

**ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE**

**THE EPIDEMIOLOGY OF CATTLE TRYPANOSOMOSIS, HELMINTHE AND
TSETSE FLIES IN SELECTED AREAS OF KELEM WOLLEGA, OROMIA, ETHIOPIA**

**MSc THESIS
BY
SOLOMON KEBEDE**

**JUNE 2012
DEBRE ZEIT, ETHIOPIA**

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial
fulfillment of the requirements for the Degree of Master of Veterinary Science in Tropical
Veterinary Epidemiology

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SOLOMON KEBEDE**

Board of External Examiners:

Signature

1. Professor Getachew Tilahun

(Aklilu Lema- Institute of pathobiology, Adiss Abeba Univeristy)

2. Dr. Genene Tefera

(Institute of Biodiversity Conservation)

3. Dr. Derege Gudet

(Kality SIT Center)

Academic Advisor:

Dr. Reta Duguma (DVM, MSc, Assist. Proffesor)

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LIST OF ABBRIVATIONS

CI	Confidence Interval
DNA	Deoxyribo nucleic Acid
ELISA	Enzyme Linked Immuno Sorbent Assay
EPG	Egg Per Gram
FAO	Food and Agricultural Organization
GIT	Gastro Intestinal Tract
<i>G.ff.</i>	<i>Glossina f. fuscipes</i>
<i>G.m.m.</i>	<i>Glossina m. submorsitans</i>
<i>G.p.</i>	<i>Glossina pallidipes</i>
<i>G.t</i>	<i>Glossina tachinoides</i>
Hg	Hemoglobin
ILCA	International Livestock Center for Africa
ILRAD	International Laboratory for Research on Animal Disease
m.a.s.l.	meters above sea level
NTTICC	National Tsetse and Trypanosomosis Investigation & Control Center
OIE	Office International des Epizooties
PAs	Peasant Associations
PATTEC	Pan African Tsetse and Trypanosomosis Eradication Campaign
RBC	Red Blood Cell
<i>T. c.</i>	<i>Trypanosoma congolense</i>
<i>T. v.</i>	<i>Trypanosoma vivax</i>
<i>T.c+v</i>	Mixed infection of <i>T. congolense</i> and <i>T. vivax</i>
WBC	White Blood Cell
WHO	World Health Organization
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
SPSS	Statistical Package for Social Sciences
USD	United State Dollar

ABSTRACT

An epidemiological study of bovine trypanosomosis, GIT helminthes and their concurrent infection was carried out from November 2011 to April 2012 in Dale Sedi and Hawa Gelan districts, Kellem wollega zone, Oromia National Regional State, Western Ethiopia. The objectives of the study were to determine the prevalence of trypanosomosis, GIT helminthes and their concurrent infection and associated risk factors, to determine the apparent densities and distributions of tsetse and other biting flies and to find out the association of trypanosomosis, GIT helminthes, and their concurrent infection to PCV. The study methodology comprises cross-sectional studies by using parasitological, entomological and coprological surveys. A total of 1,244 animals were examined in the two districts and the prevalence of trypanosomosis in Dale Sedi was 4.7% (3.3%-6.2%) differ significantly ($P < 0.05$) from Hawa Gelan district 9.7% (6.9%-12.5%). Out of 1,108 cattle examined for coprological examination, 36.2% ($n = 401$) were found to harbor one or more helminthes. Out of which 313 (28.2%), 74 (6.7%), 12 (1.1%) and 45 (4.1%) were found to be infected with *Fasciola*, *Paramphistomum*, *Oocyst* and *Strongyle*, respectively. The prevalence of concurrent infection of Trypanosomiasis and GIT helimenthes (*Fasciola*) is 23 (2.1%). The mean PCV of trypanosoma infected bovines (21.57 ± 6.49) was significantly ($P < 0.001$) lower than that of non-infected bovines (25.50 ± 4.40), the mean PCV of helminthes infected bovines (24.88 ± 4.55) was significantly ($P < 0.05$) lower than that of non-infected bovines (25.49 ± 4.75), the mean PCV of *Trypanosoma* and *Fasciola* concurrently infected bovines (19.74 ± 5.55) was significantly ($P < 0.001$) lower than that of *Fasciola* infected bovines (24.62 ± 4.35) and also lower than that of *Trypanosoma* infected bovines (22.81 ± 6.80) but there was no significant difference ($P > 0.05$) among them. Analyses of the obtained results suggest that concurrent infection markedly fall the PCV and the pathologic effects of concurrent infections due to *Trypanosome* and *Fasciola* were predominantly influenced by the *Trypanosome* infection. Four tsetse fly species are found abundantly (*G. fuscipes fuscipes* and *G. tachinoides*) riverine species are predominating over the other two species *G. pallidipes* and *G. morsitans submorsitans*, the savannah group.

Keywords: Anemia, Bovine, Concurrent infection, Districts, GIT helminthes, Trypanosomosis, Tsetse flies

1. INTRODUCTION

Although animals are known to harbor single parasitic infection, mixed parasitic infections with various species or several types of parasite is a common phenomena in most cases of natural infection (Clark, 2000; Cox, 2001). Grazing animals are usually exposed to concurrent infections and the presence of one parasite may affect other parasites within the same host system (Nwosu *et al.*, 2006). In the field, animals are exposed to multiplicities of parasites among which are trypanosomes and gastro-intestinal tract (GIT) helminthes that cause considerable animal health problems in many part of the world (Kaufmann *et al.*, 1992; Dwinger *et al.*, 1994).

Trypanosomosis is the widespread protozoan disease complex affecting cattle and other wide range of hosts, including humans in the sub-Saharan Africa. The course of the disease may run from a chronic long lasting to an acute and rapidly fatal situation depending on the vector parasite-host interactions. It is characterized mainly by intermittent fever, progressive anemia and loss of condition of susceptible hosts which if untreated leads to heavy mortalities (Bourn *et al.*, 2001).

Among livestock diseases tsetse-transmitted trypanosomosis have been incriminated as the predominant elements in the extreme deterioration of animal resources in sub-Saharan region that holds the continent's greatest potential for expanded livestock production (Maser, 2005). The devastating effects of tsetse-transmitted trypanosomosis on the livelihoods of African communities have reached unprecedented levels across much of sub-Saharan region. Accordingly, researches on the socio-economic impacts of the disease have revealed that over 3 million heads of various livestock species in Africa are lost per year by deaths due to trypanosomosis. Furthermore, over 35 million doses of trypanocidal drugs are bought annually to treat animals against the disease and more than 70 million heads are at risk of contracting the disease (Bett *et al.*, 2004).

Tsetse transmitted animal trypanosomosis is a disease complex caused by *Trypanosoma congolense*, *T. vivax*, and *T. brucei brucei* or mixed infection with one or more of these

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Tsetse transmitted animal trypanosomosis is a disease complex caused by *Trypanosoma congolense*, *T. vivax*, and *T. brucei brucei* or mixed infection with one or more of these

trypanosomes. The disease is most important in cattle but can cause serious losses in pigs, camels, goats, and sheep. In Ethiopia, there are five economically important animal trypanosome species. These are *T. congolense*, *T. vivax*, *T. brucei*, and *T. evansi* of livestock and *T. equiperdum* in horses (Abebe, 2005). The most prevalent trypanosome species in Ethiopia are *T. congolense* and *T. vivax*.

Trypanosomosis has serious impacts on the individual animal and national development endeavors. On an individual basis, untreated animal or human trypanosomosis will lead, at best, to a chronic debilitating condition and, at worst, to death. Furthermore, animals infected with trypanosomes are reported to have reproductive disorders (Ikede *et al.*, 1988). *T. vivax* and *T. congolense* infection in Zebu x Friesian cross bred bulls have caused highly deformed spermatozoa with multiple morphological defects. These can render the bulls unfit for breeding within a very short time (Sekoni *et al.*, 2004). Chronic diseases either due to *T. vivax* or *T. congolense* could be an important causative agent of infertility or sterility in bulls and it can cause severe losses on production performance in cattle and water buffalo (D'Ieteren *et al.*, 1998). Study conducted on cattle in the tsetse and trypanosomosis infested areas showed that African cattle herd living in tsetse infested areas is 20% less productive in herd meat and milk output than cattle herd in tsetse free areas of the some countries (Trail *et al.*, 1985).

In Ethiopia farm animals are kept on pasture throughout the year since extensive farming is widely practiced. Climatic conditions are favorable for development and survival of infective stages of helminthes parasites. Helminthosis is ranked as the major constraint to animal production causing anemia, diarrhea, and emaciation resulting in reduced weight gains, increased production cost and mortality (Tembely *et al.*, 1998). The importance of helminthosis may vary greatly from one year to the next and between geographical locations depending on the prevailing climatic conditions. Moreover, stress, poor nutrition and concurrent disease may be associated with the release of hypobiotic larvae from the dormant state leading to clinical helminthosis (Fakae *et al.*, 1994).

Anemia is the predominant sign of trypanosomosis, therefore, whenever it is observed in tsetse infested areas; it has mainly been attributed to trypanosome infections. There is however, other

equally potent and highly prevalent anemia causing pathogens that affect the cattle production. One of these is GIT helminthes infection (Kaufmann and Pfister, 1990). Infections with some parasite species, particularly *Haemonchus* but also *Bunostomum*, *Trichuris* and *Fasciola* can cause anemia. In acute haemonchosis and fascioliasis, the pathogenic effect of the parasite is often present before eggs appear in the faeces (Hanson and Perry, 1994).

Concurrent infection between trypanosome species and GIT helminthes is of particular relevance and interest in trypanosome endemic region of Africa in view of the powerful depression effect of the former on the immune response of their host (Holmes *et al.*, 1974; Scott *et al.*, 1977). However, comprehensive data on the concurrent trypanosomes and GIT nematode infections in cattle population of the tropic are scanty and little attention has been given. Meanwhile, the epidemiological picture of single trypanosomes and GIT nematode infections is lacking in Kelem Wollega zone of Oromia region. Therefore, the objectives of the current work were to:

- Determine the prevalence of trypanosomosis, GIT helminthes and their concurrent infection in the study area
- Determine the apparent density, distribution and species of tsetse flies and other biting flies
- Find out the relationship between trypanosomosis and GIT helminthes to anemia

2. LITERATURE REVIEW

2.1. Animal trypanosomosis

2.1.1. The trypanosomes and disease

Trypanosomes

Trypanosomes are unicellular microscopic elongated spindle-shaped protozoa which belong to the family *Trypanosomatidae*. All possess flagellum which arises at the posterior end of the trypanosome from a basal body at the foot of the flagellar pocket. The flagellum runs to the anterior end of the body and is attached along its length to the pellicle to form an undulating membrane. In stained specimens, a single centrally placed nucleus can be seen, and adjacent to the flagellar pocket, a small structure the kinetoplast which contains the DNA of the single mitochondria is observed (Urguart *et al.*, 1995).

The family consists of several genera and many species; all are parasitic in habit. The species which parasitize vertebrates require a vector for transmission (Adam *et al.*, 1979). Trypanosomes live and multiply in the bloodstream, lymphatic vessels, and tissues, including the cardiac muscle and the central nervous system. The most common form of *Trypanosomatidae* is the bloodstream form of the mammalian parasites (Itard, 1989).

Classification and Morphology

Trypanosomes are microscopic, elongated and flattened cells, which move with the help of a single flagellum directed towards, at the base of which is found a characteristic structure, the kinetoplast (Itard, 1989). The length and position of the trypanosome's flagellum is variable. In trypanosomes from the blood of a host, the flagellum originates near the posterior end of the cell and passes forward over the cell surface to extend freely at the anterior end. Where the

flagellum is adherent to the cell surface, its sheath is expanded and forms a wavy flange, called the undulating membrane (Adam *et al.*, 1979).

Pathogenic trypanosomes in the genus *Trypanosoma* are divided according to their development in the vector and transmission by either the saliva (inoculative) (Salivarian group) or transmission by faecal contamination (Stercoraria) (Mulligan, 1970; Adam *et al.*, 1979; Losos, 1986). The Salivarian consists of species of veterinary and medical importance (Mulligan, 1970; Adam *et al.*, 1979). The main pathogens in Salivaria group categorized into four sub genera: Duttonella (species: *T. vivax*; *T. uniformes*); Nanomonas (species: *T. congolense*; *T. simae*); Pycnomonas (*Trypanosoma suis*) and Trypanozoon (species: *T. brucei*; *Trypanosoma rhodesiense*; *Trypanosoma gaambiense*; *T. evansi*; *T. equiperdum*) according to their morphology in the blood and their pattern of development in the tsetse fly (Mulligan, 1970; Adam *et al.*, 1979).

Trypanosoma (Nannomonas) congolense

Trypanosomes of this subgenus range 8-24 μm in length. Free flagellum is absent at any state in the life cycle, which is an unusual characteristic. The flagellum thus terminates at anterior end of the parasite. The posterior end of the body is usually rounded but can be slightly pointed in longer parasites. The medium sized kinetoplast is usually marginal and sub-terminal in position. *Trypanosoma congolense* is one of the smallest trypanosomes with an average length of 12-17 μm . *Trypanosoma simiae*, the porcine trypanosome is mostly pleomorphic in its characteristics and the average length is 15-19 μm slightly longer than *T. congolense*. Nannomonas trypanosomes are very active in fresh blood films but do not tend to move far across the microscope field. They also demonstrate agglutinating properties by tending to adhere to host tissue in vivo (Molyneux and Ashford, 1983).

Trypanosoma (Duttonella) vivax

Trypanosoma (Duttonella) vivax has an average length of 20-26 μm , a long free flagellum and a large terminally placed kinetoplast, distinguishing it from the other pathogenic salivarian

trypanosomes. *Trypanosoma vivax* is a very mobile and “lively” parasite. It crosses the field of a microscope rapidly, which makes it difficult to follow its movements (Stephen, 1986).

Trypanosoma (Trypanozoon) brucei

The blood forms of *T. brucei* measure from 11-39 μm in length and they are typically pleomorphic represented by three forms. First, slender forms (average length 14-39 μm) possess a long free flagellum and a well developed undulating membrane, elongated nucleus, sub-terminal kinetoplast and narrow posterior end drawn out to a blunt point or sometimes truncated. Second, stumpy trypanosomes (average length 17-22 μm) which are stout and usually without a free flagellum, undulating membrane is well developed, nucleus rounded (displaced to the posterior end in posterior nuclear forms), kinetoplast near broadly rounded and pointed posterior end. Third, intermediate forms (average length 20-25 μm) in which the flagellum is shorter, the posterior end blunt and kinetoplast nearer to the extremity than in the slender forms. The kinetoplast in trypanozoon is smaller than in any of the other salivarian trypanosomes. Animal and human infective *T. brucei* are morphologically indistinguishable (Stephen, 1986).

Trypanosoma (Pycnomonas) suis

The total length of *T. suis* has a range from 13-19 μm with a normal distribution, indicating that this species is monomorphic. A free flagellum is typically present. Its body is very broad and short. The posterior end usually terminates in a short point but sometimes it is rounded. The small kinetoplast is usually situated near the posterior end and in the majority of cases occupies a marginal position while the voluminous nucleus lies in the anterior part of the body and the undulating membrane is conspicuous (Mulligan, 1970).

2.1.2. Tsetse and Trypanosomosis Challenge

Trypanosomosis is a disease resulting from infection with the genus *Trypanosoma* transmitted primarily by tsetse fly and also by other haematophagous flies. Trypanosome parasitizes all classes of vertebrates including human beings and it is predominantly a parasite of blood.

Animals affected with trypanosomosis become anaemic and weak; lose weight and body condition, reduced productivity and often mortality rates are high (Urquart *et al.*, 1995).

In Africa

Nearly 10 million km² of Africa is infested by tsetse flies. Part of this large area is composed of fertile land that is left uncultivated, a so-called green desert abandoned by humans and cattle. Eradicating the tsetse and trypanosomosis, the disease it carries, would allow rural Africans to reclaim areas of their continent and greatly increase food production. The tsetse fly transmits a deadly parasite, trypanosome that attacks the blood and tissue of its victims. It causes African Animal Trypanosomosis (AAT) known as Nagana in livestock and sleeping sickness in humans (FAO, 2002). The Glossina, encompasses around 30 species and sub-species members of tsetse flies. These flies are confined to a belt of tropical Africa extending from the Southern Sahara (Latitude 15° N) and in the South (Latitude 20-30° S). The species are restricted to various geographical areas according to habitat. The three main groups, named after the commonest species in each group, being *Palpalis*, *Morsitans* and *Fusca*, found to be in riverine, savannah and forest areas, respectively. The first two groups because of their presence in the major livestock rearing areas are the most important from the veterinary point of view.

The disease in human (sleeping sickness) caused by *T. b. gambiense* and *T. b. rhodesiense* is always fatal if left untreated. It causes malaise and associated waves of parasitaemia in affected individual (Jordan, 1986). Tsetse keeps people poor by preventing them from producing the food they need to survive. In fact, tsetse and trypanosomosis are major impediments to the development of sustainable agricultural systems in the region, hitting the poorest of the poor rural people in the most indebted countries in Africa (FAO, 2002).

Trypanosomosis is one of the most devastating diseases in Sub-Saharan Africa, killing 80% infected victims. The World Health Organization of the United Nations (WHO) reports over 60 million people, mainly living in rural areas of Sub-Saharan Africa, are at risk of becoming infected with the disease. Out of the estimated 500,000 people already infected 25,000 die every year and the situation is rapidly deteriorating with more than 40,000 new cases being registered

every year, excluding the many unreported cases from inaccessible rural areas (surveillance covering only 5-7 % people at risk).

