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ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

STUDIES ON BOVINE TRYPANOSOMOSIS AND EFFICACY OF SELECTED TRYPANOCIDAL DRUGS IN KONSO DISTRICT, SOUTHERN ETHIOPIA

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Debre Zeit, Ethiopia

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A thesis submitted to the School of Graduate Studies of Addis Ababa University, in the partial fulfillment of the requirements for the attainment of the degree Master of Veterinary Science in Tropical Veterinary Parasitology

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TABLE OF CONTENTS

LIST OF TABLES

PAGES

LIST OF FIGURES

PAGES

LIST OF ABBREVIATIONS

AAT	African Animal Trypanosomosis
ABC	Adenine nucleotide Binding Cassette
AMP	Adenosine Monophosphate
ANOVA	Analysis of Variance
A-T	Adenine-Thymine base pairs
CAHWs	Community Animal-Health Workers
CD	Curative Dose
DIC	Disseminated Intravascular Coagulation
ED	Effective Dose
EDTA	Ethylene Diamine Tetra Acetate
ELISA	Enzyme-Linked Immuno-Sorbent Assay
FAO	Food and Agriculture Organization of the United Nations
FITCA	Farming In Tsetse Controlled Areas
IBAR	Inter-African Bureau for Animal Resources
ICPTV	Integrated Control for Pathogenic Trypanosomes and their Vectors
IFA	Indirect Fluorescent Antibody test
ILRI	International Livestock Research Institute
ISMM	Isometamidium
kDNA	kinetoplast Deoxy ribonucleic Acid
MEP	Mitochondrial Electrical Potential
OAU	Organization of African Unity
PAAT	Program against African Animal Trypanosomosis
PCR	Polymerase Chain Reaction
RTTCP	Regional Tsetse and Trypanosomosis Control Programme
SIT	Sterile Insect Technique
SPSS	Statistical Packages for Social Sciences
VSGs	Variant Surface Glycoproteins

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First and foremost, great exaltation be to the supreme and Almighty God, the owner and forecaster of everything. In the absence of Lord's act as a fortress, it would have been hardly possible to successfully accomplish this dissertation under such a wilderness and cumbersome research modality. "Say to God, how awesome are your deeds!" [Psalm 66^3].

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ABSTRACT

Studies on bovine trypanosomosis and efficacy of selected trypanocidal drugs involving field and experimental investigations were undertaken in Konso district, southern Ethiopia, from September 2007 to April 2008, with views to determine the prevalence and socioeconomic impacts of bovine trypanosomosis; assess the efficacies of selected trypanocidal drugs; and evaluate the propensity of Konso community to devote public resources to integrated tsetse/trypanosomosis control approaches. Questionnaire interviews, cross-sectional and experimental study designs were applied to collect relevant information. A structured questionnaire was designed and posed to randomly selected households and key informants to identify areas with high suspicion of drug resistance. Questions addressed main issues like: herd structure and major livestock health problems; socioeconomic impacts of trypanosomosis; the sources, usage pattern and suspected failure of trypanocidal drugs, etc. Open-ended and close-ended questionnaire interviews were administered to randomly selected households to evaluate the propensity of the community to a holistic integrated disease control. In order to identify areas with high trypanosome infection pressure and risk of drug resistance, initial prevalence study was conducted in representative sample of cattle by examination of monthly blood samples through micro- haematocrit centrifugation and Buffy coat methods. The relationship between parasitological prevalence of trypanosomal infections and herd mean PCV was investigated through haematological examination during the rainy and dry seasons. In order to assess the therapeutic and prophylactic efficacies of the common trypanocidal drugs, ten zebu calves (*Bos indicus*) were experimentally infected with randomly selected field isolates of *T. congolense* and, when parasitaemic, treated with Diminazene aceturate and Isometamidium chloride at dose rates of 3.5 and 0.5 mg/kg body weight, respectively. Experimental animals were monitored for clinical and parasitological parameters on regular basis for over three months. The study results revealed trypanosomosis to be a major threat to livestock production with contrasting arrays of socioeconomic impacts; significant reductions in cattle production losses after tsetse control and a corresponding rise in mean holdings of draft oxen and use of animal traction over the same period; an indiscriminate use and increasing tendencies in mean annual expenditure on trypanocidal drugs at the household level. Contingent valuation study disclosed animus propensity of

integrated tsetse/trypanosomosis control; household size, wealth status and educational background of household heads to be the major determinants influencing willingness to support disease control. Cross-sectional study suggested an overall prevalence of 17.8 % and 14.2 % during rainy and dry season, respectively, reflecting its significant temporal and spatial variation ($p< 0.001$); and *T. congolense* to be a dominant trypanosome species hampering livestock sub-sector in Konso district. Regression analyses on haematological findings disclosed a significant reduction ($p<0.05$) in the herd mean PCV with an increase in the prevalence of trypanosomosis; and that the reduction in herd PCV was significantly higher during dry season than in rainy season ($p<0.001$), suggesting that trypanosomosis is less-well tolerated during dry months. Results of drug sensitivity testing revealed the presence of *T. congolense* populations exhibiting resistance to Diminazene aceturate and, possibly to Isometamidium chloride. In conclusion, the absence of improved veterinary service and indiscriminate use of poor-quality trypanocidal drugs have proven to boost the risk of drug resistance in Konso district. In light of the high likelihood of trypanocidal drug resistance in Ethiopia, the present findings could be a useful tool to improve trypanocidal drug usage strategies in the field, and could form baseline information to undertake holistic assessments of drug resistance across tsetse-infested areas of Ethiopia. It is recommended that integrated disease control approaches be adopted with chemotherapy restricted to clinically sick animals, and legislations be devised and harmonized to ensure the quality of trypanocidal drugs.

Keywords: Community participation; Drug resistance; Integrated approach: Sensitivity tes Southern Ethiopia; Livestock; *T. congolense*; Trypanocidal drugs.

1. INTRODUCTION

Sub-Saharan Africa has been reputed to hold the greatest opportunity for expansion of continental ruminant biodiversity, which plays pivotal roles in the development of sustainable livelihoods mainly for rural communities. However, rampant livestock diseases in general, and especially tsetse-transmitted African animal trypanosomoses have been incriminated as the predominant elements in the extreme deterioration of livestock resource across the continent (FAOSTATA 2005). Until recently, the deleterious effects of African animal trypanosomosis continue to curtai sustainable livestock development across much of sub-Saharan Africa (Shaw, 2004).

As a key component to improve the productive opportunities of rural communities in tsetse infested areas, the control methods against animal trypanosomosis have been aimed principally a using suitable trypanocidal drugs as the most important tactics in destroying trypanosome However, these drugs are limited in number and have been under extensive administration fo over 40 years with little/no regular monitoring (Holmes *et al.*, 2004). Consequently, recent ca surveys conducted in some sub-Saharan countries, including Ethiopia, have revealed that almo all of the commercially available trypanocidal drugs are gradually losing their efficacy due to t development of multiple resistance by trypanosomes (Mc Dermott *et al.*, 2003).

Therefore, resistance to trypanocidal drugs is increasingly recognized as a major constraint sustainable livestock production. Furthermore, the spread of trypanocidal drug resistance to point where therapeutic and prophylactic failure may occur over large areas is probably greatest risk to the future use of the currently existing few trypanocidal drugs in tsetse-infes areas of sub-Saharan Africa (OIE, 2004).

In Ethiopia, trypanosomosis is the most prevalent and the biggest constraint to livest production, where about 220,000 km^2 of fertile land in south and southwestern parts of country are infested with various *Glossina* species (ILRI, 2002). Socio-economic and ecolo constraints involved in initiating and maintaining vector control strategies have compelled Ethiopian livestock sub-sector to primarily rely on the use of the salts of just three trypano compounds, namely, Diminazene, Isometamidium and Homidium (Mc Dermot *et al.*, 2

Meanwhile, few experimental studies conducted in different tsetse-infested zones of the count using tests both in ruminants and mice, have revealed the occurrence of varying degrees resistance in trypanosomes to the commonly applied trypanocides (Peregrine *et al.*, 200 Yeshitila *et al.*, 2006; Miruk *et al.*, 2008).

Here, most of the experimental studies conducted to assess the efficacy of trypanocidal dru have involved experimentally infected mice where it was possible to demonstrate the gener status of resistance to the drugs used in cattle. However, research has indicated that, the exa curative and prophylactic doses in cattle for an individual trypanosome isolate could not b directly extrapolated from the results in mice (Kone, 1999).

Given the fact that neither the single-dose nor the multiple-dose tests used in mice are able t accurately predict the curative and prophylactic doses of trypanocidal drugs for cattle infecte with a particular trypanosome isolate, it is necessary to ascertain whether or not treatment with manufacturer's recommended dosage is likely to be successful in cattle infected with this isolate Nevertheless, most of the currently available information on trypanocidal drug resistance i derived from small number of case reports and does not give any indication of the exact situatio of the problem across tsetse-infested zones of Ethiopia.

On the other hand, in spite of the long-term supply and indiscriminate application of trypanocide of doubtful quality, mainly by unskilled persons over decades, adequately quantified informatio is not available for most areas in the southern region of Ethiopia, and particularly for Konso district, about the success of treatment with these drugs.

As a foreground step it was, therefore, essential to undertake cross-sectional studies in efforts to identify areas with high infection pressure and high suspicion of drug resistance. Furthermore, *in-vivo* experimental investigations are explicitly required to ascertain the success of treatment with trypanocidal drugs in cattle infected with trypanosome isolates, as a valuable step to generate baseline data as an integral part to the rapid assessments of the true prevalence and probable impacts of trypanocidal drug resistance across tsetse-infested areas of Ethiopia.

Therefore, this research work was conducted with due emphasis to:

1. Undertake cross-sectional study to determine the monthly prevalence of bov trypanosomosis;

2. Assess the therapeutic and prophylactic activities of selected trypanocidal drugs village cattle experimentally infected with field isolates of *T. congolense*; and

3. Apply contingent valuation technique to assess the propensity of Konso community, an aid to define appropriate integrated strategies to control drug resistance in the field

2. LITERATURE REVIEW

2.1. African Animal Trypanosomoses

African animal trypanosomoses remain to be the most prevalent and the biggest impediments to the sustainable developments of livestock sub-sector in sub-Saharan Africa. This disease complex is particularly important in south and southwestern parts of Ethiopia where it poses severe socioeconomic impacts to the national livestock sub-sector (ILRI, 2002). In the following section, brief accounts are made on the major pathogenic trypanosomes affecting livestock together with their pathogenic effects, socioeconomic impacts, and the importance of *Glossina* species in their epidemiology under the context of sub-Saharan Africa.

2.1.1. Major animal trypanosomes

Trypanosomosis is a parasitic disease complex caused by several species of unicellula flagellated protozoan parasites of the genus known as *Trypanosoma* that affect several mammal including humans. The disease (commonly referred to as nagana) infects various species o mammals but, from an economic point of view, tsetse-transmitted trypanosomosis is particularl important in cattle. However, the disease can also cause serious losses in pigs, camels, goats, an sheep. Infection of cattle by one or more of the three African animal trypanosomes results in sul acute, acute, or chronic disease characterized by intermittent fever, anaemia, occasional diarrhe and rapid loss of body condition and often terminates in death (Majiwa *et al.*, 2001).

Trypanosomes are predominantly haematophagous parasites though they can also exist in otl tissues (skin, lymph nodes, tissue fluid, CNS, etc.) where they can give rise to distinctive seq of trypanosome infection (Leak, 1999). Taxonomically, trypanosomes are sarcomastigoph belonging to family trypanosomatidae and genus *Trypanosoma* containing different species v varying degrees of pathogenecity. Although trypanosomes can affect many vertebr throughout the world, the species that are overwhelmingly important as seriously pathogeni domestic livestock comprise *T. vivax, T. congolense, T. brucei, T. evansi, and T. equiper*

Among these trypanosomes, the most pathogenic trypanosomes for African domestic ruminai are *T. vivax* and *T. congolense*, both of which are transmitted primarily by tsetse flies (Holmes *al.*, 2004; OIE, 2004).

2.1.2. Morphology and host ranges

A sound knowledge of the basic features of the various trypanosome species helps mainly in t identification of each species and, therefore, the exact underlying cause of the diseas Trypanosomes could be identified morphologically during parasitological examinations throug standard techniques. The fundamental structures used for the identification of these parasites to species level are described in Figure 1 below.

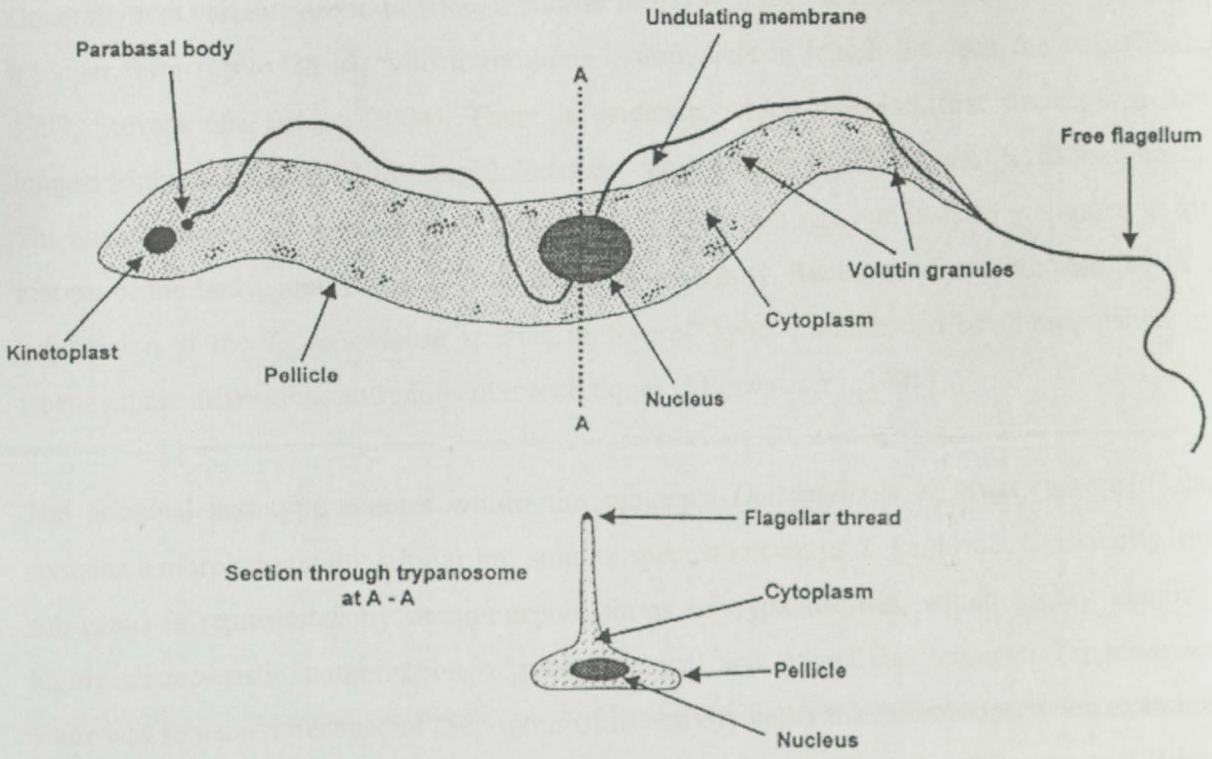


Figure 1: Basic morphological features of typical bloodstream trypanosomes

Source: (Uilenberg, 1998)

A bloodstream trypanosome consists of a single cell varying in size from 8 to over 50 μm. There are distinct differences in appearance, shape and size between the various species of trypanosomes, allowing specific identification of these parasites into particular classes (Stevens and Brisse, 2004). Generally the various trypanosomes affecting African livestock are classified into four major sub-genera, namely *Duttonella*, *Nannomonas*, *Trypanozoon*, and *Pycnomonas*.

Members belonging to the subgenus *Nannomonas* comprise *T. congolense* and *T. simae*, which are the smallest of the pathogenic trypanosomes, with a length of 8-24 μm. This subgenus is principally represented by small trypanosomes that have inconspicuous undulating membrane and a medium-sized kinetoplast, which is generally marginally positioned (Maudlin *et al.*, 2004). Here, the blood forms are monomorphic, in that they lack a free flagellum. Generally two variants are to be seen, a shorter form (9-18 μ), the typical *congolense* type and a longer form (up to 25 μ), with individuals intermediate in length between the two (Leak, 1999; Stevens and Brisse, 2004). There is evidence, which indicates that strains with the longest forms, the so-called 'dimorphic' strains, cause a more severe form of trypanosomosis. The nucleus is centrally placed; the Kinetoplast is of medium size and is usually situated at the margin of the body, just in front of the posterior extremity. Recent studies have resulted in a subdivision of the *T. congolense* species in several 'types', which can be distinguished by isoenzymatic differences and molecular techniques (Majiwa *et al.*, 2001).

The principal and type species within the subgenus *Duttonella* is *T. vivax*, where it also contains a morphologically similar but smaller species known as *T. uniforme*. Classically, this sub-genus is represented by monomorphic forms of trypanosomes, which highly exhibit a highly characteristic combination of morphological and subcellular features. *Trypanosoma vivax* was so named because of the vigour of its activity under the microscope when examined in fresh preparations. The parasite moves rapidly across the field of view (Stevens and Brisse 2004).

This parasite, as seen in the blood of mammals, is also essentially monomorphic, with a free flagellum. Its length, including the free flagellum, varies from 18 to 31 μm. The nucleus

centrally placed whereas the kinetoplast is terminal or almost so. The size of kinetoplast is much larger than in any of the other pathogenic trypanosome species, and this is a major distinguishing feature. The posterior extremity is swollen and blunt. The undulating membrane is inconspicuous. *T. uniforme* are small trypanosomes (from 12 to 20 μm), otherwise similar to *T. vivax*. Trypanosomes of this subgenus are parasitic predominantly in wild and domestic ungulates in Africa and Latin America, their development in *Glossina* being confined to the proboscis. But, significantly, they do not readily infect laboratory animals (OIE, 2004).

The subgenus *Trypanozoon* is the most homogeneous group of Salivarian trypanosomes, represented conventionally by species that are morphologically indistinguishable but which exhibit distinct epidemiological, pathological and genetic characteristic features. This group comprises five members namely: *T b. brucei, T. b. gambiense, T. b. rhodesiense, T. evansi* and *T. equiperdum*. In Africa, the most important species responsible for severe livestock losses is *T. brucei* (Stevens and Brisse, 2004). Morphologically this species is polymorphic (Uilenberg, 1998), with three main forms, all of which have a small kinetoplast and a conspicuous undulating membrane:

a) **Long slender forms** (23-30 μm in length) with a free flagellum, which may be up to one half of the length of the organism. The posterior end is pointed and the nucleus is central. The kinetoplast is placed up to 4 μm in front of the posterior extremity.

b) **Short stumpy forms** (17-22 μm in length) normally without a free flagellum, but in which there may occasionally be individuals with a short free flagellum. The kinetoplast is usually sub-terminal. The position of the nucleus varies greatly.

c) **Intermediate forms**, varying in length between the two previously mentioned types. A free flagellum, of varying length, is always present. The nucleus is centrally placed and the posterior end is usually bluntly pointed. The kinetoplast is close to the posterior extremity.

During the course of the infection, there is a change in the trypanosome population from the long thin forms, through the intermediate host, to the short stumpy forms, and this altered appearance is accompanied by a change in the type of respiration, as the trypanosome prepares

for its period within the tsetse fly. The short stumpy forms are adapted to living and developi in the tsetse, while long thin forms are the true mature blood forms which die in the gut of t insect (Majiwa *et al.*, 2001; Maudlin *et al.*, 2004).

Trypanosoma evansi is typically represented almost exclusively by thin trypomastigot comprising slender and intermediate forms corresponding to those in *T. brucei*. The slend forms have a long free flagellum and a narrow posterior extremity. The intermediate forms have shorter free flagellum and a short pointed posterior extremity, with the kinetoplast lying near th end. In conclusion, it is now generally recognized that no consistent morphological morphometric differences can reliably distinguish the subspecies of *T. brucei* or, indeed, th species within *Trypanozoon* (Stevens and Brisse, 2004).

Depending on the stage and site of development within their invertebrate vectors, the maj trypanosomes described above are further classified into two basic groups, namely sectio Salivaria and section Stercoraria (Table 1). Most pathogenic trypanosomes belong to th Salivarian group where their development is completed in the anterior part of the digestive tra within the vector so that the mode of transmission to the mammalian host is entirely inoculativ type through the proboscis the moment vectors take their blood meal. On the other hand trypanosomes under section Stercoraria undergo development on the terminal segment of th digestive tract within the vectors and transmission of the parasite is contaminative type enhance through the excreta of the vectors (Harry *et al.*, 2004; Stevens and Brisse, 2004).

Table 1: Classification of the major trypanosomes based on development sites in *Glossina*

No.	Subgenus	Species of trypanosomes	Site of development in *Glossina* species
1	*Duttonella*	*T. vivax* and *T. uniforme*	Proboscis
2	*Nannomonas*	*T. congolense and T. simae*	Mid gut and then proboscis
3	*Pycnomonas*	*T. suis*	Mid gut and salivary glands
4	*Trypanozoon*	*T. gambiense, T. rhodesiense, T. brucei*	Mid gut and salivary glands

Source: (Stevens and Brisse, 2004)

The most pathogenic trypanosomes for African domestic ruminants are *T. vivax* and *T. congolense*, both of which are transmitted primarily by tsetse flies. However, it has been generally found that the pathogenecity and clinical manifestations considerably vary according to the species and strain of the parasite, physiological conditions of the animal and degree of challenge by vectors (Hargrove *et al.*, 2003).

As to the host rages, various domestic livestock species such as cattle, sheep, goats, pigs, horses, camels, dogs, cats, and donkeys are susceptible to African animal trypanosomosis and may suffer syndromes ranging from sub-clinical, mild or chronic infection to acute fatal disease. Rats, mice, guinea pigs, and rabbits are susceptible and useful laboratory species. On the other hand, more than 30 species of wild animals can be infected with pathogenic trypanosomes, and many of these remain carriers of the organisms (Eisler *et al.*, 2004).

2.1.3. Biology

African animal trypanosomes conform exclusively to a compulsory heteroxenous mode of life cycle occurring between mammalian hosts and invertebrate fly vectors. They get access to bloodstream and body fluid of mammalian host by becoming injected along with saliva of the vectors during blood meal. Here, they undergo a series of multiplication through syngamy resulting in the formation of haploid trypanosomes that afterwards multiply by longitudinal division. Trypanosomes could be transmitted either cyclically or through mechanical means. In cyclical transmission, pathogenic trypanosomes undergo substantial morphological and metabolic changes within the vector (Stevens and Brisse, 2004).

