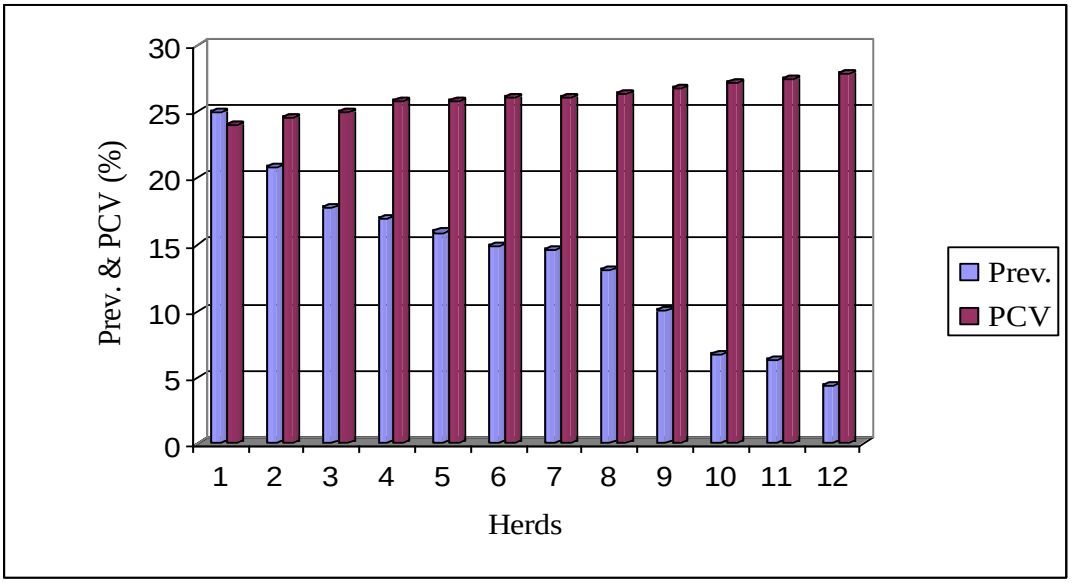

Figure 12: The mean PCV values and herd prevalence of trypanosome infection in the herds during the dry season in Amaro special district, SNNPR

The overall average PCV value of herds (24) was 26.23 % which significantly varied with the prevalence of trypanosomosis in herds (20.77 %) with negative correlation (r= -0.43) statistical significant ($p<0.05$). From 1136 animals of 24 herds examined, 236 animals were positive for trypanosome infection from these infection due to *T. congolense* were detected in 144 (61.02 %) animals of the total trypanosome infections and the remaining were due to *T. vivax* 57 (24.15 %),*T. brucei* 19 (8.05 %) and mixed infection *(T. c* and *T. v*) 16 (6.78 %).

4.5. The risk factors on trypanosome prevalence

4.5.1. Age

The study animals were categorized into different age-groups namely, calf (<1year), young (1-3years) and adult (>3years) which showed 9.09 %, 21.53 % and 24.39 % infection, respectively. The proportion of *T.congolense* was higher in adult animals. More over, when the age of animal increased the prevalence was also increased. There was a statistically significant difference ($X^2=23.54$, $p<0.05$) in trypanosome infection between different age groups.

4.5.2. Sex

A comparison of trypanosome infection between male and female was made. The overall prevalence in male and female were 21.19 % and 19.37 %, respectively. The prevalence of trypanosomes in male was higher than female .However, there was no statistically significant difference (X^2 =0.317, p>0.05) in trypanosomes infection between male and female animals.

4.5.3 Interaction of risk factors

Logistic regression analysis was used to get the level of interactions between risk factors. According to analysis the odds ratio of the risk factors of trypanosomosis in cattle with different age groups and sex in late rainy season was 2.38 odds ratio times higher trypanosomes infection in late rainy season than dry season. Trypanosomes infection in low altitude (<1500 m.a.s.l) was 0.86 odds ratio times higher than mid altitude (>1500m.a.s.l). There was statistically significant difference (p<0.05) between odds ratio of late rainy season and dry season.

4.6. Longitudinal studies

4.6.1 Parasitological findings

Cross sectional study revealed 19.1 %, 23.5 % and 26.6 % of trypanosomes infections at Gamule, Jello and Golbe PAs, respectively. The species of trypanosomes, which infected cattle, were determined prior to treatment with parasitological methods indicated in cross sectional study. Among the infected animals 40 (57.97 %), 15 (21.74 %).8 (11.59 %) and 6 (8.69 %) were infected with *T.congolense, T.vivax, T.brucei* and mixed infection *(T.c&T.v*), respectively (Table 18). The study animals were monitored for 90 days (day 15, 30, 60 and 90). Parasitological and hematological results are shown in Annex 3.

After Isometamidium chloride block treatment animals were monitored for 90 days. Parasitological examination revealed that on day 15, 8 (11.60 %) animals were infected with trypanosome and *T congolense* contributed for 75 % of infection. On day 30, 11 (18.03 %) more animals were detected to have trypanosome infections and *T. congolense* contributed 77.77 % for infections.