Trypanosomosis kills 3 million livestock animals each year and reduces the productivity of sick animals. About 50 million animals are at risk from Nagana. Domestic livestock in Africa are important as sources of protein (meat and milk), animal traction and investment (social security) and measure for enhancing agricultural or crop production (Erkelens *et al.*, 2000). The Food and Agricultural Organization of the United Nation (FAO) has estimated that 35 million doses of trypanocidal drugs (worth about USD 35 million) are bought every year in futile efforts to maintain livestock free of the disease.

The annual losses directly attributed to trypanosomosis, in terms of reduced meat and milk production and in terms of the costs related to treating the disease or controlling the vector, have been estimated at US \$ 1.2 billion. This figure rises to over USD 4.5 billion per year, if losses in potential crop and livestock production attributable to the disease are considered and excludes the losses attributable to the effects of sleeping sickness in humans. Majority of the areas that are infested with tsetse flies are most suitable for livestock and crop production extending from Senegal in the north to South Africa in the south. These areas, however, are virtually devoid of cattle and other domestic livestock (Figure 1).

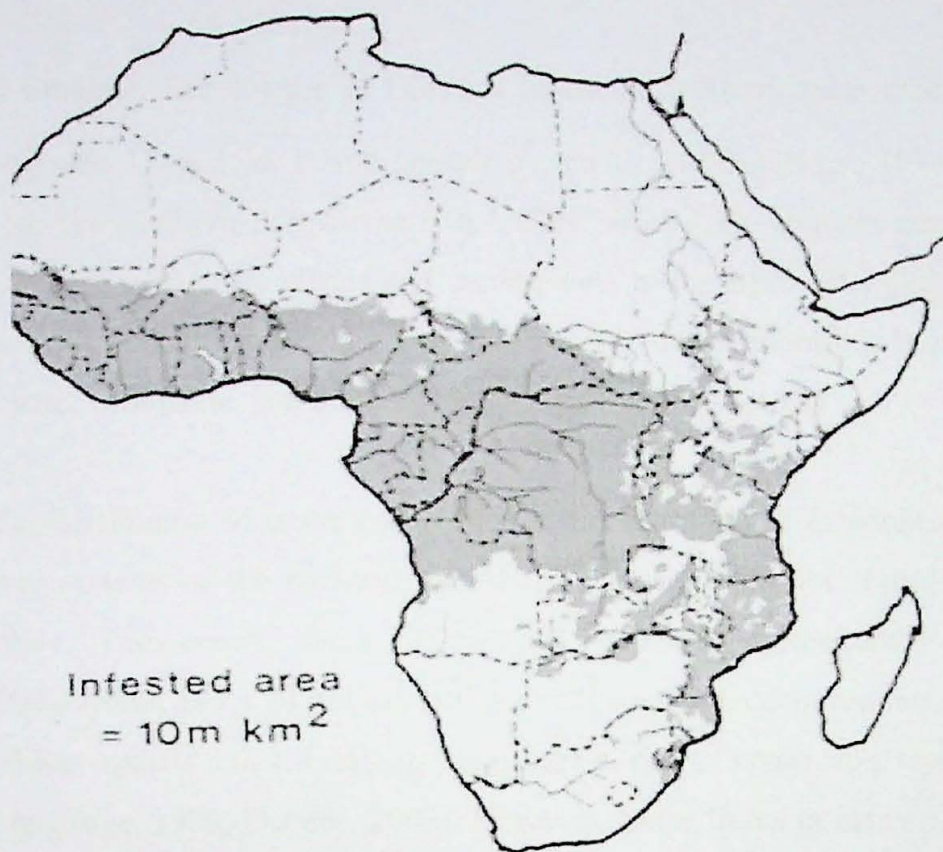


Figure 1: Distribution of tsetse flies in Africa

(Source: WFP, 1998).

Reports of reinfestation of areas that had previously been cleared of the tsetse fly are frequent. The numbers of cases recorded of the disease in man and domestic animals have reached unprecedented levels. Despite this situation, no vaccine against the disease is available and no new drugs are being developed. Some of the drugs used to treat sleeping sickness are highly toxic. All drugs currently used to treat trypanosomosis have been rendered largely ineffective by widespread drug-resistance. The future availability of drugs against trypanosomosis is uncertain since their continued production is threatened for commercial reasons. The only market is Africa where the purchasing power of the consumers affected is poor and rapidly deteriorating (PATTEC, 2001).

In Ethiopia

In Ethiopia, five species of *Glossina* infest the western and southern parts of country between longitude 33° and 38° E and latitude 5° and 12° N (Langridge, 1976). The infested area extends from the southern part of the Rift Valley, around the southwestern corner of the country and along the western lowlands and escarpments to the Blue Nile. Tsetse, through the disease they transmit, largely prevent keeping of cattle and cause enormous losses wherever they come into contact with cattle, and there by hindering rural development.

The distribution of tsetse and related trypanosomosis in Ethiopia is associated with the major river systems of the country; the Abay/Didessa, Omo/Gibe, Baro/Akobo and the southern rift valley. They occupy about 135,000-220,000 km² of agriculturally suitable land (Slingenbergh, 1992), where about 14 million head of cattle, an equivalent number of small ruminants, nearly 7 million equines and 1.8 million camels are at risk of contracting trypanosomosis at any one time (Langridge, 1976; Debebe, 2003). However, tsetse fronts in many places are unstable and tsetse-animal interface is constantly moving. Consequently, new areas are being invaded and settled communities are being continually evacuated by the advancing tsetse (Abebe, 2005).

Among the five species of tsetse reported in Ethiopia, only four (*Glossina morsitans submorsitans*, *Glossina pallidipes*, *Glossina tachinoides* and *Glossina fuscipes fuscipes*) are widespread and have significant economic importance (Debebe, 2003; Abebe, 2005). Whereas *Glossina longipennis* is of minor economic importance. *G. pallidipes* almost always associated with extensive and fragmented thickets including evergreen species. *G. m. submorsitans* is usually found in deciduous woodland and wooded grassland; often mix together with evergreen vegetation. *G. f. fuscipes* and *G. tachinoides* are common to valley forests, thickets and fringing vegetation on streams, rivers and lake shores (Langridge, 1976). *G. longipennis* inhabits in dry acacia, thorny bush and is very active after sunset and before nightfall. The geographic distributions of the different species of tsetse are presented in Table 2.

In Ethiopia, the most important trypanosomes in terms of severe economic loss in domestic livestock are the tsetse-transmitted species, which includes *T. congolense*, *T. vivax* and *T. brucei*.

The other trypanosoma species of economic importance are *T. evansi* of camels and *T. equiperdum* of horses. The *T. brucei* subspecies, *T. b. rhodesiense*, causes human sleeping sickness (Abebe, 2005). Geographic distributions of pathogenic trypanosomes in Ethiopia are depicted in Figure 2.

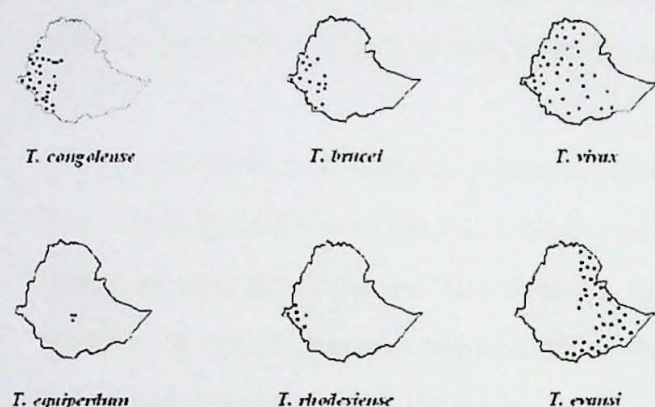


Figure 2: Geographical distribution of pathogenic trypanosomes in Ethiopia

Source: Abebe (2005)

2.1.3. Epidemiology

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

Table 1: Tsetse infested regions of Ethiopia

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

(exhaustion, lactation and parturition) also play a role in the severity of the disease (Eisler *et al.*, 2003).

Transmission

The transmission of the disease is either cyclically by tsetse flies or mechanically by hematophagous flies. Transmission by tsetse fly is a complex mechanism in which the fly remains lifelong carrier. In the vector trypanosome changes through several morphological distinct stages (amastigote, promastigote and epimastigote) until it reaches trypomastigote (metacyclic stage), which is infective for mammals (Stephen, 1986, Urquhart *et al.*, 1996).

Trypanosomosis is a disease, which is cyclically transmitted by different species of tsetse flies. The tsetse fly becomes infected with trypanosomes when feeding on an infected animal. Once the trypanosomes are ingested they lose the surface coat, develop a mitochondrion and undergo a number of developmental stages before they become infected, once more, for the mammalian host these developmental stages are known as trypomastigote, epimastigote and metacyclic forms (ILRAD, 1988). Mechanical vectors cause mechanical transmission of trypanosomosis (*T. vivax*) that is accomplished through interrupted blood meals and can occur through tsetse flies and other biting flies (Tabanids and Stomoxys). Mechanical transmission requires only the blood that contains infectious trypanosomes be transmitted mechanically and occasionally be found outside tsetse infested areas (Langridge, 1976).

Pathogenesis

The pathogenesis of the disease caused by the African trypanosomes differs according to the species causing the infection. *T. vivax* and *T. congolence* appear to be strictly parasites of the blood plasma and produce tissue injury primarily by the anaemia associated with infection. *T. brucei* is more widely distributed in the host affecting the intercellular fluids of the body cavities (Robertson, 1976). Here, although anaemia occurs, it is considered to be of secondary importance to the extensive degenerative, necrotic and inflammatory changes. The pathogenesis of

trypanosomosis includes: chancre, lymphadenopathy, anaemia, and tissue damage (Mulligan, 1970).

Chancre

The fly bite deposits metacyclic trypanosomes in dermal connective tissue of skin and here a local inflammatory reaction, the 'chancre' develops. All three cyclically transmitted species eventually undergo a chancre (Vickerman and Barry, 1982) although the local skin reaction is less severe with *T. vivax* infections (ILRAD, 1986). From the chancre, the trypanosomes enter the draining lymphatics and then the bloodstream. *T. congolence* multiplies in the tissue of the chancre as a morphologically distinct phase before it invades the bloodstream (Vickerman and Barry, 1982). The chancre phase completed, *T. congolence* and *T. vivax* remain largely intravascular parasites (Losos and Ikede, 1972), the former localizing in small blood vessels where it attaches to the endothelium. Both species are now known to occur also in the lymphatics. *T. brucei* may secondarily escape from the bloodstream into the soft connective tissues and multiply in the tissue fluid (Vickerman and Barry, 1982).

Lymphadenopathy

Following enlargement of the lymph nodes draining the chancre, generalized enlargement of lymph nodes and splenomegally develop. This is associated with marked proliferation of lymphoid cells in these organs. Numerous large active germinal centers are present and there is a marked increase in the number of plasma cells in the medullary cords of the lymph nodes and in the splenic red pulp. Marked hypergammaglobulinaemia accompanies these changes (Morrison *et al.*, 1981).

Anaemia

Anaemia plays the major role in the pathogenesis of bovine African trypanosomosis. The development of anaemia is a well recognized sign of trypanosome infection in cattle (Losos and Ikede, 1972). In most early cases, there is an acute onset of anaemia corresponding clearly with

the detection of parasites in the bloodstream (ILRAD, 1987). The initial fall in packed cell volume (PCV) value is associated with the first wave of parasitaemia in the blood. During this period the anaemia is extravascular and is possibly the result of increased red cell destruction by erythrophagocytosis in the spleen, lungs, haemal nodes and bone marrow as a result of direct traumatic effect on red blood cells thereby increasing red blood cell fragility (Mamo and Holmes, 1975; ILRAD, 1984). In cattle subjected to a single needle or fly challenge, the packed cell volume (PCV) progressively decreases by about 40-50% over the first 4-6 weeks (Morrison *et al.*, 1981). During this period the anaemia is haemolytic due to production of toxins like haemolysins (lytic factor associated with protein of low molecular weight which has anaphylatoxin activity and the release by autolysing trypanosomes of endogenous phospholipases)(Holmes and Jennings, 1976; Morrison *et al.*, 1981).

There is evidence that immune sensitizations of red blood cells occur in animals infected with trypanosomes. The trypanosomal antigens can attach to red blood cell membranes and thereby make them susceptible to phagocytosis. The contribution of splenomegally to red cell membrane was suggested that pooling of red cells which may occur in the enlarged extra-sinusoidal compartments having detrimental effect leading to increased osmotic fragility and secondly, enhanced phagocytic activity of reticulo-endothelial system (RES) which may lead to clearance of both sensitized and normal red cells (Holmes, 1987). Haemodilution also contributes to the anaemia of African trypanosomosis by a disproportionate increase in the plasma volume as demonstrated in cattle (Mamo and Holmes, 1975) and laboratory animals (Holmes and Jennings, 1976). This phenomenon was commonly observed to occur during acute phases of the disease. Death may occur within 4-12 weeks after infection (Morrison *et al.*, 1981).

Cattle that survive the acute process, progress into a chronic anaemia. This may still result in death, or in either spontaneous recovery or survival with persisting low grade anaemia. This chronic phase is characterized by low and transient parasitaemia or complete absence of detectable parasites in the blood. Death results from congestive heart failure (Morrison *et al.*, 1981).

Bleeding disorders are commonly associated with trypanosome infections and may become a major aspect of the pathogenesis of trypanosomosis in cattle with a haemorrhagic syndrome associated with *T. vivax* infection (Wellde *et al.*, 1983; Holmes, 1987). In this type of infection, the trypanosomes rapidly reach high numbers in the bloodstream and there is a severe drop in the number of red blood cells and platelets, a dramatic enlargement of the spleen and extensive haemorrhaging, both of the external mucous surfaces and the internal viscera and especially of the gut. Cattle may die of this disease in two weeks (Wellde *et al.*, 1983; ILRAD, 1984; Olubayo and mugera, 1985).

Tissue Damage

As might be expected from the blood-borne nature of trypanosome infections, most tissues and organs are damaged during the course of infection although some are more consistently and severely infected than others. While necrosis is not a major feature of the disease in cattle, tissue cell damage and degeneration may be marked. The nature of the cellular infiltrate and possibly the mechanisms involved in cell injury would appear to depend on the difference in tissue invasiveness between species of trypanosomes. One vital organ which is consistently damaged by all three species of trypanosomes is the heart. The changes which occur in the heart also reflect to some extent what occurs in other tissues and organs. Other vital organs which are commonly affected include the skeletal musculature, central nervous system, endocrine organs and the reproductive tract (Robertson, 1976; Morrison *et al.*, 1981; Hall, 1985; Abebe, 1991).

Clinical Features

Acute infections may be seen occasionally in all domestic animals notably with *T. vivax* in cattle leading to death after 1-3 weeks (Robertson, 1976). Regardless of the species of trypanosomes and the species of the host, the principal clinical signs are intermittent fever, an increasing degree of anaemia and progressive loss of condition and the disease is seen more commonly as a chronic form (Robertson, 1976; Hall, 1985). Infected animals are dull, have a staring lustreless coat, lose weight and are easily exhausted, lagging behind the herd, superficial lymph nodes are enlarged

and prominent, cattle infected with *T. vivax* often show photophobia and excessive lacrimation (Robertson, 1976; Murray *et al.*, 1983; Hall, 1985; Losos, 1986).

Pathology

Post-mortem examination of animals after acute trypanosomosis may show extensive small haemorrhages involving mucous and serous surfaces, areas of emphysema in the lungs and mild gastroenteritis. After more chronic infections, the carcass may be anaemic and emaciated with an enlarged spleen and lymph nodes (Robertson, 1976) subcutaneous oedema and accumulations of pericardial and thoracic fluid containing trypanosomes are found particularly in horses and dogs infected with *T. brucei* (Seifert, 1996). Degenerative changes have been observed in testis and epididymis of sheep, goats, and cattle infected with *T. congolense*, *T. brucei* and *T. vivax*. Pathological changes of pituitary and adrenal glands with associated endocrine dysfunction have been observed in Boran (*Bos indicus*) cattle infected with *T. congolense* (Abebe, 1991).

2.1.4. Diagnosis

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

Clinical diagnosis

In general, diagnosis of trypanosome infection based on clinical signs alone is rather difficult, but haematological parameters like PCV could be reliable indicators of the progress of the disease. Intermittent fever can be observed due to the variation in parasitaemia and if the animal survives, the disease becomes chronic and there is development of anaemia and emaciation. Therefore, fever, anaemia and loss of body condition are important parameters used routinely for tentative

diagnosis of trypanosomosis in areas where this disease is endemic and laboratory service are not available (Uilenberg, 1998). Definitive diagnosis of the disease is ultimately dependent on the detection of the trypanosome in blood samples from infected animals.

Parasitological diagnosis

Parasitological diagnosis is the direct demonstration of the parasite in blood or less commonly in other body fluids using a microscope. The scarcity of the parasites and the fluctuating nature of the parasitaemia limit the use of the laboratory tests based on demonstration of trypanosomes in accessible body tissues such as the peripheral blood (Doyle, 1977). Therefore, several techniques for the concentration of blood trypanosomes have been developed, which increase the chance of trypanosome detection.