In the fly vector, trypanosomes go through a cycle of development to finally enter the mid-gut and transform through a lengthwise division into epimastigote form. They penetrate the hemocoel through the peritroph and mid-gut to move into the salivary glands where, after restructuring the surface glycoprotein coat, they develop into the infective forms called metacyclic trypomastigote. These are injected into mammalian blood along with saliva when fly vectors take blood meal. It is that the cycle is completed (Hargrove *et al.*, 2003; Hargrove, 2004).

After animals acquire the infection, immune response is activated to mount antibodies against the surface coat to destroy the parasites, but a very severe problem of this situation is the fact that trypanosomes have multiple genes coding for different surface proteins that allow the organism with new coat to elude the immune response of the host. This phenomenon is known as antigenic variation (Barry and Carrington, 2004). There are certain specific strategies that allow trypanosomes to evade the immune responses of their mammalian hosts. Trypanosomes, as protozoan parasites mainly within the vasculature of their mammalian hosts, are challenged and killed by specific immune responses, but they produce a steady trickle of variants unrecognized by the host's response (Donelson, 2003).

The basis of the trypanosome system of antigenic variation is the protective coat underlying the plasma membrane of the parasite. Antigenic variation is clonal in origin and occurs rapidly when individual trypanosomes spontaneously undergo the switch to a new coat and continue to proliferate. As antigenic variation is centrally linked to the growth and transmission of trypanosomes, it is important to understand its underlying molecular and genetic mechanisms. Although the overall rate of antigenic variation is probably constant throughout the infection, the whole process of this phenomenon is imprecise when examined at the level of the infecting trypanosome population (Barry and Carrington, 2004).

2.1.4. Pathogenic effects and clinical manifestations

Initial replication of trypanosomes takes place at the site of inoculation in the skin, causing a small but complex swelling and sore known as chancre. Trypanosomes then spread to the lymph nodes and blood and continue to replicate. *Trypanosoma congolense* localizes in small blood vessels and capillaries. On the other hand, *T. brucei brucei* and *T. vivax* localize also in tissue. Antibodies developed to the glycoprotein coat of the trypanosomes lyse these parasites and result in the development of active immune complexes. Antibody, however, does not clear the infection, for the trypanosomes have genes that can code for many different surface-coat glycoproteins and change its surface glycoprotein to evade the antibody (Barry and Carrington, 2004). Thus, there is a persistent infection that results in a continuing cycle of

trypanosome replication, antibody production, immune complex development, and changing surface-coat glycoproteins (Maudlin *et al.*, 2004).

The pathogenesis of animal trypanosomosis depends on three main factors, namely anaemia; tissue lesions notably myocarditis and myositis; and immunosuppression. In susceptible livestock breeds, the development of anaemia is a cardinal sign of trypanosomosis, and increased red blood cell breakdown commences with the development of parasitaemia, the level and duration of which often determine the severity of the anaemia (FAO, 2003). Within a week of infection with the haematic trypanosomes (*T. congolense* and *T. vivax*) there is usually a pronounced decrease both in the packed cell volume (PCV), haemoglobin and red blood cells, and within 2 to 3 months the PCVs may drop to below 30 percent of their preinfection values. On the other hand, the pathogenesis of tissue damage depends on the species of trypanosome involved and its tissue invasiveness. Since *T. congolense* and *T. vivax* are mainly haematic parasites, they induce changes in the endothelium of capillaries, and so indirectly damage adjacent tissues (Bengaly *et al.*, 2002).

Immunologic lesions are significant in trypanosomoses, and it has been suggested that many of the lesions (e.g., anaemia and glomerulonephritis) in these diseases may be the result of the deposition of immune complexes that interfere with, or prevent, normal organ function. The most significant and complicating factor in the pathogenesis of trypanosomosis is the profound immunosuppression that occurs following infection by these parasites. This marked immunosuppression lowers the host's resistance to other infections and thus results in secondary diseases, which greatly complicate both the clinical and pathological features of trypanosomosis (Taylor and Authie, 2004).

Apart from the above clinical manifestations, the other complications invariably present are intermittent fever, oedema and loss of condition. Abortion may be seen, and infertility o males and females may be a sequel. The severity of the clinical response is dependent on th species, the breed of affected animals, the dose and virulence of the infecting trypanosome Stress, such as poor nutrition or concurrent disease, plays a prominent role in the diseas process (Barret *et al.*, 2004). Haemorrhagic *T. vivax* stocks have been isolated from Ea

hyper-acute disease, characterized by high parasitaemia, severe anaemia and haemorrhages, which have been related to intravascular disseminated coagulation (DIC). Cattle may die within 2 weeks or, under favourable conditions, rapidly self-cure after 2 months (Machilla and Thuranira, 2004).

Because simultaneous infections with more than one trypanosome species are very common and simultaneous infection with trypanosomes and other hemoparasites (*Babesia* spp., *Theileria* spp., *Anaplasma* spp., and *Ehrlichia* spp.) frequently occurs, it is difficult to conclude which clinical signs are attributable to a given parasite. Few adequately controlled studies have been made, and thus a "typical" clinical response to each trypanosome is difficult to reconstruct (Bett *et al.*, 2004). Altogether, however, the cardinal clinical manifestation observed in African animal trypanosomosis is anaemia (Taylor and Authie, 2004).

2.2. Epidemiology of African animal trypanosomosis

2.2.1. Transmission and distribution

Trypanosomes spread between animal hosts through the medium of tsetse flies or via mechanical transmission (biting flies, injections), causing a serious and fatal disease in domestic livestock but of low pathogenecity to the African wildlife with which they have co-evolved. In general, the development and distribution of African animal trypanosomosis is governed by interplay o factors intervening among the mammalian hosts, the parasites and their fly vectors. Hence, th epidemiology of African animal trypanosomosis depends on interaction among these thre factors. The fact that the disease affects various domestic animals as well as a greater variety c wildlife (the principal reservoirs of the disease) makes the epidemiology of tsetse-transmitte trypanosomosis extremely complicated (Matovu *et al.*, 2003).

Because of the focal nature of the disease, African animal trypanosomosis varies spatially. It determined mostly by vector-related variables such as: the host-fly contact; the prevalence trypanosomal infections in the vector; the density of flies in an area; and the coefficient transmission of a trypanosomal infection or the proportion of infected bites that give rise

transmission of a trypanosomal infection or the proportion of infected bites that give rise to infection. Furthermore, the degree of risk to which domestic livestock are exposed to trypanosomosis depends on the species and strain of trypanosomes, animal's immune status and the presence of concurrent infections (OAU, 2001).

2.2.2. The importance of *Glossina* species

In Africa, the primary vector for the three pathogenic trypanosomes (*T. congolense*, *T. vivax*, and *T. brucei)* is the tsetse fly. These trypanosomes replicate in the tsetse fly and are transmitted through tsetse fly saliva when the fly feeds on an animal (Stevens and Brisse, 2004). For this reason, the important variables in the epidemiology of trypanosomosis and probably the most important component of challenge are tsetse-related factors, particularly the density of tsetse population (Eisler *et al.*, 2004; Hargrove, 2004).

In tropical Africa, the epidemiology of animal trypanosomosis is governed especially by the distribution of tsetse flies (*Glossina* species). Here, tsetse ecology is confined roughly between 15° N-25°S latitude where the disease as well occurs. However, the distribution of mechanically transmitted trypanosomosis (through biting flies, injections, etc.) is wider than the above limit (Bett *et al.*, 2004). When we deal with tsetse-transmitted trypanosomosis, much depends on the distribution and the vectorial capacity of *Glossina* species responsible for transmission. The three main species of tsetse flies for transmission of trypanosomes are *Glossina morsitans*, which favours the open woodland of the savanna; *G. palpalis*, which prefers the shaded habitat immediately adjacent to rivers and lakes; and *G. fusca*, which favours the high, dense forest areas (Bett *et al.*, 2004). Of the three groups of *Glossina*, the savannah and riverine classes are the most important ones since they inhabit areas suitable for grazing and watering (Hargrove, 2004).

Although the infection rate of *Glossina* with trypanosomes is usually low, ranging from 1 – 2(% of the flies, each is infected for life, and their presence in any number makes the rearing o cattle, pigs and horses extremely difficult. In areas where savannah tsetse is the vector, the ris of contracting the disease is widespread. On the other hand, when the riverine species are th

dominant species, transmission occurs particularly along rivers with dense vegetation along the banks. The proportion of a tsetse population found infected with pathogenic trypanosomes depends not only on its vector capability, but also on the host on which it mainly feeds (ICPTV, 2003).

From the above descriptions, it is evident that tsetse fly as the primary vector of animal trypanosomosis in sub-Saharan Africa is incriminated as the predominant and continuing threat to the efforts aimed at improving the livelihoods of rural communities through amelioration of livestock sub-sector (Matovu *et al.*, 2003; Sinyangwe *et al.*, 2004). For this reason, recent and ongoing international programs addressing the problem of tsetse-transmitted animal trypanosomosis, such as the Farming in Tsetse Controlled Areas (FITCA) and the Regional Tsetse and Trypanosomosis Control Programme (RTTCP) initiatives place great emphasis on the disease and vector control (Hargrove *et al.*, 2003; Barret, 2004).

2.3. Socio-economic impacts of African animal trypanosomosis

The greatest opportunity for expanding crop production, livestock production and productivity lies in the forested portion of humid zone and the sub-humid part of sub-Saharan Africa. Furthermore, it is in the same zones that the greater half of the ruminant population of the continent (over 232 million heads of cattle and 343 million heads of sheep and goats) predominates (FAO and IAEA, 2002). This huge continental resource, through improved production strategies, has been proved to play a significant role in the development of a sustainable and environmentally sound agriculture in efforts to ensure food security and effective utilization of natural resources across the continent (Afework *et al.*, 2004; Shaw, 2004).

Nevertheless, there are several factors that influence the production and productivity o livestock kept mainly under extensive and poor management systems in sub-Saharan Africa Here, the production potential of its livestock sub-sector is primarily curtailed by seasonal fee scarcity, underdeveloped veterinary infrastructure and high prevalence of rampant livestoc diseases (Machila and Thuranira, 2004). As the result, the continent is faced with th

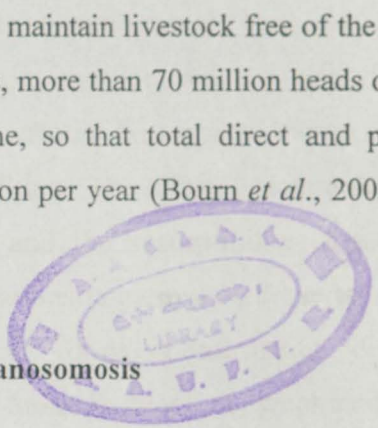
challenge of satisfying a dramatic increase in the demand of its population for livestock products particularly milk and meat (Budd *et al.*, 2001; ILRI, 2002).

From veterinary stand point, animal diseases in general, and particularly those of parasitic origin, have been incriminated as the predominant elements in the extreme deterioration of animal biodiversity in sub-Saharan region that holds the continent's greatest potential for expanded livestock production. Especially, the devastating effects of tsetse-transmitted animal trypanosomosis on the livelihoods of African communities and on the productivity of their livestock have reached enormous levels across much of the region (Bett *et al.*, 2004). The impacts of this problem extend over a greater proportion of sub-Saharan Africa where it threatens more than 70 million livestock and partly makes Africa to produce about 70 times less animal protein per unit area than Europe (FAOSTATA, 2005). Furthermore, trypanosomosis reduces cattle density by 37 % to 70 % and the offtake of meat and milk by about 50 % (FAO, 2003).

Trypanosomosis has direct impacts on livestock productivity, livestock management and human settlement. Through those direct impacts, the disease has indirect impacts on mixed agriculture as well as human welfare. Changes in livestock management, human settlement and crop agriculture also result in changes in land use, vegetation cover, the environment and human welfare. And all of these have implications for resource use patterns, investments in natural capital (e.g. planting of tree, shrubs and herbaceous legumes, construction and maintenance of conservation structures), social institutions that govern resource use (formal and informal conventions, norms and rules) and, once again, human welfare (Gilbert *et al.*, , 2001; Shaw, 2004).

Basically, the direct and indirect socio-economic impacts of nagana are often difficult to quantify. Nevertheless, the socio-economic impact of the disease and the expected socio-economic impacts of control interventions are essential components of planning for cost-effective control (Barret, 2004). Sustainable control can only be achieved when the benefits accruing from the control intervention are larger than its cost. In this case, the impacts that are the easiest to quantify are the direct effects of the disease on livestock productivity. Yet, few

Altogether, however, it has been revealed that the socio-economic impacts of tsetse-transmitte trypanosomosis have reached unprecedented levels across much of sub-Saharan Africa. F instance, over 3 million heads of cattle and other domestic species in Africa are lost every year b deaths caused by trypanosomosis, and about 35 million doses of trypanocidal drugs (worth US 35 million) are bought per annum in futile efforts to maintain livestock free of the disease (Har *et al.*, 2004). In addition to these devastating effects, more than 70 million heads of livestock a at risk of contracting the disease at any one time, so that total direct and potential loss attributable to the disease worth over USD 4.5 billion per year (Bourn *et al.*, 2001; Stitch *et a* 2003).



2.4. Approaches to diagnose African animal trypanosomosis

The primary reason for the diagnosis of animal trypanosomoses is for the application appropriate therapeutic and prophylactic measures. Moreover, diagnosis of this disease aids target and monitor tsetse control or eradication operations, investigations into the efficacy chemotherapy and particularly trypanocidal drug resistance, and for pathophysiologica epidemiological and socio-economic studies (Eisler *et al.*, 2004). Under such circumstances, th type of diagnostic test used in the detection of infections caused by the animal trypanosomos will vary according to the epidemiological characteristics of the disease and the strategy f control of the disease (OIE, 2004).

In areas where a high prevalence of tsetse-transmitted trypanosomosis occurs, even tests of lo diagnostic sensitivity will suffice if chemotherapy or chemoprophylaxis is administered on a he basis. However, in many situations where mechanically transmitted trypanosomosis is foun drugs are often administered therapeutically to individual infected animals and it is essential th more sensitive diagnostic tests be used in order to detect active infections (Harry *et al.*, 2004).

2.4.1. Clinical diagnosis

In areas where trypanosomosis is an endemic problem, clinical symptoms and post mort lesions are important considerations in diagnosing the disease especially in combination with

epidemiological history of the disease. Moreover, systematic surveys aiming at assessment of th seasonal occurrence of trypanosome species and eradication schemes of the vector through highl effective diagnostic approaches may be far more precise (Delespaux *et al.*, 2003).

Clinical signs of acute bovine trypanosomosis include anaemia, weight loss, roughness of the ha coat, enlargement of peripheral lymph nodes, pyrexia, abortion, reduced milk yield and, in th absence of treatment, death. Hence, trypanosomosis should be suspected when an animal in a endemic area is febrile, anaemic and in poor condition. The clinical picture depends to som extent on the species of infecting trypanosomes and the susceptibility of the bovine hos Diagnosis of the disease based on clinical manifestations is complicated due to the fact that th disease may have acute, chronic or sub-clinical forms. Confirmation depends on th demonstration of the trypanosomal organism in the animal's blood or lymph node smears usin the parasitological methods available (Bourn *et al.*, 2001; Mc Dermott *et al.*, 2003).

2.4.2. Parasitological techniques

The conventional techniques of microscopic examination for the presence of trypanosomes a still widely used, but newer and far more sensitive methods are beginning to supplant them. simple parasitological technique is to examine fresh blood through a combination of micr haematocrit centrifugation and Dark-ground/Buffy coat microscopic study techniques. Th method is simple and inexpensive, and if trypanosomes are found, the disease is diagnosed on t spot with the detection limit usually around 10^4 trypanosomes per ml of blood (Delespaux *et a* 2005). The parasite concentration techniques have the added advantage that the packed c volume, and hence the level of anaemia, can be determined at the individual animal and/or he level (OIE, 2004).

Thin and thick blood smears fixed in methanol or acetone and stained with Giemsa may be us in the laboratory to detect blood parasites and determine the trypanosome species involv respectively. Early in infection, blood smears are optimal for the demonstration of *T. congolen* These techniques are not sensitive enough to detect low parasite levels, characteristic of disease in large animals at the chronic level and, as a result, several techniques for

concentration of blood trypanosomes have been developed, which increase the chance o trypanosome detection (Holmes *et al.*, 2004). Stained lymph node smears are a very good metho for diagnosis especially for *T. vivax* and *T. b. brucei*. In chronic *T. congolense* infection, th parasites localize in the microcirculation of the lymph nodes and in other capillary beds, allowin diagnosis by examination of lymph node smears or smears made with blood collected from th ear (Barrett *et al.*, 2004).

The sub inoculation of blood into rodents, usually mice or rats, is particularly useful in revealin sub patent infections. The laboratory animals are injected intraperitoneally with 0.2–5 m (depending on the size of the rodent) of freshly collected blood. Artificial immunosuppression o recipient animals by irradiation or drug treatment will greatly increase the chances of isolatin the parasite (FOA, 2003). They are bled three times a week for at least 2 months and collecte blood is examined using the wet film method. Here, animal inoculation is more sensitive tha direct examination of the wet blood film. Nevertheless, the method is not practical as it expensive and diagnosis is not immediate (Eisler *et al.*, 2003; OIE, 2004).

The method is highly sensitive in detecting *T. b. brucei* infections. However, some *T. congolen* strains are not easily transmitted and *T. vivax* rarely infects laboratory rodents. Also anim inoculation should be avoided as it raises serious animal welfare concerns (OIE, 2004). Exce for *T. vivax,* injecting laboratory animals such as rats and mice with infected blood can reveal lo or sub-patent parasitaemias of certain species and strains of the pathogenic trypanosom (Delespaux *et al.*, 2005).

2.4.3. Serological techniques

The diagnosis of trypanosomes has been improved since the 1980s by serological diagnost methods and DNA-based techniques. Thus, currently a number of serological and molecul tests are available and presently used for the diagnosis of trypanosomosis. Serological tes generally detect specific antibodies developed by the host against the infection or th demonstrate the presence of circulating parasitic antigens in the blood of the host by the use characterized specific antibodies (Delespaux *et al.*, 2003).

On the other hand, the principle of molecular tests is based on the demonstration of th occurrence of sequences of nucleotides, which are specific for a trypanosome subgenus, specie or even type or strain (Eisler, *et al.,* 2003; Shaw, 2004). Several antibody detection technique have been developed to detect trypanosomal antibodies for the diagnosis of anima trypanosomosis, with variable sensitivity and specificity. The methods of choice comprise th indirect fluorescent antibody test (IFAT), which allows comparatively easy and large quantitie of the antigen by making smears of laboratory animals, and the indirect enzyme-linke immunosorbent assay (ELISA) for the detection of trypanosomal antibody. The identification c major antigens of trypanosomes, and their production as recombinant molecules or syntheti peptides, is leading to the current development and validation of new tests based on the use c defined molecules (Geysen *et al.*, 2003; Maser, 2005).

The diagnosis of trypanosomes by DNA-based molecular techniques is either based c hydridization profiles of parasite DNA with DNA probes or polymerase chain reaction (PC technology. A DNA-probe is a known DNA sequence which can be obtained by cloning or l PCR with labelled nucleotides (enzymes or isotopes). DNA probing entails exposing a denatur DNA sample fixed on nitro-cellulose to a labelled DNA-probe under specific salt a temperature conditions. If the complementary DNA sequence is present in the sample, the prob will bind to it and remain on the nitro-cellulose where they can be visualized (Desquesnes a Davila, 2002; Geysen *et al.*, 2003).

A PCR method has been developed as a tool for the diagnosis of infections with Afric trypanosomes in humans and animals, as well as tsetse flies. Recently PCR restriction fragme length polymorphism (RFLP) assays have been developed that allow the identification of Trypanosoma species as single or mixed infections using one single test (Delespaux *et al.* 200 Although with very high sensitivity and specificity, serological and molecular tests are high sophisticated demanding senior professional staff, expensive commercial kits and, thus, primar used as tools for research, for monitoring trypanosomosis control programmes and for surve not so much for routine diagnosis of the disease in the field (Delespaux *et al.*, 2005; Shaw, 200

2.5. Strategies to control African animal trypanosomosis

Controlling African animal trypanosomosis is a key component of poverty alleviation in tsetse infested areas, through improving productivity of livestock, as a set out to improve productiv opportunities and living conditions of the rural poor. Combating African animal trypanosomosi presents a highly challenging and complicated task and is hampered by several factors such a great fluctuation in the apparent tsetse density and variations in the composition of varian surface glycoproteins (VSGs) of trypanosomes that allows the parasite to escape the host' immune system (Harry *et al.*, 2004).

The fact that the deleterious effects of trypanosomosis have reached unprecedented levels o African scene has forced researchers and policy makers to devise various methods to mitigate th problem at large-scale and in an integrated manner (ICPTV, 2003). Here, the control an prevention of tsetse-transmitted African animal trypanosomosis depends on methods directed t the vectors, the host and the parasites. Each of these approaches is useful but has sever important limitations, such as expense, environmental pollution, drug resistance and other relate problems (Bossche and Deken, 2004).

2.5.1. Use of trypanocidal drugs against trypanosomes

As African animal trypanosomosis is primarily transmitted through the cyclical involvement *Glossina* species, a programme to eradicate tsetse flies from some 10 million km^2 of the contine is highly ambitious. Obviously, it will be complex, take many years and possibly cost Afri some USD 20 billion (Gilbert *et al.*, 2001). Until recently, the great majority of control metho against trypanosomosis have, therefore, been aimed primarily at protecting animals by the use suitable trypanocidal drugs as indubitably the most important tactics in destroying the parasit These compounds could be used either as chemoprophylactic entities or chemotherapeu formulations (Geerts *et al.*, 2001; PAAT, 2003).

The three anti trypanosomal compounds upon which treatment and prophylaxis of ca trypanosomosis currently depends are Isometamidium chloride, Homidium chloride/bromide

Diminazene aceturate. On the other hand, Quinapyramine, Suramine and Melarsomine ar primarily used as therapeutic drugs for infections caused by *T. evansi* in equines, camels an buffaloes, although Quinapyramine is also used for prophylactic purpose (Eisler *et al.*, 2001).

Current use of trypanocidal drugs for the treatment and control of African animal trypanosomosi is generally practiced according to one or a combination of the following defined treatmer strategies:

1. **Routine and/or strategic block treatment:** these are carried out using prophylacti drugs, notably isometamidium, at predetermined intervals on the basis of perceive duration of prophylaxis, or when a challenge reaches predetermined levels (Holmes *al.*, 2004).