On day 60, 9 (18 %) additional animals were found infected, of which 81.81 % with *T. congolense* infection. On day 90, 6 (14.63 %) more animals showed trypanosome infections, of which *T. congolense* contributed 66.67 %. Overall trypanosome infections were detected in 34 (49.28 %) animals during 90 days period of post Isometamidium chloride block treatment and *T.congolense* contributed for 76.47 % of total infections (Table 18). Within 90 days trypanosomes infection (relapsed) accounts in PA Gamule 36.1 %, Jello 33.6 and Golbe 37.4 % but, there was no statistically significant ($p>0.05$) different between PAs in trypanosomes infection. All post Isometamidium chloride block treatment parasitaemic animals were later treated with Diminazene aceturate at 7.0 mg /kg bw and excluded from the study

Table 18: Therapeutic efficacy of Isometamidium chloride on trypanosome infection in naturally infected cattle in Amaro special district, SNNPR

Parameters	Days after ISMM chloride treatments						
	0	15	30	60	90	Total detected Post treatment	Prevalence (%)
Number of animals	69	69	61	50	41	69	
Infection (%)	100	11.60	18.03	18	14.63		49.28 %
T.congolense	40	6	9	7	4	26	76.47
T.vivax	15	2	2	1	2	7	20.59
T.brucei	8			1		1	2.94
Mixed *(T.c,T.v)*	6					-	
Total	69	8	11	9	6	34	

There was statistically significant difference ($p<0.05$) in occurrence of trypanosomes infection between days of examination after ISMM block treatment.

4.6.2. Hematological findings

There was a statistically significant ($p<0.05$) increase in the average PCV of all examined cattle after ISMM treatment. However, on day 60 of post Isometamidium chloride block treatment the haematocrit value of examined cattle decreased due to development of trypanosomes infection (Table 19).

Table 19: The mean PCV value of Isometamidium chloride treated animals in Amaro special district, SNNPR

Days after treatment	Number of cattle examined	Mean PCV (%)	95 % Confidence interval
0	69	21..46	20.63-22.30
15	69	23.28	22.62-23.93
30	61	25.36	24.76-25.96
60	50	26.90	25.97-27.83
90	41	24.98	23.86-26.09

5. DISCUSSION

In order to improve the welfare and security of rural communities in Africa rapid method for assessing risk and diagnosing urgent problems are needed to control both human and animal diseases (Vlassoff, 1999). Therefore questionnaire survey to collect information from community respondents, rapid survey of tsetse abundance, the prevalence of trypanosome infection in village livestock (Snow and Rawlings, 1999) and study on curative and prophylactic action of Isometamidium chloride was important, the result of which could lead to some conclusion and necessary suggestions.

The result of questionnaire survey revealed that trypanosmosis was the most important problem for agricultural activity and animal production in Amaro special district. About 100 % of respondent farmer's livelihood depends on mixed farming. The result of questionnaire also revealed that the disease was known in the area for about 38 years, and still considered as a main disease. Since then trypanosomosis hampered animal production and agricultural activity and resulted a considerable socioeconomic loss through mortality and morbidity of draught power animals which in turn affects crop production. The present findings are in agreement with reports of FAO/WHO/OIE, (1982). In the regions infested with tsetse flies, chronic trypanosomosis causes a severe reduction in animal productivity reflected in poor growth, low milk yields, reduced capacity as work animals and infertility.

The disease trypanosomosis, locally called as "Gendi" was reported to be the most important livestock constraint limiting overall agricultural activity and livestock production. Though 95 % respondents claimed that erratic rain was the primary constraint for agricultural activity, 100 % of them indicated trypansomosis to be the next important constraint. The present result is in agreement with repot of Itard (1989), which indicated that trypanosomosis was the major constraint in animal production in Africa. Many works (Tewelde, 2001; Afewerk, 1998) in the western and north western parts of Ethiopia revealed that tsetse transmitted trypanosomosis was the primary problem for livestock productivity and agricultural development. Swallow, (2000) indicated that animals in tsetse infested area has lower calving rate, milk yield, calf mortality and more treatment with trypanocidal drugs and trypanosome susceptible animals can be devastated by sudden exposure to high levels of trypanosome risk. The productivity of the survivors in terms of draft power, milk production, growth and birth rate is lowered by 10–40 %.

According to questionnaire survey, 70.5 % of sick animals were treated by owners, where as other works revealed 97.5 % of sick animals treated by owners and smugglers with high frequency and thereby aggravate drug resistance problem in Boloso Sore district Wolayta Zone, SNNPR (Daya, 2004). In Kolashara PA of Arbaminch Zuria, each peasant was armed with his own syringes and needles to treat his cattle with trypanocidal drugs (Woldeyes and Aboset, 1997).

Majority (92 %) of the respondents indicated that trypanosomosis occurred throughout the year but high infection rate was observed during late rainy season and low infection rate observed during the dry season. The present findings are in agreement with results of Tewelde (2001) and Afewerk (1998). Prevalence of trypanosomosis was high in late wet season and low in dry season in Boloso Soro district (Daya, 2004). Absence of tsetse control activity generally makes farmers prone and dependable on the use of trypanocidal drugs for many years.

Diminazene aceturate and Isometamidium chloride were used by respondents. Among them 87 % used Diminazene aceturate and 13 % used Isometamidium chloride to treat their animals. The proper dosage of trypanocidal drug was used by 95 % of respondents for oxen and milking cows but 85 % respondents treated the rest group of animals by underdose of the drugs. Similar reports but less in percent was also reported by Tewlde (2001) and Afewerk (1998), about 57 % and 43 % of the drugs applied by the farmers themselves and other uncertified personnels.

About 94.3 %of the treatment was given for clinical cases and 5.7 % for non clinical cases. Almost similar results reported in the upper Didessa valley of Ethiopia (Vanden Bossche 2001; Tewelde, 2001) where 85 % of the treatment was given for clinical cases. Similar results were reported by Vanden Bossche, *et al.*, (2001) was indicated that majority of farmers prefer to use Diminazene aceturate than Isometamidium chloride and most of the time treatment was given for clinical cases not only for trypanosomosis and oxen and cows took priority for treatment.