Dark ground or phase contrast buffy coat technique

The buffy coat zone prepared in a microhaematocrit capillary tube filled with 70 μ l of blood and centrifuged for 5 minute at 10,000 revolutions is examined for trypanosomes by cutting the capillary tube to include 1mm of erythrocytes and 1cm of the plasma. The buffy coat is poured on a slide and covered with a 22x22mm coverslip. The preparation is examined using a microscope with a phase contrast and dark ground illumination. The use of 10x eyepiece in combination with a 25x objective gives optimal viewing, by allowing large visual fields and sufficient magnification for ready identification of trypanosomes. This technique is the most sensitive of the parasitological tests for the detection of *T. congolense* and *T. vivax* detecting trypanosomes to an estimated level of just over 102 parasites per ml (Murray *et al.*, 1977). In addition, species identification based on size and movement is easier (Paris *et al.*, 1982). Trypanosomes can be identified and the level of parasitaemia estimated using a scoring system (Murray *et al.*, 1983). The PCV is measured before examination of the blood for parasitaemic detection.

Haematocrit centrifugation technique (Woo, 1970)

A microhaematocrit capillary tube containing 70 µl of blood is centrifuged for 8 minute at 10,000 revolutions as for measurement of PCV. Two rectangular pieces of glass from a standard microscope slide (1.2 mm thick) are fixed 1.5 mm apart on a microscope slide. The prepared capillary tube is then placed in the slot and a drop of immersion oil put on top of the capillary tube. The oil fills the space between the capillary tube and the two pieces of glass, thus reducing the effect of light diffraction. By slowly rotating the tube the Buffy coat plasma junction is examined using a long working distance (6.7mm) objective which allows considerable depth of focus through the capillary unlike the standard objective where the average working distance is approximately 0.5mm. Depending on the trypanosome species the analytic sensitivity for this method is $1-5 \times 10^2$ trypanosome per ml of blood.

Capillary concentration technique

Because of the tendency of *T. congolense* to be retained amongst red blood cells (RBC), this technique was designed to create a large differential density between the RBC and the parasite. This was achieved by mixing infected blood with a strongly hypertonic nontoxic medium (Walker solution). On centrifugation, the denser red cells separate from the trypanosomes, which display normal motility. Equal volume of diluents and blood are mixed on a micro titer titration plate. After being allowed to stand for a minimum of 15 minutes plain capillary tubes are three quarter filled from the wells, sealed and spun for 2 minute in a microhaematocrit centrifuge. The capillary tubes are placed on a clean microscope slide and the Buffy coat zone covered with a few drops of diluents beneath a cover slip. This technique is more sensitive than haematocrit centrifugation test in the detection of *T. congolense*. However, it needs more time to prepare the samples and PCV cannot be measured at the same time (Walker, 1972).

Serological diagnosis

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

Antibody ELISA

Luckins and Mehltz (1976) used micro plate-ELISA system in their study of bovine trypanosomosis and found that cattle developed positive ELISA values after infection but it was not possible to differentiate between *T. vivax*, *T. congolense*, *T. brucei* or *T. rhodesiense*. The serological tests in current use suffer from a lack of well-defined antigens necessary for designing simple and accurate tests that are easily adaptive for field use. Secondly, the detection of antitrypanosomal antibodies in serum cannot distinguish between an active infection and a past infection. The length of time taken for antibodies to disappear from circulation after a successful therapy of cattle is not yet clear. Thirdly, the present serological tests are not sufficiently specific to reveal conclusively the identity of the infecting trypanosomal species (Nantulya *et al.*, 1987).

Antigen ELISA

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

Polymerase chain reaction

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

2.1.5. Impacts of trypanosomosis on productivity and reproductive performance

Impact on livestock productivity

Trypanosomosis is characterized by intermittent fever, anemia and lymphadenopathy often followed by death in untreated cases (Mulligan, 1970). In 38 countries of Africa, 30% of the total of approximately 147 million cattle and 50 million people in the continent are said to be at risk from trypanosomosis (Murray and Gray, 1984; Kuzoe, 1991).

The direct impact of trypanosomosis is anemia as a result of which the animal becomes morbid and its ability to perform function other than maintenance is reduced. In addition, trypanosomes reduce the effectiveness of the animals' immune system. It has been reported that trypanosomosis kills 3 million animals each year and reduces productivity of sick animals (Erkelense *et al.*, 2000). Another estimate has shown that trypanosomosis reduces the total number of livestock in affected area by between 10 and 50%. With an output elasticity of 0.2, we can predict that trypanosomosis would reduce agricultural gross domestic product by 2% to 10% (Trail *et al.*, 1985).

Adult cattle infected by trypanosomes undergo also weight loss, abortions and infertility. In young animals, reduced growth rate, reduced calves survival rate and delayed onset of puberty is common in herds found in areas of high trypanosomosis challenge (Osaer *et al.*, 1999). These all subsequently result in loss of meat and milk protein to the population and consequently failure to

fulfill the demand for exports of meat and dairy products (ILCA, 1985). The annual loss in meat production alone in Africa is estimated at 5 billion USD in 1984 (Murray and Gray, 1984).

Impact on reproductive performances

In male animals, inflammation of testicles is reported in trypanosomosis caused by *T. b. brucei* that leads to degeneration of the seminiferous tubules, slowed spermatogenesis and aspermia, and in severe cases bulls become infertile (Ikede *et al.*, 1988). Decline in semen quality and quantity and spermatozoal abnormalities were observed in goats infected with *T. evansi* and in most severe cases, there were aspermia and atrophy of testicles (Sekoni, *et al.*, 1990; Ngeranwa *et al.*, 1991). In another study, it has been indicated that Zebu x Friesian crossbred bulls infected with *T. vivax* and *T. congolense* developed highly deformed spermatozoa with multiple morphological defects, which can render these bulls unfit for breeding within a very short time (Sekoni *et al.*, 2004). Chronic diseases either due to *T. vivax* or *T. congolense* could be important causatives of infertility or sterility in Zebu x Friesian crossbred bulls.

In female animals, abortion, long intervals between calving/lambing, irregular estrous cycles and infertility are common in chronic cases. Although abortions are often reported from cows infected with trypanosomes, parasites are rarely detected in the fetus. Persistent corpus luteum, a condition normally associated with endometritis, occur in *T. congolense* infected cattle and goats. Cystic ovaries and endometritis have been reported from cattle infected with *T. vivax* (ILCA, 1985). In other study it has been demonstrated that infection with pathogenic *T. vivax* during late pregnancy influenced the outcome of pregnancy in both susceptible Galana and trypano-tolerant Orma Boran heifers, resulting in premature births, perinatal loss, retained placentae, low birth weights and a prolonged period to the onset of postpartum ovarian activity.

Indirect impacts

There are also some indirect costs associated with the use of curative and prophylactic treatments. Estimates have shown that African livestock producers and governments spend at least 30 million USD per year to treat (25-35 million doses) animals exposed to trypanosomosis (Jahnke *et al.*, 1988; Borne, 1996). In addition, the impact extends to restricted access to fertile and cultivable

areas (about 10 million square kilometer of land in Africa and up to 220,000 square kilometer of fertile land in Ethiopia) (FAO/WHO/OIE, 1982; Slingenbergh, 1992), imbalances in land use and exploitation of natural resources and compromised growth and diversification of crop-livestock production systems.

2.2. Major GIT helminthes of cattle

2.2.1. Major GIT nematodes of cattle

Nematodes parasites are major causes of production loss, impaired GIT function via competition for the hosts essential nutrients and damages during their feeding. These effects could be manifested by sever clinical signs such as anemia, edema, diarrhea and anorexia resulting in poor general performance, even mortality particularly in the young, aged and immune suppressed individuals (Fox, 1997; Eysker and Ploeger, 2000).

Mode of host damage by GIT nematode

GIT nematodes that cause decreased feed ingestion and utilization by the host

This group includes mainly *Teladorsagia spp.*, *Trichostrongylus spp.*, *Cooperia spp.*, *Strongyloides papillosus*, *Oesophagostomum spp* and *Nematodirus spp*. The pathological changes observed due to these parasites include: tunnel under mucosal epithelium of abomasum (*Teladorsagia spp .*, *Trichostrongylus axei*) and small intestine, erosion of mucosal epithelium of abomasum and intestine, catarrhal enteritis, villous atrophy in anterior small intestine, hyperemia and edema. In extreme cases, diphtheritic enteritis and exudates hinder absorption (Dunn, 1978; Soulsby, 1986; Urquhart *et al.*, 1996; Radostits *et al.*, 2000).

The manifestation of these lesions is marked alteration in the influx and efflux of water and electrolytes (chlorine and sodium ions) in the bowel and morphological and biochemical changes in the epithelial cells and their microvilli (Soulsby, 1986) leading to protein losing enteropathy,

accompanied by excessive mucus production, diarrhea, weight loss, hypo proteinaemia (Smith and Sherman, 1994; Urquhart *et al.*, 1996), sometimes pica (Troncy, 1989) and in chronic cases this form is almost indistinguishable from malnutrition (Dunn, 1978).

GIT Nematodes that remove the host's tissue and blood

This group of parasite includes *Haemonchus* spp., *Chabertia ovina*, *Gaigeria pachyscelis*, *Bunostomum trigonocephalum* and *Trichuris* spp. In most cases, the predominating syndrome is said to be progressive debilitating anemia (Smith and Sherman, 1994), but hyper acute and acute infection of *Haemonchus contortus* and acute infection of *Gaigeria pachyscelis* could lead to death without any more clinical manifestation than acute anemia (Dunn, 1978; Soulsby, 1986; Troncy, 1989; Smith and Sherman, 1994; Bowman, 2003; Radostits *et al.*, 2000).

The losses caused by *Haemonchus contortus* are more severe and important due to its extreme pathogenicity, wide geographical distribution in tropics (Dunn, 1978; Troncy, 1989). *Gaigeria pachyscelis* is a highly virulent nematode that sucks blood and can cause death of a host even in small burden as few as 24 parasites (Soulsby, 1986). *Chabertia ovina* is a plug feeding parasite of large intestine which causes loss of blood when it draws plugs of mucosa by its wide mouth, and its blood sucking is said to be accidental, and loss due to hemorrhage at the biting site is voluminous (Soulsby, 1986; Troncy, 1989). *Bunostomum trigonocephalum* is a blood sucker causing progressive anemia (Dunn, 1978; Soulsby, 1986) and blood sucking habit of *Trichuris ovis* was indicated by Troncy (1989).

The clinical manifestations due to these nematodes vary from sub-clinical to bleeding to death (Dunn, 1978; Soulsby, 1986; Troncy, 1989; Urquhart *et al.*, 1996) and could be generally summarized as pale mucus membrane, edema on the ventral aspect of the body, bottle jaw, weakness, wool falls out in patches, prostration and death.

GIT nematodes that cause marked nodular reaction

According to Soulsby (1986), these nodules are due to leucocytes, especially eosinophils and foreign body giant cells collected around the parasites and the focus becomes encapsulated by fibroblasts. Whenever there is massive infestation, the number of nodules will be numerous while the colon contains few adult worms. Extensive nodules on the intestinal walls and mucosa interfere with absorption, bowel movement, digestion and when these suppurative nodules rupture to peritoneal surface causing suppurative peritonitis and multiple adhesions (Soulsby, 1986; Smith and Sherman, 1994).

Morphology and Biology

Nematodes are multicellular organisms with unsegmented with elongated body and externally covered by cuticle. The sexes are separate, with females generally larger than males. The cuticle may be modified to form inflation, expansion and papillae. The mouth opening in its simplest form is only a pore, whereas in highly specialized forms it leads to a bucal capsule of variable size (Hendrix, 1998).

The oesophagus is filariform type. In the male nematodes, the caudal end may terminate on a cuticular expansion, the copulatory bursa, with bursal lobes and rays which are useful in identification. In the case of *Haemonchus* species asymmetrical dorsal lobe is a characteristic. Accessory male organs are sometimes important in identification, especially of the trichostrongyloids, the two most important being the spicules and gubernaculums. The spicules are chitinous organs, usually paired, which are inserted in the female genitalia opening during copulation. The gubernaculums, also chitinous, is a small structure which acts a guide for the spicules (Dunn, 1978; Urquhart *et al.*, 1996).

The life cycle of most of GIT nematode is similar, the cycles are direct, that is these nematodes do not require other animals to complete their life cycles (Urquhart, *et al.*, 1996). The life cycle is direct with a single host. Females are oviparous. The eggs passed in the external environment, under favourable conditions (temperature and humidity) hatch to first stage larvae (L₁). The L₁

develop and moult to second stage larvae (L₂), and then the L₂ in turn moult to third stage larvae (L₃), which is the infective stage. The first three larval stages are free-living constituting the pre-parasitic phases. Within GI tract of the host the L₃ moult to fourth stage larvae (L₄), which transform to a young adult stage (L₅) which eventually becomes mature and start to lay eggs (Soulsby, 1986; Urquhart *et al.*, 1996).

Adult nematodes inhabit the gastro-intestinal tract. Eggs produced by the female gastro intestinal nematodes are almost similar. They are elliptical with oval shell. Depending on the species, the dimension of these eggs varies (55-100µm x 25-35µm). The only exception is the case of *Nematodirus* egg, which is much larger measuring 150 -230µm (Troncy, 1989). The eggs embryonate and hatch into first-stage larvae (L₁), which in turn moult into second-stage larvae (L₂) shedding their protective cuticle in the process. The L₂ larvae moult into third-stage larvae (L₃), but retain the cuticle from the previous moult during this stage, the larvae leave faecal pellet and available on nearby pasture waiting for a host (Soulsby, 1986).

These double-cuticles L₃ is the infective stage. The time required for the eggs to develop into infective larvae depends on temperature. Under optimal conditions (high humidity and warm temperature), the developmental process requires about 7 to 10 days. In cooler temperature the process may be prolonged. Ingesting of the L₃ infects ruminants. Most larvae are picked up during grazing and pass to the abomasum, or intestine, ex-shedding the extra cuticle in the process. The L₃ of the *Trichostrongyle* group penetrates the mucous membrane (in the case of *Haemonchus* and *Trichostrongylus*) or enters the gastric glands (*Ostertagia*). During the next few days the L₃ moults to the fourth stage (L₄) and remains in the mucous membrane (or in the gastric glands) for about 10 to 14 days. They then emerge and moult into a young adult stage (L₅). Most *Trichostrongyles* mature and start egg production about 3 weeks after infection (Urquhart *et al.*, 1996). The parasitic part of the life cycle of *Oesophagostomum* requires about 6 weeks completing the life cycle. The infective L₃ penetrate the lamina propria of the intestinal wall and the host response to the infection, which surrounds the L₃ results in the formation of fibrous nodules.

The larvae emerge into the lumen of the intestine after about 2 weeks and mature in the following 4 weeks. If animals previously infected, the larvae may spend a prolonged period of time (3-5 months) in the nodules. Eventually many of the larvae will die and the nodules may become calcified. The L₃ larvae of *Bunostomum* infect ruminants when they are ingested or penetrate the hosts skin. Following skin penetration, the larvae are carried in the venous blood through the heart to the lungs, where they penetrate the alveoli, are coughed up and then swallowed, and so pass to the small intestine. Here they moult and mature in 8-9 weeks after infection (Reinecke, 1989). The infective larval stage of *Trichuris* is contained within the egg. The larva is released after the egg is ingested by the host (Charles, 1998). According to Anene, (1993) adult female nematodes produce eggs. The period between the infection of an animal by ingestion of infective L₃ larvae and the first egg production by the adult female parasite is called the prepatent period. This period is different for different species of nematodes which varies from one to three weeks. For some other gastro-intestinal parasites, the prepatent period is about 3-4 weeks (Egwang and Slocombe, 1981).

Different species of nematodes have different egg-producing capacities. The individual female *Cooperia*, for example, produces many eggs but is not very pathogenic. Females of *Trichostrongylus* are quite pathogenic but produce few eggs. This means that the number of nematode eggs in a faecal sample is not an accurate indication of the amount of damage being done by gastro-intestinal parasites (Urquhart *et al.*, 1996).

Table 2: Daily egg production per female of some gastro-intestinal nematodes

No	Species	Daily egg production/female
1	<i>Haemonchus</i>	5000-15000
2	<i>Ostertagia, Trichostrongylus</i>	100-200
3	<i>Cooperia</i>	1000-3000
4	<i>Nematodirus</i>	50-100
5	<i>Oesophagostomum, Chabertia</i>	5000 - 10000

Source: O' grady and Slocombe (1980).

Epidemiology

The study of Epidemiology is based on an intimate knowledge of the life cycle as well as the complex host parasite relationship in the parasitic stage. In nematode parasite the emphasis is on the seasonal incidence, which has been mentioned previously. Many factors are known to influence the transmission and prevalence of nematode infection in grazing ruminants (Urquhart, *et al*, 1996). Broadly, the influencing factors that can determine the occurrence of ruminant GIT nematodiosis could be mentioned as summarized below by Smith and Sherman (1994) and Stromberg (1997).

Environment-Parasite interaction

Nematode eggs are deposited on the pasture in faecal pellets and pass through developmental stages in that faecal matter. L₃ moves out of the faeces and onto herbage. The development from egg to L₃, the survival of all stages to that point, the translation of L₃ onto herbage and the duration of survival of L₃ determine the infectivity of pastures. All of the factors are themselves dependent on climatic conditions (Urquhart *et al.*, 1996).

Hypobiosis

Ostertagia spp, *H. contortus* and *T. axei* can arrest their development at the early L₄ stage. This phenomenon is probably not primarily a host immune response but a survival technique developed by the parasite. Inhibition is not a characteristic of intestinal trichostrongylosis (Troncy, 1989).