2. **Monitoring and treatment of individual infected animals:** animals in a particul area are monitored using standard parasitological methods (thin film, Buffy coat haematocrit centrifugation methods), and infected animals are treated using therapeutic drug, usually Diminazene aceturate (FAO, 2003).

3. **Monitoring and treatment of clinical cases:** this strategy is similar to monitoring a treatment of infected animals, but here, not all infected animals are treated. Cattle a treated with curative drugs only when their PCV falls below a predetermined thresh level, or if clinical signs of trypanosomosis are detected (Holmes *et al.*, 2004).

However, drugs used for the treatment and prophylaxis of animal trypanosomosis have be under extensive administration for the last 40 years with little/no regular monitoring. In line w this, most of livestock owners do not have adequate knowledge and experience on the diagno and appropriate drug usage even in areas with high prevalence of trypanosomosis, trypanocides are used in the absence of diagnosis or used to treat for conditions for which t are ineffective (Mc Dermott *et al.*, 2003). A further factor is the fact that the choice between use of therapeutic and prophylactic drugs is made on the basis of cost per dose, with understanding the advantages of prophylactic drugs in appropriate circumstances (Homes *et* 2004).

As the result of the above scenario, recent case surveys conducted in some sub-Saharan Afri countries have revealed the occurrence of varying degrees of resistance to the available a trypanosomal compounds. For this reason, the heavy reliance of livestock owners on trypanoci drugs, the alarming emergence of resistance to the existing anti-trypanosomal compounds and unlikelihood of new trypanocides appearing in the foreseeable future together with the l adoption of other alternatives to the use of trypanocidal drugs in aggregate create a great dilem in the management of African animal trypanosomosis in Sub-Saharan Africa (Bett *et al.*, 2004)

Furthermore, the cost of development of new compounds has increased enormously in keep with inflation and, the market for recovering the enormous investments needed to develop n compounds to the commercial stage is limited and mostly poor (Bett *et al.*, 2004). In this ca the high cost of drug production, estimated at USD 200-800 million, compared to the low va of the market for animal trypanocides, estimated at USD 20-30 million, is a strong disincent for the private sector to invest in the development of new drugs (De Koning *et al.*, 2004). Thus is evident that control of trypanosomosis will depend in the foreseeable future on the use of existing few trypanocidal drugs. The challenge, therefore, remains to make optimal use of three relatively old compounds until new methods of treatment emerge, possibly throu unanticipated cross-reactivity with new broad-spectrum anti-protozoal compounds such as th currently being developed for the treatment of malaria and cryptosporidiosis (Holmes *et* 2004; OIE, 2004)

2.5.2. Control of the vector

Parallel to the application of trypanocidal drugs on infected animals, continuous effort has b put towards controlling tsetse flies as part of an integrated approach to keep the size of ve population down to a level where the trypanosomosis problem is tolerable. Several approache fly control have been used with varying degrees of success. Many methods widely used for ts control in the past have ceased to be used in the last 10-20 years, either because they v ineffective, or because they have become environmentally unacceptable (Bossche and De 2004).

Some of the conventional methods currently employed to control the vectors are: selective spraying of the vegetation support of the flies; use of artificial bait devices such as insecticide impregnated traps and targets; application of small quantities of persistent powerful pyrethroid insecticides on animals; and the use of the so-called sterile insect technique (SIT) after rearing and releasing male flies (Bourn *et al.*, 2001; OAU, 2001).

Discriminative bush clearing, extensively used in early tsetse fly eradication campaigns, has been locally useful because it eliminates the breeding places of the tsetse. But, to be completely effective, bush clearing requires destruction of vast areas of bush and forest, which is ecologically unacceptable. It is still a useful procedure when used locally in conjunction with other control methods. Game elimination, and thus elimination of the main source of blood-meals for the tsetse, was used in early eradication campaigns. This was an ineffective and wasteful procedure. Today, the method has been abandoned, to a large extent on environmental and ethical grounds (PAAT, 2003). On the other hand, ground and aerial spraying with insecticides and the use of synthetic pyrethroids on cattle have lowered fly densities in some areas, but widespread use would require considerable international cooperation and expense. Widespread application of insecticides has the tremendous disadvantage of also eradicating many other arthropods, several of which are desirable (ICPTV, 2003).

There has been a substantial amount of research for more environmentally acceptable techniques because the widespread use of persistent insecticides or drastic habitat modification is no longer acceptable. These include the use of insecticide impregnated traps or targets, non-impregnated traps and the use of 'pour-on' insecticides applied to cattle (Bossche and Deken, 2004). More recently, the use of 'pour-on' insecticides applied to cattle has been a widely applied technology. Whilst still depending upon insecticides, the quantities used are much smaller, and the types used are predominantly synthetic pyrethroids with high toxicity for *Glossina* spp. but low mammalian toxicity. Furthermore, they are not widely distributed in the environment but are much more closely directed at the specific target organism (Hargrove, 2004; Majiwa *et al.*, 2001). Application of the sterile male technique (SIT) received considerable attention in the 1980's where large numbers of male flies, usually sterilized by irradiation, are released and compete with wild males to mate with female flies. This novel approach has been used successfully

Burkina Faso, Tanzania, Nigeria and, most recently, in Zanzibar where it eradicated *Glossina austeni* from the 1600 km^2 Unguja Island (PAAT, 2003). Though currently being implemented in Ethiopia, this method of control is proved costly, technically demanding and only suitable for relatively small, isolated areas of tsetse infestation (Gilbert *et al.*, 2001).

In general, although vector control techniques have some environmental adversity, banning the more primitive methods and adopting the modern strategies has proved to be very efficient. Thus, the policy of the Program against African Animal Trypanosomosis (PAAT) is to encourage the improved vector control alternatives so as to exterminate this menace and explicitly bring about a sustainable livestock production across tsetse-infested areas of sub-Saharan Africa (PAAT, 2003).

2.5.3. Use of innate resistance of the host

It has long been known that there are differences in susceptibility to trypanosomosis between various livestock breeds in Africa. Trypanotolerance in taurine (humpless) cattle breeds such as those of the N'Dama and other West African shorthorn breeds is particularly well known and widespread in sub humid and humid regions of Western Africa. Susceptibility studies have shown the N'Dama to be the most resistant breed followed by the smaller west African short horned cattle, but the large and more recently introduced Zebu has proved the most susceptible breed to trypanosomosis. These West African local breeds of livestock offer another solution to the problem of trypanosomosis as they perform relatively better than other breeds under high levels of tsetse and trypanosomosis challenge (Murray *et al.*, 2004).

Though trypanotolerant livestock breeds are realistic and environmentally friendly, these animals are generally smaller than the preferred zebu breed and, for preference reasons, have been less adopted in most parts of the continent. The high cost of transporting these animals to other sub Saharan countries is also another problem with the use of trypanotolerant livestock breeds as alternative approaches in Africa (Murray *et al.*, 2004; Taylor and Authie, 2004).

2.6. Development of resistance to trypanocidal drugs

In the vast majority of countries where trypanosomosis is a major and endemic problem, the use of trypanocidal drugs is the principal method of controlling the problem but the heavy reliance of farmers on drugs for disease management makes them very vulnerable to the emergence of drug resistance, as drug resistance is an inevitable sequel to continuous drug use (De Koning *et al.*, 2004). Thus, the problem of drug resistance in trypanosomes appears to be spreading geographically into many regions in which trypanosomosis prevails (Shahi et *al.*, 2002).

2.6.1. Mechanism and genetic basis of resistance to trypanocides

Drug resistance may be defined as loss of sensitivity by a strain of an organism to a compound to which it had previously been susceptible. So, it implies failure of treatment and prevention, and if no other active drugs are available the animal has to rely on its immune defenses alone to combat the disease (Holmes *et al.*, 2004). Drug resistance covers both host- and parasite- related factors. Host-related factors include poor distribution of a drug to infected tissues or intracellular sites, variation in drug metabolism between individuals, and diminished activity of a drug in animals with a suppressed immune system. On the other hand, parasite-related factors contributing to resistance include: reduced drug accumulation in the parasite, a change in enzyme target through an increase in its levels and affinity, increased metabolite production or retention and alteration in drug metabolism, and use of alternative pathways to bypass the site of inhibition (Geysen *et al.* 2003; Maser, 2005).

An understanding of the mode of action of trypanocidal drugs and the mechanisms of drug resistance by trypanosomes is important so as to identify the potential and novel drug targets and provide directions towards new chemotherapeutic strategies in efforts to reduce the development of resistance to trypanocides. Some of the most important factors influencing the development of resistance to trypanocidal drugs are shown in Figure 2 below.

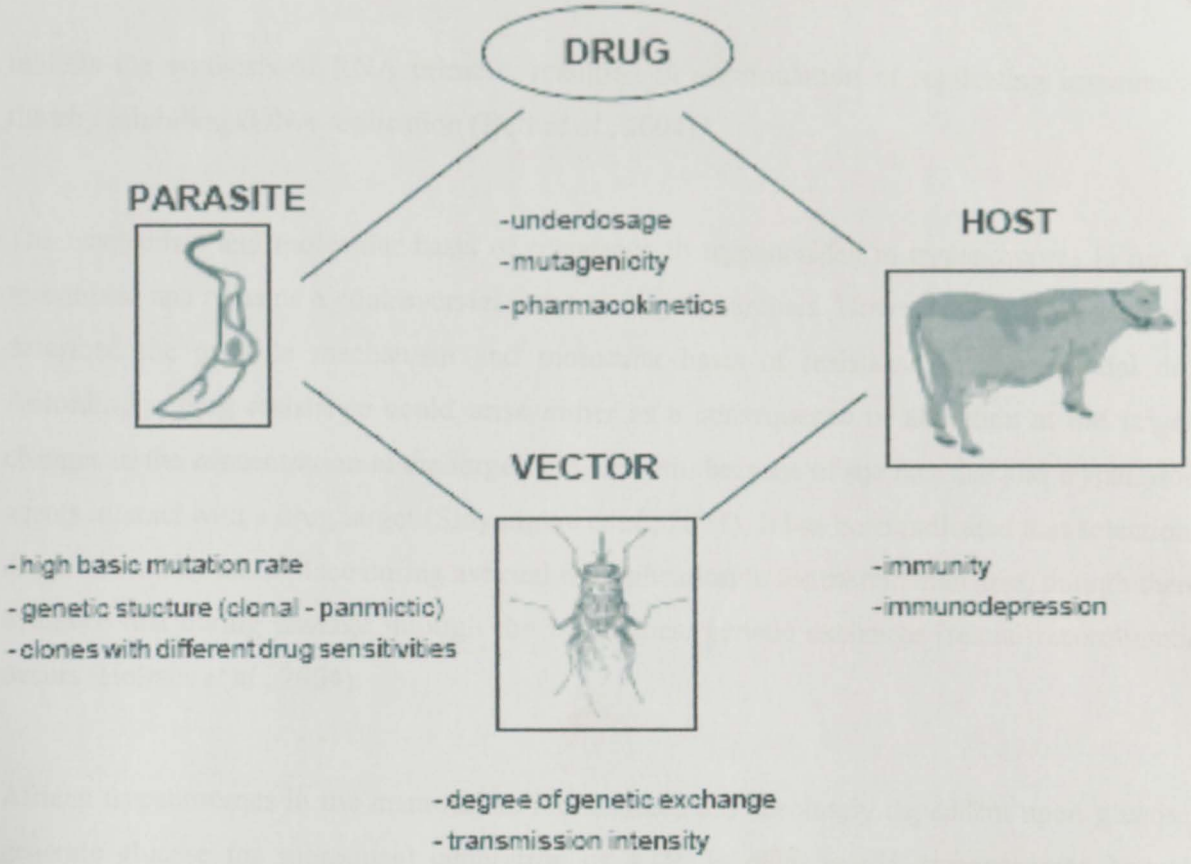


Figure 2: Factors affecting the development of resistance to trypanocidal drugs
Source: (FAO, 2003)

The primary mode of action that is currently accounting for the molecular mechanisms of anti-trypanosomal activity of phenanthridinium drugs is blockade of nucleic acid synthesis through intercalation between DNA base pairs, inhibition of RNA and DNA polymerases and incorporation of nucleic acid precursors into DNA and RNA (Sinyangwe *et al.*, 2004). Although the mechanism of Isometamidium and Homidium salts for anti-trypanosomal action is not well understood, it has been shown that these drugs interfere with glycosomal functions, the function of an unusual adenosine monophosphate (AMP) binding protein, trypanothione metabolism and the replication of kinetoplast DNA (De Koning *et al.*, 2004). Diminazene aceturate exerts its pharmacologic effects through binding to trypanosomal kinetoplast DNA. This binding via specific interaction with trypanosomal DNA sites rich in adenine-thymine (A-T) base pairs inhibits the synthesis of RNA primers, resulting in accumulation of replicating intermediates thereby inhibiting kDNA replication (Bett *et al.*, 2004).

inhibits the synthesis of RNA primers, resulting in accumulation of replicating intermediates, thereby inhibiting kDNA replication (Bett *et al.*, 2004).

The mechanism and molecular basis of resistance to trypanocides in trypanosomes is not well recognized and remains a controversial issue among researchers. However, few researchers have described the possible mechanism and molecular basis of resistance to trypanocidal drugs. Accordingly, drug resistance could arise either as a consequence of alteration at the target or changes in the concentration at the target site, or both, because of the fact that anti trypanosomal agents interact with a drug target (Sinyangwe *et al.*, 2004). It has been indicated that selection by drugs essentially takes place during asexual multiplication in the mammalian host, though there is evidence that during passage through the tsetse flies, genetic exchange (sexual recombination) occurs (Holmes *et al.*, 2004).

African trypanosomes in the mammalian bloodstream are absolutely dependent upon glucose to generate glucose for subsequent generation of ATP. In other words, trypanosomes have lost anabolic pathways such as purine synthesis and rely entirely on salvages of purine bases or nucleosides from their hosts to make nucleic acids. P-glycoproteins are prominent representatives of a family of adenine nucleotide binding cassette (ABC) that is widely represented in trypanosomes and other protozoa, fungi, and metazoans. Trypanosomal purine transporters are therefore potential drug targets (i.e. the specific delivery of toxic compounds to the parasite through transporters that are absent in the host). Conversely, mutations in nutrient transporters can cause drug resistance by reducing drug import (Matovu *et al.*, 2003).

Research has also revealed that the maximal uptake rates (V_{max}) of isometamidium in resistant *T. congolense* were significantly lower than in sensitive populations (Mulugeta *et al.*, 1997). A gene deletion study, which attempted to investigate the role of TbAT1 (cloned strain of *T. brucei*) in drug uptake and resistance in *T. brucei* by genetic knockout of TbAT1 demonstrated the total absence of P2-type transport in TbAT1-null bloodstream form and indicated that loss of TbAT1 reduced the sensitivity of trypanosomes to melaminophenyl arsenicals (Matovu *et al.*, 2003).

Whether or not drug-resistant trypanosomes are less pathogenic than susceptible ones remains a controversial issue. Several authors have observed a loss of virulence and/or a loss of fitness in drug-resistant trypanosomes. To date, few studies have accurately assessed the impact of drug resistant trypanosomes on livestock productivity, although it is generally assumed that uncontrolled infections will have a severe impact on both survival and productivity (OIE, 2004). A useful study to assess the impact of drug-resistant trypanosomes on the productivity of local cattle was carried out in the Ghibe valley, south Ethiopia, where a high prevalence of multiple drug resistance was already reported (Codjia *et al.,* 1993).

Therefore, it is worth to deduce that much more work remains to be done in order to properly elucidate the mechanism of resistance to the currently available trypanocidal drugs. Such studies, as well as being of great value in their own right, may also provide novel methods for the detection of drug resistant trypanosomes in the future (OIE, 2004). Recent advances in the manipulation of the gene structure and/or expression, such as gene knockouts, over expression and RNA interference followed by observation of their effects on the trypanosome phenotype, are useful strategies which would provide us valuable information on the genetic bases of drug resistance in trypanosomes (FAO, 2003; Holmes *et al.*, 2004).

2.6.2. Assessment of resistance to trypanocidal drugs

Several standardized methods have come into view to identify drug resistance in trypanosomes. At present, two types of technique are commonly used for this purpose, namely *in vivo* methods (tests in ruminants and mice) and *in vitro* assays (OAU, 2001). Standardized protocols for these tests have been developed which should allow a better comparison of data on a temporal and spatial basis (Eisler *et al.*, 2001).

Tests in ruminants could provide direct information from studies in ruminant using recommended doses of trypanocides. The tests commonly used in these animals consist of infecting a group of cattle or small ruminants with the isolate under investigation and later, when the animals are parasitaemic, treating them with various levels of a trypanocide. The animals are then regularly monitored over a prolonged period (up to 100 days) to determine the effective dose and the curative dose (Harry *et al.,* 2004). It is preferable to use at least three animals in each group, because it has been shown that results obtained after inoculation and treatment of one animal are not always reliable (Eisler *et al.*, 2001). The animals are then regularly monitored over a prolonged period (up to 100 days) to determine the effective dose (ED), i.e. the dose that clears the parasites from the circulation, and the curative dose (CD), i.e. the dose that provides a permanent cure. For these studies, the cattle or small ruminants must be kept in fly-proof accommodation or in a non-tsetse area in order to eliminate the risk of re infection during the study (Mc Dermott *et al.*, 2003).

This test is useful because of the difficulty in extrapolating the curative dose in cattle from the results of tests in mice (Kone, 1999). A useful indication of the level of resistance can be obtained from studies in ruminants by recording the length of time between treatment and the detection of breakthrough populations of trypanosomes. The shorter the period, the greater the level of resistance. The main constraints to this technique are that not all populations might grow equally well and that sensitive isolates might overgrow resistant ones when inoculated. Moreover, the long duration of follow-up and the cost (purchase and maintenance of the animals are expensive) make this method usually impractical to examine a large number of isolates (Holmes *et al.*, 2004).

Due to the high costs of tests in the definitive host, tests in laboratory mice have become the preferred method, at least for testing those isolates of *T. congolense* which grow well in mice. After expansion of an isolate in a donor mouse, groups of five or six mice are inoculated with trypanosomes and twenty-four hours later, or at the first peak of parasitaemia, each group except the control group is treated with a range of drug doses. Thereafter, the mice should be monitored three times a week for 60 days. Although test in mice has several feedbacks, it makes use of the advantage of being cheaper and easier to conduct (Eisler *et al.*, 2003).

A standardized and simplified method was described for the investigation of trypanocidal drug resistance using laboratory animals (Eisler *et al.*, 2001). The first is a single-dose mouse test, which is a simplified approach to the use of mice for investigation of drug resistance. It is intended to compare and characterize areas in terms of the extent of drug resistance in *T. congolense* or *T. brucei* by examination of as many isolates as possible, rather than to characterize individual stabilates. This method has shown to be useful in the investigation of trypanocidal drug resistance on an area-wide basis (Geerts *et al.*, 2001). The standardized multi-dose test is to obtain more detailed information by determining the CD50 and CD80 values for a given trypanocidal drug (Eisler *et al.*, 2001).

A great deal of progress has been made in the field of *in vitro* assays to determine the drug sensitivity of trypanosomes. Here, many techniques are used for this purpose, such as identifying genetic markers for ISMM resistance using polymerase chain reaction (PCR), trypanocidal drug ELISAs in combination with parasite detection tests, and the use of a mitochondrial electrical potential (MEP), etc (Holmes *et al.*, 2004). Due to the vast limitations of the currently available tests to validate drug resistance in trypanosomes, an alternative approach in the future may be made to identify genetic markers for drug resistance, which might be developed into reagents for the identification of resistant trypanosomes using the polymerase chain reaction (PCR). A PCR-based test could provide a rapid and convenient tool, suitable for large-scale epidemiological surveys of livestock. Developments of such tests require the identification of genetic mutations that may be associated with drug resistance in livestock-infective trypanosomes (Desquesnes and Davila, 2002).

Longitudinal parasitological data can be used to detect resistance problems in cattle herds under natural tsetse challenge. There are a number of disadvantages, however. Firstly, the true prevalence of drug-resistant infections seems to be underestimated. Secondly, it is retrospective by at least six months. Finally, the technique is quite expensive, if a longitudinal study is not carried out for other purposes (Bett *et al.*, 2004). Another method for the assessment of trypanosomosis risk and the level and prevalence of resistance to Isometamidium chloride has been developed using cattle populations under natural challenges in the field (Eisler *et al.*, 2000).

This protocol compares new trypanosome infections in a group of cattle treated with Isometamidium chloride to an untreated group. The rate at which new infections occur in the two groups is assessed by a comparison of their survival curves over an 8 – 12-week period. This may give a rapid and accurate assessment of isometamidium resistance and the impact of drug use relative to no treatment. This method has been successfully applied to assess isometamidium resistance of trypanosomes in cattle in western Ethiopia (Tewelde *et al.*, 2004). One of the disadvantages of this study is that it cannot be used to assess the resistance situation with regard to Diminazene aceturate (Mc Dermott *et al.*, 2003).

The other possibility is a block treatment study, whereby a group of naturally trypanosome-infected animals are treated with prophylactic doses of isometamidium and monitored for relapse of infections. This technique can be used to assess the period of prophylaxis conferred by isometamidium in naturally trypanosome-infected animals under field condition, such that the data has direct relevance to the field, which is not always the case with data obtained in mice (Afewerk *et al.*, 2004). The disadvantage is that the animals are left after treatment to graze in their natural environment, where there is high tsetse-challenge.

Consequently, the risk of re- infection after treatment cannot be eliminated, and it is not possible to know whether the trypanosomes detected in the animals originated from the population present in the animal at the time of treatment or from tsetse feeding on these cattle after treatment. Therefore, further confirmation may be required by isolating the trypanosomes from the animals with relapse infection and testing them in mice or in natural hosts in a fly proof stable (Mc

Dermott *et al.*, 2003). Besides, this test is not suitable for Diminazene aceturate because this drug is eliminated from the blood of treated animals in a few days time (Harry *et al.*, 2004).
Since it has been shown that the rate of isometamidium accumulation in *T. congolense* is a good indicator of the degree of drug resistance and since the mitochondrial electrical potential (MEP) appears to be closely linked with the rate of drug uptake, it might be possible in the near future to develop a quantitative *in vitro* test to evaluate the results. If such a test could be carried out using a small number of trypanosomes, it might provide a rapid indication of the level of resistance of a given trypanosome isolate. It is hoped that this test could be conducted on whole blood samples and would not, therefore, suffer from the same limitations as other *in vitro* tests referred to in the earlier headings (OIE, 2004).