In the study area the average frequency of treatment per year was about 12 times for cow and oxen. It was higher than the result of Muturi (1999) at Merab Abaya, South Ethiopia (2-9 times) and Afewerk (1998) at Pawe, North West Ethiopia (3.1 times). Uilenberg (1997) reported that the number of treatment over a year reflects the magnitude of trypanosome challenge in an area.

Glossina pallidipes is the only species of tsetse fly found in the study area of Amaro special district, SNNPR with a mean apparent density of 1.62 tsetse flies/trap/day in the late rainy season and 0.66 tsetse fly/trap/days in the dry season during in the present research work.
Biting flies, Tabanids 2.37 flies/trap/day and Stomoxys 1.55 flies/trap/day were caught during the late rainy season where as during dry season 1.12 and 1.03 flies/trap/day were caught, respectively. The highest catches of flies were in bush land in late rainy and dry season. Most of the tsetse flies were caught in lowlands (below 1500 m.a.s.l) and the apparent density decreases as altitude increases ($p<0.05$). These findings agreed with works by Langridge (1976),

Tikubet and Gemechu (2000) and Leak *et al*., (1999) indicated that climate, was largely influenced by altitude and had an important impact on tsetse population. Different species of tsetse flies require particular vegetation type that would provide an optimal condition for growth and survival and vegetation is also important that provides shelter to their host (Leak, 1999). The highest catches of *G.pallidipes* were in bushes and wooden grass land in the Southern Rift Valley of Ethiopia (Veryesen *et al*., 1999).

The apparent density of different flies was statistically significant higher during the late rainy season. Similar results were reported by Msangi (1999), Mohammed Ahmed and Dairri (1987) and Leak et al, (1987). Williams *et al.*, (1992) stated as temperature drops the flies would tend to spread out into more open areas. Leak (1999) also indicated that during rainy season as vegetation grows and a more suitable habitat formed, the flies start to disperse to other parts of valley.

Sex ratio and age composition of the flies were assessed and higher number of female and adult flies were recorded during the present study. Out of a total of 375 tsetse flies, 224 (59.73 %) were females and 151 (40.23 %) were males. The other biting flies caught were 576 Tabanids, 469 Stomoxys and 2102 other muscid flies.

Present finding is similar to the results of some workers. Leak, (1999), Mohammed Ahmed and Dairri (1987), Msangi, (1999) showed that in unbiased sample female flies would comprise between 70-80 % of the mean population.

The mean age of fly population was 31 days in the late rainy season and 26 days in the dry season with wing fray analysis. Wing fray analysis for the estimation of the age of fly population was also reported by Msangi (1999) as compared to ovarian dissection provided the flies are collected frequently less than 24 hours.

The highest prevalence of trypanosomes infection was found in the low altitude areas along Jello river in PAs Golbe and Jello compared to the mid altitude areas. The seasonal occurrence of the disease is also consistent with the distribution of the vectors of trypanosomosis and hence it was higher during the late rainy season.

The overall trypanosome infections prevalence in cattle was 20.77 % and *T.congolense* and *T.vivax* contributes 61.02 % and 24.15 % for trypanosomosis infections, respectively.
The present result revealed the majority of infections were due to *T.congolense* (61.02 %). Similar result was reported by Muturi (1999) at Merab Abaya distrct, South Ethiopia (66.10 %), Afewerk (2001) at Pawe, North West Ethiopia (60.9 %) and Abebe and Jobre (1996) for the tsetse infested area of Ethiopia (58.50 %).

This result was almost similar with the findings of different workers (Afewerk, 1998; Tewelde, 2001; Muturi, 1999) which reported a prevalence of 17.2 %, 21% and 17.5 % in Meketel district, in upper Dedessa valley and Southern Rift Valley of tsetse infested regions respectively and the dominant species was *T.congolense.*

In the lowland areas tsetse apparent density for *G.pallidipes* during the dry and wet season were 1.47 and 0.61 and therefore the proportion of *T.vivax* infection would have been expected to be higher than in the midland where tsetse apparent density during the dry and wet season were 0.6 and 2.9 (Msangi, 1999). But their observation confirms that animal in the midland area are grazing in the lowland areas during the day time. In cattle which is affected by the species of tsetse to which cattle are exposed. *G. morsitans* and *G. pallidipes* are efficient in the transmission of *T.congolense* than *T.vivax* in East Africa (Langridge, 1976).

Higher infection rate was observed in male animals in the present study, but the difference was not statistically significant ($p>0.05$). Similar results reported by different workers (Afewerk, 1998; Muturi, 1999; Tewelde, 2001).

Other findings indicated that lactation stress result in higher prevalence of infection in lactating cows than non-lactating cows (Rowlands *et al*., 1995). The possible suggestion to the present findings would be that male animals are more exposed to stress due to long distance travel for draught purposes and exposed to areas where the tsetse challenge is high.

Age was found to be a risk factor in the present findings and higher rate of infection were observed in adult animals above three years of age in both seasons and altitudes. Present work findings are in agreement with other workers. Rowlands *et al.,* (1995) in Ghibe valley indicated that suckling calves did not go out with their dams but graze at home steads until weaned off. Young animals are also naturally protected to some extent by maternal antibodies (Fimmen *et al.,* 1992). This could result in low prevalence of trypanosome infection in young age.

The prevalence of trypanosomosis in low altitude areas in late rainy season (31.77 %) and in dry season (14.45 %) have been found statistically significant higher than the mid altitude areas (17.32 % and 12.68 %, respectively). Odds ratio indicated that the risk of trypanosomosis in the mid altitude areas were 0.86 times lower than lowland areas. Similar findings reported by Muturi (1999) in North Omo Zone.