Ingested larvae are more likely to be inhibited at some particular times of the year. For instance, in case of *H. contortus*, some ingested larvae are immediately rejected, depending on the immune status of the host. In such case, the proportion which becomes inhibited instead of developing directly to adults increases from near zero in January to an overwhelming majority in winter in summer rainfall zones. This seasonal inhibition occurs independently of the immune response of the animal and appears to be a result of an environmental effect on the larvae during the free-

living stage. Infective larvae ingested in spring and summer has the ability to develop directly to adults, if not rejected by the host. The inhibition of larvae ingested in autumn and winter appears to be an evolutionary adaptation which favors the parasite by delaying the egg laying phase of the life cycle until after winter when a higher proportion of eggs will be able to develop and complete their life cycles (Barger, 1987).

Maximum inhibition of *Ostertagia* spp and *T. axei* occurs for larvae ingested in late winter and early spring. Without treatment effective against inhibited larvae, it is assumed that development of these parasites resumes in autumn, leading to contamination of pastures in autumn and winter, when eggs have a higher biotic potential than in spring. Firm proof of the normal fate of inhibited *Ostertagia* spp and *T. axei* is lacking. Inhibited larvae of any genus do not cause any pathogenic effects while they remain inhibited (Stromberg, 1997).

Climate and Season

In the cool tropical environment of Ethiopia, Tembley *et al.*, (1998) recovered a large number of nematode parasites from ruminants during wet season. Similarly peak infection rate of GIT nematodes were reported during the rainy season and lower infection rate during dry season of the year in southern Nigeria (Fritsche *et al.*, 1993).

This seasonal fluctuation may be due to a number of factors which are responsible for the numbers and availability of infective stage. These may be conveniently be grouped as factors affecting contamination of the environment, and those controlling the development and survival of the free living stage of parasite. The number of parasite eggs found in the faces is influenced by number of adult parasites established in the gastro-intestinal tract, level of host immunity, age of the host, species of parasite, stage of infection, parturition, consistency of the faces (Urquhart, *et al.*, 1996; Stromberg, 1997).

Grazing Behavior of Hosts

Grazing behavior of the host is one of the influencing factors affecting the epidemiology of GIT nematodes. Sheep grazing close to ground are more exposed to massive numbers of infective larva compared to free ranging goats that are less exposed to the infective larva since their feeding behavior includes a large component of browsing at levels well above the ground (Smith and Sherman, 1994).

Flock Management

Overstocking and prolonged grazing on the same pasture lead to over grazing (loss of available herbage per animal), and hence lowers plane of nutrition. It also encourages large amount of fecal deposit on the grazing field and potentially to higher level of infectivity per unit area. Due to this overstocking and prolonged grazing on a plot of land, besides affecting the growth rate of animals, it may exacerbate the pathogenic effect of acquired infections by lowering the protective immunity (Troncy, 1989; Ndamukong *et al.*, 1994; Thamsborg *et al.*, 1996).

Feed availability and quality

Nutrition plays a great role in worm burden control. Poorly fed animals are more susceptible and carry more worm burden due to their failure to overcome of infection. In tropical Africa where ruminants are kept under grazing management, the feed availability and quality mainly depends on the season of the year. Wet season being good both for feed availability and the quality is also conducive for parasite-larvae survival and development on pasture, while dry season is bad both to the host and parasite larva. But during dry season, accumulation of flock along water courses for the search of water and green grass favors disease transmission and spread between flocks (Troncy, 1989; Teklay, 1991).

Development and survival of infective larvae in the environment

The development of larvae in the environment depends upon the warm temperature and adequate moisture. In most tropical and sub-tropical countries, temperatures are permanently favorable for larval development in the environment. Exceptions to this are the highland and mountainous regions throughout the world, and the winters of southern Africa and Latin America where temperatures may fall below those favorable for the development of *Haemonchus* larvae (Anene, 1993). The ideal temperature for larval development of many species in the microclimate of the tuft of grass or vegetation is between 22°C and 26 °C. Some parasite species will continue to develop at temperatures as low as 5 °C or at much slower rate. Development can also occur at higher temperatures, even over 30 °C, but larval mortality is high at these temperatures. The ideal humidity for larval development in this microclimate is 100%; the minimum humidity required for development is about 85%. The survival of larvae in the environment depends upon adequate moisture and shade. Desiccation from lack of rainfall kills eggs and larvae rapidly and is the most lethal of all climatic factors (Troncy, 1989; Urquhart *et al.*, 1996).

Larvae may be protected from desiccation for a time by the crust of the fecal pat in which they lay or by migrating into the soil. Infective larvae may survive for up to 6 weeks or even longer in the manure pats, which act as a reservoir of infections during dry periods. The development of infective larvae ingested by an animal during adverse environmental conditions may be temporarily arrested in the abomasal or intestinal mucosa. This suspension of development helps some nematode parasites to survive in the dry seasons. Among the three larval stages in the environment (L₁, L₂ and L₃), it is the L₃, which has a protective sheath that is the most resistant to moisture, temperature and sunlight to make themselves accessible to ingestion by ruminants, the larvae have to migrate or be transported from the faeces in which they were deposited on the ground to any nearby herbage. Such movement occurs in two ways: horizontal migration/transport and vertical migration/transport (Urquhart *et al.*, 1996; Stromberg, 1997).

2.2.2. Fasciola

The disease

The disease, fasciolosis, is caused by digenetic trematodes/flukes of the genus *Fasciola*, commonly referred to as liver flukes. The two species most commonly implicated are *F. hepatica* and *F. gigantica*. Meanwhile, *F. hepatica* has a worldwide distribution but predominates in temperate zones and cooler areas of high altitude in tropics and subtropics while *F. gigantica* is found in many regions of the world, mainly with wide range of distribution in tropical regions (Urquhart *et al.*, 1996; Okewole *et al.*, 2000).

Even though all ruminants are susceptible to *Fasciola* specie, frequent infection occurs in sheep, cattle and buffalo and has economic importance. Goats, camel, horses, deer and many other species of herbivore can be infected; however, the parasite is of less economic importance on a global scale in these hosts (Mira and Ralph, 1989; Torgerson and Claxton, 1999).

Fasciola hepatica is wide spread in areas with altitude range of 1800 to 2000 masl while *F. gigantica* appear to be most common in areas below 1200 masl; in between these altitude limit both species co-exist as the ecology is conducive for both snails so that mixed infection is envisaged (Urquhart *et al.*, 1996; Malone and Yilma, 1998)

F. hepatica and *F. gigantica* causes major economic losses in livestock predominantly in East Africa. *F. hepatica* was to be shown the most important fluke species in Ethiopia where it is distributed to over three quarter of the nation except in the arid-ecosystem lying in northeast and east of the country. Meanwhile the distribution of *F. gigantica* was localized mainly in the western humid zone of the country that encompasses approximately one fourth of the nation (Malone and Yilma, 1998).

The presence of fasciolosis in Ethiopia has been known a long time and is responsible for causing considerable losses in livestock production. Several researchers have reported its prevalence and economic significance. Moreover, in recent years, small-scale irrigation and water conservations

are being expanding in many parts of the country, which has created habitats for fluke spreading vector and concomitant increased occurrence.

The parasite morphology

The genus *Fasciola* is a digenean parasite of ruminants and other mammalian hosts, which undergoes sexual and asexual generation parasitizing alternative hosts. *Fasciola hepatica* is leaf shaped worm of variable size ranging from 20 to 30 mm long and 10 to 13mm wide, dark brown in color, marginal zone of vitelline is easily seen with two suckers close together at the anterior end, broader in the anterior than in the posterior and cone shaped (Elmer and Glenna, 1983). The cuticle is covered with backwardly directed spines that wear off with age, but are always visible microscopically in the hollow of the shoulder and on the anterior cone through which trauma of hepatic parenchyma is caused (Bowmann *et al.*, 2003). The flukes are easily recognized grossly and the only confusion likely is with *F. gigantica*. *F. gigantica* is similar with *F. hepatica* but larger in size ranging from 25 to 75 mm long and 3 to 12 mm wide, and has less prominent shoulders (Elmer and Glenna, 1983; Urquhart *et al.*, 1996).

The eggs of both *F. hepatica* and *F. gigantica* are similar in that both are oval, operculated and yellowish- brown in color measuring 130 to 150µm by 60 to 90µm and 156 to 197µm by 90 to 104µm, respectively, and have a thin shell containing a morular mass (Soulsby, 1986; Hendrix, 1998).

The snail intermediate host

The principal intermediate hosts of *Fasciola* species are *Lymnaea truncatula* for *Fasciola hepatica* and *Lymnaea natalensis* for *F. gigantica*. All require water especially for breeding but spend much of their time out side. They need high oxygen, and are never found in deep water (Radostitis *et al.*, 1994; Urquhart *et al.*, 1996; Bowmann *et al.*, 2003). *Lymnaea natalensis* is truly aquatic where as *L. truncatula* is amphibious (Troncy, 1989). The lymnaeid snails that serve as intermediate hosts require neutral soils that remain reasonably moist throughout the year and tend to flourish where winter areas are not so cold as to destroy the eggs and juvenile stages thus

permitting the parasite population to survive its season of hardship in both definitive host and the environment (Bowmann *et al.*, 2003).

Fasciola hepatica uses a mud for the intermediate host survival so that infection with this species is usually associated with herds and flocks grazing in the mud areas during wet season or on marshy land. On the other hand, *F. gigantica* uses water snails for its intermediate host. Therefore, infestation with this species is associated with livestock grazing in wet and irrigated lands (Spithil *et al.*, 1999).

According to Urquhart *et al.* (1996) and Andrews (1999), *L. truncatula* is the most common and widely distributed snail in the world. *Lymnaea truncatula* are capable of withstanding summer drought or winter freezing for several months by hibernating deep in the mud. Optimal conditions includes slightly acidic pH environment and a slowly moving water medium, they feed mostly algae and the optimal temperature range for development is 15 to 22⁰c, below 10⁰c development ceases. *Lymnaea natalensis* is aquatic and associated with permanent water channels or dams. Development with the snails is completed at the end of rainy season (Urquhart *et al.*, 1996; Bowmann *et al.*, 2003).

Life cycle

The life cycle of *Fasciola* species consists of five phases from egg laying up to adult worm formation in succession: passage of eggs from the final host, hatching in the environment, development in the intermediate host, encystment and ingestion of the larvae by the final host (Andrews, 1999).

The life cycle of *Fasciola hepatica* is typical of the family Fasciolidae. It is indirect where the parasite requires snails as intermediate host. The principal intermediate hosts of *Fasciola* are *Lymnaea truncatula* for *F. hepatica* and *Lymnaea natalensis* for *F. gigantica* in Africa (Radostitis *et al.*, 1994, Urquhart *et al.*, 1996).

Fasciola eggs are laid in the bile ducts, pass down into the intestine and then pass out in the faeces. They develop and hatch releasing motile ciliated miracidia. This takes nine days at optimal temperature of the 22-26 °C and little development occurs below 10 °C (Hendrix, 1998).

The mechanism of hatching is a complex process, depending on responses to several external and internal stimuli. Lights, and tonicity of the environmental fluid, are among the external factors and the miracidium itself contributes an enzyme, which acts upon the eggshell, and in particular, upon the cement sealing down the operculum which is present in trematode eggs (Radostitis *et al.*, 1994).

The liberated miracidium has a short life span and must find a suitable snail within three hours for successful penetration. In infected snails, development proceeds through sporocyst and radiae stages to final stages in the intermediate host, the cercariae. The cercariae are shed from the snail as motile forms, which attach themselves to firm surfaces such as grass blades and encysted there to form the infective stage, metacercariae. It takes a minimum of 6 to 7 weeks for completion of the development from miracidium to metacercaria, although under unfavorable circumstances a period of several months is required. Infection of snail with one miracidium can produce over 4000 metacercariae. This phenomenon is called paedogenesis, which is essential point in the life cycle of trematodes. Metacercariae ingested by the final host excyst in the small intestine, migrate through the gut wall, cross the peritoneum and penetrate the liver capsule. The young flukes tunnel through the parenchyma for 6-8 weeks then enter the bile ducts where they migrate to larger ducts (Radostitis *et al.*, 1994). The prepatent period for *F. hepatica* is from 10-12 weeks and for *F. gigantica* is 13 to 16 weeks. The longevity of *F. hepatica* in untreated sheep may be years, while in cattle is usually less than one year (Urquhart *et al.*, 1996).

Pathogenesis and clinical Signs

Several workers described the pathology and pathogenesis of fasciolosis in detail during the 1960s; however, there are still undiscovered pathological changes in particularly to *Fasciola* species infection in domestic ruminants (Behm and Sangster, 1999). Adult liver flukes live in the bile ducts of the final host (Radostitis *et al.*, 1994) while immature flukes are found in the liver

parenchyma where it causes severe pathological lesions; however, occasionally aberrant flukes are found and become encapsulated in other organs such as lung (Urquhart *et al.*, 1996).

Abrasions caused by spines and the prehensile action of the suckers appear to account for the damage caused in the liver. Death of the host is a consequence of the hemorrhage induced by this damage; however, the precise causes of the pathology are still unknown, though information on histopathology and examination of caecal contents are bases for the knowledge of pathogenesis (Behm and Sangster, 1999).

The pathogenesis of fasciolosis may vary depending upon the development of the parasite in the liver and the species of the host involved. Most commonly, the pathogenesis of fasciolosis has two phases: the first phase occurs during immature fluke migration in the liver parenchyma while the second phase occurs when the adult parasite establishes in the bile ducts. The first phase is associated with liver tissue damage and causes hemorrhage while the second phase results by the haematophagic activity of adult flukes and damage to the biliary mucosa, which causes cholangitis by their cuticular spines (Radostitis *et al.*, 1994).

Several clinical syndromes may be associated with liver fluke infection, depending on the numbers and stages of development of the parasite and on the presence or absence of bacteria: *Clostridium novyi*. The disease causes a wide range of clinical signs but none of the syndromes is pathognomonic. Depending on the number of metacercariae ingested, the disease could be manifested in acute, sub-acute or chronic forms. Acute form of the disease occurs during invasion of the liver by recently ingested metacercariae that causes trauma and is inflated by the migrants tunneling about in the liver and cause inflammatory reaction, which results in heavy fatal clinical illness (Bowmann *et al.*, 2003). It occurs 2-6 weeks after the ingestion of a large number of metacercariae, usually, over 2000, which invariably causes severe hemorrhagic trauma during of immature fluke migration in the liver parenchyma (Urquhart *et al.*, 1996).

In the subacute form of the disease 500-1500 metacercariae are ingested over longer a period of time, of which some might have reached the bile ducts, cause cholangitis, and others still migrating, cause less severe lesions. This is manifested as a rapid and severe hemorrhagic anemia

with hypoalbuminemia, rapid loss of condition, submandibular oedema and ascites may be present (Radostitis *et al.*, 1994; Bowmann *et al.*, 2003).

Chronic fasciolosis is the most common form in cattle, sheep, goats and other hosts (Kauffmann, 1996). It is associated with the presence of adult flukes in the bile ducts and is characterized by the classical clinical signs: gradual losses of condition, progressive weakness, anemia and hypoproteinemia with development of edematous subcutaneous swelling, especially in the intramandibular space and over the abdomen (Bowmann *et al.*, 2003).

Epidemiology of the disease in domestic ruminants

Fasciolosis is principally cosmopolitan endemic infection and of greatest economic importance in ruminants while in human occurs by chance and focal in distribution (Dalton, 1999). In developed countries, the incidence of fasciolosis can reach up to 77%. However, in tropical regions, it is considered the single most important helminth infection of cattle with prevalence 30% to 90% in Africa, 25% to 100% in India and 25% to 90% in Indonesia (Spithill, *et al.*, 1999). The incidence in Ethiopia is known to be relatively high (Graber, 1975; Bahru *et al.*, 1979).

Factors that substantially influence its occurrence, dissemination and epidemiology of *Fasciola* species are the host, parasite and environmental risk factors or their combination. Availability of suitable definitive host, snails intermediate host, altitude, temperature and soil types are important factors for *Fasciola* species existence (Bowmann *et al.*, 2003).

Host factor

The genus *Fasciola* has adapted to alternative hosts in which susceptibility of various species and breeds of animals to the parasite vary and are genetically determined (Urquhart *et al.*, 1996). Evidence suggests that sheep and cattle may be considered the main host species, pigs and donkeys being secondary (Mas-Coma *et al.*, 1999). However, cattle, sheep and goats are more susceptible to internal parasites than other types of farm livestock (Schoenian, 2003); some ruminants show resistance against establishment of *Fasciola* species. For example, *Bos indicus*

cattle appear to be more resistant than *B. taurus* to infection with *F. hepatica* (Torgerson and Claxton, 1999). The Indonesian Thin Tail Sheep have high resistance against *Fasciola gigantica* but not against *F. hepatica* (Roberts *et al.*, 1997). Khallaayoune *et al.*, (1991) reported particularly marked infection variations in the Timahdin breed in sheep in Morocco. It was indicated that the major mechanism of resistance was manifested against immature parasites while Merino Sheep did not acquire resistance against *F. hepatica* (Radostitis *et al.*, 1994; Urquhart *et al.*, 1996).

Infection depends on several factors related to the vectors, biology and management of the flocks and herds. In Europe, the molluscs population reaches maximum density between November and March and start decreasing beginning April. During the rainy season, from June to October only few snails remain to ensure survival of the species (Mira and Ralph, 1989).