2.6.3. Current status of resistance to trypanocides

Drugs used for the treatment of animal trypanosomosis are generally subject to lower standards of quality control than those used in human disease. The release of preparations that contain low quantities of active drug provokes ideal conditions for the selection of drug resistance as well as leading directly to therapeutic failure (Barrett *et al.*, 2004) So far, resistance to one or more of the common trypanocidal drugs used in cattle has been reported in at least 13 countries within sub-Saharan Africa. In more of the countries in which the studies have been conducted, multiple resistance was reported to both Diminazene aceturate and Isometamidium chloride (Geerts and Holmes, 2001).

But the currently available information on drug resistance is derived from limited numbers of case reports and does not give any indication of the true situation of resistance in a region or a country as systematic surveys have not been fully conducted (Eisler *et al.*, 2004). This problem of drug resistance in trypanosomes currently appears to be spreading geographically into many regions in which the disease occurs partly due to the spread of many generic products with doubtful quality (Holmes *et al.*, 2004). The table in the following section summarizes the list of published reports in which a number of trypanosome isolates has been examined in some countries across sub-Saharan Africa.

Table 2 : Reports of resistance to standard doses of the common trypanocidses used in cattle in some African countries

Country	Trypanosome species	Drug/s to which resistance is developed	Reference
Nigeria	*T. vivax*	D, H, I, Q	Ilemobade (1990)
	T. congolense	D, H, I	Clausen (1992)
Burkina Faso	*T. congolense*	D, I	Matovu (1997)
	T. congolense	D, H, I, Q	Clausen (1992)
Uganda	*T. brucei*	D	Ainanshe (1992)
	T. rhodesiense	I	Kalu (1995)
	T. congolense	D, H	Mohamed (1992)
	T. congolense	I	Mohamed (1992)
Zimbawe	*T. brucei*	DFMO	Iten (1995)
Zambia	*T. congolense*	D, I	Matovu (1997)
	T. vivax	I	Sinyangwe (2004)
Sudan	*T. vivax*	I	Arakawa (1991)
	T. rhodesiense	H	Ahmed (1992)
	T. brucei	D, H, I	Ahmed (1992)
	T. congolense	D, H, I	Mohamed (1992)
Kenya	*T. congolense*	H, I, Q	Iten (1995)
Somalia	*T. congolense*	D, I	Ainanshe (1992)
Ethiopia	*T. congolense*	D, I, H	Mulugeta (1997)
	T. congolense	D, I	Tewelde (2004)
	T. congolense	D, I, H	Codjia (1993)

D = Diminazene; DFMO = Difluorometylornithine; H =Homidium); I = Isometamidium; Q = Quinapyramine

Source: (Holmes *et al.*, 2004)

2.7. The need for integrated tsetse/trypanosomosis control approach

A review of the literature with special reference to the control of animal trypanosomosis in Africa has led to the conclusion that each of the control strategies in use even in the present situations has several limitations. Currently, the limitations to the effectiveness of chemoprophylaxis and chemotherapy have been the extensive administration and, consequently, a widespread development of multiple resistances to the few drugs on market (Holmes *et al.*, 2004). Therefore, resistance to the drugs used for the control and prevention of African animal trypanosomosis is increasingly recognized as a major constraint to livestock production in Sub-Saharan Africa (Shaw, 2004). Furthermore, the unlikelihood of new trypanocides appearing in the foreseeable future together with the low adoption of other alternatives to the use of trypanocidal drugs create additional dilemma in the management of African animal trypanosomosis (Bett *et al.*, 2004).

Economic and ecological constraints on trypanosomosis control are still evident so that the high cost involved in a continuing program of eradicating tsetse flies is often beyond the reach of individual country. Furthermore, tsetse fly eradication remains an unreliable measure when continued surveillance is not guaranteed since this will not necessarily result in the eradication of trypanosomosis, as some trypanosomes like *T. vivax* and *T. b. evansi* can be transmitted mechanically by biting flies (Sinyangwe *et al.*, 2004). Though realistic and environmentally friendly, trypanotolerant livestock are generally smaller than the preferred Zebu breed and, for preference reasons, have been less adopted in most parts of the continent. The high cost of transporting them also creates another problem (Murray *et al.*, 2004).

On the other hand, illegal introduction of poor-quality trypanocidal drugs and recurrent theft of vector control devices (traps/targets) have been amongst the major constraints to a successful management of tsetse/trypanosomosis in most areas of Africa where the problem is endemic. This problem is particularly serious in tsetse-infested zones of Ethiopia where:

1. Black market for trypanocidal drugs by drug smugglers is well experienced; and
2. Most of the traps/targets provided by different governmental and non-governmental organizations for tsetse suppression have been destroyed through recurrent theft, bush fire and other vandals under different circumstances.

The above scenario suggest the need for a method which would sustain disease management strategies through guarding vector control devices, and ensuring the provision of cost-effective, quality drugs to the suffering community.

Furthermore, despite enormous advances that have been made in the area of immunology over the last thirty years, the prospect of a vaccine against trypanosomosis in any species is no longer closer. Under such a circumstance, the almost unlimited antigenic variation during infection by one single strain of trypanosome and the antigenic strain diversity within each of the several trypanosome species and types are the main obstacles preventing vaccine development. Therefore, little progress has been made in the development of a vaccine against trypanosomes and, further, no effective vaccine is likely to be marketed in the near future (FAO, 2003; Geerts and Holmes, 2001).

Though trypanotolerant livestock breeds are realistic and environmentally friendly, these animals are generally smaller than the preferred Zebu breed and, for preference reasons, have been less adopted in most parts of the continent. The high cost of transporting these animals to other sub-Saharan countries is also another problem with the use of trypanotolerant livestock breeds in Africa (Murray *et al.*, 2004).

Therefore, the several limitations described above for the various control measures used to mitigate tsetse-transmitted trypanosomosis indicate that there is no stand-alone method to effectively handle the problem, and that international coordination is required for combating this disease complex. For this reason, to fully exterminate the interacting causes of trypanosomosis so as to ameliorate livestock production as an alternative to pathways out poverty for the rural poor in sub-Saharan Africa, it is essential to devise and adopt disease control strategies that would entail the various conventional approaches (Sinyangwe *et al.*, 2004).

Shortly put, in countries with few monetary and foreign exchange resources, use o environmentally friendly and sustainable community-based vector control schemes, application c trypanocidal drugs only on sick animals, and the use of trypanotolerant livestock, are to b

favoured such that control should be flexible and integrate all available methods which are suitable in a particular situation (Bossche and Deken, 2004; Hargrove *et al.*, 2003).

2.8. Guidelines on the control of resistance to trypanocidal drugs

The factors responsible for the development of resistance to anti-trypanosomal compounds are not well known as described earlier. The exposure of parasites to sub-therapeutic drug concentrations, owing to under-dosing, has been considered as the most important factor for the development of resistance (Barry and Carrington, 2004). It has been generally accepted that resistance genes are present in a very small proportion of the population and that these pre-existing resistant individuals are selected by drug pressure. Therefore drug resistance in trypanosomes is likely to occur under circumstances where there is large-scale drug use; inadequate dosing; and using correct dosing with drugs that are slowly eliminated from the body. Furthermore, some trypanocidal drugs are well-known mutagenic compounds and might induce mutations, the most resistant of which are certainly selected under drug pressure (Maudlin *et al.*, 2004).

Up to now the most important guidelines on the avoidance or delay of the development of drug resistance were considered to be: i) Use of the "sanative pair" of drugs (ISMM or Ethidium and Diminazene); and ii) Avoidance of the exposure of trypanosomes to subtherapeutic drug concentrations. It is clear, however, that the application of these guidelines may not be sufficient to maintain the efficacy of the existing drugs, especially since they lack recommendations concerning a reduction of the treatment frequency (FAO, 2003). Based on current knowledge in the field of trypanocide resistance, the following recommendations are proposed in order to delay the development of resistance to the available trypanocidal drugs:

1. **Use of the correct dose:** Under-dosing is one of the major causes of resistance development. Sub-therapeutic drug concentrations exert a strong selective pressure for the emergence of resistant clones that pre-exist in the trypanosome population. Unfortunately under-dosing occurs very frequently since farmers have the tendency to under-estimate the weight of their animals when they have to treat them (Mc Dermott *et al.*, 2003).

2. **Reducing the number of treatments:** It is widely agreed that the most efficient way to delay the development of drug resistance remains the reduction of selection pressure through decreasing the number of treatments. This is of particular importance in areas of high tsetse challenge, which are commonly associated with reduced periods of chemoprophylaxis (Harry *et al.*, 2004). It is, thus, strongly recommended that in high tsetse challenge areas, exclusive reliance on drugs for the control of trypanosomosis, and mass treatments at short intervals should be avoided. More attention should be given to integrated control measures involving the vector as well as the parasite (Holmes *et al.*, 2004).

3. **Avoiding exposure of the whole parasite population to a drug:** Animal trypanosomosis is commonly controlled with mass treatments which can be highly successful over many However, this form of treatment exerts a strong selection pressure on the trypanosom population. The higher the proportion of the trypanosome population exposed to the sub therapeutic trypanocidal drug doses, the higher the selection pressure. The percentage of th total parasite population that is exposed to the drug at the time of treatment might, thu have an impact on resistance development to trypanocides (OIE, 2004).

In conclusion, because it is very unlikely that new trypanocidal drugs will be released on to th market in the near future, it is essential to try to maintain the efficacy of the currently availab drugs. Here, the most important and most efficient measure is to adopt an integrated disea management strategy. Furthermore, better data, instead of case reports, are required on both t true prevalence of trypanocide resistance and its probable impact on the productivity of livestoc In order to allow a reliable comparison of the data on a temporal and spatial basis, it is of cruc importance that tests for drug resistance are carried out across Africa according to standardiz protocols (Eisler *et al.*, 2003).

3. MATERIALS AND METHODS

3.1. Description of the study area and population

The present study was conducted in Konso district of southern Ethiopia, with most material and logistic assistance offered by the local Bureau of Agriculture and Rural Development. Konso district is a location about 600 Kms distant southwards from Addis Ababa, the capital of Ethiopia and at approximately 370 Kms from regional head-Awassa- on the way to south Omo (Annex I).

3.1.1. Human and livestock population

In the present study area, more than 250,000 people reside who secure subsistence livelihood through mixed agricultural farming practices, as elsewhere in Ethiopia. On the other hand, above 150,000 heads of small east African zebu cattle and more than 500,000 heads of small ruminants (sheep and goats) maintained under traditional village management system with multiple ownership are raised mainly in the low lying areas. Significant numbers of other livestock (poultry, equine, etc.) also exist in the area. Livestock are raised for several purposes, including agricultural activities, home consumption and as a source of additional monetary incomes through sales of live animals in times of need.

3.1.2. Climatic condition and vegetation

The study site has an area of about 200,000ha, with more than 70% being lowland and the remaining a middle zone. Its landscape consists of a heterogeneous topography where mountainous, undulating and rugged topographic terrain dominates particularly in the mid altitude. In this section of the area, the greater half of human population resides resulting i simultaneous episodes of over-crowding, over-ploughing, soil erosion, land degradation an overall resource deterioration. On the other side, the lowlands are the mainstream for the va majority of livestock species and act as a home for the production of a great variety of cere crops.

The area has an altitude of 550-2300 m.a.s.l., with a pronounced rainy season occurring between February and May, and a short rain falling between July and November. The average rainfall is about 750mm per annum. Most of the precipitation falls in April and May, the lowest occurring between October and November. Temperature is generally the lowest from April to June, and the highest from December to March, the annual range of temperature falling between 20^0c and 34^0c. Konso is part of the great southern rift valley system where the vegetative physiognomy is dominated by thorn bushes with some taller Acacia trees, wooded grasses and savanna grassland.

Segen, Yanda and Woito rivers flow through the relatively plain and productive lowlands where the altitude drops to about 500-950 m.a.s.l. These rivers currently serve as the main sources for irrigation-based agriculture along the river basins. It has been indicated that increased agricultural activities as well as continuous hunting by farmers have greatly hampered the existence of wild fauna. Therefore, only few wild animals such as Dik-dik, monkey, apes and warthog are found in considerable numbers (Gemechu *et al.*, 1998).

3.1.3. Disease challenge to livestock sub-sector

The lowlands of Konso area have been reputed to simultaneously harbour the great majority o livestock population and many infectious and protozoal diseases, ecto- and endo-parasti infestation together with various nutritional disorders of animals in different seasons across th year. Among the diseases, tsetse-transmitted animal trypanosomosis has been recorded to be th main impediment to the development of subsistence agriculture in the area, with a mean month prevalence of about 19.5 %. The high infestation of low-lying areas by tsetse flies was register more than a decade back. Moreover, *Glossina pallidipes* was found to be the only tsetse speci prevalent in the study site and the greater section of the southern rift valley system of Ethiop (Trumper, 1994).

Although not yet documented and quantified through systematic surveys, it was believed that t absence of a concerted action among different stockholders to alleviate major animal hea problems, superimposed by frequent shortage of pasture and water, have seriously hampered productivity of livestock sub-sector within the area so that annual losses attributable to livest

diseases have been significantly higher. Nevertheless, the catastrophic morbidity and mortality rates, including low production and productivity particularly in cattle, have been attributed to tsetse-transmitted African animal trypanosomosis (Gemechu *et al.*, 1998).

In order to mitigate the deleterious effects of this menace, the Bureau of Agriculture and Rural Development launched a tsetse control programme between 2003 and 2004, using application of a synthetic pyrethroid formulation (deltamethrine 1 %) to the back of cattle in selected areas with high risk levels of trypanosomosis. Between 2005 and 2006, the Southern Ethiopia Tsetse Eradication Project (STEP) in collaboration with the regional government conducted a tsetse suppression programme by a combination of deployment of insecticide-impregnated targets and application of deltamethrine formulations to the back of cattle. However, socioeconomic surveys were not undertaken so as to assess the impacts and achievements following this control intervention.

3.1.4. Livestock management and farming practices

Small east African zebu cattle tended in large herds by groups of cattle owners graze on communal grasslands around riverbanks in the lowlands. Natural grasses are the main sources of cattle feed while supplementation with crop residues after harvesting is also common. An integrated farming is the dominant form of production where rain-fed agriculture is the commonest production system. Crops such as maize, sorghum, millet, soybean, cotton, 'teff', and different vegetables are produced in both the lowlands and mid-altitude. Agricultural technology employed is the same as that in other parts of Ethiopia. The Bureau of Agriculture and Rural Development often enhances the delivery of agricultural inputs such as fertilizer, insecticides and pesticides.

Although Konso people, both sexes, have long been witnessed extremely hard workers, the highly rugged topographic terrain of the mid-altitude with poor soil fertility and the long-term occurrence of erratic rainfall have aggregately frustrated crop production for several decades. Therefore, recurrent drought and poverty have been common phenomena and the socio-economic

progress of the community has been severely impeded. For this reason, Konso society has so far been subjected to multi-factorial problems.

Currently, however, irrigation-based agricultural projects are being intensively implemented in the lowlands along river basins, which are believed as the key components of poverty alleviation through improving the productive opportunities and living conditions of the rural community. Moreover, the regional government has recently put emphasis on aspects to improve animal productivity through strategies aiming at reducing livestock health problems.

3.2. Study design and sample size determination

In order to successfully accomplish the current research work, combinations of questionnaire interviews and cross-sectional as well as experimental study were conducted.

3.2.1. Questionnaire survey

Field investigations were conducted between September and October, 2007 in selected sites o the study area, with a view to identify areas with high trypanosomosis risk, and those sites highl suspected with drug resistant trypanosome populations. To this effect, two agro-ecologicall distinct study sites, namely, Jarso and Gumaide were selected for subsequent administration o questionnaire interviews. These sites were the mainstreams where more than three-fourth of th livestock resource was maintained, and a contrasting veterinary service delivery system w experienced.

Here, Jarso was a livestock-rearing site that was relatively far from vet clinic and, therefore, h poor animal health delivery system so that cattle owners often purchased drugs from dr smugglers and open markets. On the other hand, Gumaide site was in close proximity to the lo veterinary service, hence with relatively better animal health service delivery. However, previ experience suggested that some CAHWs in this area often provide subnormal treatment doses.

Meanwhile, a structured questionnaire was designed and applied to a randomly selected sample of 100 households, and 4 purposely selected focus groups of 36 key informants (8-10 farmers per group). Therefore, a sample comprising 136 farmers (50 % from each site) was organized so as to acquire baseline information on such important issues as: herd composition, socioeconomic activities, the major livestock health problems and their management strategies; source and usage strategies of trypanocidal drugs; and suspected failure of trypanocidal activities (Annex II). In this study, the questionnaire for subsequent administration to randomly selected household heads and key informants was properly designed and pre-tested in the field in order to modify questions for clarity, and so as to shorten the average time needed to interview a respondent.

Currently, tsetse/trypanosomosis control programs become effective and sustainable through a strong participation of local communities and a concerted action among many other stockholders (Dransfield and Brightwell, 2004). It is beloved that prior evaluation of the potential breadth o the target community to this intervention system is a pre-requisite. Accordingly, in order t evaluate the propensity of Konso community to devote public resources to integrate tsetse/trypanosomosis control approaches so as to sustain disease management strategies throug guarding vector control devices, and ensuring the provision of cost-effective, quality drugs to th suffering community, a questionnaire with both open-ended and semi-close ended question (Annex III) was designed and applied to a total sample of 136 selected households and focu groups (50 % from each study site).

3.2.2. Cross-sectional study to determine the prevalence of bovine trypanosomosis

In efforts to determine the monthly parasitological prevalence of bovine trypanosomosis in t study cattle population, a cross-sectional study was conducted in the rainy season (Septembe October, 2007) as well as the dry months (February-March, 2008) in both of the agro-ecolog zones. A total of 7 herds (3 and 4 herds from Jarso and Gumaide, respectively) comprising of 4 cattle heads were sampled during each season in respective sites to determine the montl prevalence of bovine trypanosomosis (Table 3).

Table 3: Structure of cattle herds and herd sizes included in cross-sectional studies in Konso district, southern Ethiopia.

Agro-ecologic categories	Study areas and sampling sites		Herd size per site
Lowland (560-890 m.a.s.l.)	Jarso	Faro	82
		Morteta	72
		Salla	76
Middle altitude (1760-1990 m.a.s.l)	Gumaide	Lultu	62
		Segen	64
		Birbirsa	60
		Becho	66
Total			482

The sample size required to conduct cross-sectional study was determined based on simple random sampling technique, with a previous prevalence rate of 19.5%; a precision level of 5% and a confidence interval of 95%.

For this purpose, the following formula was employed (Thrusfield, 2005):

$$N = \frac{T^2 \times P_{exp}[1-P_{exp}]}{L^2}$$

Where;

N: the sample size to be determined;

T: student's t-value at 95 % confidence level;

P_{exp}: expected prevalence of trypanosomosis in the study area; and

L: accepted absolute error/level of precision

Therefore, a total of 964 cattle head were selected and sampled during the entire visiting perio The selected animals were, then, ear-tagged in order to facilitate easy identification of eac animal during subsequent monthly visits. Parameters like age, sex, breed, body weight, PC parasitaemia, history of previous treatment, etc. were recorded in a monthly data-recording she

(Annex IV). For estimating body weights of individual animals during sampling visits, the measurement of heart-girth was used.

Monthly blood samples from randomly selected cattle were collected into heparinized capillary tubes by puncturing marginal ear-veins using a sterile lancet, and one end of each capillary tube was sealed with a cristaseal. The collected blood samples were, then, examined through a combination of micro-haematocrit centrifugation (at 12,000 r.p.m for 5 minutes) and a Dark-ground/Buffy Coat microscopic study technique (under 40x objective lens). Packed cell volume values of each blood sample were estimated using a micro-haematocrit reader.

Animals with lower PCV readings (≤ 24 %) were weighed and treated with Diminazene aceturate at the dose rate of 3.5mg/kg body weight. For blood samples in which trypanosomal parasites were detected during microscopic examination, thin smears were prepared, stained with Giemsa solution and examined under oil immersion (100x objective lens) for species identification of trypanosomes (Uilenberg, 1998).

3.2.3. Estimation of apparent tsetse density

In order to determine the current apparent density of tsetse flies and other vectors, and assess their relative importance in bovine trypanosomosis in the study areas, biconical traps odour-baited with cow-urine were deployed at an interval of about 100 meters during both the rainy and dry season through the study sites. For this purpose, a total of 80 biconical traps (40 traps at Jarso and 40 at Gumaide) were deployed in areas like grazing lands, along river banks, wet and dense areas, etc. Cow-urine as an odour attractant was regularly brought and placed in locally available containers, which were permanently kept under each trap and regularly topped up with urine on weekly basis. All the traps were coated with grease in order to prevent the trapped flies from being prayed out by ants and other predators. The traps were emptied at an interval of twenty-four hours.

Identification of the flies into their species level was carried out on the basis of morphologica features (Leak, 1999), such as specific colourations of their abdominal and tarsal segments o

their front legs. To facilitate this technique, a magnifier hand lens was employed. Distinguishing tsetse flies from other mechanical vectors was based mainly on their external appearances and microscopic structures like the hatchet cell on their wings-a structure unique for tsetse. Sexing of tsetse flies was based on the genital structure of respective sexes. The number of fly catches was properly recorded at every 24 hours interval.

3.2.4. Trypanocidal drug sensitivity testing in cattle experimentally infected with field isolates of *T. congolense*

In order to assess the therapeutic and prophylactic efficacy of the commonly used trypanocidal drugs (Diminazene aceturate and Isometamidium chloride), field isolates of *T. congolense* were randomly collected from parasitaemic cattle at Jarso study site. For this purpose, ten calves of about 5 to 6-month old were selected from small east African zebu cattle, obtained from, and kept at, Durro experimental site (2268 m.a.s.l.). Then, *in-vivo* experimental infection with the trypanosome isolates (one stabilate per five calves) was made by intravenous injection. The main features of the experimental calves at the time of inoculation are summarized in Table 4.