The present study also reveals that the apparent density of tsetse flies varies significantly between altitude levels and concurrently the risk of trypanosomosis also varies as *G.pallidipes* were found with higher apparent density in lowlands. The increase in apparent density of tsetse flies led to an increase in trypanosome prevalence in the study area which resulted in the observed difference in trypanosome prevalence during the two sampling season (late rainy and dry season).

Trypanosome infection and mean PCV obtained between parasitaemic and aparasitaemic animals had statistical significant difference ($p<0.05$). Present findings is in agreement with the work done in Ghibe, southwest Ethiopia, indicated PCV less than 26 % required treatment.

Rowlands *et al.,* (2001) in Ghibe observed that with an increase in PCV value, the proportion of positivity decreases and hence mean PCV was a good indicator for the health status of herds in an anemic area. The lower mean PCV value in parasitaemic animals than the aparasitaemic animals was reported by several authors (Leak, 1987; Afewerk; 1998; Muturi, 1999; Tewelde, 2001). No statistical significant ($p>0.05$) difference in the mean PCV value of animals was found between the late rainy season (24.75 % in the lowland area and 25.64 % in the mid land) and the dry season (27.46 % in the low land areas and 27.37 % in the mid land).

The mean PCV values of parasitic animals in the low lands areas in late rainy season was 21.06 % and in the mid land areas 21.90 % while for aparasitaemic animals 26.47 % and 26.43 %, respectively ($p<0.05$).The PCV profile in herd level analysis indicated that the herd average PCV were dependent on herd prevalence that regression coefficient were negatively correlated (-0.245).

The same result reported by Vanden Bossche and Rowlands (2001) that regression analysis of herd's average PCV of parasitological positive herds decreased with increasing prevalence of trypanosomosis. The development of anemia is one of the most typical sign of trypanosomosis caused by *T.congolense* in susceptible cattle breeds (Murray and Dexter, 1988).

The level of anemia of PCV usually gives a reliable infection of the disease status and productive performance of an infected animal (Trail *et al*., 1991).The knowledge of relation ship between prevalence of trypanosome infection and herd average PCV could be useful tool for the assessment of impact of control intervention However, the herds mean PCV is affected by factors other than trypanosomosis (Conner, 1994).

As anemia is the classical symptom of the disease pathogencity (Murray *et al*., 1977; Seifert, 1996) the low PCV in parasitaemic animals could have contributed in reducing the mean PCV for cattle in the low land area and mid land areas. Conner (1994) indicated anemia associated with trypanosomosis causes weakness, lethargy and lack of stamina which ultimately reduce efficiency of working animals The consequence of anemia is one of the most typical signs of trypanosome caused by *T. congolense* in susceptible cattle breed (Murray and Dexter ,1998; Abebe,1991)

During cross sectional study 69 positive cattle were screened out from 3 PAs ,and given blanket treatment with Isometamidium chloride at day 0 then after, at day 15 ,8/69 (11.60 %) relapse of infectious post treatment with prophylactic dose 1 mg /kg bw of Isometamidium chloride was demonstrated. At day 30, 11/61 (18.03 %) relapse through infections were identified. At day 60, 9/50 (18 %) and at day 90, 6/41 (14.63 %).Overall 34/69 (49.28 %) relapses through infections were demonstrated within 90 days after treatment and the dominant cases were due to *T. congolense* (76.47 %).

The present findings of 18.03 % recurrent parasitaemic and 81.82 % of *T.congolense* infections with in 30 days of treatment was higher than the finding of Afewerk *et al.,* (2001). Parasitaemia reappeared in 6 out of 46 cattle (13 %) within 4 weeks, 18 out of 46 (38 %) within 8 weeks and 25 out of 50 cattle (50 %) within 12 weeks of treatment, where by *Trypanosoma congolense* accounted for 80 % of the overall relapse infections after treatment with prophylactic dose of Isometamidium chloride in Metekel district, North-west Ethiopia.

Muturi (1999) showed that 30 % of recurrent parasitaemic with in 4 weeks time of Isometamidium chloride, which is higher than present findings. During 60 days post Isometamidium block treatment 18 % of trypanosome infections were demonstrated and *T.congolense* contributed 77.77 % of infections.

Trypanosome infections at Pawe reported by Afewerk (2001) were 3.6 % and Tewelde (2001) also reported the infection of 4.51 % at Chelelekétu, 5.72 % at Kolu and 10.08 % at Burka of South west Ethiopia in 60 days time of post Isometamidium block treatment. At present study with post Isometamidium block treatment, 49.28 % trypanosome infections were detected and *T.congolense* contributed 76.47 %. This result was almost similar to the result reported by Afewerk (1998) at Pawe, North west Ethiopia.

Similar works carried out in South west Ethiopia (Codjia *et al.*1993; Leak *et al*., 1993; Rowlands *et al*., 1993) also indicated that *T. congolense* was the most prevalent drug resistant trypanosome species in Ethiopia. Ninety days following isometmidium chloride block treatment study suggest that there is an indication of resistance of trypanosome due to *T. congolense* against Isometamidium chloride. Resistance against this drug was strongly suspected when more than 25 % of Isometamidium chloride treated cattle become parasitaemic within 8 weeks of exposure (Eisler *et al*., 2000).

The period of Isometamidium chloride prophylaxis in infected cattle in the field was less than 1 month as reported by Afewerk (1998) North West Ethiopia.