The low incidence of fasciolosis in goats could relate to their grazing behavior. Goats naturally are browsers and usually prefer to feed on higher plants than grazing grasses (Devendra and Marca, 1983; Georgi, 1985; Payne, 1990). The epidemiology of the disease is also influence by the grazing habit of the animals. Animals grazing in wet marshy areas, favored by the fluke's snails are more likely to become infected.

Adult flukes can survive for as long as 6 months up to 2 years in the livers of infected cattle but sheep can live for longer period. The egg production figures for *F. hepatica* are impressively high; in light infection in sheep (up to 50 flukes), each fluke produces an average of 25,000 eggs per day over a long period. Eggs production is responsible for high degrees of pasture contamination and thus greatly influences the epidemiology of the disease (Fairweather *et al.*, 1999).

Climate and environmental factors

Parasite distribution in the environment is extremely variable (Hanson and Perry, 1994). However, despite the variability, for *Fasciola* species to complete their life cycle the environment must provide a consistent set of suitable conditions of moisture and temperature for the development of the larval stages and the development of the intermediate host itself (Torgerson

and Claxton, 1999). Generally, *F. hepatica* is found at an altitude of above 2000 meters above sea level (masl) while *F. gigantica* found below 1200 masl (Radostitis *et al.*, 1994; Malone and Yilma, 1998). Media with slightly acidic PH are considered optimal for the development of Lymnaeidae (Urquhart *et al.*, 1996).

In many parts of the tropics as well as in temperate areas the numbers of larval stages of parasitic helminths found on the pasture follow seasonal fluctuations. Investigations carried out on *Fasciola* larvae ecology in parts of sub-Saharan Africa, the rate of development and the longevity of eggs and larvae vary with temperature, rainfall and humidity in different geo-ecological regions and may have an influence on the seasonal availability and abundance of the free-living stages of *Fasciola* parasites. The survival of *F. hepatica* infection on tropical pasture is variable depending on the climate and degree of shade, but the infective larvae are relatively resistant for 1-3 months on pasture (Hanson and Perry, 1994). On the other hand, European temperate climate is unfavorable for the survival of *Fasciola gigantica*, while *Fasciola hepatica* prefers cool climate (Urquhart *et al.*, 1996).

Effect of temperature on snail, intermediate host, is important to consider as epidemiological factor. When temperature rises above 10°C and is maintained above that level, a significant multiplication of snails and fluke larval stage occurs. Maximum growth of snails occurs at 18-27 °C. *L. truncatula* can grow to sexual maturity in 3 to 4 weeks, depending on the amount of food available and the number of parasites in the snail. It is stated that *L. truncatula*, the mud snail, may undergo a prolonged state of aestivation, if the environment dries out, during which transmission of the parasite is suspended but when the rains return, there can be rapid recolonization of the environment (Torgerson and Claxton, 1999). The minimum temperature for the development of *L. Natalensis*, intermediate host for *F. gigantica*, is greater than that required for the intermediate host of *F. hepatica*. Development of cercariae does not occur below 12°C, but grows in 73 days at 15°C and 25 days at 30°C (Al Habbib and Al Zako, 1981).

The optimal moisture condition for snail breeding and the development of *F. hepatica* with snails are provided when rainfall exceeds transpiration and field saturation is attained. Such conditions are also essential for the development of fluke eggs, miracidia, searching for snails and dispersal

of cercarial being shed from the snails. Typically, long wet seasons are associated with a higher rate of infection. However, large number of cysts more likely infects sheep during dry periods, following a wet season. Due to a reduction in availability of pasture, animals are forced to graze in swampy areas or in areas where the water has receded, thereby contaminating the vegetation resulting in heavy infection with metacercariae in *Fasciola* species (Hanson and Perry, 1994; Torgerson and Claxton, 1999).

Metacercariae have been shown to survive for more than one year in the laboratory but under pasture condition, it is likely that high level of infection does not persist for such long period (Soulsby, 1986); however, both *F. hepatica* eggs and metacercariae can survive over the winter and play an important role in the epidemiology (Urquhart *et al.*, 1996). At 12-14°C, up to 100% of metacercariae can survive for 6 months while only 5% survive for 10 months in pasture lands and up to 3 months in harvested hay in endemic highland areas. Metacercarial survival is reduced in hot condition (Torgerson and Claxton, 1999; Njau and Scholtens, 1991).

Moreover, discovering the multiple causation of the disease might be predicted through Geographical Information System (GIS) and satellite sensor technology emerging new tool for epidemiological studies on human and animal cases, especially vector-borne diseases with strong environmental determinants. The new tool was applied in Ethiopian condition to predict prevalence of fasciolosis because of presence of suitable information on the disease and natural habitats in a defined ecosystem (Malone and Yilma, 1998).

In Ethiopia, the general pattern is that the low-lying areas in the highland have poor drainage and the soil seems to be acidic and there is suitable temperature, moisture and snails intermediate host. This condition favors the development of fasciolosis. Various researchers recorded the epidemiology of fasciolosis in different areas in Ethiopia. The following table summarizes prevalence of fasciolosis in different parts of Ethiopia (Table 1).

Table 3: Prevalence of fasciolosis in different parts of Ethiopia

Area	Fasciola Prevalence (%)	Authors
Debre Berhan	64.23	Zerihun (2006)
Upper Blue Nile	37.2	Solomon (2005)
Assela	32.9	Dinka (1995)
E. Gojjam	50.56	Beyazn (1995)
Nekemte	18.99	Wassie (1994)
Debre Berhan	80.0	Dange (1994)
Bale	34.6	Abduljebar (1994)
W.Shoa	26.22	Yadeta (1994)
Kombolcha	45.26	Mulugeta (1993)
Soddo	15.77	Abdul (1992)
Chillalo	60.2	Zerfu (1991)
Assela	32.0	Wondowosson (1990)
Kallu	15.77	Girmay (1988)
Bahre Dar	60.2	Fekadu (1988)
Nekemte	32.0	Abebe (1986)
Gondar	40.6	Gebreyesus (1986)
Hollota	82.78	Yilma (1985)

Source: Legesse (2008)

2.2.3. Diagnosis of GIT helminthes

In the absence of laboratories, clinical signs are relied upon when diagnosing helminthosis in animals. In general, heavy helminth infection in animals is characterized by anaemia, diarrhoea, soiling of the hind quarters, submandibular oedema, ascites, lethargy, inappetance, staring hair coats and progressive weight loss in chronic cases. However, the other signs are not pathognomonic for helminthosis.

Quantitative methods have been developed for the diagnosis of helminthosis. Approximately 5g of faeces should be collected from the rectum using plastic gloves and examined fresh. Since eggs embryonate rapidly, the faeces should be stored in a refrigerator unless the examination is carried out within a day of collection (Urquhart *et al.*, 1996). These coprological methods involve concentration of faecal worm eggs for detection and quantification and they include: the flotation technique and the sedimentation technique and the McMaster method (MAFF, 1986).

2.3. Blood and importance of determining hematological values

Blood is one of the most important and frequently examined indexes of physiological and pathological alterations in animals and humans (Andrews *et al.*, 1990). Whole blood comprises fluid and cells. The fluid component is plasma and comprises about 90 % water and 10 % dissolved constituents, such as proteins, carbohydrates, vitamins, hormones, enzymes, lipids, salts, waste materials, antibodies and other ions and molecules. The cellular component is made up of red blood cells (RBC), or erythrocytes; white blood cells (WBC), or leukocytes; and platelets, or thrombocytes (Colville, 2003). The trypanosome infections are generally characterized by anemia, leucopenia, thrombocytopenia as well as biochemical aberrations such as hypoglycemia, elevated blood urea nitrogen and hyperglobulinaemia (Anosa, 1988).

2.3.1. Blood cells (erythrocyte and leukocyte)

The red blood cells are derived from the erythroblasts, which are continuously being formed from the primordial stem cells in the bone marrow. In developing into a mature red cell, the primordial stem cell moves through a series of stages: erythroblast to normoblast to reticulocyte and finally to erythrocyte. During its transformation from normoblast to reticulocyte, the red blood cell loses its nucleus and hemoglobin synthesis begins at the erythroblast stage and continues until the cell becomes an erythrocyte. The four polypeptide chains of hemoglobin attached to a heme unit, which in turn, surrounds an atom of iron that binds oxygen. Therefore, the function of red blood cell, facilitated by the hemoglobin molecule, is to transport oxygen to the tissues. In addition, hemoglobin binds some carbondioxide and carries it from the tissues to the lungs (Porth, 1990).

The leucocytes, or white blood cells, which are larger and less numerous than the red blood cells, develop from the primordial stem cell located in the bone marrow and lymphoid tissue serve to protect invasion by foreign agents. There are two types of white blood cells, granular (neutrophils, eosinophils, and basophils) and non granular leukocytes (monocytes and lymphocytes) (Porth, 1990).

2.3.2. Importance of determining hematological parameters

The red blood cell count (RBC) measures the total number of RBCs in cubic millimeter of blood. The hemoglobin (grams per 100 ml of blood) measures the hemoglobin content of the blood. The packed cell volume (PCV) which can be determined by the micro-haematocrit centrifugation technique is used as a quantitative expression of anemia. Packed cell volume is a most useful index in assessing the progress of trypanosomiasis and the degree of anemia. Additionally, PCV is a reliable indicator of the extent and severity of infection as well as the performance of the host (ILCA, 1986). The mean corpuscular volume (MCV) reflects the volume or size of red cells. The MCV falls in microcytic anemia and rises in macrocytic anemia. The mean corpuscular hemoglobin concentration (MCHC) is the concentration of hemoglobin in each cell and it decreases in hypochromic anemia. Mean cell hemoglobin (MCH) refers to the mass of red cell and is less useful in classifying anemia. Determination of PCV, MCV, MCHC, and MCH, signify not only anemic disorders, but also show imbalances induced by a deficiency of ions and plasma proteins in the blood (Coles, 1986; Josh *et al.*, 1991).

2.3.3. Factors affecting hematological parameters

Various factors influence the values of hematological parameters in both healthy and diseased animals. These include sex, species or breed of the animal, age of the animal and altitude, and physiological factors. Physiologic factors to be considered includes: age of the animal, breed or species of the animal (Coles, 1986).

Sex

Males tend to have slightly higher RBC count, Hg concentration and PCV than females in different species of animals. The differences however, were not statistically significant and of no practical importance (Bekele 1987; Sekar *et al.*, 1990; Jain, 1993; Sherman and Mary 1994). The slight sex difference existed in leukogram was also reported (Coles, 1986; Fufa, 1999) and showed that females have higher WBC count than males.

Species/breed

Species variation is marked, ranging from a predominance of lymphocytes in bovine to a preponderance of segmented neutrophils in canine blood. The influence of breed on hematological parameters has been reported by Coles (1986), Jain (1993) and Fufa (1999). For example, Zebu cattle have more erythrocytes; smaller mean corpuscular and packed cell volumes and a lower hemoglobin level than the Scotch Highland breed (Coles, 1986).

Age

The occurrence of significant age difference in both total WBC and differential leukocyte counts were reported by Jain (1993) where the total WBC count and absolute lymphocyte number were highest in young growing animals with gradual reduction in advancing age. New borne have large erythrocytes of fetal origin, which is later, replaced by smaller cells with reduced MCV value. The erythrocyte size will become representative of the normal adult of that species by the age of 2-12 months and the extent of decrease in lymphocyte with age is useful when interpreting the degree of stress imposed by diseases. In sheep the number of erythrocytes increases from approximately 7.5 million/ μ l at one week of age to over 14 million/ μ l at eight weeks of age. In the goat, the total erythrocyte count is relatively low at birth (7 to 8 million/ μ l) , reaches its highest level at about eight months of age and gradually falls to stabilize at about 11 million/ μ l by three years of age. The MCV is higher at birth (45 fl) and declines as the animal ages (Coles, 1986).

Altitude

Animals at high altitude have a greater number of erythrocytes, larger PCV values and higher concentrations of hemoglobin than comparable animals raised at lower altitude (Coles, 1986).

2.4. Anemia

Anemia is the reduction in the number of erythrocytes, haemoglobine or both in the circulating blood. Many classifications of anemia have been proposed and the two most common and widely accepted are morphologic and etiologic classifications. Anemia is classified morphologically utilizing MCV (Mean corpuscular volume), MCH (Mean corpuscular haemoglobine) and MCHC (Mean corpuscular haemoglobine concentration)(coles, 1986)

Morphologically anemia can be Normocytic normochromic, Macrocytic normochromic, Macrocytic hypochromic and Microcytic hypochromic. Etiologically anemia may be placed in four general categories including blood loss (haemorrhagic anemia), anemia due to excessive destruction of erythrocytes or shortened erythrocyte life span, anemia due to depression of bone marrow and nutritional deficiency anemia (Coles, 1986).

2.4.1. Trypanosome induced anemia

The mechanism involved in trypanosomosis to induce anemia may be complex and different mechanisms have been described as the cause of anemia during trypanosome infections. The major cause of which has been attributable mainly to extra vascular haemolysis due to phagocytosis of erythrocytes by an expanded mononuclear phagocytic system (MPS) resulted from massive intravascular presence of living, dying and dead trypanosomes as well as from resulting antigen antibody complexes (Murray and Dexter, 1988b; Anosa, 1997).

Intravascular haemolysis may be of a minor feature (Esiebo *et al.*, 1982), haemololytic factors (trypanosomes haemolycins, such as lipid soluble factors, plasma factor, enzymes including

trypanosome proteases having proteolytic activity and sialidase activities (Esiebo *et al.*, 1982, Knowless *et al.*, 1989), autoerythrocytelysis and /or destruction during trypanosomosis.

Active phospholipase has been shown to be generated during autolysis of trypanosomes and have direct effect on red cell membrane and cause the release of large quantities of free fatty acids (FFA) particularly linoleic acids and are hemolytic. Apart from the possible localized harmful effect of FFA; trypanosomal phospholipase could have a direct pathological effect on red cell membrane (Murray and Dexter, 1988b).

2.4.2. GIT helminthes induced anemia

The abomasal parasites of ruminants that have been associated with anemia are *H. placei* in cattle and *H. contortus* in sheep and goats. Infection of *Trichostrongylus axei*, *Ostertagia ostertagi* and *O. circumcincta* have also been reported to be associated with anemia but it much more likely to be a marginal nutritional anemia associated with in appropriate and excessive loss of plasma proteins in to the alimentary tract (Jennings, 1987; Urquhart *et al.*, 1996).

2.4.3. Other causes of anemia

Blood loss anemia: is associated with acute, sub acute and chronic hemorrhage. Anemia is not manifested immediately following per acute hemorrhages but appears when the blood volume returns to normal. Acute hemomorage usually follow trauma or surgical procedure unless blood loss is adequately controlled. Sever coagulation defects may also produce acute hemorrhage such as that occurring in poisoning associated sweet clover, warfarine or brackernfern, and in animal with coagulopathies (Coles, 1986).

External parasites such as ticks, blood sucking lice and certain type of fleas may produce anemia. Chronic blood loss resulting in anemia may occur in association with gastrointestinal lesions, ulceration of the digestive tract, neoplasm or coagulation defects that are not extremely sever (Shalm *et al.*, 1975). Other blood parasites, bacterial infections, viral infections, chemical agents, poisoning plants and metabolic diseases may also result anemia by destruction of erythrocytes. In

addition hemolytic anemia may be associated with iso-immunization phenomena or auto immune reactions (Coles, 1986).

Non regenerative anemia is associated with the depression of bone marrow and is caused by several factors including physical, chemical and infectious agents. Such anemia also occurs as a secondary reactions associated with primary disease conditions. These kinds of anemia are said to be hypoplastic or aplastic in nature and are not uncommon. They are characterized by the lack of adequate reticulocyte response (Coles, 1986).