Table 4: Major characteristics of experimental animals at the time of inoculation with *T. congolense* isolates in Konso district, southern Ethiopia

Animal categories	ID No.	Sex	Age in days	Body weight (kg)	PCV value (%)	*T. congolense* isolate inoculated
Treatment group	K0101	M	159	88.5	26	ET/07/Konso 59
	K0102	M	176	98.0	28	ET/07/Konso 59
	K0103	F	158	88.0	27	ET/07/Konso 59
	K0104	M	167	92.9	25	ET/07/Konso 59
	K0105	F	181	100.7	28	ET/07/Konso 59
	K0106	F	163	90.7	24	ET/07/Konso 114
	K0107	M	171	96.8	27	ET/07/Konso 114
	K0108	M	178	99.3	26	ET/07/Konso 114
	K0109	F	175	98.5	27	ET/07/Konso 114
	K0110	M	180	100.1	26	ET/07/Konso 114
Control group	K0111	M	174	97.0	28	_
	K0112	M	170	96.5	25	_
	K0113	F	169	94.2	27	_
	K0114	M	179	100.1	29	_
	K0115	M	171	95.8	26	_

Experimental design

A month prior to the commencement of the experimental work, experimental animals wer moved to a fly-proof accommodation and treated with long-acting oxytetracyline, anthelmint (Albendazole, 2500mg), ivermectin, and Diminazene aceturate at recommended dose rates. Aft two weeks, the animals were examined for presence of trypanosomes in their blood. Startir from this day and continuing until the end of the experiment, PCV and parasitaemia we monitored three times per week by examination of peripheral blood from marginal ear-vei through Dark ground/Buffy coat microscopic technique. Moreover, the calves were al examined, on daily basis, for clinical conditions throughout the study period (Eisler *et al.*, 2001

Isolation and inoculation of trypanosome isolates

Parasitological examinations of blood samples, by a combination of micro-haematocrit centrifugation and Buffy coat methods, were conducted in the field at Jarso site in order to identify cattle naturally infected with heavy scores of trypanosomal parasitaemia. Thin blood smears stained with Giemsa solution were prepared and examined for species identification of trypanosomes. Two adult cattle heads (ID No. K59 and ID No. K114) were randomly selected from the cattle having heavy scores of parasitaemia (*T. congolense strain*). The isolates were named as ET/07/Konso 59 and ET/07/Konso 114, according to (FAO, 2003).

Blood samples from the jugular veins of these cattle were collected into EDTA- treated vacutainer tubes, placed in liquid nitrogen and carried to the site of the experiment (Durro). After confirming the viability of the trypanosome stabilates microscopically (Annex V), the isolates were injected into the jugular veins of the experimental calves that were found negative for trypanosomal parasites upon previous examination.

Treatment and monitoring

The experimental calves inoculated with the trypanosome isolates were regularly monitored for clinical and parasitological parameters. When the first peak of parasitaemia was detected, they were weighed and treated, on the same day, intramuscularly with Diminazene aceturate at a dose rate of 3.5 mg/kg body weight. For calves in which relapse/breakthrough infections were detected after the treatment with Diminazene aceturate, clinical and parasitological monitoring was carried out every three other day for 45 days in order to obtain basic information on the pathogenecity of drug resistant trypanosomes.

Based on the consideration of parasitological and clinical examination, the relapsed calves whose PCV values had revealed a fall by one-fifth of their value at the time of relapse, and those with significantly deteriorated clinical manifestations, were intramuscularly treated, on day 60, with Isometamidium chloride at a dose rate of 0.5 mg/kg body weight. All experimental animals were monitored and followed up for 100 consecutive days (December 15/2007-March 24/2008) when they were maintained on natural grass supplementation and water *ad libitum*.

3.3. Data management and statistical analysis

Both qualitative and quantitative data, collected through a combination of questionnaire interviews, cross-sectional as well as experimental study designs were handled properly in MS Excel spread sheets and analyzed carefully.

Information that was generated through questionnaire survey on livestock management practices basic health problems and usage strategies of trypanocidal drugs was summarized and analyzed in the form frequency distribution and percentage expressions in diagrammatic forms. For the determination of mean annual costs incurred by trypanocidal drug treatment at the level of households, cattle were first categorized into young (4 years of age and below) and adult (above 4 years) cohorts and mean treatment costs for single injection were estimated accordingly for each cohort. Furthermore, average holdings of cattle per household were estimated in each site. Finally, the mean annual expenditure on trypanocidal drugs per households was estimated as the function of the two variables.

In order to quantify the socioeconomic impacts currently posed by tsetse/trypanosomosis on livestock production and other agricultural indicators, data collected during the present survey time were analyzed and compared with the corresponding values in 1997/8 (Gemechu *et al.* 1998). Data obtained through application of contingent valuation technique were analyzed in efforts to ascertain household-level factors that were hypothesized as influencing respondents' willingness to contribute cash and/or labour time to integrated tsetse/trypanosomosis control. Therefore, several factors were selected and defined as explanatory variables, whereas money and labour time were defined as dependent variables.

A series of simple linear regression models were, then, fitted to the data and analyzed for potential monthly contribution of money, then for labour time and finally for both money and labour contributions. In this case, the occurrence of each type of contribution was considered to follow a mutually exclusive event. Here, money was defined as the maximum amount of Ethiopian Birr that respondents would be willing to volunteer per month, and labour was defined as the maximum number of days that respondents would be willing to volunteer each month. For ease of mental constructs, an integrated tsetse/trypanosomosis control strategy was considered

be one that encompasses reduction of tsetse population through deployment of traps/targets, application of insecticides to the back of cattle, and legal provision of quality trypanocidal drugs to local communities.

In cross-sectional study, the monthly prevalence of bovine trypanosomosis and hematological values were presented and analyzed. Monthly prevalence of bovine trypanosomosis was expressed as the number of parasitologically positive animals through Buffy coat microscopic study to the total number of animals examined (%) at a particular visiting time. Therefore, the magnitude of trypanosomal infections was determined in terms point prevalence of the disease in respective sites. Hematological findings were expressed as percentage of the red blood cells to the total blood content centrifuged. In order to compare the monthly prevalence of bovine trypanosomosis within cattle herds and between the two agro-ecologic areas, a normal distribution test (z-test) was applied. The herds mean PCV between parasitaemic and aparasitaemic animals in both sites was also compared using the same technique.

On the other hand, linear regression analysis model was employed to assess the relationship between parasitological prevalence of trypanosomal infections and herd average PCV. This relationship was established using the estimates of herd average PCV readings as dependent variable and the prevalence of trypanosomal infections as an explanatory variable.

In the case of estimating the apparent fly density, the number of flies caught was determined by counting their species, sex and other relevant variables. Fly population in respective study sites was estimated in terms of the relative fly density, which was calculated as fly number per trap per 24 hours. One-way ANOVA test was applied to determine the significant variations in the mean daily catches of the respective fly vectors.

For data obtained from drug sensitivity testing in experimentally infected animals, information on the occurrence of relapse infections was carefully analyzed and interpreted. The average time between treatment and the occurrence of relapse infections was determined so as assess the efficacy of Diminazene aceturate and Isometamidium chloride. Information on deterioration of clinical conditions and parasitological parameters was summarized to estimate the pathogeneci

of drug-resistant trypanosome strains. The prevalence of relapse/breakthrough infections in experimental calves was calculated as the number of animals with relapse/breakthrough infections on the day of monitoring divided by the total number of animals examined at that particular day. Hence, interpretation of the results on this entity was made based on the standardized experimental protocols described for drug sensitivity testing in cattle (Eisler *et al.*, 2001).

In this study, a 95 % confidence level was employed for most of the quantitative data, in order to extrapolate sample results to the target population in the study area. All the data used for description as well as inference purposes were analyzed by the help of Statistical Package for Social Sciences (SPSS, Version 15.0) and Microsoft Excel progarmmes of the computer software.

4. RESULTS

4.1. Questionnaire survey

Structured questionnaire interviews were administered to a total of 136 households and key informants in order to acquire baseline information on socioeconomic activities and other essential issues pertaining to livestock health and resistance to trypanocidal drugs, as perceived by farmers. Response rate of the questionnaire interviews was 100 %. The main findings of the present questionnaire survey are summarized under the following headings:

4.1.1. Socioeconomic characteristics of sampled households

The great majority of the sampled households (97.8 %) who survive now in the study areas are from the indigenous ethnic group (Konso community) where over half of them are in the productive age category (25-35 years), with the mean household size of 6.83 (3-12). However, the household heads involved in this study were relatively older in Gumaide sites than those in Jarso area, although the difference was not statistically significant ($p>0.001$). Indeed, majority of the sampled households (about 89 %) in both study sites had little/no formal education (Table 5).

Table 5: Socioeconomic peculiarities of sampled households in Konso district, southern Ethiopia.

Age category (% age classes)		Ethnic composition		Major means of livelihood (%)		Formal education (%)		Mean livestock holdings/household*	
25-35 years	58.8	Konso	97.8	Mixed farming	96.3	0 years	88.9	Cattle	8.5 (1.6)
36-45 years	31	Oromo	1.5	Off-farm job	2.9	1-3 years	9.6	Sheep/goat	9.7 (1.4)
>45 years	10.2	Amhara	0.7	Trade	0.8	> 3 years	1.5	Draft oxen	4.3 (0.91)

*, Standard deviations were reported in the parentheses.

On the other hand, nearly all of the respondents (99.3 %) indicated that they thrive on a subsistence mixed crop-livestock farming system, where draft oxen play a role of great importance in tilling croplands. According to the testimony of interviewed farmers, livestock are used for direct home consumption, agricultural activities and as a source of monetary income through the sale of live animals in times of need.

4.1.2. Livestock management practices and major animal health problems

The interviewed respondents indicated that cattle were often tended in communal herds and allowed for free grazing mainly on natural grasslands, though supplementation with crop residues (aftermath) following harvesting times was also common. Cattle usually grazed around rivers and thereabout, obtaining water from small local ponds and rivers, with an average distance of 2.5 kilometers from homesteads to main rivers. It was revealed that livestock feed was relatively abundant during rainy months of the year (April-October) whereas feed deficits become severely common in the dry season (December-March).

Livestock owners wholly (100 %) disclosed that there have long been multifactorial livestock health problems in their respective localities, with animal diseases and recurrent drought being the commonest ones among several others. Among the several diseases affecting livestock in the areas, respondents incriminated animal trypanosomosis, contagious caprine pleuropneumonia (CCPP)/ contagious bovine pleuropneumonia (CBPP), various endo- and ecto-parasites as the most important livestock health problems posing severe annual losses to livestock sub-sector (Table 6).

However, as indicated by results of the study, most of the annual livestock losses were attributed to animal trypanosomosis, a serious disease affecting several livestock species (Table 6 and Figure 3). In most of farmers' perceptions, animal trypanosomosis was an endemic livestock problem occurring, at least, since the last 15 years, with higher challenges occurring during rainy months of the year (April-October). The interviewed respondents described that animal trypanosomosis was characterized, among several other signs, by depression, rough hair coat, reduced appetite, emaciation, weakness and, thus, a reduction in the working power of their draft

oxen. The great majority of livestock owners attributed the transmission mode of trypanosomosis to flies, but none of them were able to distinguish tsetse from biting flies.

Table 6: Farmers' perceptions of the main diseases responsible for annual livestock losses in Konso district, southern Ethiopia

Category of livestock losses	Frequency of main diseases (%) causing livestock death (n = 136)				
	Animal trypanosomosis	CCPP /CBPP	Endo-parasites	Ecto-parasites	Unknown causes
Crude death rate	76.4	13.2	3.6	6.1	0.7
Adult mortality	89.6	9.1	0.4	0.8	0.1
Calf/lamb mortality	63.9	22.8	5.3	7.2	0.8
Abortion	91.9	8.0	0.0	0.0	0.1
Stillbirth*	84.5	12.4	1.4	0.2	1.5

*: Expressed as percentage of pregnant female animals

Sixty seven percent of the households witnessed that there has been a decreasing tendency in the occurrence and impacts of animal trypanosomosis, at least, since the last 8 years. Nevertheless particularly in Jarso site where highly promising irrigation-based agricultural projects have currently been under implementation as setouts to improve food security, most of the respondents claimed that there had never been any improvement in the occurrence and impacts of trypanosomosis in their localities. For this reason, they were desperate that if such a problem remained unsolved, their livelihoods would be highly hampered.

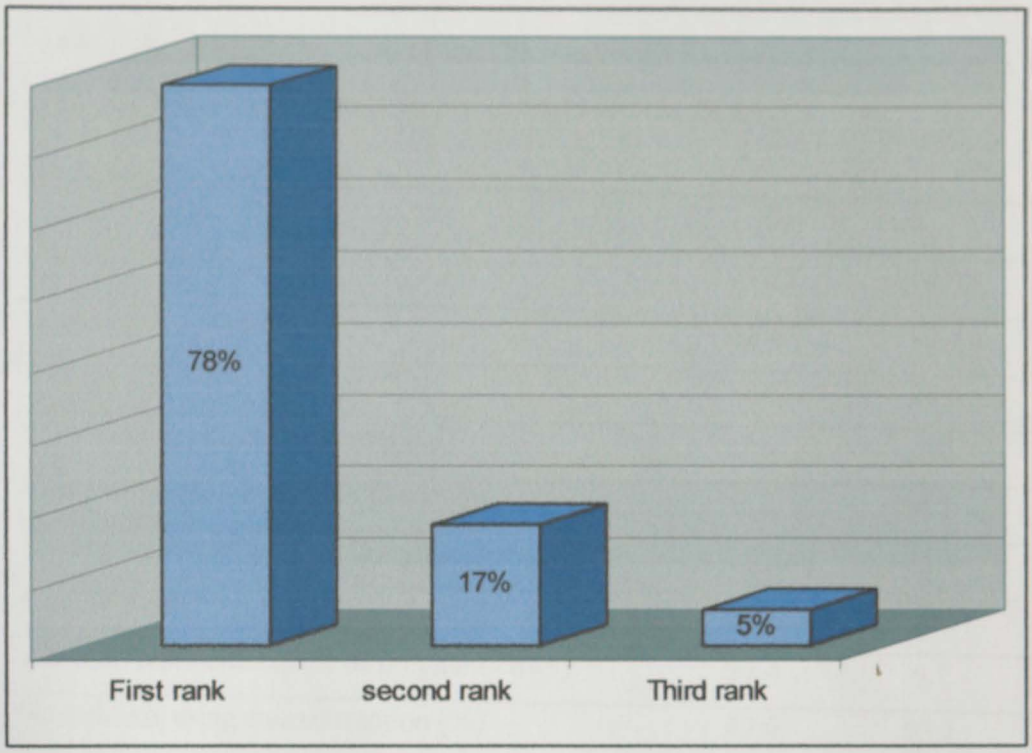


Figure 4: Relative importance of animal trypanosomosis to other livestock diseases, as ranked by farmers in Konso district, southern Ethiopia (n = 136).

4.1.3. Socioeconomic impacts attributed to animal trypanosomosis

Livestock owners perceived that the most important livestock production trait highly affected by animal trypanosomosis was cattle mortality, irrespective of sex-age groups. By comparing current annual cattle losses due to trypanosomosis with the corresponding figures in the previous survey years (1997/8), the results revealed that the percentage of households with cattle, and those using animal traction were increased by about 34 % and 51 %, respectively, during the next 10 years (Table 7). Furthermore, the overall proportion of cattle dying due to trypanosomosis consequences were reduced from 23.5 % to 11.8 %, whereas both stillbirth and abortion rates were diminished more than three folds over the same period.

Table 7: Farmers' perceptions of the changes in the impacts of trypanosomosis and its control o cattle production in Konso district, southern Ethiopia

Health indicators and other focus points	Survey years		Changes (%)
	1997/8[a]	2007/8	
Households with cattle (%)	58.6	92.7	+34.1[b]
Households with draft oxen (%)	61.0	94.6	+33.6[b]
Mean holding of cattle /household	3.6	8.5	+4.9[c]
Mean holding of draft oxen/household	1.2	4.3	+3.1[c]
Overall cattle mortality from trypanosomosis	23.5	11.8	-11.7
Calf mortality	58.1	15.3	- 42.8[b]
Abortion and stillbirth (% of pregnant cows)	27.4	9.1	-17.7
Households using animal traction (%)	42.0	93.1	+51.1[b]
Average period of field traction by oxen (hours)	3.2	6.8	+3.6

[a]: Survey data from Gemechu *et al.* (1998); [b]: changes were significant at 0.001 level; [c]: changes wer significant at 0.05 level.

As indicated in the table above, in all categories of respondents (individual vs. group), th socioeconomic impacts imposed by tsetse/trypanosomosis were invariably ranked as:

1. A reduction in the overall health condition of herds, causing high livestock losses;
2. A diminution in working power of draft oxen, resulting in shorter period of fiel traction;
3. Lesser/no access to cultivable lands and grazing areas due to an increase in nuisanc flies.

On the other hand, the results revealed that the successful tsetse control over the past few yea resulted in significant reduction in annual livestock production losses and improved access t pasture and water resources. However, about 7 % of the interviewed respondents have contende that tsetse/trypanosomosis could not influence the expansion of arable lands and access to pastu and water.

4.1.4. Use of trypanocidal drugs and the problem of drug resistance

Respondents all in all (100 %) reported Diminazene aceturate and Isometamidium chloride to be the most common trypanocidal drugs used for the treatment of cattle against trypanosomosis. As indicated by results of the present study, Konso community most frequently used curative trypanocidal drugs (69.8 %) than prophylactic drugs (26.5 %). Seventy-nine percent of the interviewed households witnessed that they have been using these drugs at least for the last 15 years, and this phenomenon of drug usage showed an increasing tendency for the last 10 years (Table 8). On the other hand, most of the farmers (98 %) claimed that they had quitted the use of Homidium tablets over the past 8 years because of its lower trypanocidal effects and abortion problems associated with its use in pregnant animals.

Table 8: Changes in the use and costs of trypanocidal drugs as perceived by livestock owners in Konso district, southern Ethiopia

Focus points on trypanocidal drug usage pattern		Survey years		Changes (%)
		1997/8[a]	2007/8	
Livestock owners using trypanocides (%)		79.51	92.30	+12.79[b]
Proportion of cattle treated per year at household level		74.63	79.81	+5.18[b]
Mean annual incidence of treatment		9.85	7.50	-2.35
Mean cost of single injection	Young (≤ 4 years)	1.63	2.70	+1.07
	Adults (> 4 years)	3.25	5.40	+2.15[b]
Mean annual expenditure on trypanocidal drugs (Birr)	Per head of cattle	32.01	40.50	+8.49[c]
	Per household	86.00	274.75	+188.75[c]

[a]: Survey data from Gemechu *et al.* (1998); [b]: changes were significant at 0.001 level; [c]: changes were significant at 0.05 level.

According to the testimony of the households sampled, majority of the farmers (86.9 %) often treated only sick cattle, focusing mainly on matured cows and oxen. Moreover, the report indicated that nearly 70 % of the farmers acquired trypanocidal drugs from open market

CAHWs and drug smugglers (Figure 4) where most of the treatments of livestock against animal trypanosomosis were delivered by non-professional persons (Figure 5).

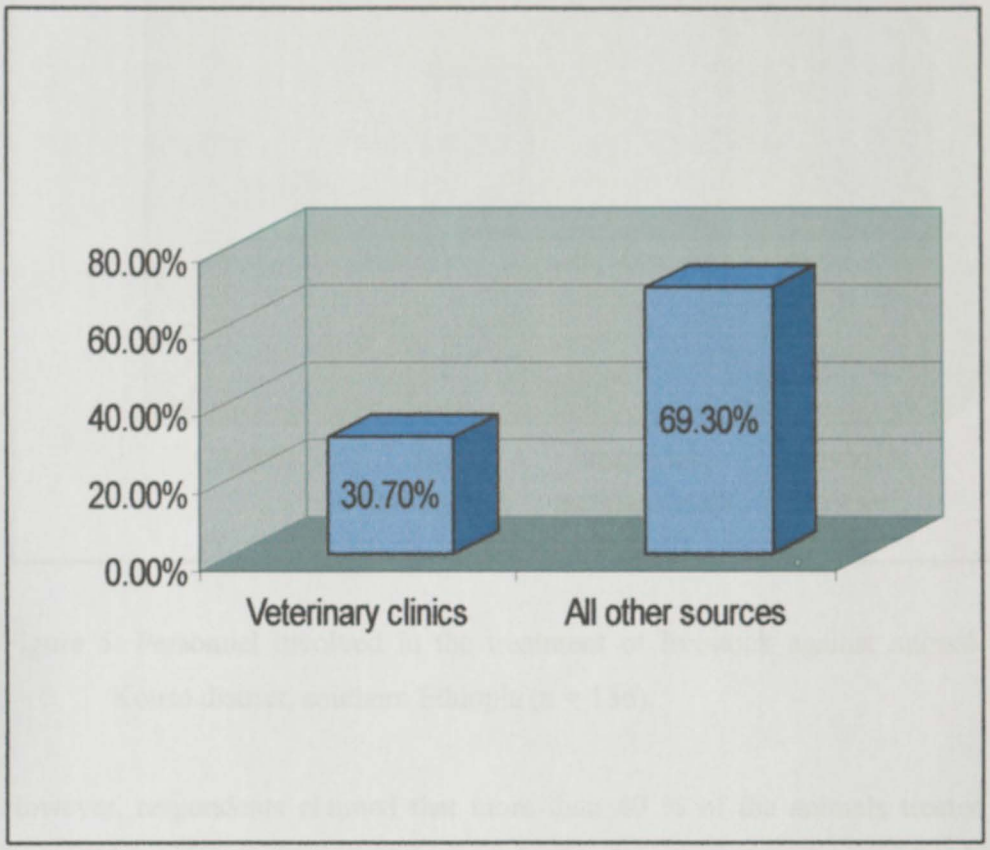


Figure 4: The main sources of trypanocidal drugs used to treat animal trypanosomosis in Kons district, southern Ethiopia (n = 136).

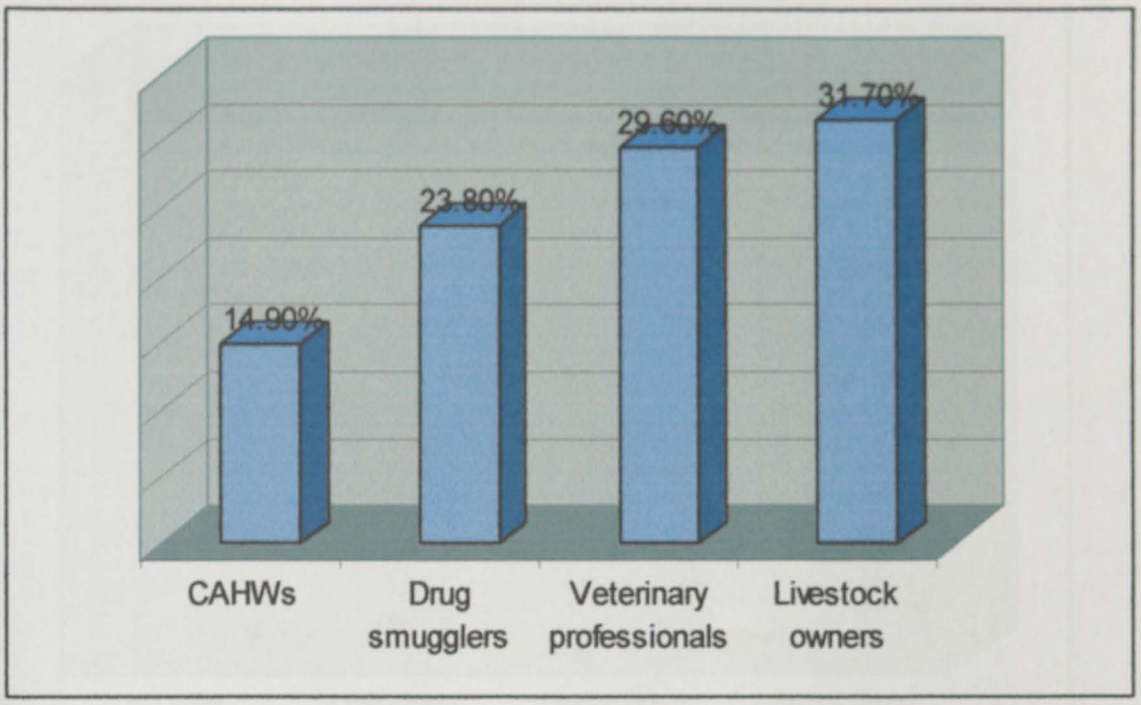


Figure 5: Personnel involved in the treatment of livestock against animal trypanosomosis i Konso district, southern Ethiopia (n = 136).