Parasitaemia reappeared in 6 out of 46 cattle (13 %) within 4 weeks, 18 out of 46 (38 %) within 8 weeks and 25 out of 50 cattle (50 %) within 12 weeks of treatment, where *Trypanosoma congolense* accounted for 80 % of the overall relapse infections at Metekel district, North-west Ethiopia. Rowlands *et al*. (1993) from South west Ethiopia indicated that 1mg/kg bw ISMM was less effective and reappeared the parasite within 28 days. It is because of high level of multiple drug resistance infections appeared to be expressed at the level of individual trypanosome; chemotherapeutic agents only did not control trypanosomosis at Ghibe on long term basis (Codjia *et al*., 1993). Clausen *et al*., (1992) and Greets and Holmes (1999) stressed that the prolonged and frequent use of trypanocides in high challenge areas resulting high selection pressure for resistance as well. The epidemiology of drug resistance population of trypanosome is dynamic when the incidence is progressively spread within the population. Transmission of resistant trypanosome by tsetse do not change the strains resistant after passage, the trait stable for long time and spread by cattle movement and or spread of tsetse population (Moloo and Kutuzu, 1990).

The experimental work to monitor the drug resistance in pathogenic trypanosomes and other associated factors which influence the development of resistance are of the vital importance and must be taken into the consideration. Although, the prophylactic efficacy with isometmidium over 3 months was not observed during the study, however the observations usually require a long follow up and farmer's intervention on the study animals.

6. CONCLUSIONS AND RECOMMENDATIONS

The result of the present research work revealed that trypanosomosis is the most important constraints for agricultural activity and animal production in the low land and mid lands of Amaro special district that cripples the livelihoods of livestock owners by causing ill health and death to their animals. Only one species of tsetse, *G. pallidipes*, the main vector of pathogenic trypanosome was encountered in the study area. Biting flies such as Tabanids and Stomoxys were caught and contributed for the transmission of trypanosomosis.

Limt of *G. pallidipes* caught at altitude1550 m.a.s.l. It can be said that the control and monitoring of *G. pallidipes* can be done by NGU traps in the area which were used in the study area for controlling program by Agriservice Ethiopia Amaro Integrated Food Security program office. According to questionnaire survey 70.5 % of sick animals were treated by owners with high frequency and there by aggravate drug resistance problem in the study area. The situation is getting worse as the control and the prevention of trypanosomosis is facing a challenge due to limitation of vector control activities and the development of drug resistance in the area. The prevalence of bovine trypanosomosis was found to be 20.77 %. However, in late rainy and dry seasons the prevalence was 27.55 % and 13.79 %, respectively. The prevalence of trypanosomosis was higher in low altitude areas compared to mid altitude ($p<0.05$) in both seasons. The prevalence of trypanosome infection was positively correlated with apparent density of *G. pallidipes*. The mean PCV values of different seasons are negatively correlated with the prevalence of trypanosomosis of corresponding seasons. *T. congolense* contributed higher infections in cattle in all seasons of study area. The results of drug therapeutic efficacy studies in the field have shown that the period of prophylaxis conferred by Isometamidium against trypanosomes, mainly of *T. congolense* species is less than a month. It can be assumed that the widespread use and misuses of drugs contributed to the development of drug resistance in the population of *T. congolense* parasites in the study area. Since there is little prospect for the development of new trypanocidal drugs in the near future, control of trypanosomosis in the study area and other tsetse infested part of Ethiopia needs to focus on the careful use of the available anti trypanosomal compounds.

Taking into consideration the above conclusions the following recommendations are forwarded:

- In the study area interventions against tsetse and *Bovine* trypanosomosis must be seen and considered in the broader context of poverty alleviation and food security through livestock and agricultural development. Community awareness creation about the disease control strategies specially risk of misuse of trypanocidal drugs should be undertaken.
- Proper and strict follow up of trypanocidal drug use should be implemented by concerned stake holders'.
- The distribution and degree of drug resistance has to be carefully monitored so as to work out the best possible therapeutic strategies and alternative control measures.
- Systematical expansion of farming land can decrease tsetse population resulting in dercreaseing the prevalence of trypanosomosis.
- Further studies on the economic impact of trypanosomosis and drug resistances will have essential roles for the over all control of tsetse transmitted trypanosomosis in the Amaro special district.

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8. ANNEXES

Annex 1: Questionnaire set to interview farmers about herd structure, diseases and usage of trypanocidal drugs and agricultural constraints

Region_____ P.A_____

Woreda_____ village_____

Name_____date_____

a/LIVESTOCK MANAGEMENT AND FARMING SYSTEM

1 .How many animals do you have?

a/Cattle_____ female calves_____

oxen_____ bulls_____

cows_____

male calves_____

Heifer_____

b/sheep_____

c/Goat_____

d/Equine_____

Donkey_____

Horse_____

Mule_____

How did you get the animal to begin with a) gift/dowry b) buying c) share rearing d) other (specify)

2 How do you manage your animals in different season?

a / during dry season

Free grazing_____ tether_____ stall feeding_____

b/during wet seasons free grazing_____ tether_____ stall

feeding_____

3/Where do cattle graze in different seasons and how far is it?

Month_____ site _____ distance_____

Month_____ site _____ distance_____

Month_____ site _____ distance_____

4/where is watering points perennial or seasonal?

If it is perennial: river_____ ponds_____ streams_____ irrigation canals _____

5/where is watering points in different seasons and how far is it?

Months_____ site _____ distance_____

Months_____ site _____ distance_____

Months_____ site _____ distance_____

6/In which season does the availability of water and forage scarcity occurs?

After rainy seasons _____months_____

During dry seasons _____months_____

During the rainy seasons_____ months_____

7/What about the feed abundance in different seasons ?