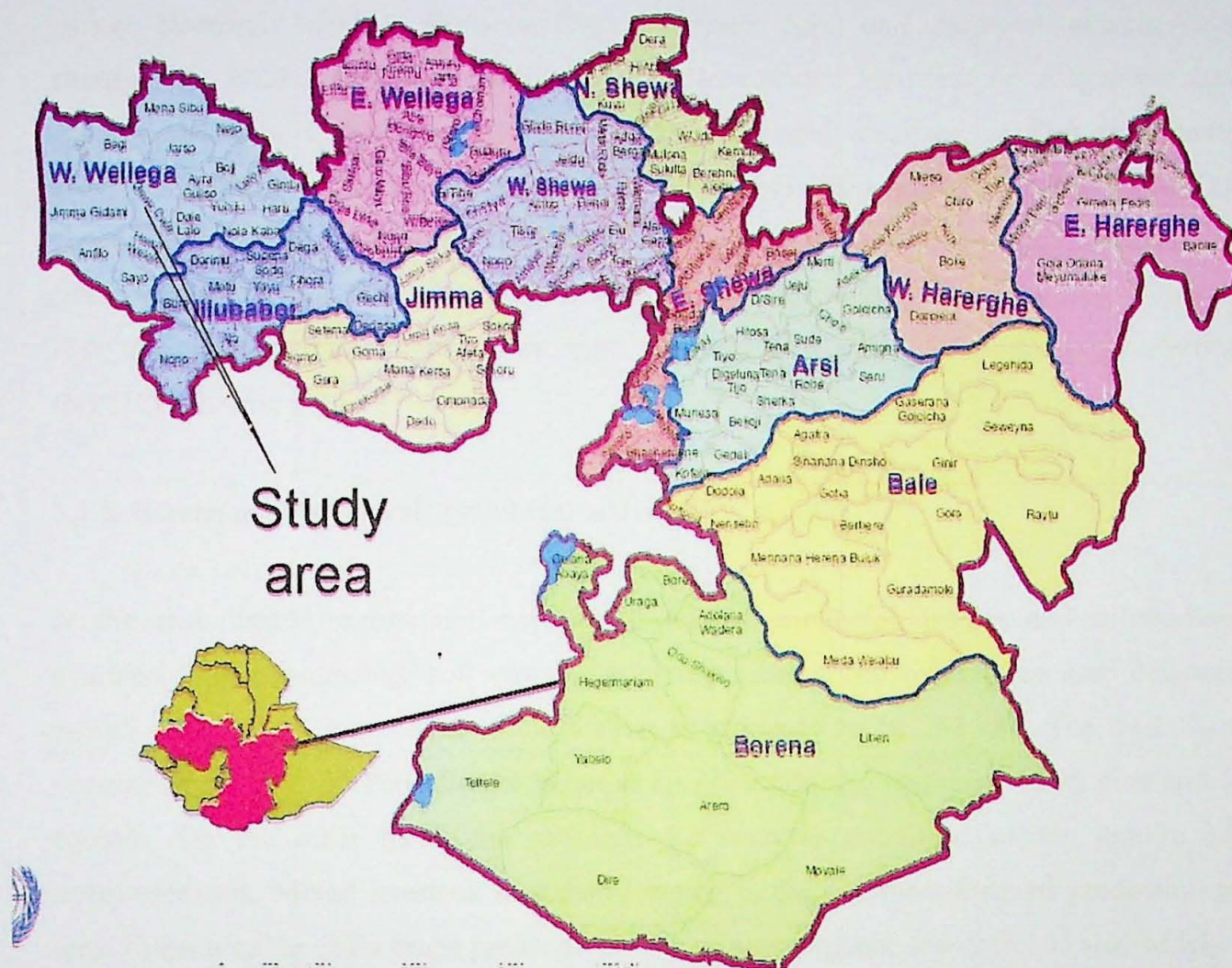
Anemia due to nutritional deficiencies seldom occurs as single entities in domestic animals. It is most commonly associated with disease conditions that result in anorexia, debilitation, or a metabolic alteration affecting either digestion or absorption of nutrients. Mineral deficiency such as, Cu, maybe Se deficiency and mineral toxicity like Cu toxicity can also cause anemia (Radostits *et al.*, 2007).

3. MATERIALS AND METHODS

3. 1. Study Area

The present study was conducted in two districts namely Dale Sedi and Hawa Gelan from Kelem Wollega Zone, Oromia Regional State, and Western Ethiopia in collaboration with National Tsetse Fly and Trypanosomosis Investigation and Control Center (NTTICC). The Kelem Wollega zone is bordered by Gambella regional state in the south, Benishangul Gumuz regional state in the west, west Wollega zone in the north and Illuababor in the east. According to the survey report of NTTICC (2004) seven districts of Kelem Wollega (namely Anfillo, Sayo, Hawa Gelan, Yemalegi Walal, Gawo Dalle, Dalle Sadie and Lalo kille) which lie between 08°N 25' 56" to 08°N 58'05" and 034°E 33'41" to 035°E 28'48" geographical positions are infested by tsetse flies. The area receives a mean rain fall of 1200-1400ml and mean temperature 20-25°C. Of the seven Districts Hawa Gelan and Dalle Sadie were selected for the current study. They were selected by convenience sampling method based on previous information on tsetse and trypanosomosis challenge. Hawa Gelan district which is located 515km away from Addis Ababa towards the west part of Ethiopia. The study area has an altitude of 1,200 to 2,200 m above sea level; temperature range from 15.5 to 32.5°C with the annual average being 24°C. The area receives an average annual rainfall of 700 to 1,100 mm. The district covers an area of 79,849 km² hectares. The area is located at the limit of forest and savanna zones in Birbir water shade which is one of Baro Akobo River system which is a tributary of the Nile River. The riparian vegetation of the Birbir River and its tributaries is infested with *G. pallidipes* and *G. tachinoides* (Abebe, 2005). In the Dale Sedi district, which has relatively high human population (60 people/km²); some segments of riparian vegetation have been obliterated by high human activity causing the disappearance the potential habitat for the tsetse flies. Hawa Gelan has a moderate human population (CSA, 2004). Contact between tsetse fly and cattle occur when cattle are herded along the Rivers.

Figure 3: The study area in western part of Oromia regional state, Ethiopia



Source: <http://www.ocha-eth.org/Maps/downloadables/OROMIYA.pdf>

3.1.1. The wild life and natural vegetation

Wild animals which have been seen and recorded in this district include baboon, colobus monkey, vervet-monkey, African buffalo (*Syncerus caffer*), bush pig (*Potomacheorus porcus*), warthog (*Phacoeheorus aethiopicus*), antelop, bushbuck (*Tragelaphus Scriptus*), crocodile, hippopotamus, gazelle, hyena, and snakes. The tall riverine tree species found are *Pygeum africanum*, *Aningeria adolfriederici*, *Cordia africana*, *Ficus thonning*, *Ficus Vasta*, *Albizia grandibracteata*, *Albizia gummifera*, *Millettia ferruginea*, *Albizia shimperiana*, *Juniperus Procera*, *Croton machrostachyus* *Sapium ellipticum*, *Stereo spermum Kunthianum*, *Manilkara*

butugi Dombeya torrida, Drachena Spp., Pheonix Spp. and claasena anisata,, Justica shimperiana, prunus Africanus , Acassia Spp., carissa edulis; Maytenus sp., Calpurnea digitata, Ricinus communis, Coffea Arabica, Telcea Nobilis; Vernonic amygdacina, Bersama abyssinica; Maytenus senegaensis, Ekebergia capensis; Erthrina abyssinica and Grewia ferruginea. Ground cover include Rubus spp., Agerratum Spp., Sudan grass, Oxytenanthera spp., Solanacea spp., pteridophyte spp. Garecina glutea and Syzygium guineense. Hyparrhenia elongate, Hyparrhenia rufa, Hyaparrhenia spp., Pennisetum spp., Panicum maximum and Andropogen abyssinicus (NTTICC, 2006).

3.1.2. Human population and agricultural activities

In the mid-altitude region, the majority of human population resides and a simultaneous overcrowding, overgrazing, soil erosion, land degradation and overall resource deterioration prevail. The human population of the district is estimated to be 111,784. The total livestock population of the Dale Sedi district is about 29,722 cattle, 5,700 sheep, 4,895 goat and 1,840 equines. On the other hand, the lowlands are scarcely populated mainly due to animal trypanosomosis. Mixed livestock and crop farming is the dominant form of production in the area. In the area the main crops produced are maize and sorghum and coffee is also widely used as the main cash crop (CSA, 1998). The area is closely situated to settlements, forest and woodland next to Ketto River, a tributary of the Birbir River which is a favourable environment for tsetse distribution.

3. 2. Study population

Animals included in this study were cattle that were kept under traditional extensive husbandry system with communal herding during the day and return to their owner's farmstead in the evening in the study area.

3. 3. Sampling method and sample size determination

The sampling method applied in this study was a multi-stage random sampling starting from Zone-to-districts-to-PAs-to-villages-to-herd and then finally to individual animal. In fact, the districts were selected purposely based on the extent of the existing problems, the complaints of farmers and the level of ecological favorability to high tsetse challenge in the areas. Two districts were purposively selected because of differences in environmental characteristics, namely Hawa Gelan and Dale Sedi districts. In each district two agro-ecologies based on their geographical difference (*e.g.* altitude as lowland and mid-altitude) were sampled. Then four PAs from the Hawa Gelan and five PAs from the Dale Sedi were selected purposively. Therefore, a total of nine representative PAs were selected based on agro-ecology representation, cattle production potential and accessibility. Random selection was made on households and each of study cattle from each household's cattle population. Thus, household and cattle in the selected PAs have equal chances to be selected for this study. The sample size required to conduct cross-sectional study was determined based on simple random sampling technique, for this purpose, the following formula was employed (Thrusfield, 2005):

$$n = \frac{1.96^2 \times P_{ex} (1 - P_{ex})}{d^2}$$

Where **n** is the required sample size, **P_{exp}** is expected prevalence; **d** is the level of precision (5%), 1.96 to indicate 95% confidence level.

Assuming estimated trypanosomosis prevalence of 50% and with a desired accuracy of 5% at the 95% confidence level, an estimated sample size of 384 cattle was obtained for each zone (Cannon and Roe, 1982). To improve the precision and the reliability of the results, more than the required numbers of samples were collected (802 cattle and 442 cattle in Dale Sedi and Hawa Gelan districts, respectively). In each districts, the number of animals selected in each village was based on proportional weighting.

3. 4. Study Design and Sampling Methodology

A cross-sectional study was used to determine the prevalence of bovine trypanosomosis and the prevalence of GIT helminths, and the apparent density, distribution and species of tsetse flies and other biting from November 2011 to April 2012.

Trypanosomosis and anemia Survey: To determine the prevalence of bovine trypanosomosis and to estimate the potential risk factors associated with the disease in the study areas, a cross-sectional parasitological survey was used. A total of 1,244 blood samples were collected after properly securing the animal and aseptically by puncturing with the tip of lancet on the margin of ear vein. Subsequently, a pair of heparinized capillary tubes were filled three fourth of their length. Then parasitological examination was performed according to the following two procedures. First, the capillary tubes were sealed by using cristaseal at one end. The blood in the capillary tubes were centrifuged at 12,000 revolutions per minute (rpm) for five minutes and consequently the packed cell volume (PCV) or anemia of each sample were determined by using a hawksley micro-haematocrite reader (Murray *et al.*, 1977). Second, the capillary tubes were cut 1cm below the buffy coat intersection to include the top layer of red blood cells. The content of the capillary tubes was then expressed on to a clean microscope slide, mixed and covered with 22 x 22 mm cover slip. Then, the slide was examined using a microscope with 25 x objective to determine the prevalence of trypanosomosis using the buff coat /phase contrast/ dark ground technique (BCT) as described by Murray *et al.* (1977). Confirmation of trypanosome species by morphological characteristics was done after staining the blood smear with Giemsa and examination with oil immersion microscopy with 100x power of magnification (Murray *et al.*, 1977).

Entomological Survey: to assess the apparent densities, distributions and species of tsetse flies and other biting flies the entomological data was collected in the study period of the year. Mono-conical traps baited with acetone, octenol and three-week-old cow urine (Brightwell *et al.*, 1992) was used to assess the fly density. Site selection was done to include suitable tsetse habitats like savannah area, river valleys, livestock grazing areas and watering points and wild game reserve areas.

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In all study sites a total of 155 mono-conical traps (Annex 5) was deployed early in the morning and maintained in position for 48 hours at ten different sites in the study period. The cages from these traps were emptied after 48 hours. Caught tsetse flies and other biting flies were counted, identified, sexed and live flies were dissected for trypanosome detection using the methods of Lloyd and Johnson, (1924) and for other biting flies according to their morphological characteristics such as size, color, wing venation structure and proboscis at the genus level (Walle and Shearer, 1997). During trapping, acetone and octenol were dispensed from open small vials through an approximately O-sized hole while cow urine from open bottles was inserted with filter paper to facilitate odour diffusion. All odours were placed on the ground about 15cm upwind of the traps. The trap poles were greased to avoid the entry of insect predators like ants. Sexing was done just by observing the posterior end of the ventral aspect of the abdomen by stereoscope: male flies were identified by enlarged hypopygium in the posterior ventral part of the abdomen. Tsetse apparent density was the mean catches in traps deployed expressed as the number of tsetse fly catch/trap/day (Leak *et al.*, 1987).

Coprological examination: The objective was to collect equal number of sample to trypanosomosis but due to escape of the animals or owner unwillingness anymore, 1,108 faecal samples were collected from rectum with new, unused gloves from each animal sampled for trypanosomosis. Collected samples were put into fecal pots, labeled and kept cool prior to transportation to the laboratory where they were preserved in 10% formalin for later examination in the Bedelle Regional Laboratory. The fecal samples were examined by both qualitative (flotation and sedimentation techniques) and quantitative (modified McMaster) techniques. The sedimentation technique as described by MAFF (1986) was used to detect the presence of stomach and liver fluke eggs (trematodes) and floatation technique for strongyle eggs (nematodes) in the samples. The presence of coccidian oocysts was also recorded. Fecal egg count was determined using modified McMaster technique; each nematode egg counted represents 50 eggs per gram of feces. The nematode eggs present were identified in general terms as strongyle eggs, since relevant nematode genera produce eggs that are similar in appearance and cannot be discriminated easily, except for the eggs of *Nematodirus*, *Strongyloides* and *Trichuris* species. The intensity of infection was classified as light (50-200 EPG), moderate (201-800 EPG) and heavy infections (> 800 EPG) as described in MAFF (1986).

Risk factors: different factors influence the impact (outcome) of trypanosomosis, helminthes and anemia in individual animals. In this study district, altitude, age, sex, body condition and others were investigated for presence of association with outcomes in the study area. GPS was used to determine altitude to assess its relation with the prevalence of trypanosome, GIT helminthes and anemia. The body condition status of the animals was assessed on the criteria (Nicholson and Butterworth, 1986). Accordingly, animals were considered as poor when they show marked emaciation, transverse process project prominent, spines appear sharply, individual dorsal spines are pointed to the touch, hips, tail, head and ribs are prominent. Good body condition score were animals with smooth, well covered and heavy deposits of fat, which is clearly visible on tail, head, brisket, dorsal spines, ribs and hooks and as medium when they are found between the two.

3. 5. Data collection and analysis

All the relevant data was collected and recorded in excel for analysis. These include owner of the animal, district, village, longitude, latitude, altitude, age and sex of the animals, physiological status of the animals, body condition score, species of trypanosome detected, GIT helminthes, identification of the animals by farmer, parasitaemic and aparasitaemic animals, PCV values. Animals found positive for trypanosomes and PCV level lower than 21 were treated by anti-trypanosomal drug. Altitude records were stratified into two, lowland (<1500 m.a.s.l.) and midland (≥ 1500 m.a.s.l.). SPSS 16 software was used to analyze the data. Chi-square was used to test for significance of prevalence in relation to risk factors. The strength of association was measured using odds ratios for different variables vs. outcome by Chi-square test. Univariate and multivariate logistic regression was also employed to estimate the strength of association between the outcomes (diseases) and the risk factors. The mean PCV values of parasitaemic and aparasitaemic were compared for significance by Student t-test. In all cases differences between parameters were declared for significance at 95% confidence interval with a probability level of 0.05 or less.

4. RESULTS

4.1. Parasitological Survey

4.1.1. Prevalence of trypanosome

Out of a total 1,244 bovines sampled 81 (6.5%) were found to be infected with trypanosomes. The prevalence of trypanosomosis in Dale Sedi and Hawa Gelan district was 4.7% (3.3%-6.2%) and 9.7% (6.9%-12.5%), respectively. There was a statistically significant ($p < 0.05$) difference in the prevalence of trypanosome infection between the two districts. The overall finding indicated that *T. congolense* was the predominant species identified (71.60 %) followed by *T. vivax* (16.05%), mixed infection of *T. congolense* and *T. vivax* was (7.41%) and *T. brucei* (4.94%).

Table 4: Prevalence of trypanosomosis infection in two districts of western Wollega, Oromia

Districts	Infected	Total	Trypanosomes spp. diagnosed				Prevalence (%)
			<i>T.congolense</i>	<i>T.vivax</i>	<i>T.brucei</i>	<i>T. c + v</i>	
Dale Sedi	38	802	26(3.2%)	7(0.9%)	4(0.5%)	1(0.1%)	4.7%
Hawa Gelan	43	442	32(7.2%)	6(1.4%)	0(0.0%)	5(1.1%)	9.7%
Total	81	1244	58(4.7%)	13(1.0%)	4(0.3%)	6(0.5%)	6.5%

4.1.2. Risk factors for trypanosomosis

As shown in Table 5 the assumed risk factors were analyzed against trypanosome prevalence. Accordingly, districts, age, sex, altitude, body condition score and functional status of the animal were observed. Among these districts, altitude, sex and age showed significant difference ($P < 0.05$).

Table 5: Univariate analysis of the association between trypanosome infections in cattle with different risk factors

Risk factors	No examined	No of +ve	Prevalence(95%CI)	OR(95%CI)	P-value
Districts					
Dale Sedi	802	38	4.7% (3.3%-6.2%)	1	
Hawa Gelan	442	43	9.7% (6.9%-12.5%)	2.167(1.378-3.408)	0.001
Altitude					
Low land	652	57	8.7 % (6.5%-10.92%)	1	
Mid land	592	24	4.1 % (2.5%-5.7%)	0.479(0.270-0.720)	0.001
Sex					
Female	389	15	3.9% (1.9%-5.8%)	1	
Male	855	66	7.7% (5.9%-9.5%)	2.086(1.175-3.703)	0.012
Age					
< 3years	185	5	2.7% (0.3%-5.1%)	1	
≥ 3years	691	50	7.2% (5.3%-9.2%)	2.78(1.11-6.98)	0.023
Body condition					
Poor	407	28	6.9% (4.4%-9.4%)	1	
Medium	546	31	5.7% (3.7%-7.6%)	0.903(0.506-1.62)	0.731
Good	291	22	7.6% (4.5%-10.6%)	0.736(0.418-0.1296)	0.288
Functional status					
Before puberty	261	10	3.8% (1.5%-6.2%)	0.78(0.276-0.219)	0.633
Lactating	189	8	4.2% (1.3%-7.1%)	0.86(0.292-2.547)	0.788
Plugging	671	57	8.5% (6.4%-10.6%)	1.81(0.763-4.298)	0.178
Pregnant	123	6	4.9% (1.0%-8.7%)	1	

Districts: The prevalence of trypanosome infection varied significantly between the Dale Sedi and Hawa Gelan district ($P = 0.001$, $\chi^2 = 11.66$ at 1 d.f) and animals in the Hawa Gelan were at higher risk by 2.17 times Odds from Dale Sedi (95% CI = 1.38-3.41).