However, respondents claimed that more than 40 % of the animals treated with trypanocid drugs did not recover from trypanosomosis, despite repeated treatments. In addition, results of th questionnaire survey on trypanocidal dosage regime revealed that statistically significa proportions of the total trypanocidal drugs were applied below the manufacturers' recommende doses (Figure 6).

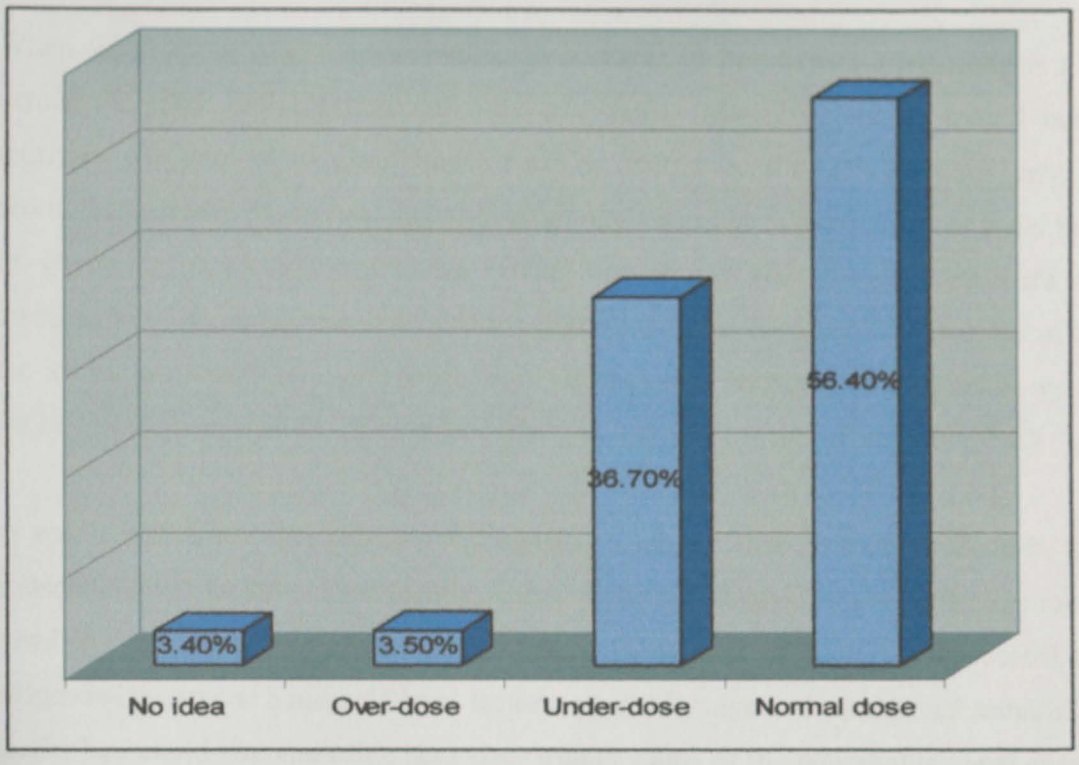


Figure 6: Dosage patterns of trypanocidal drugs as applied by livestock owners for the treatmer of animal trypanosomosis in Konso district, southern Ethiopia

4.1.5. Application of contingent valuation technique to assess people's propensity

Results on the application of contingent valuation revealed the animus propensity (100 %) Konso community to take active parts in a sustainable, integrated approach for the control tsetse/trypanosomosis. The potential monthly contributions, mainly for labour time, we generally high across all the sampling sites. About 35 %, 58 % and 12 % of the sampl households showed a willingness to volunteer monthly contributions in terms of money, labou and both money and labour time, respectively.

On average, sampled households who volunteered only cash or labour contributions indicated propensity to pay 4.35 Ethiopian Birr and 7.82 labour days per month. Among those w volunteered both money and labour contributions, a mean monthly willingness of 3.42 Ethiopi Birr and 4.61 days was indicated.

When asked about what measures ought to be taken to protect tsetse traps/targets from loss o damage by theft and other vandals, the households wholly agreed to control bush-fire an motivate one another to guard traps/targets as would be for individual property. They als promised to detect, expose and punish drug smugglers either in local courts or under the authorit of government/project implementers. Those households who secure subsistence livelihood through pure mixed farming indicated the highest average monthly contributions of labour, bu the lowest propensity for cash contribution. On the other hand, the reverse trends were observe for households engaged in off-farm jobs (daily labour work, trade, office work, etc.).

It was hypothesized that people's willingness to support disease control would be affected b household-level factors. Consequently, linear regression analysis on these factors revealed that whether the respondents would be willing to volunteer cash or labour contributions wa influenced by several household-level factors, especially: age and educational status of household heads; household size and cattle herd size; wealth status of the households ; land ownership an size of cropland (Table 9).

Table 9: Linear regression of determinants influencing the propensity of the community t support integrated disease control in Konso district, southern Ethiopia (n = 136)

Major determinants (Household-level factors)	Coefficient of regression (β) and its significance*		
	Money (Birr)	Labour-time (days)	Money and labou
Age of household head	0.77[a]	0.45[b]	0.47[a]
Educational status of household head	0.81[a]	0.53[b]	0.57[b]
Household size	0.55[b]	0.79[b]	0.69[a]
Wealth status (capital) of household	0.66[a]	0.50[b]	0.67[a]
Cattle herd size	0.59[a]	0.83[a]	0.61[b]
Size of cropland	0.62[b]	0.76[b]	0.89[a]

*: [a], Values were statistically significant at 0.001 level; [b], Values were statistically significant at 0.05 leve

4.2. Cross-sectional study on bovine trypanosomosis

Cross-sectional study on bovine trypanosomosis was conducted on randomly selected villag cattle heads. For this purpose monthly blood samples were examined through a combination c micro-haematocrit centrifugation and Buffy coat microscopic studies.

4.2.1. Parasitological prevalence of bovine trypanosomosis

Blood samples from a total of 964 heads of communally managed village cattle were examined a 7 sampling sites (Morteta, Salla, Faro, Birbirsa, Lultu, Segen and Becho) during the rainy as we as the dry seasons. Major aspects of the cross-sectional study are summarized in Table 10 belov Accordingly, out of a total of 230 heads of cattle at Jarso and 252 heads at Gumaide whose bloo samples were examined during rainy months (September-October, 2007), trypanosome parasite were detected in 45 (19.6 %) and 43 (17.1 %) animals, respectively. The corresponding record during dry months (February- March, 2008) for these agro-ecologic areas were 36 (15.6 %) an 33 (13.1 %) animals, respectively.

Table 10: Parasitological results of cross-sectional study at seven sampling sites of two agro ecologic areas in Konso district, southern Ethiopia (N = 964).

Agro-ecologic areas and sampling sites		Sample size per season		Mean parasitological prevalence (± 1 S.E	
		Rainy season	Dry season	Rainy season	Dry season
Jarso	Faro	82	82	18.6 ± 1.2	13.2 ± 0.5
	Morteta	72	72	19.9 ± 0.2	14.3 ± 1.1
	Salla	76	76	20.4 ± 0.1	17.3 ± 0.4
Subtotal		230	230	19.6 ± 0.4	15.6 ± 2.1
Gumaide	Lultu	62	62	14.7 ± 1.4	13.2 ± 1.9
	Segen	64	64	14.8 ± 1.2	12.9 ± 0.8
	Birbirsa	60	60	15.9 ± 0.6	13.7 ± 0.7
	Becho	66	66	14.5 ± 0.1	12.6 ± 0.3
Subtotal		252	252	17.1 ±1.1	13.1 ± 1.3

The trypanosomal infections varied among the sampling sites of the two areas, especially during the rainy season. Among the seven sampling sites, the highest mean parasitological prevalence (20.4 %) was recorded at Salla site in Jarso whereas the lowest (12.6 %) was detected at Becho site in Gumaide. On the other hand, among the positive animals detected at both the study sites throughout the entire visiting period, 133 (84.47 %), 19 (12.10 %) and 5 (3.43 %) cases were due to *T. congolense*, *T. vivax* and mixed infections (due to *T. congolense* and *T. vivax*), respectively. In each case, the monthly prevalence of *T. congolense* infection was significantly higher ($p<0.001$) than the infections due to *T. vivax* and mixed species.

Comparing trypanosomal infection on spatial basis, the mean monthly prevalence was significantly higher ($p<0.001$) in Jarso than those in Gumaide during rainy months. Temporally however, the mean monthly prevalence during dry season did not show statistically significant differences between the two areas ($p>0.01$). Nevertheless, the reduction in mean parasitological prevalence of trypanosomal infection from rainy season to dry season at both sites revealed statistically significant changes ($p<0.05$).

4.2.2. Haematological examination

The average PCV readings and distribution patterns of parasitologically positive and negative animals are summarized in Table 11, Figures 7 and 8 below.

As indicated in Table 11, the average PCV readings of both parasitaemic and aparasitaemic animals in Gumaide area were slightly higher than the corresponding average values in Jarso site. Thus, the differences in PCV values for parasitaemic and aparasitaemic animal categories were statistically significant ($p<0.05$). On the other hand, the overall patterns of mean PCV readings for both parasitaemic and aparasitaemic animals are depicted in the following figures (Figure 7 and 8).

Table 11: Haematological results of cattle herds at seven sampling sites of Konso district southern Ethiopia

Sampling sites	Sample size per season		Mean PCV (± 1 S.E)	
	Rainy season	Dry season	Parasitaemic animals	Aparasitaemic animals
Faro	82	82	26.4 ± 0.2	28.1 ± 0.8
Morteta	72	72	25.7 ± 0.1	27.0 ± 1.3
Salla	76	76	25.1 ± 0.5	27.3 ± 0.5
Subtotal	230	230	25.7 ± 0.3	27.5 ± 0.5
Lultu	62	62	27.1 ± 0.5	29.2 ± 0.2
Segen	64	64	26.7 ± 0.7	28.7 ± 0.5
Birbirsa	60	60	26.8 ± 0.3	28.9 ± 1.1
Becho	66	66	26.9 ± 0.4	28.8 ± 0.3
Subtotal	252	252	27.3 ± 0.2	28.9 ± 0.6

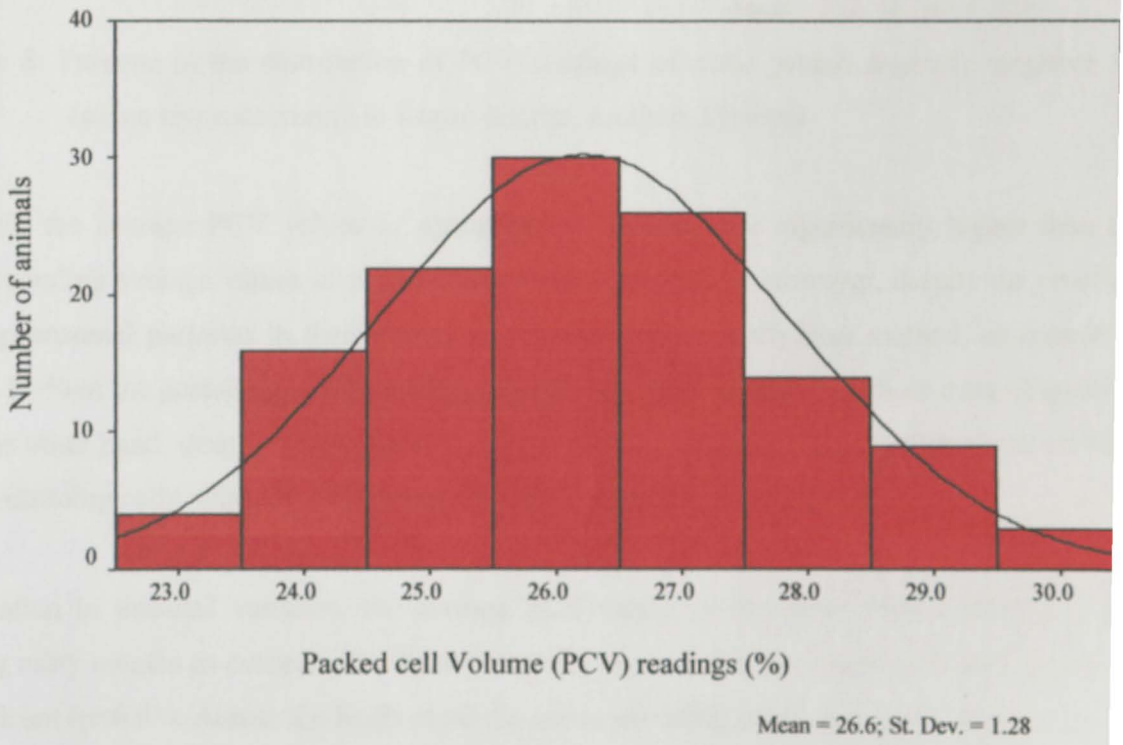


Figure 7: Patterns in the distribution of PCV readings of parasitologically positive animals in Konso district, southern Ethiopia.

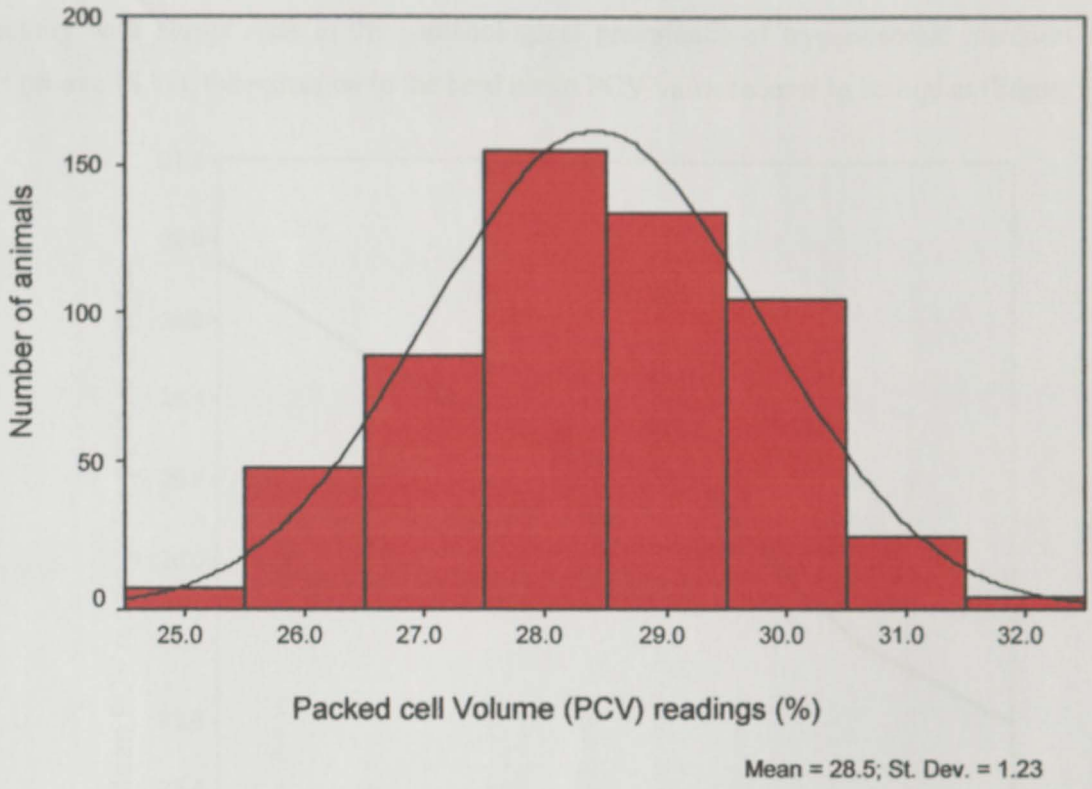


Figure 8: Patterns in the distribution of PCV readings of cattle parasitologically negative f bovine trypanosomosis in Konso district, southern Ethiopia

Overall, the average PCV values of aparasitaemic animal were significantly higher than t corresponding average values of parasitaemic animals ($p<0.001$). However, despite the presen of trypanosomal parasites in their blood as witnessed by the Buffy coat method, an overall about 15 % of the parasitologically positive animals had PCV values of 28 % or more (Figure 7 On the other hand, despite the absence of trypanosomal parasites in their blood, about 10 % the parasitologically negative animals had PCV scores below 26 % (Figure 8).

In relation to seasonal variation, the average PCV values of the herds were relatively high during rainy months as compared to those values for dry months, the variation being statistica significant ($p<0.05$). Across the herds at all the seven sampling areas, a negative association w demonstrated between the herd average PCV readings and parasitological prevalence trypanosomal infections.

Especially with abrupt rises in the parasitological prevalence of trypanosomal infection in the herds (above 18 %), the reduction in the herd mean PCV values seems to be higher (Figure 9).

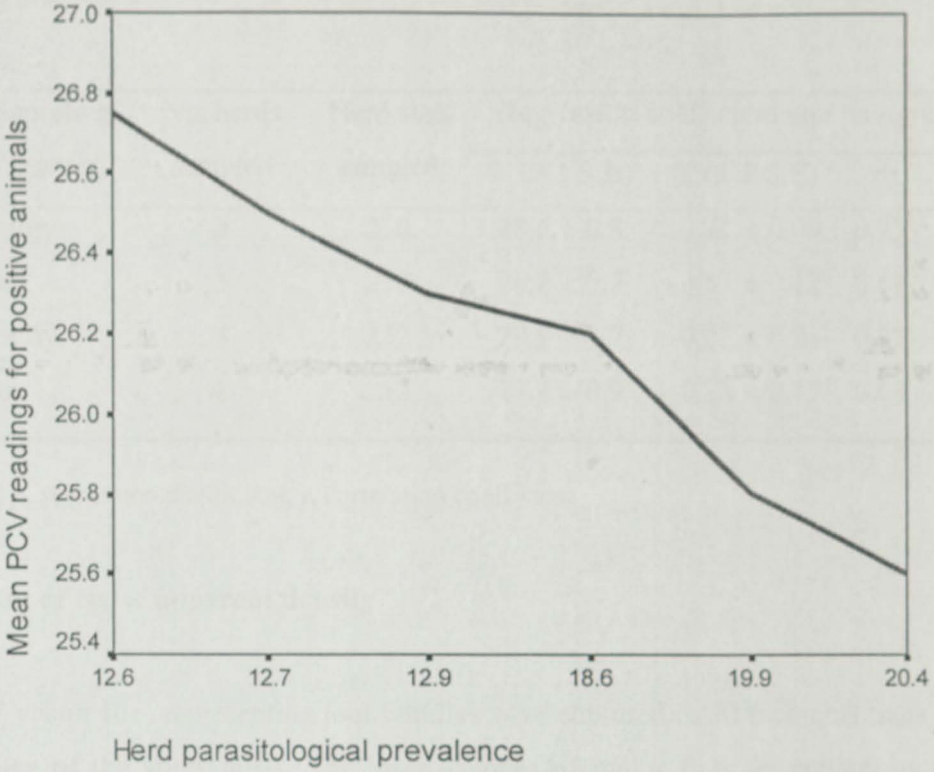


Figure 9: The relationship between herd mean PCV and the parasitological prevalence o trypanosomal infections in cattle in Konso district, southern Ethiopia.

In addition, linear regression analyses of herds mean PCV readings on the prevalence o trypanosomal infection revealed that the PCV value of parasitologically positive anima significantly decreased ($p<0.001$) with increases in the prevalence of trypanosomal infectio (Table 12).

Table 12: Linear regression of herd means PCV on the prevalence of trypanosomal infection in two agro-ecologic areas of Konso district, southern Ethiopia, during the rainy and the dry seasons

Sampling area	Sampling season	No. herds sampled	Herd size sampled	Regression coefficient and its significance*			
				α (± 1 S.E)	β (± 1 S.E)	r	P-value
Jarso	Rainy	3	230	28.6 ± 0.8	-0.21 ± 0.09	0.73	0.0004
	Dry	3	230	26.2 ± 0.5	-0.43 ± 0.12	0.69	0.0001
Gumaide	Rainy	4	252	29.9 ± 0.7	-0.37 ± 0.05	0.82	0.0001
	Dry	4	252	27.4 ± 0.9	-0.28 ± 0.17	0.63	0.0002

*: α, intercept; β, regression coefficient; r, correlation coefficient

4.3. Estimation of tsetse apparent density

A total of 287 vector flies representing four families were captured in 80 biconical traps deployed in selected sites of the study areas. The mean catches of major flies (expressed as the mean number of flies caught per trap per day) in each season of respective sites are summarized in Table 13 below.

Table 13: Summary of the major fly vectors trapped at seven sampling sites in Konso district, southern Ethiopia

Sample areas	Sample Seasons	Composition and mean catches (fly/trap/24hours) of main vectors*							
		Glossina		*Tabanus*		*Stomoxys*		*Hippobosca*	
		Total	Mean	Total	Mean	Total	Mean	Total	Mean
Jarso	Rainy	67	2.95	26	1.31	21	1.05	17	0.85
	Dry	36	1.80	8	0.42	5	0.25	3	0.15
Gumaide	Rainy	39	1.94	16	0.80	13	0.65	9	0.45
	Dry	15	0.75	7	0.35	3	0.15	2	0.10
Total		157	1.86	57	0.71	42	0.53	31	0.38

*: Significantly higher mean catches recorded for *Glossina* compared to other vectors in both seasons ($p < 0.05$)

As indicated in Table 13, *Glossina* species formed the predominant proportion of the fly catches (54.47 %) throughout the entire survey period, followed by *Tabanus* (19.86 %) , *Stomoxys* (14.63 %) and *Hippobosca* (10.80 %). Thus, the results of the analysis of one way-ANOVA for mean catches of the flies indicated that the mean daily catches of *Glossina* species were in statistically significant excess ($p < 0.05$) than the corresponding means of other vectors both during the rainy and during the dry seasons in all the sampling sites.