High_____ medium _____low_____

Months_____Months_____Months_____

8/What are your main sources of income in descending order (Live stock, Cereal crops, Coffee, Enset, Chat, Fruits and others)

1_____2_____3_____4_____

5_____

9/What is the importance of keeping cattle? (Meat production, milk production, manure,

Paying do wry, draught power and others).

1_____ 2_____ 3 _____

4_____ 5_____ 6_____

10/Milk production (average in liters) of an individual cow _____lit.

Perday_____average day's _____of lactation

11/Draught oxen work out put _____hours per day _____work days per year

12/How many cattle have you lost or introduced in to your herd since last year

Animal category	Cattle with drawn				Cattle introduced		
	Sold	Slaughtered	Died	Gifted out	Born	Purchased	Gifted in
Oxen							
Cows							
Female calves							
Male calves							
Heifer							
Bull							

13/When did you start crop cultivation _____years ago

14/What is the size of your cropland?

a/When you are starting cultivation _____

b/Now_____

15/What is reason for such a trend (decrease, increase, un changed) of your cropland holding?

_____

16 / Main crop practiced in your farmland

a/teff_____hectare d/ Coffee_____

b/Maize_____ e/ Cassava_____

c/Enset_____ f/ Sweet potato_____

17/How much Birr do you obtain from crop production per year _____

Livestock production per year _____

Forest products per year_____

Trading pre year_____

18 / do you use improved in puts for crop production _____yes or no if, yes which

one specify_____

Do you use commercial fertilizer or manure? _____

19/What are your major problems in crop production? And, how do you rank them in

descending order of importance

Arable land _____, draught power_____, erratic rain_____, Animal

Disease_____and other_____

B/LIVE STOCK DISEASES

1/What are the most common livestock diseases affecting your animals?

_____

_____

_____

_____

_____

_____

2/Does Trypanosomosis occur in this area? Yes_____or No_____

If yes what do you call it by local name? _____

2.1/What are the main clinical sign observed when an animal affected by

Trypanosomosis?

3/Wich live stock species affected most by Trypanosomosis in descending order?

1 /_____ 2/_____

3/_____ 4/_____

4/In which season does the disease occur commonly?

High month's _____ seasons_____
Medium months_____seasons_____

Low months_____seasons_____

5/When did you know the problem of Trypanosomosis in this area?
a/Just when they start to live their (Years) _____

b/After 10 years of settlement _____

c/After 15_20 years of settlement _____

d/We do know the exact time _____

6/Is the trend of Trypanosomosis is increasing, decreasing, both, the same

7/Do you know tsetse flies? a / Yes_____ b / No_____

8/Do you know that tsetse flies transmit Trypanosomosis

a/Yes_____b / No_____

9/What is the local name of tsetse flies? _____

10/Where do you get tsetse flies different seasons?

Month_____site_____

Month_____site_____

Month_____site_____

11/In which seasons do this flies are most abundant?

Season_____months_____

Season_____months_____

Where is this flies population very high?

a/In grass land areas _____ b/ In bush land _____c / In areas close to river and

watering points_____ d / In cultivated land_____ e / In wooden land_____

13/What are the main control measures of Trypanosomosis?

a/Treatment of sick animals by trypanocidal drugs_____

b/Application of deltamethrin on back of animal _____

c/Use of both in combination_____

d/Use of traditional medicines_____specify_____

e / Feeding well affected animals_____

14/Where are the common trypanocidal drugs source a / Veterinary clinics_____?

b/Local farmers _____ c / Drug smugglers_____d / From private Vet.

Pharmacy_____

15/Who are giving the treatment? a / Yourself_____b / veterinary personnel's_____

c/Drug smugglers_____

16/Which trypanocidal drug do you use commonly to treat your anima l?
Name_____color_____dose for cow_____

For oxen,_____for heifer,_____for bull ,_____for calf,_____

17/When did you start treatment of animals with with trypanocidal drugs? Specify in

Years_____

18/How many times do you treat each animal in a year?

Cow_____Ox_____Heifer_____Bull_____Calves_____

Goat_____sheep_____

19/For how long do you delay to treat your animal after observing clinical symptoms

_____

20/What about availability of money to purchase trypanocidal drugs in different seasons

(high, medium, low)

Month_____availability _____

Month_____availability _____

Month_____availability _____

21/Treatment with trypanocidal drugs a / effective_____b / not effective_____
If it is not effective what is the reason behind that?

_____

22/Are their traditional method of treatment and management practices for controlling and prevention of Trypanosomosis

_____

_____

_____

Thank you

Name of ntervieor_____

Signature_____

Date_____

Annex 2: Wing fray analysis for the estimation of average age of tsetse population in the late rainy season and dry season in Amaro special district, SNNPR

Wing fray number		
	No of flies for the category	
Wing fray number	Late rainy season	Dry rainy season
1	7x1=7	4x1=4
2	7x2=14	6x2=12
3	11x3=33	11x3=33
4	12x4=48	5x4=20
5	16x5=80	4x5=20
6	35x6=210	11x6=66
Mean wing fray number	4.45=31 days	3.7=26 days

Key

1= Perfect wing with no damage

6= Highly damaged wing with excessive wearing

4.45= was calculated from the sum of each category product divided by the sum of actual number of flies, 31days=the equivalent average age of the tsetse population read from table of wing fry analysis

Annex 3: Parasitological and hematological results in the ISMM block treatment study from day 0 to day 90 in Amaro special district ,SNNPR