Sex: The prevalence of trypanosome infection varied significantly between male and female ($P = 0.001$, $\chi^2 = 6.56$ at 1 d.f) and male animals were at high risk by 2.09 times Odds from female (95% CI = 1.18-3.70).

Age: Age was categorized into two groups based on estimated age ranges: young (< 3 years) and adult (≥ 3 years). The prevalence of trypanosome infection varied significantly between young and adult ($P = 0.023$, $\chi^2 = 5.18$ at 1 df) and adult animals were at high risk by 2.78 times Odds from young (95% CI = 1.18-3.70).

Body condition: Based on their body condition score, study animals were categorized into poor, medium and good body conditions. But there was no significant difference ($P > 0.05$) between body condition scores.

Functional status of the animal: Functional status of the animal was also considered as a variable during data collection, study animals were categorized into age before puberty, lactating, plugging and pregnant. But there was no significant difference ($P > 0.05$) among the functional status.

Altitudinal prevalence: In this study altitude of the sites ranged from 1334 m.a.s.l to 1620 m.a.s.l. Prevalence of trypanosome infection was analyzed according to the agro-ecological category: lowland (below 1500 m.a.s.l) and midland (above and equal to 1500 m.a.s.l). The prevalence of trypanosome infection varied significantly between the lowland and midland areas ($P = 0.01$, $\chi^2 = 11.2$ at 1 d.f) and animals in the midland area were at lower risk by 0.479 times odds from lowland areas (95% CI = 0.27-0.72).

Table 6: Multivariate analysis of hypothesized risk factors that were significant using univariable logistic regression analysis as shown in table 5

Risk factors	B	Odd ratio	(OR 95% CI)	Standard error	P value
Altitude (lowland/ midland)	0.866	2.378	(1.16-4.86)	0.365	0.018
Age (< 3yea/r $\geq$ 3year)	-1.239	0.29	(0.11-0.77)	0.50	0.013
Sex (female/ Male)	-5.86	0.557	(0.306-1.01)	0.30	0.054

All risk factors were analyzed against trypanosome prevalence by univariate logistic regression. Accordingly, districts, age, sex and altitude were selected for multivariate logistic regression because they have P value less than 0.25 but during multivariate analysis only altitude and age showed significant ($P < 0.05$) association with trypanosomosis (Table 6).

4.1.3. Hematological examination

Statistical analysis was made using independent t-tests to compare mean PCV value of infected and non-infected animals and other variables as shown in Table 7. When the findings were compared, the mean ($\pm$ SD) PCV value of all tested animals was 25.25 ± 4.66 . The mean PCV of infected bovines (21.57 ± 6.49) was significantly ($P < 0.001$) lower than that of non-infected cattle (25.50 ± 4.40). Using a PCV value of 24–46% as a normal value (Radostits *et al.*, 2007), 62.0% of the trypanosomosis infected and 31.4% of the non- infected animals were found to be anemic. The mean PCV of poor body condition bovines (21.57 ± 6.49) was significantly ($P < 0.05$) lower than that of good body condition (26.19 ± 4.51). On the other hand, no significant ($P > 0.05$) variation of mean PCV was observed between different age groups, animals of lowland (24.98 ± 4.63) and mid land (25.14 ± 4.37) and also between male and female sex group.

Table 7: Mean PCV value of cattle under different factors

Factors	n	Mean PCV%	[95% conf. interval]	Stand dev	t-test	P value
Trypanosome inf.						
Infected	81	21.6	(20.13,23.00)	6.4	1	
Non-infected	1163	25.5	(25.25, 25.76)	4.4	7.5	0.000
Sex						
Male	855	25.2	(24.87, 25.50)	4.7	1	
Female	389	25.4	(24.92, 25.84)	4.6	0.7	0.487
Altitude						
Low land	652	25.3	(24.97, 25.72)	4.9	1	
Mid land	592	25.1	(24.78, 25.49)	4.4	0.8	0.428
Age						
< 3years	185	24.8	(24.19, 25.36)	4.05	-1.5	
≥ 3years	1059	25.3	(25.04, 25.61)	4.76	-1.7	0.053
Body condition						
Poor	407	24.98	(24.53, 25.43)	4.63	-	
Medium	546	24.94	(24.54, 25.33)	4.72	-	0.988
Good	291	26.19	(25.68, 26.72)	4.51	-	0.002

4.2. Entomological survey

To assess tsetse apparent density, distribution and species prevailing as well as other biting flies in the study area 155 mono-conical traps were deployed. A total of 930 tsetse flies were captured in the study period out of which 25.1% (233) belong to *Glossina pallidipes*, 14.6% (136) to *G. morsitans submorsitans*, 29.8% (277) to *G. f. fuscipes* and 30.5% (284) to *G. tachinoides*. Out of 3749 total fly caught during the study period, tsetse fly 930 (24.8%), Stomoxys 2819 (75.2%) and 65 (1.73%) tabanus. In this area four tsetse fly species were found abundantly (*G. fuscipes fuscipes* and *G. tachinoides*) which belongs to riverine species were predominating over the other two species *G. pallidipes* and *G. morsitans submorsitans*, the savannah group. The apparent densities of 0.76, 0.90, 0.44 and 0.92 for *G. pallidipes*, *G. f. fuscipes*, *G. m. submorsitans* and *G. tachinoides* were recorded respectively. The apparent density of 9.09, and 0.21 were obtained for Stomoxys and tabanus, respectively, in the study area. The apparent densities recorded in

altitudes greater than 1500 m.a.s.l. was lower than that obtained in lower altitudes in the areas but there is no significance difference between them (Table 8).

Table 8: Mean fly species trapped per trap as assessed in two different altitudes

Altitude		Glosina Species					Biting Flies	
m.a.s.l		<i>G.m.smorsitans</i>	<i>G. pallidipes</i>	<i>G.f.fuscipes</i>	<i>G. tachinoides</i>	Total	Stomoxys	Tabanus
<1500	Mean	1.08	1.77	1.30	2.11	6.23	13.18	0.30
	S D	2.38	3.53	2.53	4.47	11.21	13.97	0.55
>1500	Mean	0.62	1.20	2.43	1.43	5.72	19.58	0.58
	SD	1.93	2.19	2.99	2.98	6.41	16.54	0.77
Total	Mean	0.88	1.52	1.79	1.83	6.02	18.19	0.42
	SD	2.21	3.04	2.78	3.92	9.47	15.09	0.66

4.3. Coprological results

Out of 1,108 cattle examined, 36.2% (n = 401) were found to harbor one or more helminthes species. Out of which 313 (28.2%), 74 (6.7%), 12 (1.1%) and 45 (4.1%) were found to be infected with *Fasciola*, *Paramphistomum*, *Oocyst* and *Strongyle*, respectively. The overall finding indicated that *Fasciola* was the predominant species identified (78.1%) followed by *Paramphistomum* (18.5%), *Strongyle* (11.22%) and *Oocyst* (2.99%). The infection of helminthiasis include more than one types of parasite was found in 3.46% of the examined animals. Of this the concurrent infection of *Strongyle* and *Fasciola* constitute the highest followed by *Fasciola* and *Paramphistomum*(Table 9).

Table 9: The prevalence of different GIT helminths v_s different variables

Variables	No. exam.	No. positive	Types of parasite in genus			
			<i>Fasciola</i>	<i>Paramphistomum</i>	<i>Strongyle</i>	<i>Oocyst</i>
Districts						
Dale Sedi	708	291(41.1%)	237(33.5%)	54(7.7%)	30(4.2%)	2(0.3%)
Hawa Gelan	400	110(27.5%)	76(19%)	20(5%)	15(3.8%)	10(2.5%)
Altitude						
Low land	567	160(28.2%)	116(20.5%)	29(5.1%)	20(3.5%)	10(1.8%)
Mid land	541	241(44.5%)	197(36.4%)	45(8.3%)	25(4.6%)	2(0.4%)
Sex						
Male	760	270(35.5%)	205(27%)	54(7.1%)	28(3.2%)	11(1.4%)
Female	348	131(37.6%)	108(31%)	20(5.7%)	17(4.9%)	1(0.3%)
Age						
< 3years	159	60(37.7%)	48(30.2%)	11(7%)	20(5.3%)	7(1.8%)
≥ 3years	949	431(45.4%)	265(27.9%)	63(6.63%)	25(2.63%)	5(0.5%)
Body condition						
Poor	380	126(33.2%)	93(24.5%)	24(6.3%)	20(5.3%)	7(1.8%)
Medium	471	178(37.8%)	140(29.7%)	35(7.4%)	18(3.8%)	3(0.6%)
Good	257	97(37.7%)	80(31.2%)	15(5.9%)	7(2.7%)	2(0.8%)

4.3.1. Risk factors

Among the two agro ecological zones, significantly ($P < 0.001$, $\chi^2 = 31.96$ at 1 d.f) high gastrointestinal helminths infections were found in midland than lowland areas and animals in the midland area were at high risk by 2.23 times odds from lowland areas. Infection with *Fasciola* was significantly higher ($P < 0.001$, $\chi^2 = 34.77$ at 1d.f) in midland (36.4%) compared to lowland (20.5%) agroecology. No significant difference was observed in prevalence of GIT helminthes between male and female cattle ($P > 0.05$). Relatively higher prevalence of helminthiasis was observed in female animals (37.6%) compared to male (35.5%). Likewise, prevalence of GIT helimenthes did not show significant association with body condition of the animals ($P > 0.05$). Among the two districts significantly ($P < 0.001$, $\chi^2 = 20.48$ at 1d.f) higher prevalence of helminthiasis was found in Dale Sedi than Hawa Gelan and animals in the Dale Sedi were at higher risk by 1.90 times Odds from Hawa Gelan. Infection with *Fasciola* was significantly

higher ($P < 0.001$, $\chi^2 = 26.42$, $df=1$) in Dale Sedi (32.9%) compared to Hawa Gelan (21.3%) as shown (Table 10).

Table 10: Univariate analysis of the association between GIT helminthes infections in cattle with different risk factors

Variables	No. exam.	No. positive	Odd ratio	P value
Districts				
Dale Sedi	708	291(41.1%)	1.90(1.17-3.08)	0.009
Hawa Gelan	400	110(27.5%)	1	
Altitude				
Low land	567	160(28.2%)	1	0.002
Mid land	541	241(44.5%)	2.23(1.33-3.74)	
Sex				
Male	760	270(35.5%)	1	0.051
Female	348	131(37.6%)	1.93(1.06-3.52)	
Age				
< 3years	159	60(37.7%)	1	0.114
≥ 3years	949	431(45.4%)	1.36(0.93-1.98)	
Body condition				
Poor	380	126(33.2%)	1	0.991
Medium	471	178(37.8%)	0.996(0.544-1.83)	
Good	257	97(37.7%)	0.672(0.361-1.25)	

Table 11: Multivariate analysis of hypothesized risk factors that were significant using univariable logistic regression analysis as shown in table 10

Risk factors	B	Odd ratio	(OR 95% CI)	Standard error	P value
Altitude (midland/ lowland)	1.11	3.02	(1.39-6.55)	0.395	0.005
Sex (female/ Male)	-0.6	0.54	(0.29-1.03)	0.324	0.062
Age (< 3yea/r ≥3year)	0.551	0.243	(1.08-2.09)	1.74	0.053

All risk factors were analyzed against GIT helminthes prevalence by univariate logistic regression. Accordingly, districts, sex, age, altitude and physiological status were selected for multivariate logistic regression because they have P value less than 0.25, but during multivariate analysis only altitude showed significant ($P < 0.05$) association with GIT helminthes (Table 11).

Table 12: Mean cattle PCV as assessed between different helminthes infections as a risk factor

Factors	N	Mean PCV%	[95% conf. interval]	Stand dev	t-test	P value
Helminthes						
Infected	401	24.88	(24.43,25.32)	4.55	-2.09	0.036
Non infected	707	25.49	(25.14,25.84)	4.75	-2.12	
Fasciola						
Infected	316	24.29	(23.78,24.80)	4.57	-4.37	0.000
Non infected	795	25.65	(25.32,25.97)	4.68	-4.41	
Strongyle						
Infected	45	25.53	(24.34,26.72)	3.96	0.39	0.69
Non infected	1063	25.25	(24.97,25.54)	4.72	0.46	
Oocyst						
Infected	12	26.25	(24.49,28.01)	2.77	0.73	0.46
Non infected	1096	25.26	(24.97,25.53)	4.70	1.23	

4.3.2. Hematological examination

The mean PCV of helminthes infected cattle (24.88 ± 4.55) was significantly ($P < 0.05$) lower than that of non-infected bovines (25.49 ± 4.75) and also the mean PCV of *Fasciola* infected cattle (24.29 ± 4.57) was significantly ($P < 0.001$) lower than that of non-infected (25.65 ± 4.68). On the other hand, no significant ($P > 0.05$) variation was observed between mean PCV of animals infected by *Strongyle* and non infected animals and also between animals infected by *Oocyst* and non-infected one (table 12).

Table 13: Mean PCV comparison among single and mixed *Trypanosoma* and *Fasciola* infected cattle and *Trypanosoma* and *Fasciola* single infected cattle.

Factors	n	Mean PCV%	[95% conf. interval]	Std dev	t	P value
Concurrent v _s single inf.						
<i>Trypanosoma</i> + <i>Fasciola</i>	23	19.74	(17.34,22.14)	5.55	-5.08	-
<i>Fasciola</i> infected	293	24.62	(24.12,25.12)	4.35	-4.12	0.000
<i>Trypanosoma</i> infected	48	22.81	(20.84,24.79)	6.80	-2.03	0.481
Single v _s single inf.						
<i>Fasciola</i> infected	293	24.62	(24.12,25.12)	4.23	3.34	
<i>Trypanosoma</i> infected	48	22.81	(20.84,24.79)	6.74	2.47	0.001

Statistical analysis was made using independent t-test to compare mean PCV value of *Fasciola* infected and *Trypanosoma* and *Fasciola* concurrently infected animals, the mean PCV value of *Trypanosoma* infected and *Trypanosoma* and *Fasciola* concurrently infected animals and also to compare the mean PCV value of *Fasciola* and *Trypanosoma* singly infected animals as shown in Table 13. When the findings were compared, the mean PCV of *Trypanosoma* and *Fasciola* concurrently infected bovines (19.74±5.55) was significantly ($P < 0.001$) lower than that of *Fasciola* infected bovines (24.62±4.35). The mean PCV of *Trypanosoma* and *Fasciola* concurrently infected bovines (19.74±5.55) was also lower than that of *Trypanosoma* infected bovines (22.81±6.80) but there was no significant difference ($P > 0.05$) among them. The mean PCV of *Trypanosoma* singly infected bovines (22.81±6.80) was significantly ($P < 0.001$) lower than that of *Fasciola* infected bovines (24.62±4.35).

The prevalence of concurrent infection of *Trypanosoma* and GIT helimenthes (*Fasciola*) is 23 (2.1%). 32.4% of trypanosomosis infected animals concurrently infected by Fasciolosis.

Table 14: Degree of EPG at the study districts

Districts	EPG Category (%)			
	Low	Moderate	Severe	Total
Dale Sedi	28 (4.0%)	3 (0.4%)	0 (0%)	31
Hawa Gelan	18 (4.5%)	4 (1.0%)	1 (0.2%)	23
Total	46 (4.2%)	7 (0.6%)	1 (0.1%)	54

The degree of EPG of nematodes and oocysts in most of the study animals was low (Table 14) and also there was no significant association observed between the EPG and PCV. In addition only a single concurrent infection was observed between *Trypanosoma* and EPG of GIT nematode and oocyst.

5. DISCUSSION

The overall prevalence of bovine trypanosomosis in the study area was 6.5%. This result was lower as compared to a range of studies conducted previously in Ethiopia; for example, Wondesen (1986) in Bunno (18%); Abebe and Jobre (1996) at tsetse infested areas of Ethiopia (17.67%); Yohanes (1997) in Metekel District (17.20%); Afework (1998) at Pawi, North West Ethiopia (17.20%); Abiy (2002) in Goro District (19.01%) and Shimelis *et al.* (2005) in Dembecha and Tabitehenan (12%). Relatively, low prevalence of trypanosomosis in these areas may be related to the tsetse distribution and these areas are located at the fringe of advancement of the vector where there is relatively low fly-animal contact as compared to high tsetse challenge and partly attributed to the fact that it was conducted during the dry season. In addition, there was intervention of parasite and vector control by NTTICC, which has contributed in reducing the prevalence. On the other hand, prevalence difference was seen among districts, where Hawa Gelan district showed significantly higher prevalence 9.7% as compared to Dale Sedi (4.7%) district. This indicates that animals found in Hawa Gelan districts were 2.17 times more likely to be infected with the disease than those of Dale Sedi. Mamoudou *et al.* (2006) reported that there was high prevalence (20%) in tsetse belt area as compared to the fringe (13%) and much less (5.2%) in the plateau of Cameroon. The finding is in agreement with current study.