For all the flies, the proportion of female population revealed a higher preponderance (65.8 %) over the male population (34.2 %). Furthermore, mean daily catches of the various vectors considerably varied both spatially and temporally, with the highest mean catches recorded in Jarso area. Across the sampling sites, the overall fly catches were relatively higher in the rainy months than the corresponding figures in the dry season.

In this study, all the *Glossina* species captured at each sampling site, irrespective of season totally belonged to only sub-group, namely *G. pallidipes*. In this case, however, the mean daily catches of this fly during the rainy season were significantly higher ($p < 0.05$) than its corresponding values during the dry season. In addition to this, the variation in the mean catche

of *Glossina* species between Jarso and Gumaide areas, both during the rainy and dry month revealed statistically significant differences ($p<0.001$). However, the corresponding records di not reveal statistically significant spatial and temporal differences ($p>0.05$) for the rest fl vectors.

4.4. Trypanocidal drug sensitivity testing in experimentally infected calves

Trypanocidal activities of Diminazene aceturate and Isometamidium chloride were assessed i ten calves experimentally infected with field isolates of *T. congolense* collected from randoml selected cattle at Jarso study area. The first peaks of parasitaemia in the experimental calves wer detected between 13-15 days following intravenous inoculation of the trypanosome isolates unde investigation. During this period, the infected calves manifested typical clinical signs c trypanosomosis such as: depression, fever, inappetance, swelling of pre-scapular and pre-femor lymph nodes, rough hair coat, and overall reduction in PCV.

Until day 24, which is 9 days after intramuscular treatment with Diminazene aceturate, neither c the calves revealed relapse/breakthrough infections. However, 12 days after treatment wit Diminazene aceturate (on day = 27), relapse infection was detected in one of the calves (ID No K0101) and the same phenomenon were also manifested in an additional calf (ID No. K0104) 1 days following treatment (on day = 30). Afterwards, this relapse persisted until the time when th calves were treated with the second trypanocidal drug, namely Isometamidium chloride (on da 60). On the other hand, no relapse/breakthrough trypanosomal infections were detected in any c the remaining eight calves until the termination of the experiment (Table 14).

Table 14: Patterns of relapse infections of *T. congolense* isolates in experimentally infected calves after treatment with Diminazene aceturate (day = 15) in Konso district, southern Ethiopia.

Animal ID	Days to the first detection and persistence of relapse/breakthrough infection*					
	18th	21st	24th	27th	30th	60th
K0101	b	b	b	a	a	a
K0102	b	b	b	b	b	b
K0103	b	b	b	b	b	b
K0104	b	b	b	b	a	a
K0105	b	b	b	b	b	b
K0106	b	b	b	b	b	b
K0107	b	b	b	b	b	b
K0108	b	b	b	b	b	b
K0109	b	b	b	b	b	b
K0110	b	b	b	b	b	b

*: a, Relapse detected or persisted on the specified day; b, relapse neither detected nor persisted on the specified day

In this experiment, an overall relapse/breakthrough infection rate of about 20 % (2/10) was detected, corresponding to an average relapse time of 13.5 days. Monitoring of the relapsed calves for clinical as well as parasitological parameters revealed that, until re-treatment with Isometamidium chloride, deteriorations mainly in haematological values and body weight gains were observed. Nevertheless, linear regression analysis of the effects of persistent trypanosomal infections on mean PCV and body weight gain in relation to the initial conditions of these parameters in the relapsed calves revealed that the reduction in these parameters was not statistically significant ($p>0.05$) for the experimental period (Table 15).

Table 15: Linear regression of mean PCV and body weight gain of experimental calves on the persistence of *T. congolense* isolates in Konso district, southern Ethiopia

Major health parameters	Regression and correlation coefficients and their significance*			
	α (± 1 S.E)	β (± 1 S.E)	r	P-value
Mean PCV (%)	27.2 ± 1.5	-0.01 ± 0.02	0.35	0.062
Mean body weight gain (gm)	248 ± 1.3	-0.03 ± 0.04	0.28	0.078

*: α, intercept; β, regression coefficient; r, correlation coefficient

On the other side, despite deterioration of the clinical and haematological conditions during the first few weeks post inoculation of the trypanosome isolates under investigation, there was a progressive improvement in these parameters following treatment with Diminazene aceturate and Isometamidium chloride. On the basis of deterioration in both the clinical and hematological conditions described above, the relapsed calves were treated, 45 days later (on day = 60) with Isometamidium chloride at the dose rate of 0.5mg/ Kg body weight.

Accordingly, relapse/breakthrough infections were not detected microscopically in either of the calves from day 63, until the termination of the experiment (Table 16).

Table 16: Patterns of relapse infections of *T. congolense* isolates in experimental calves after treatment with Isometamidium chloride in Konso district, southern Ethiopia.

Animal ID	Detection and persistence of relapse /breakthrough infection*										
	63rd	66th	69th	72nd	75th	78th	84th	90th	93rd	97th	100th
K0101	n	n	n	n	n	n	n	n	n	n	n
K0104	n	n	n	n	n	n	n	n	n	n	n

*: n, Relapse infection neither detected nor persisted on the specified day.

5. DISCUSSION

Tsetse-transmitted African Animal Trypanosomosis (AAT) is a disease complex with profoun social economic consequences on African scene. In endemic area of sub- Saharan Africa, the ris of the disease explicitly influences livestock management practices among livestock owner shaping their choices about the breed and overall composition of the herds. Therefor understanding the perceptions and knowledge of livestock owners about the impacts c trypanosomosis are valuable steps in the formulation of assumptions about livestock productivity

The aspect of primary importance in the present study is the evidence that animal trypanosomosi was the most important threat to livestock production in Konso district of Southern Ethiopia. Th perception by livestock owners that trypanosomosis primarily constrains livestock production ha also been reported in other areas of Ethiopia (Swallow, 1999); the agropastoral zone of Yal (Kamuanga *et al.*, 2001a) and Mouhoun valley of Burkina Faso (Kamuanga *et al.*, 2001c Similarly, reports of socioeconomic surveys and temporal comparison of livestock herds unde varying risk levels across sub-Saharan Africa revealed that most of the annual livestock losses ar attributed to trypanosomosis (Swallow *et al.*, 2000).

Majority of the respondents, especially those in Gumaide site, reported a declining tendency i the occurrence and impacts of animal trypanosomosis over the last few years. This indicates th dramatic effects associated with tsetse control operation through deployment of traps/targets an application of 'pour-on' formulation to the back of cattle. On the other hand, the contention b many livestock owners, in Jarso site, that the disease problem remained apparent till the preser could be explained by the fact that in dry months livestock are confined to graze around the mai rivers where tsetse flies concentrate posing great challenges. In addition to this, the relativel higher insufficiency of livestock feed in Jarso area could exacerbate the intolerance of livestoc to trypanosomosis during this period, as compared to that in Gumaide.

The foregoing conclusion is in accordance with inferences drawn from other studies about th adverse effects of poor nutrition on the production and productivity of livestock kept unde tsetse/trypanosomosis challenge (De Clercq, 1997; Van den Bossche and Rowlands, 2001).

Everything else being equal, it can be hypothesized that a successful tsetse/trypanosomosi control would lead to a rapid increase in cattle population and, consequently, a rapid rise in th percentage of households with cattle and draft oxen. The results presented in the current study o the livestock health and productivity indicators generally support the above standard hypothesi Thus, the important aspects, here, are the significant increases in the proportion of household with cattle and the corresponding rise in mean holdings of draft oxen. This scenario favourabl supports the findings that farmers under high trypanosomosis risk raise only 25-60 % of th number of cattle kept by farmers in nearby areas of low risk (Swallow, 2000). Similarly, it ha been indicated under various situations that the primary impact of an intervention is a reductio in the prevalence of trypanosomosis, and a corresponding rise in livestock health and productivit (Kamara *et al.*, 2000).

The evidence that high calf mortality, abortion and stillbirth rates were recorded in the previou ten years, but reduced by more than three folds in the post intervention period, consistently goe with most empirical studies that have shown significant impacts of trypanosomosis and of i control on livestock birth rates (Bauer *et al.*, 2000; Swallow, 2000). Furthermore, the decline i overall crude mortality rates in adult cattle, during the post intervention period, could b attributed to the effective tsetse control and the treatment of sick animals, which might hav combated trypanosomosis-related problems. This evidence favourably compares with thos findings obtained under health and productivity studies under similar disease conditions in oth parts of Ethiopia (Ahmedin and Hugh-Jhones, 1995) and Tanzania (Doran and Bossche, 2000 Here, it is worth noting, however, that the compromising effects of other infectious diseases an miscellaneous health disorders are not studied and, it is recommended that these have to b addressed through properly designed surveys.

On the other hand, the significant changes observed in the proportion of households with da oxen, and the higher average periods of field traction by draft oxen after the disease contro could be attributed to the improvements in animal health that accompanied tsetse/trypanosomos control. The expanded use of animal traction indicates better investments in draft cattle improve labour productivity in cropping activities. In general, this relationship between disea

control and animal traction use is in agreement with other studies indicating that, oxen in areas high trypanosomosis risk and incidence cultivate lower hectares of cropland, as compared to oxe under low trypanosomosis risk (Swallow, 1999). In conclusion, the rise in cattle holdings, th relative reduction in livestock production losses and overall improvement in other socioeconom indicators in Konso district are in accordance with the evidence from other studies about th relationship between livestock production and variation in the risk of animal trypanosomos (Kamuanga *et al.*, 20001c).

The current survey has revealed significant increases in the percentage of livestock owners usir trypanocides, and mean annual expenditure on these drugs. Indeed, these results on drug usag pattern are contrary to the hypothetical assumption that a successful tsetse/trypanosomos control would result in lower incidence and, thus, less expenditure on trypanocidal drugs household level during the period of intervention. This phenomenon can be explained, partly, b the fact that livestock owners desire to avert, or minimize, disease risk through repeate treatments and that the control activity improved livestock productivity resulting in bett availability of monetary incomes. A similar situation was observed in Northern Cote d'Ivoir with more farmers in tsetse controlled areas using trypanocidal drugs than those in non tsets controlled areas (Pokou *et al.*, 1998).

Another possible explanation for the persistence tendency in the incidence of annual treatment despite effective disease control, could be the occurrence of trypanocidal drug resistance. Th situation was partly ascertained by analysis of data on trypanocidal drug dosage regime, whic disclosed that block treatment of sick animals without prior diagnosis is a standard approach i the management of animal trypanosomosis at the farmers' level in the study area. Moreove delivery of poor-quality drugs and administration of over-diluted quantities mostly by unskille persons were identified as the common treatment strategies. However, the increasing tendenci in the misuse of trypanocidal drugs are not without serious feedbacks, as it is uneconomic an may lead to drug resistance (Holmes *et al.*, 2004).

The majority of livestock owners witnessed a more inclination to the use of curative trypanocid drugs than prophylactic ones over the last decade, and this trend indicated inadequate knowled on appropriate drug usage. In a similar manner, surveys in the Zambia have shown that farme

administer most of the trypanocide treatments, with a strong tendency to use curative drugs tha prophylactic ones (Van den Bossche *et al.*, 2000). Generally, it has been revealed that mos livestock owners do not have adequate knowledge on the diagnosis and appropriate drug usage s that trypanocides are used in the absence of diagnosis. Moreover, the choice between the use o therapeutic and prophylactic drugs is made on the basis of cost per dose, without understandin the advantages of prophylactic drugs under essential circumstances (Holmes *et al.*, 2004) Therefore, the similar situation expressed by livestock owners in the present area adds to th complex circumstances, which could booster drug resistance in sub-Saharan Africa (FAO, 2003).

The evidence by the respondents that most treatments are confined to sick animals, with majo emphasis on mature cows and oxen, reflects the preference of livestock owners to treat the mos productive animals as a priority. It has been indicated that the governments of most Africa countries now lack institutional capabilities to provide adequate public veterinary services, an over 60 % of the treatments are delivered by cattle owners (Holmes *et al.*, 2004). Furthermore there are evidences from other parts of sub-Saharan Africa indicating that, farmers treat not al their animals for economic reasons (Kamuanga *et al.*, 2001c) and that, irrespective of the drug used, the most productive animals receive the majority of treatments (Sinyangwe *et al.*, 2004 Van den Bossche and Rowlands, 2001).

One major aspect in the present study was the application of contingent valuation technique t evaluate the property of Konso community to support an integrated tsetse/trypanosomosi control. This study strategy is a valuable step because organizational capacity of the community their willingness to initiate and sustain disease control interventions are crucial consideration with the commencement of community-based programmes (Kamara *et al.*, 2000). Furthermore there is a strict consensus that mobilization and working with the community are the mos important components of every substantive program in tsetse control. For this reason, contingen valuation surveys have proved useful tools in studies of the community to support disease contro (Dransfield and Brightwell, 2004).

The animus propensity (100 %) indicated by Konso residents in playing active roles in th initiation and sustainability of integrated tsetse/trypanosomosis control explicitly reveals

potential for reliable livestock disease control strategies in the study area. Furthermore, th higher potential contributions among the households suggest a general enthusiasm for bette tsetse/trypanosomosis control strategy involving community participation. In the Ghibe valley o southwest Ethiopia, where a tsetse control programme was initially spoiled by recurrent theft bushfire and other vandals, a willingness evaluation was applied to assess the potential depth o the local community to support a re-designed intervention. The results revealed higher potentia contributions mainly in terms of labour times (Gewado, 2004; Swallow and Mulatu, 1995). Th present findings in this regard also compare favourably with other studies under contrastin situations across Africa (Kamara *et al*., 2000).

The highest potential money contributions volunteered by those household heads engaged in off farm activities and the educated class of the community may indicate the more access of thes people to monetary income and the lesser discretion over time allocation to labour involvement On the other hand, the highest potential labour contribution volunteered by those households wh secure subsistence livelihood through pure farming could also be explained in relation to th lower monetary resources and higher discretion for labour involvement. Survey results o evaluating the propensity and determinants of the willingness of local community to suppor tsetse control in southern Burkina Faso and Kenya have reached similar conclusions (Kamara *e al*., 2000; Kamuanga *et al*., 2001b).

The present study indicated that *Glossina* species constitutes a predominant section of the fl population in the spatial and temporal distribution over other vectors, with female se preponderating over their male counterparts. The dominance of *Glossina*, mainly at Jarso area could be explained by the presence of suitable savanna dominating the vegetative physiognom in this area. In addition, the preponderance of female flies over the male population indicates th impeding challenge of tsetse and other vectors, and the corresponding risk of anima trypanosomosis. The higher parasitological prevalence of bovine trypanosomosis recorded a Jarso sampling sites also confirms this scenario.

Indeed, a significantly higher reduction in the apparent fly density ($p<0.001$) was recorded in th present survey period, compared to the corresponding values in the past 10 years. This scenari

reflects the attractive achievements attained following successive vector control. Furthermore human settlement and mixed crop-livestock farming have been more intensively expanded in the lowland areas particularly over the last seven years, with concomitant bush clearing, which might have resulted in the destruction of fly habitats. Obviously, the adverse effects of human settlement, indiscriminate deforestation and expansion of mixed agriculture on the habitat and population dynamics of savanna tsetse have been explicitly described in sub-Saharan Africa (ICPTV, 2003; Vale and Torr, 2004).

All the above findings consistently add to other entomological studies about the seasonal and ecological dynamics in the population of tsetse flies and mechanical transmitters (Hargrove 2004); the higher confinement of *Glossina morsitans* group into habitats with savanna vegetation (Hargrove *et al*,, 2003); and the significant impacts of female fly population on the vector challenges and associated risk of animal trypanosomosis (Rogers and Robinson, 2004;, Vale and Torr, 2004). On the other side, the recorded evidence that no *Glossina* species other than *G. pallidipes* were recorded in all the sampling areas, during both seasons, further strengthens previous entomological findings in the same area and the STEP-target areas of the southern rift valley (Gemechu *et al*, 1998; Trumper, 1994).

In this study, a cross-sectional study on the parasitological prevalence of bovine trypanosomosis was undertaken as a foreground step. A spatial variation in the trypanosomal infection was observed among the sampling areas, with higher infection manifested at Salla site in Jarso area. This scenario was obviously expected because livestock in these sites spend the majority of grazing times in the savanna grasses around the rivers, where infestation with the dominant fly vector (*G. pallidipes*) is higher. Moreover, veterinary services are poor where most treatments are given by livestock owners that often involve under-dosing, eventually resulting in drug resistance. In addition to this, as Jarso site is less accessible there were little vector control efforts. However, livestock in Gumaide site are at relatively less risk of the disease, mainly owing to better veterinary services and a more consolidated vector control operation.

On the other hand, the recorded seasonal variation in the prevalence of trypanosomal infection in the study areas reflects the changes associated with temporal alterations in the apparent density of

fly vectors. The present findings on the apparent fly density support this trend, where highly varied mean fly catches were observed in relation to season of sampling. The higher dependence of tsetse-transmitted animal trypanosomosis on the temporal and spatial variations in vector population has been described across several areas of sub-Saharan Africa (Hargrove *et al.*, 2003; Hargrove, 2004).

Despite effective tsetse suppression measures over the last few years, the present cross sectional study found no statistically significant declines in the overall parasitological prevalence of trypanosomal infections (15.6 %), as compared to the previous reports (19.5 %) (Gemechu *et al.*, 1998). Although it has been generally inferred that a successful tsetse control could obviously bring about drops in tsetse apparent density and animal trypanosomosis in an area (Rowlands *et al.*, 2001; Swallow *et al.*, 2000), a number of factors really influence this aspect, as it is the case with the present study. As discussed earlier, illegal drug supply and administration of over-diluted drug dosages mostly by unskilled people could lead to inevitable development of drug resistance. Furthermore, vector control devices in Jarso were recently destroyed by vandals which explains the higher fly apparent density and, therefore, apparently high prevalence of the disease. This present finding is comparable with other field studies, which described the over-estimation of animal trypanosomosis due relapse infections owing to drug resistance (Rowlands *et al.*, 2001), and the effects of spoiled tsetse control on the risk of the disease (Swallow *et al.*, 2000).

The evidence that *T. congolense* is the dominant species in the present study area is in accordance with most of the previously conducted cross-sectional studies in the southern rift valley of Ethiopia (Gemechu *et al*, 1998; Trumper, 1994) and in the Ghibe valley (Rowlands *et al*, 2001). In general, it has been proved that *T. congolense* is the most prevalent and virulent trypanosome species in Eastern Africa, although certain hemorrhagic *T. vivax* strains prevail in this area (Taylor and Authie, 2004). Furthermore, the relatively lower prevalence of *T. vivax* could be due to the recurrent use of trypanocidal drugs that could have depressed its incidence. It has been also shown that cattle herds could more readily develop protective immunity and tolerate this parasite than *T. congolense* in East Africa (OIE, 2004).

In the present cross-sectional study, the lower PCV readings in parasitaemic cattle and the high corresponding values in aparasitaemic animals reveals an inverse relationship between herd PC and trypanosomal infection (Figure 7). It also reflects the depressive effects of anim trypanosomosis on normal physiologic aspects of livestock. This relationship is in agreeme with findings from other studies under similar situations (Van den Bossche and Rowlands, 2001

Despite the absence of trypanosomal parasitaemia in their blood, the evidence that son aparasitaemic animals had PCV values below 26 % could possibly be due to the compounde effects of concurrent infections by haematophagous helminth parasites. On the other side, t parasitaemia observed in some animals with PCV values above 27 % could be explained either relation to the presence of very recent infections in these animals, or to the variation among cat in the tolerance to trypanosomosis. Similar findings to this scenario were reported from studies the Ghibe valley (ILRI, 2002; Rowlands *et al*., 2001).

On the other hand, the finding that the overall herd mean PCV values for Gumaide site exceed the corresponding mean values for cattle at Jarso is inconsistent with the inferences drawn fr other studies. In a study of the relationship between parasitological prevalence of trypanosom infection and herd average PCV in eastern Zambia, removal of sampling area from multi linear regression models had not resulted in a significant change in the fit (Van den Bossche a Rowlands, 2001).

However, the season of sampling had a profound effect on the herd average PCV and its relati with trypanosomal infection. This phenomenon can be explained as follows. First, severity anaemia is influenced by plane of nutrition (Taylor and Authie, 2004). Among the areas, po pasture and high temperatures pose recurrent nutritional stress in Jarso site mainly in dry seas Therefore, the observed seasonal effects on the association between herd PCV and trypanosom infection are very likely to be due to poor nutrition during the dry season. Second trypanosomosis seems to be less well tolerated during the dry season, as partly explained results on the questionnaire interviews that indicated a higher proportion of trypanocidal dr treatments being administered during the dry season. This pattern favorably goes in line w inferences drawn from other studies (Van den Bossche *et al*, 2000).

The results of the *in-vivo* experimental study confirmed the conclusions drawn from the initi questionnaire survey about the diminished trypanocidal activities of the most frequently us drugs in the study area. As presented in earlier headings, trypanosomosis remains a maj problem in areas with high infection pressure (Mc Dermott and Coleman, 2001), and ma errors are committed in calculating the correct doses of trypanocidal drugs owing to t involvement of unskilled persons in the treatment of livestock (Geerts and Holmes, 200 Therefore, the many temptations to over-dilute these drugs could result in sub-therapeutic dr concentrations leading to inevitable emergence of drug-resistant trypanosome strains (Holmes *al.*, 2004).

The fact that the present test was conducted in a fly-proof accommodation and in non tsets endemic area has reliably avoided the confounding effects that could be attributed to the risk re-infection during the study period. In addition, a relatively large number of experimental calv were employed and this was very essential, since previous research has shown that resu obtained after inoculation and treatment of small number of animals are not always reliable, a provide more of qualitative results (Eisler *et al.*, 2003).

In this test, the detection of relapse infections in some of the experimental calves followi treatment with Diminazene aceturate at the dose of 3.5 mg/Kg body weight is clearly indicati of the presence of at least sub-populations of trypanosome isolates resistant to this drug. Th conclusion steams from the fact that Diminazene aceturate could maintain therapeutic blo levels until 22 days following treatment, unless resistance is present. This finding on the relap date in resistant strains is comparable with inferences drawn from a previous study at the Ghi valley, where trypanosomes resistant to Diminazene aceturate have relapsed after over 14 da following treatment (Codjia *et al.*, 1993; Peregrine *et al.*, 2000).