N0	Owners Name	ID N0	Sex	Age	Day 0		Day 15		Day. 30		Day 60		Day 90 Tryp	
					PCV	Tryps. spp.	PCV	Tryps spp	PCV	Tryps spp	PCV	Tryps. spp.	PCV	Tryps spp
1	Zelekr Boru	Ga1	F	8	30	*Tc Tv*	30	*Tc*		0		0	0	0
2	Zelekr Boru	Ga2	F	5	15	*Tc*	20	0	22	*Tc*		0	0	0
3	Zelekr Boru	Ga3	M	2	24	*Tv*	25	0	26	0	24	*Tc*	0	0
4	Zelekr Boru	Ga4	M	8	30	*Tc*	30	0	32	0	35	0	24	0
5	Tadele Reba	Ga5	F	6	25	*Tc*	26	0	28	0	28	0	30	0
6	Shande Elema	Ga6	F	6	24	*Tc*	25	0	24	*Tc*		0	0	0
7	Tariku Reba	Ga7	F	2	20	*Tc*	24	0	25	0	28	0	22	0
8	Demeke Lima	Ga8	F	10	20	*Tc*	22	0	23	0	25	0	28	0
9	Dembelash Deressu	Ga9	F	3	22	*Tb*	23	0	24	0	26	0	28	0
10	Duke Bochola	Ga10	F	10	20	*Tc*	20	*Tc*		0		0	0	0
11	Tadele Reba	Ga11	M	4	21	*Tc*	24	0	25	0	34	0	27	0
12	Bitacha Reba	Ga12	F	3	22	*Tc Tv*	24	0	26	0	27	0	28	0
13	Ayele Boru	Ga13	M	8	20	*Tc*	23	0	25	0	28	0	26	0
14	Abdurished Abdela	Ga14	F	2	24	*Tb*	24	0	23	*Tc*		0	0	0
15	Abdurished Abdela	Ga15	M	1	18	*Tv*	23	0	24	0	26	0	28	0
16	Adamu Alito	Ga16	F	12	15	*Tv*	23	0	24	0	26	0	18	*Tc*
17	Adamu Alito	Ga17	M	4	18	*Tc*	22	0	24	0	28	0	27	0
18	Jemal Baraka	Ga18	M	6	21	*Tc*	25	0	26	0	28	*Tv*	0	0
19	Adunga Katsula	Ga19	M	5	16	*Tv*	16	*Tv*		0		0	0	0
20	Mogose Boru	Ga20	F	8	18	Tc	21		22		23	*Tc*	0	0	0

N0		ID N0	Sex	Age	Day 0		Day 15		Day. 30		Day 60		Day 90	
					PCV	Tryps. spp.	PCV	Tryps spp	PCV	Tryps. spp	PCV	Tryps spp.	PCV	Tryps spp
21	Luku Algosa	Ga21	F	5	21	*Tc*	23	0	25	*Tc*				
22	Eygezu Bahare	Ga22	M	7	18	*Tb*	22	0	23	0	24	*Tc*		
23	Ebrahim Harun	Je1	M	3	20	*Tc*	22	0	24	0	26	0	26	
24	Brehanu Sebena	Je2	M	8	25	*Tc*	25	0	28	0	28	0	24	
25	Aragaw Mekonen	Je3	M	4	21	*Tc*	21	0	22	*Tc*		0		
26	Aragaw Mekonen	Je4	M	5	24	*Tv*	23	*Tv*		0		0		
27	Getachew Zenebe	Je5	M	5	25	*Tc*	26	0	28	0	3	0	25	
28	Getachew Zenebe	Je6	F	4	20	*Tv*	22	0	25	0	26	0	28	
29	Getachew Zenebe	Je7	F	2	22	*Tc Tv*	23	*Tc*		0		0		
30	Getachew Zenebe	Je8	F	8	23	*Tc*	24	0	26	*Tc*	24	*Tc*		
31	Orgade Oromo	Je9	F	7	22	*Tb*	22	0	24	0	26	0	18	Tc
32	Orgade Oromo	Je10	F	2	26	*Tc*	26	0	28	0	28	0	26	
33	Orgade Oromo	Je11	F	2	20	*Tc*	20	0	24	0	26	0	24	Tv
34	Endris Sherda	Je12	M	6	22	*Tc*	20	0	24	0	22	*Tc*		
35	ToziydeMengesha	Je13	F	4	24	*Tv*	25	0	26	0	28	0	28	
36	Wotema Wosisa	Je14	M	6	25	*Tc*	24	*Tc*		0		0		
37	Geberu Gobena	Je15	F	4	25	*Tb*	26	0	28	0	30	0	27	
38	Grtachew Zenebe	Je16	M	6	28	*Tc*	28	0	30	0	32	0	31	
39	Mekonene Solata	Je17	F	8	20	*Tv*	22	0	26	0	28	0	25	
40	Mekonene Solata	Je18	M	2	21	*Tc*	21	0	24	0	22	*Tc*		

N0			Sex	Age	Day 0		Day 15		Day. 30		Day 60		Day 90	
		ID N0			PCV	Tryps spp.	PCV	Tryps spp	PCV	Tryps. spp	PCV	Tryp .spp.	PCV	. Tryp spp
41	Endrias Sherda	Je19	F	1	24	*Tc*	25	0	26	0	28	0	27	0
42	Dubale Bedesa	Je20	M	5	18	*Tc*	18	0	23	0	25	0	24	0
43	Dubale Bedesa	Je21	M	6	23	*Tv*	24	0	26	0	28	0	27	0
44	Dubale Bedesa	Je22	M	8	26	*Tc*	27	0	30	0	32	0	28	0
45	Dubale Bedesa	Je23	M	2	18	*Tv*	21	0	24	0	28	0	30	0
46	Tadele Bonja	Je24	M	10	23	*Tb*	24	0	24	0	22	*Tc*	0	0
47	Admasu Geberu	Je25	M	10	20	*Tc*	27	0	28	0	30	0	27	0
48	Leben Tilahun	Je26	F	1	25	*Tc*	26	0	30	0	35	0	16	*Tv*
49	Ashenafi Zeke	Go1	M	6	18	*Tc*	20	0	24	0	26	0	25	0
50	Ashenafi Zeke	GO2	F	3	20	*Tc*	24	*Tc*		0		0	0	0
51	Tamerat Tefera	Go3	M	1	21	*Tc*	23	0	26	0	24	*Tc*	0	0
52	Abraham Toche	Go4	M	6	20	*Tv*	23	0	26	0	28	0	24	0
53	Chebuko Chewa	Go5	M	6	18	*Tc Tv*	20	00	22	*Tc*		0	0	0
54	Gemede Mushute	Go6	F	1	19	*Tc*	21	0	24	0	25	0	23	0
55	Arab Ashenafi	Go7	M	6	25	*Tc*	26	0	28	0	24	0	17	*Tc*
56	Negashe Zeleke	Go8	F	5	16	*Tb*	20	0	22	0	22	*Tb*	0	0
57	Tserede Menjo	Go9	M	4	20	*Tc*	23	0	25	0	26	0	25	0
58	Derese Mushute	Go10	F	4	26	*Tc*	28	*Tc*		0		0	0	0
59	Mizan Aklilu	Go11	M	5	13	*Tc*	18	0	20	*Tc*		0	0	0
60	Samuel Tadesse	Go12	M	4	15	*Tv*	18	0	25	0	28	0	26	0

61	Lema Adem	Go13	M	5	18	*Tv*	21	0	22	0	24	0	22	0
62	Bezabehi Tsegaye	Go14	F	1	21	*Tv*	24	0	25	0	26	0	25	0
63	Mekuria Zewede	Go15	M	7	23	*Tc Tv*	25	0	26	0	25	*Tv*	0	0
64	Ayele Suka	Go16	M	4	22	*Tv*	23	0	25	0	20	*Tc*	0	0
65	Ademasu Azela	Go17	M	3	20	*Tb*	24	0	26	0	28	0	23	0
66	Agafari Kerbeche	Go18	M	6	25	*Tc Tv*	26	0	28	0	29	0	25	0
67	Agafari Kerbeche	Go19	M	4	25	*Tc*	26	0	29	0	30	0	24	0
68	Agafari Kerbeche	Go20	M	5	21	*Tc*	22	0	23	0	22	0	18	*Tc*
69	Mulugeta Mushute	Go21	M	6	23	*Tc*	24	0	23	*Tc*		0	0	0

Key

Tc =Trypanosoma concolense

TV=Trypansoma vivax

Tb=Trypanosoma brucei

M=Male

0= Negative result or excluded from the study

F=Female

Ga=Gamule PA

Je=Jello PA

Go=Golbe PA

Annex 4: An ox with recurrent parasitaemic with *T.congolense* infection at Golbe PA, Amaro special district, SNNPR

Annex 5**:** Picture showing when field personnel's were returned from trap removing in Jijolla PA Amaro special district, SNNPR

Annex 6: NGU trap deployment in bush land vegetation in Globe PA, Amaro special district SNNPR

9. CURICULUM VITAE

Personal identification

Name Teshome Assefa
Birth place Bale Goba, Oromiya Regon
Birth date March 8, 1966
Sex Male
Nationality Ethiopian
Marital status married
Religion; Christian of the Orthodox Church
Profession Veterinarian
Occupation; team leader, animal health department
Language skill
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Amharic writing, reading and speaking
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Educational back ground

1972- 1975 Dinsho Elementary School, Dinsho, Oromiya

Achievement certificate

19726-19757 Shashemene Junior Secondary School, shashemene, Oromiya

Achievement certificate

1978-1981 Shashemene Comprehensive High School, Oromiya (ESLCE)

Achievement certificate

1982-1983 Awassa Junior College of Agriculture

Achievement, Diploma in Animal Science and Technology

1987-1992 Ukraine Agricultural Academy, Kiev

Achievement Doctor of Veterinary Medicine

2005-2008 (Summer Program, faculty of veterinary Medicine

Achievement MSc degree in Tropical veterinary Medicine

Training

23/9/1985-17/11/1985 Training on the organizational of cooperative .at Yekatit 25 Cooperative Institute, Ardayta, Oromiya

24/3/2003-22/04/2003 Training course on Veterinary public Health, at the Faculty of Veterinary Medicine Addis Ababa University

Achievement Certificate

22/1/2007-19/04/2007 training on entitled: Advanced general Veterinary Pathology at the Faculty of Veterinary Medicine, Addis Ababa University

Achievement Certificate

Work Experience

1984-1986 Animal husbandry technical experts in Ginner and Kokossa district.Oromiya

1994-1999 Field veterinarians in Amaro special district, SNNPR

2000-2008 Team leader of district Animal Health Department

Paper writing

Diagnosis and Treatment Sub clinical Mastitis in cows under dry period and post partum.

DVM Thesis, Ukraine Agricultural Academy, Kiev, 1992.

Epidemiology, Diagnosis and Current Control Options of Bovine Trypanosomosis in Ethiopia

A paper presented for the course, Seminar on Current Topics in Tropical Veterinary Parasitology Addis Ababa University, Faculty of Veterinary Medicine, Debre zeit, 2007; Ethiopia.

Study on Bovine Trypanosomosis, Tsetse Challenge and Efficacy of Isometamidium Chloride in Amaro Special District Southern Eyhiopia. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre zeit, 2008; Ethiopia

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10. SIGNED DECLARATION SHEET

This thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name: Teshome Assefa

Signature _____

Date of submission _____

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

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