As there is an increasing number of case reports from other trypanosomosis-endemic areas Ethiopia, disclosing a range of prevalence of *T. congolense* resistant to Diminazene aceturate a Isometamidium chloride (Afework, 2004; Codjia *et al.*, 1993; Mulugeta *et al.*, 1997; Rowlands *al.*, 2001; Tewelde *et al.*, 2004), the demonstration of resistance to Diminazene aceturate (20 manifested by the current *T. congolense* isolates in Konso distric, was unsurprisingly an expect

outcome. Furthermore, it has been observed under longitudinal studies that there was association between the initial trypanosome prevalence and the occurrence, and thus the degre of drug resistance (Mc Dermott *et al.*, 2003).

Despite a considerable initial deterioration in clinical and haematological parameters of t relapsed calves, this study revealed a significant improvement ($p<0.01$) in these paramete following administration of Diminazene aceturate and Isometamidium chloride. This relationsh can be explained in relation to the following possible effects. Firstly, the trypanosome isolat under investigation might have entailed a heterogeneous population of trypanosomes a treatment with Diminazene aceturate could have eliminated the sensitive sub-population throu its therapeutic effects, so that the parasite burden was limited to the resistant populatio Furthermore, re-treatment of the calves with Isometamidium chloride could have resulted in complete elimination of the sub-population that revealed resistance to Diminazene acetura These two strategies could trigger better improvements in the conditions described abov Improvement in livestock health parameters following treatment of infected animals wi trypanocidal drugs at specified times has been observed in other experimental studies (Holmes *al.*, 2004).

Secondly, even the sub-population resistant to Diminazene aceturate could be less pathogen with insignificant impacts on productive performances of the calves. Although the findings ha been inconsistent and controversial, a limited number of studies have demonstrated a loss virulence and/or loss of fitness in drug resistant trypanosomes (Codjia *et al.*, 1993; Mulugeta *al.*, 1997). A useful study that assessed this important issue was conducted in the Ghibe valle where *T. congolense* strains demonstrated multiple resistance to all available drugs. In that stud it was deduced that, despite the occurrence of high degrees of drug resistance, local zebu cat could yield profitable productivity, so that attractive economic returns were generated for h owners (Holmes *et al.*, 2004; ILRI, 2002).

In the present experiment, the relapsed calves were treated with Isometamidium chloride, 45 da later, and neither of the calves relapsed post treatment with this drug, until the termination of experiment. This observation is in accordance with the currently available information indicat

the trypanocidal effects of treatment with Isometamidium in cattle (Eisler, 1996). On the other side, delaying of Isometamidium chloride treatment by 45 days after the administration of Diminazene aceturate was a reasonable process in light with the possible side effects that could be associated with the application of the second drug in a short period following the administration of the first trypanocidal drug (Eisler *et al.*, 2001; Holmes et al., 2004).

On the other hand, the results on the sensitivity testing of the isolates to Isometamidium chloride are generally not conclusive as to whether or not the isolates were sensitive to this drug at the specified dose. In this procedure, the possible effects of selection biases are of crucial importance, since the trypanosome population against which the second drug was administered might not be a complete representative of the original field population. A possible explanation for this scenario is the fact that by the time of testing against Isometamidium chloride, previous treatment with Diminazene aceturate might have eliminated the sub-population resistant to Isometamidium chloride. Indeed, the possible effects of selection biases in trypanocidal drug sensitivity tests following administration of the first drug have been explicitly described (Eisler *et al.*, 2004).

In nut shell, the present study revealed that *T. congolense* isolated from Konso district were found to exhibit resistance to Diminazene aceturate at recommended dose of 3.5 mg/Kg body weight. Nevertheless, the dispute on the sensitivity of the *T. congolense* isolates to Isometamidium chloride and, thus, the possible occurrence of multiple drug- resistance, should be urgently confirmed in a re-designed test using the drug under investigation. Finally, in light of the above scenario, it is recommended to intensify the principle of "sanative pairs' of trypanocidal drugs, and resistance to the available trypanocides should be monitored in the field on regular basis over time. On top of that, holistic, integrated disease control approaches should be adopted.

6. CONCLUSIONS AND RECOMMENDATIONS

Until recently, the majority of control methods against animal trypanosomosis have been aim at using suitable trypanocidal drugs as the most important tactics in destroying trypanosome However, the heavy reliance of the veterinary sector on these drugs, the alarming emergence a vulnerability to resistance together with the low adoption of integrated control approaches ha aggregately created a greater dilemma in the management of this disease complex. Therefo resistance to trypanocidal drugs is increasingly recognized as a major constraint to reliab livestock production. Furthermore, the unlikelihood of new trypanocides appearing in t foreseeable future and the spread of drug resistance to the point where therapeutic failure m occur over large areas is probably the greatest risk to the future use of these trypanocidal drugs.

As a benchmark, understanding the perceptions and knowledge of livestock owners about t socioeconomic impacts of trypanosomosis and its control are essential considerations in t formulation of assumptions about livestock productivity in relation to control of the diseas Animal trypanosomosis has proved a major and continuing threat to livestock production Konso district. Efforts to combat the devastating effects of this menace have faced sustainabili problems due to the absence of concerted coordination among different stockholders, and becau of little/no direct participation of Konso community. Therefore, treatment of sick animals witho prior diagnosis has remained to be a standard approach in the management of the disease at t farmers' level.

Nevertheless, continuous supply of trypanocidal drugs of doubtful quality by drug smuggle indiscriminate use and application of over-diluted quantities of these compounds mainly unskilled persons coupled with poor veterinary services have greatly contributed to t development of resistance to the available trypanocidal drugs. Therefore, drug resistance currently identified to be amongst the major determinants curtailing livestock production a productive opportunities of the community in Konso district.

The questionnaire survey as well as parasitological prevalence of bovine trypanosomosis in t present study provided valuable field information about the potential for the development

trypanocidal drug resistance. Furthermore, the experimental studies conducted in naïve calv revealed resistance exhibited by field isolates of *T. congolense* to Diminazene aceturate, a possibly to Isometamidium chloride. This information has proved to be useful as an integral pa to the rapid spatial and temporal assessments of the prevalence and probable impacts of dru resistance across tsetse-infested areas of Ethiopia.

Based on the present research findings and currently available relevant information pertaining African animal trypanosomosis, the following worthwhile recommendations could be forwarded

- Temporal and spatial surveys should be conducted in the southern region in order provide valuable data to the rapid assessments of the true prevalence and probab impacts of drug resistance across trypanosomosis-endemic areas of Ethiopia

- The use of sanative pairs of trypanocidal drugs should be intensified, and resistance to th available trypanocidal drugs should be monitored over time on regular basis. In additio without a high risk of trypanosome infections, the prophylactic application Isometamidium chloride should be discouraged because of the risk of unnecessary dru pressure promoting the development of resistance

- Chemotherapy and chemoprophylaxis should be combined with other control measures i an integrated manner in such a way that sustainable community-based vector contr schemes should be more intensified and application of trypanocidal drugs should b restricted to the treatment of parasitaemic animals

- Establishment and harmonization of the existing legislations on drug delivery and usage essential in order to ensure drug quality. To this effect, particular attention should be pai to training and recruitment of qualified professionals as well as the public sector, so as improve the delivery of veterinary services to rural communities.

- Initiating and maintaining representative as well as transformative communit participation should take a priority in an effort to ensure the sustainability of integrat tsetse and trypanosomosis control approaches.

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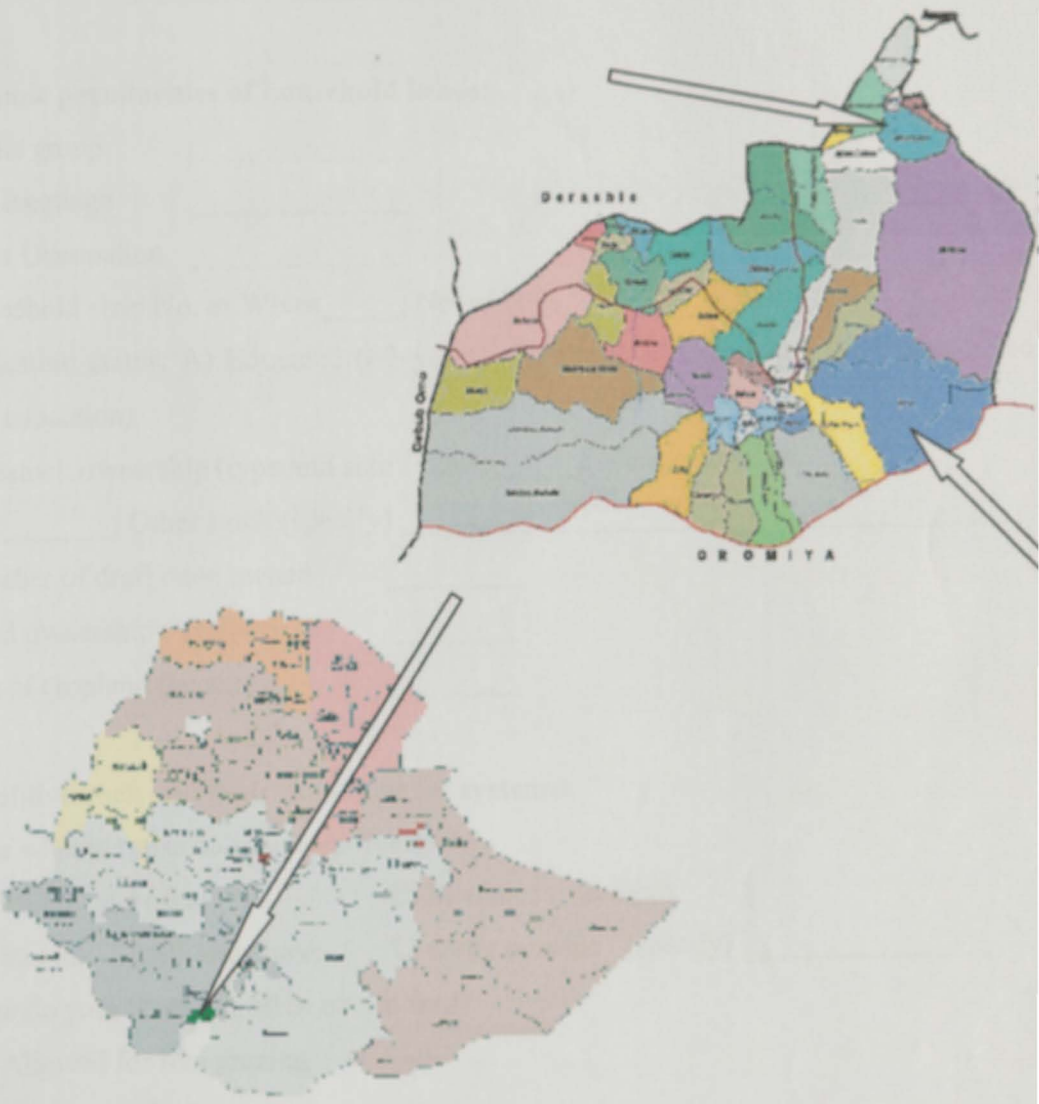
8. ANNEXES

Annex I: Map of Ethiopia including Konso district (long arrow) with two agro-ecologic area (short arrows) used for the present research work



Source: (Trumper, 1994)

Annex II: Questionnaire survey format used for the collection of information on major animal health problems and disease management systems in Konso district, Southern Ethiopia.

Date_____ Village (PA) _____

Full name of interviewee_____ Sex (M/F) _____Age (Years)____

I. Socio-economic peculiarities of household heads:

☐ Ethnic group _____

☐First language _____

☐Major Occupation _____

☐ Household size: No. of Wives_____; No. of boys_____; No. of girls_____

☐ Education status: A) Educated (>3 years) B) Can read (1-3 years) C) Illiterate (no formal education)

☐ Livestock ownership (type and size): Cattle_____; Sheep and goats_____; Equine_____; Other stock (specify) _____

☐ Number of draft oxen owned _____

☐ Land ownership (Yes/No) _____

☐Size of cropland (hectare) _____

II. The role of livestock and their management systems:

1. For what purpose do you keep livestock?

   ☐ Milk ☐ Meat ☐ Manure ☐ Draft

   ☐ Income ☐ All the above ☐ Other benefits (specify) _____

2. How do your livestock often obtain feed?

   ☐ Allowed for free grazing

   ☐ Feed on stall feed

   ☐ Both ☐ Other methods (specify) _____

3. Where do you often tend your cattle?

   ☐ Communal grassland

   ☐ Private area

   ☐ Other methods (specify) _____

4. How often are your cattle tended?

☐ In herds with other cattle

☐ As private herd alone ☐ Other methods (specify) _____

5. Where do your cattle often get water? _____

6. What is the distance of watering point from your homestead (km)? _____

7. What is the main source of feed for your cattle?

☐ Natural pasture ☐ Aftermath

☐ Both sources ☐ Other sources (specify) _____

8. If natural pasture is the major source,

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

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

III. Major health problems to the livestock sub-sector

1. Have there been problems to your livestock since the last ten years? ☐ Yes ☐ No

2. If your answer is yes, in your perception, what are the main problems? Please list them in order of their importance (from the most to the least important).

A. _____

B. _____

C. _____

D. _____

3. What are the main diseases that mostly affect livestock? Please list them in order of their importance (from the most to the least important).

A. _____

B. _____

C. _____

D. _____

4. Is trypanosomosis one of the problems to your cattle? ☐ Yes ☐ No ☐ I have no idea

5. If your answer is yes,

5.1. Which species does it mostly affect?

☐ Cattle ☐ Goats and sheep ☐ Equine

☐ All of the above species ☐ Neither of these species

5.2. In your perception, what rank does this disease take? _____

5.3. When do you think has this disease started in your area?

☐ 20 years ago

☐ 10 years ago

☐ 5 years ago

☐ I have no idea

5.4. During which season (months) does it mostly occur? _____

5.5. During which season (months) does it occur less? _____

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

☐ An increase

☐ A decrease

☐ No change

☐ I have no idea

5.7. What are the major signs that an animal affected by the disease shows?

Signs: _____

_____

5.8. How do you think is the disease transmitted to animals? _____

5.9. How many animals did you loss since the last 12 months form trypanosomosis?

Adults_____; young _____

Iv. Use of trypanocidal drugs and the problem of drug resistance

1. If your animals get sick and show signs of trypanosomosis, where do you treat them?

☐ Take to Veterinary clinics

☐ Treat at home

☐ Other methods of handling (specify) _____

2. From where do you usually obtain the medicine?

☐ From veterinary clinics

☐ From private clinics

☐ From local farmers

☐ From drug smugglers

☐ From other sources (specify) _____

3. What is/are the main types of drugs you use for the above purpose?

(Name/type/color) _____

4. Who applies the treatment?

☐ Myself

☐ Government veterinary professionals

☐ Private individuals

☐ Community animal-health workers

☐ Drug smugglers

☐ Other sources (specify) _____

5. What amount of the drugs is used?

5.1. If Diminazene aceturate:

☐ 1 sachet for 1 adult cattle

☐ 1 sachet for two adult cattle

☐ Other methods (specify) _____

5.2. If Isometamidium chloride:

☐ 1 sachet for 10 adult cattle

☐ 1 sachet for 15 adult cattle

☐ 1 sachet for 20 adult cattle

☐ Other methods (specify)_____

5.3. If Homidium:

☐ 1 sachet for 1 adult cattle

☐ 1 sachet for two adult cattle

☐ Other methods (specify)_____

6. Since when have you been using the above drugs for the treatment of your cattle?

☐ Since the last 15 years

☐ Since the last 10 years

☐ Since the last 5 years

☐ Other methods (specify) _____

7. How much do you pay for treating one adult cattle? _____ Ethiopian Birr

8. For the last one year, how much did you pay for treating your animals against trypanosomosis? _____ Ethiopian Birr

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

- ☐ Treat all cattle
- ☐ Treat only mature oxen
- ☐ Treat only mature cows
- ☐ Treat only sick ones
- ☐ Treat mature cows and oxen
- ☐ Other methods of application (specify) _____

10. Do the animals treated by the drugs get healthy?

☐ Yes ☐ No ☐ I have no idea

11. If your answer is no,

11.1. What do you think is the reason? _____

11.2. Could you tell us the interval between treatments? _____ days/months

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

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

14. Do you have any trypanocidal drug at your home?

☐ Yes ☐ No

15. If your answer is yes,

15.1. Would you show us? _____

15.2. Where did you obtain? _____

15.3. Where and how do you store them? _____

15.4. What time has elapsed since you acquired them? _____days/months

THANK YOU FOR YOUR CO-OPERATION

Annex III: Questionnaire survey format for the evaluation of people's willingness to support integrated tsetse/trypanosomosis control approaches in Konso district, Southern Ethiopia.

1. If an area-wade tsetse and trypanosomosis control program by means of traps targets, insecticidal pour-on and trypanocidal drugs entailing community participation is to be implemented in your area, would you be willing to participate?

   ☐ Yes ☐ No

2. If you would be willing to participate, what would you contribute?

   ☐ Money

   ☐ Labour

   ☐ Both

3. If you are willing to contribute money, how much you pay if the payment were to be made on monthly basis? _____ Ethiopian Birr per month

4. If you are willing to contribute labour, what are the things that you contribute?

   A. _____

   B. _____

   C. _____

   D. _____

5. What maximum number of days would you participate per month? _____ days.

6. If you are willing to contribute both money and labour to the disease control, how much money lobar would you contribute per month?

   ☐ _____ Ethiopian Birr

   ☐_____ Days

7. If you are willing to contribute neither money nor labor to the disease control, would you tell us the reason why? _____

8. If traps/targets were lost by thieves, bush fire or other vandals, what would be your role in protecting these devices? _____

9. If individuals who destroy the traps/targets were captured,

   10.1 What do you think should be done to them? _____

   10.2 Where do you think should they be seen? _____

10. If a program is designed in your area to detect and control drug smugglers, and community participation is required, are willing to participate?

☐ Yes ☐ No

11. If you are willing to participate,

11.1. What would you contribute?

☐ Detect and expose them

☐ Teach people ☐ I would do all

☐ Other methods of participation (specify) _____

11.2. In your opinion, what should be done to the drug smugglers if they are captured with drugs? _____

11.3. Where should they be seen?

☐ They should be punished by local community

☐ Implementer should take his own action

☐ They should be released freely

☐ I have no idea

THANK YOU FOR YOUR CO-OPERATION

Annex IV: A format for the collection of monthly information on parasitological prevalence of bovine trypanosomosis in selected sampling sites of Konso district, Southern Ethiopia.

Animal ID	Date of exam	Body weight (Kg)	PCV value	Parasitaemia detected upon microscopic examination				Treatment given	
				TV	TC	TB	Mixed	Name of drug	Dose (ml)

Annex V: Stabilation/Cryopreservation of trypanosomes for experimental infection.

Stabilation of trypanosomes for experimental infection encompassed of the following step-wise procedures.

1. Obtain 5-10 ml blood from jugular veins of infected animal with EDTA-treated vacutainer tubes;
2. Take 0.5 ml of 30 % glycerol and 0.5 ml EDTA blood into serum vials, to have a final glycerol concentration of 15 %;
3. Mix the contents in (2) gently;
4. Label according to FAO (2003) recommendations;
5. Leave the contents for 15 minutes to equilibrate at room temperature;
6. Insert the vials into a placticin insulated large screw plastic bottle: the cover of the bottle should be 1 cm thick;
7. Suspend the vials in the vapour phase of liquid nitrogen container and allow to cool for two hours;
8. After cooling, transfer the tubes from the cooling device to a storage canister in liquid nitrogen bath;
9. Check the viability of the stabilates as follows:
    i. Withdraw a tube and instantly throw at room temperature;
    ii. Take the tube soon and examine under microscope, check for mobility and viability
10. Infect experimental animals;
11. Check for infectivity

9. CURRICULUM VITAE

1. Personal information:

➢ Full name	Gewado Ayledo Gellebo.
➢ Sex	Male
➢ Nationality	Ethiopian.
➢ Date of birth	14th April, 1980.
➢ Place of birth	Konso Special Woreda, Karat town, Southern Nations Nationalities and People's regional State (SNNPRS), Southern Ethiopia.
➢ Marital status	Not married/single.
➢ Academic profession	Veterinarian.
➢ Current occupation	Veterinary expert in Konso Special Woreda, Southern Ethiopia
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2. Educational background:

Period (Years)	School/Institution	Academic award/s
➢ 1987-1992	Durro Elementary School, Durro	
➢ 1993-1998	Konso Senior Secondary school, Karat	Certificate
➢ 1999-2004	Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit	DVM.
➢ June, 2003-June, 2004	Attachment associate and Research fellow at ILRI sub-quarter, Addis Ababa, Ethiopia.	

3. Work Experience:

Period (Years)	Institution/s and responsibilities
➢ 1st July, 2004-30th December, 2005	Field Veterinarian and Animal Team Leader, MoA, Konso Special Woreda, SNNPRS, south Ethiopia.
➢ 1st January-30th April, 2006	Head of Animal health and production Desk, MoA, Konso, Karat.
➢ 1st May-August 30th, 2006	Deliverer and co-coordinator of training for CAHWs on tsetse and trypanosomosis control and related issues

4. Project designing output:

- Project on the theme "Towards upholding the Veterinary service quality through improving skills of Community Animal-Health Workers (CAHWs)", in collaboration with FARM Africa and Konso Development Association (KDA), Karat.

5. Language Skill:

- Konsigna — Excellent at speaking, reading and writing.
- Amharic — Very good at speaking, reading and writing.
- English — Very good at reading and writing, good at speaking.
- Afan Oromo — Able to communicate

6. Special skills:

- Computer skills in MS DOS, MS Word, MS Excel, MS Access, STATA command, SPSS and MS power point

7. Major research output:

- Gewado Ayledo, 2004. Assessment of the socio-economic impacts of bovine trypanosomosis control in the Ghibe valley, southern Ethiopia. DVM thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit.
- Gewado Ayledo, 2008. Studies on bovine trypanosomosis and efficacy of selected trypanocidal drugs in Konso district, southern Ethiopia. MVSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit.

8. Membership of recognized scientific society:

- Member of the Ethiopian Veterinary Association (EVA), Addis Ababa, Ethiopia.

9. Membership of local development associations:

- Member and lobbyer on the membership towards Konso Development Association (KDA), Konso, Karat.

10. References:

- Dr. Hagos Ashenafi (DVM, MSc, Assistant Professor), Faculty of Veterinary medicine, Addis Ababa University.
- Dr. A. K. Basu (DVM, MVSc, PhD, Associate Professor), Faculty of Veterinary medicine, Addis Ababa University.

10. SIGNED STATEMENT OF DECLARATION

I, the undersigned, declare that this thesis is my original work and 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 GEWADO AYLEDU

Signature

Date of submission 25/06/08

This thesis has been submitted for examination with our approval as university supervisors:

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Gew/ /2008

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TITLE Studies on Bovine Trypanosomosis & efficacy--

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