Among trypanosome species, *T. congolense* (71.60%) followed by *T. vivax* (16.05%), mixed infection of *T. congolense* and *T. vivax* (7.41%) and *T. brucei* (4.94%) was detected. Similar proportional trend was reported in Northwestern and Southwestern Ethiopia by (Abebe and Jobre, 1996; Afework, 1998; Tewelde, 2001; Mamoudou *et al* 2006; Mekuria and Gadissa, 2011). Such a high ratio of *T. congolense* may also suggest that the major cyclical vectors *Glossina* species are more efficient transmitters of *T. congolense* than *T. vivax* in East Africa (Langridge, 1976) and the high number of serodemes of *T. congolense* as compared to *T. vivax* and the development of better immune response to *T. vivax* by the infected animal (MacLennan, 1970; Leak, 1999). Since the transmission of *T. congolense* is cyclical, it requires the presence of tsetse flies, whereas the transmission of *T. vivax* more readily transmitted mechanically by biting flies than tsetse flies.

A number of studies have shown the effect of age on the incidence or prevalence of trypanosome infections in cattle. Rowlands *et al.* (2001) found a significant effect of age on the incidence of *T. congolense* infections in animals below 15 months of age with calves being the least infected. Trail *et al.* (1994) observed similar low infection rates in calves. In both cases, however, calves were kept at the homestead. This low prevalence is attributed to the management effect and protective maternal immunity in high challenge area. This is in agreement with the current work. The prevalence of trypanosomosis is significantly higher ($P < 0.05$) in adult animal's (7.2%) than young ages (2.7%). *T. congolense* infection was chronic diseases that increase infection rates with age. *T. congolense* infection is usually higher in adult animals than young (McDermott and Coelman, 1999).

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

Prevalence of trypanosomosis in lowland area was relatively higher than midland. In this study lowland area showed significantly ($P < 0.05$) higher prevalence and animals in mid land was 0.44 times less likely to be infected than in lowland and the finding is in agreement with many other studies (Abebe, 2005; Mamoudou *et al.*, 2006). It is assumed that there are many factors that contribute to the distribution of tsetse flies and tsetse transmitted trypanosomosis: Among these altitude with temperature is one of the major biotic factors that limit the distribution of flies.

The prevalence of trypanosomosis is higher in poor body condition animals than good body condition animals were reported by Dinka and Abebe (2005) and Radostits *et al.* (2007). In the present study, body condition was not found to be one of the risk factors. It was not in agreement with the previous studies. This finding could be due to the cause of poor body condition in trypanosomosis negative animals might be other factor like nutrition. Most of the animals in high

trypanosomosis prevalent district (Hawa Galan) have good body condition due to a better plane of nutrition and feeding practices by farmers of the district compared to the farmers of Dale Sadi.

In the present finding the overall mean PCV values for parasitaemic cattle was 21.6 while in aparasitaemic 25.5 was recorded. The same trends of mean PCV values were reported for parasitaemic and aparasitaemic animals by other researches. Muturi (1999) obtained the mean PCV values of 16.7 and 28.0 in North Omo Zone; Feyisa (2004) reported 21.65 and 25.54 in southwest Ethiopia for the parasitaemic and aparasitaemic; Takale (2006) reported 20.22 and 27.23 in selected sites of East Wollega Zone; Tekle (2007) recorded 18.8 and 24.8 in cattle, respectively; Mekuria and Gadissa (2011) obtained the mean PCV of infected bovines was 19.42 and non-infected bovines was 24.13 in Awi and Metekel zone. Therefore, the infection of trypanosome and overall mean PCV obtained between parasitaemic and aparasitaemic cattle animals have significant difference ($P < 0.001$).

It was shown that the development of anemia is the most reliable indicator of the progress of the trypanosome infection (ILRAD, 1988; Abebe, 2005). In the absence of other diseases causing anemia, low PCV-value of an individual animal is a good indicator of the presence of a trypanosomal infection (Marcotty *et al.*, 2008); because during his study period the presence of a *Trypanosoma* infection significantly reduced the PCV, independently of the age and sex of the infected animal. Bossche and Rowlands (2001) reported that the relationship between prevalence of trypanosomal infections (*T. congolense*) and average packed cell volume (PCV) in herds of communally managed adult Angoni breed cattle was investigated in four districts of eastern Zambia. In all areas, regression analyses showed that the herd average PCV decreased with increasing prevalence of trypanosomal infections. Anemia can occur because of other causes such as nutritional deficiency, worm infestation and other hemoparasite (Radostits *et al.*, 2007). Even though the mean PCV of infected bovines (21.57 ± 6.49) was significantly ($p < 0.001$) lower than that of noninfected bovines (25.50 ± 4.40) in this study, PCV value alone could not be used as a diagnostic tool for trypanosomosis. There are individual animals with trypanosomosis infection despite having high PCV value and vice versa.

The development of anaemia is one of the most typical signs of trypanosomosis caused by *T. congolense* in susceptible cattle breeds (Murray and Dexter, 1988b). The level of anaemia or the PCV usually gives a reliable indication of the disease status and productive performance of an infected animal (Trial *et al.*, 1991). Bovine trypanosomosis control aims at reducing the prevalence of infection with a concomitant increase in the average PCV (Bauer, 2001). Therefore, the knowledge of relationship between prevalence of trypanosome infection and mean PCV could be a useful tool for the assessment of impact of control on intervention. However, the mean PCV is affected by factors other than trypanosomosis (Conner, 1994) and these confounding factors are not always identifiable but they are likely to affect both trypanosomosis positive and negative animals.

The overall apparent densities of tsetse and biting flies were 2.95 and 9.3 flies/trap/day, respectively. There was significant difference ($P < 0.05$) in overall fly count among districts. Four species namely *G. m. submorsitans*, *G. pallidipes*, *G. fuscipes fuscipes* and *G. tachinoides* were identified. *G. f. fuscipes* is distributed along the large rivers of Birbir and its tributaries like Ketto, Shentema, Medalle, Kersa, Aweto and others, which cross the study area, and thus provides conducive environment (Tall gallery forests and dense thickets along the river bank) for the distribution of *G. f. fuscipes*.

Previous studies showed that as altitude increases, tsetse fly apparent density and trypanosomosis decrease (Muturi, 1999). Such a close relationship between tsetse density and incidence of infection was attributed largely to the high proportion of blood meals taken from cattle by tsetse (Van den Bossche and Staak, 1997). In the current study tsetse fly apparent density is higher in the lowland (3.01) than midland (2.86) but there is no significant difference ($P > 0.05$). The study sites from Hawa Gelan were 100% lowland. The reason for the decline of apparent tsetse fly density in the Hawa Gelan district during the survey might be due to bush fire for cultivation by settled Harer people and due to intervention of Oromia agency by application of pour on deltamethrin for every animal come to clinic for treatment. Such circumstance might have suppressed the fly density and forced the fly to move to moisture areas of the side of valley and river bank of extreme low altitude.

The overall prevalence of gastrointestinal parasites of cattle was 36.2%. The animals are infected with variety of parasites of which *Fasciola* are the most abundant. The present study revealed that overall prevalence of Fasciolosis based on coprological investigation was 28.2%. This finding agrees with previous studies observed in Tegray region by Legesse (2008) (26.8%). However, it was much lower when compared with results of Bahru and Ephrem (1979) 86 % in Kaffa, Yadeta (1994) 88.57% in western Showa who came to this result through faecal examination. This might be due to differences in climate and agroecology for the development of the snail intermediate hosts, seasonal and nutritional level variation in the study periods. The difference in prevalence and severity of the disease syndrome are evident in various geographical regions depending on the local climatic conditions, availability of permanent water and system of management (Urquhart *et al.*, 1996).

The study further revealed that sex and body conditions of the animal did not show significant association with the prevalence of GIT helminthes. The absence of association between sexes is in agreement with that of Keyyu *et al.* (2003) and Regassa *et al.* (2006). Hence both male and female are equally susceptible and exposed to the disease. However, that of body condition in agreement with Regassa *et al.* (2006) and disagrees with Keyyu *et al.* (2003). This could be explained by the fact that loss of body condition in the study animals could be due to other factors such as seasonal change of forageable feed staff and the presence of other concurrent disease conditions.

The degree of EPG in most of the study animals was low, and in agreement with various works (Deressa, 1998; Maichomo *et al.*, 2004) indicating the sub-clinical cases of gastrointestinal parasites with subsequent subsistent low degree of pasture contamination.

The mean PCV of helminthes infected (24.86 ± 4.53) was significantly ($p < 0.05$) lower than that of non-infected cattle (25.44 ± 4.72). The mean PCV of *Fasciola* infected (24.27 ± 4.66) was significantly ($P < 0.001$) lower than that of non-infected cattle (25.58 ± 4.55). The anemia in fasciolosis could be due to the activity of the migrating larvae through the liver parenchyma leading to extensive destruction and marked hemorrhage and their hematophagic activity. On the

6. CONCLUSIONS AND RECOMMENDATIONS

The current epidemiological study of bovine trypanosomosis and GIT helminthes has provided vital information on the current situation of the diseases in the study areas. The results of the present study revealed that trypanosomosis and GIT helminthes especially *Fasciola* is the most important constraints for agricultural activity and animal production in the study area during dry season. The prevalence of trypanosomosis was higher in lowland compared to midland ($P < 0.05$). In contrary, the prevalence of GIT helminthes was higher in midland as compared to lowland ($P < 0.05$). The most common trypanosome species found in the study area were *T. congolense* and followed by *T. vivax*. The infection rate was higher in adult animals when compared with young animals. The mean PCV values in the herds of parasitaemic and aparasitaemic animals had significant difference and the average PCV values were negatively correlated to the herd prevalence. The present study strongly suggest that concurrent infection markedly fall the PCV and the pathologic effects of concurrent infections due to *Trypanosome* and *Fasciola* were predominantly influenced by the *Trypanosome* infection. The information on vector flies indicated that four types of tsetse flies (*G. Pallidipes*, *G. m. submorsitans*, *G. f. fuscipes* and *G. tachinoides*) were caught during the study period. *G. f. fuscipes* and *G. tachinoides* are considered to be the main vector of the pathogenic trypanosome in the study area. Biting flies such as tabanids and muscids were also caught in the study period.

Based on the results of this the following recommendations are forwarded:

- Further epidemiological studies on single and concurrent infection of trypanosomosis and helminthes across all seasons in different agro-ecologies and animal categories with a very sensitive diagnostic technique should be conducted.
- Societies practice on safe usage of drugs and the socio-economic impact of trypanosomosis and helminthes should be studied.
- Since the concurrent infection have markedly decrease PCV than single infection of trypanosomosis, it is better NTTICC should be done integrated control measure on both trypanosomosis and GIT helminthosis.

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8. ANNEXES

Annex 1: Sedimentation technique (for trematode eggs)

Principle

The sedimentation technique is a qualitative method for detecting trematode eggs (*Paramphistomum*) in the faeces. Most trematode eggs are relatively large and heavy compared to nematode eggs. This technique concentrates them in sediment.

Application

This is a procedure to assess the presence of trematode infections. It is generally run only when such infections are suspected (from previous postmortem findings on other animals in the herd/flock area), and is not run routinely. The procedure can be used to detect liver fluke (*Fasciola*) and *Paramphistomum* eggs.

Equipment

- Beakers or plastic containers
- A tea strainer or cheesecloth
- Measuring cylinder
- Stirring device (fork, tongue blade)
- Test tubes
- Test tube rack
- Methylene blue
- Microslide, coverslips
- Balance or teaspoon
- Microscope

Procedure

- (a) Weigh or measure approximately 3 g of faeces into Container 1.

- (b) Pour 40-50 ml of tap water into Container 1.
- (c) Mix (stir) thoroughly with a stirring device (fork, tongue blade).
- (d) Filter the faecal suspension through a tea strainer or double-layer of cheesecloth into Container 2.
- (e) Pour the filtered material into a test tube.
- (f) Allow to sediment for 5 minutes.
- (g) Remove (pipette, decant) the supernatant very carefully.
- (h) Resuspend the sediment in 5 ml of water.
- (i) Allow to sediment for 5 minutes.
- (j) Discard (pipette, decant) the supernatant very carefully.
- (k) Stain the sediment by adding one drop of methylene blue.

Annex 2: Simple test tube flotation

Principle

The simple test tube flotation method is a qualitative test for the detection of nematode and cestode eggs and coccidia oocysts in the faeces. It is based on the separating of eggs from faecal material and concentrating them by means of a flotation fluid with an appropriate specific gravity.

Application

This is a good technique to use in initial surveys to establish which groups of parasites are present.

Equipment

- Beakers or plastic containers
- A tea strainer (preferably nylon) or double layer cheesecloth
- Measuring cylinder or other container graded by volume
- Fork, tongue blades or other type of stirring rod
- Test tube
- Test tube rack or a stand
- Microscope
- Microslides, coverslips
- Balance or teaspoon
- Flotation fluid

Procedure

- (a) Put approximately 3 g of faeces (weigh or measure with a precalibrated teaspoon) into Container 1.
- (b) Pour 50 ml flotation fluid into Container 1.
- (c) Mix (stir) faeces and flotation fluid thoroughly with a stirring device (tongue blade, fork).

- (d) Pour the resulting faecal suspension through a tea strainer or a double-layer of cheesecloth into Container 2.
- (e) Pour the faecal suspension into a test tube from Container 2.
- (f) Place the test tube in a test tube rack or stand.
- (g) Gently top up the test tube with the suspension, leaving a convex meniscus at the top of the tube and carefully place a coverslip on top of the test tube.
- (h) Let the test tube stand for 20 minutes.
- (i) Carefully lift off the coverslip from the tube, together with the drop of fluid adhering to it, and immediately place the coverslip on a microscope slide.

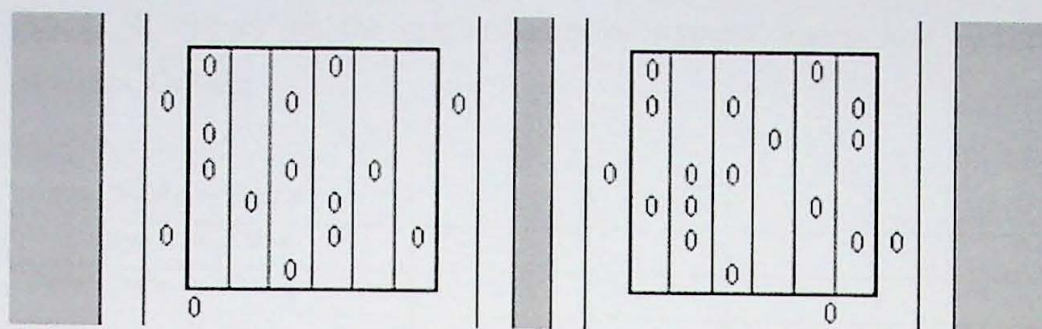
Annex 3: McMaster egg counting technique

Equipment

1. Beaker or plastic container
2. Balance
3. A tea strainer or cheesecloth
4. Measuring cylinder
5. Stirring device (fork, tongue depressor)
6. Pasteur pipettes and (rubber) teats
7. Flotation fluid (see the Appendix to this handbook for formulation)
8. McMaster counting chamber
9. Microscope

Procedure

- (a) Weigh 4 g of faeces and place into Container 1.
- (b) Add 56 ml of flotation fluid.
- (c) Mix (stir) the contents thoroughly with a stirring device (fork, tongue blade).
- (d) Filter the faecal suspension through a tea strainer or a double-layer of cheesecloth into Container 2.
- (e) While stirring the filtrate in Container 2, take a sub-sample with a Pasteur pipette.
- (f) Fill both sides of the McMaster counting chamber with the sub-sample.
- (g) Allow the counting chamber to stand for 5 minutes (this is important)
- (h) Examine the sub-sample of the filtrate under a microscope at 10 x 10 magnification.
- (i) Count all eggs and coccidia oocytes within the engraved area of both chambers.
- (j) The number of eggs per gram of faeces can be calculated as follows: Add the egg counts of the two chambers together.
Multiply the total by 50. This gives the e.p.g. of faeces. (Example: 12 eggs seen in chamber 1 and 15 eggs seen in chamber 2 = $(12 + 15) \times 50 = 1350$ e.p.g.)
- (k) In the event that the McMaster is negative (no eggs seen), the filtrate in Container 2 can be used for the simple flotation method.



Annex 4: Survey on the epidemiology of trypanosomosis and GIT helminthes in western Wollega, Oromia

Animal Number (= sample)							
Date of sample collection							
owner's name							
Region							
Zone							
District							
Kebele							
Site							
Altitude							
Latitude							
Longitude							
Number of cattle owned							
sex of the animal sampled							
Age of the animal sampled							
Role of the animal sampled**							
Milk yield (liter)							
body condition							
PCV (%)							
Trypanosome species Dx							
Helminthes DX							
> Fasciola							
> strongyle							
> oocyst							
> etc							
Helminthes count DX							
> Fasciola							
> strongyle							
> oocyst							
> etc							

** Role = Dry; Pregnant; Lactating; Heifer; Calf; Bull; Ox

Annex 5: Data collection format for the Entomological study of trypanosomosis

Seasonal collection -----

Collection site -----

Type of trap used -----

Date of collection -----

No. of Traps	Bait used	Tsetse flies								Biting flies			
										Muscids		Tabanids	
		<i>G. p</i>		<i>G. m. m</i>		<i>G. f. f</i>		<i>G. t</i>		Mos.	Stom.	Tab.	Haema.
		M	F	M	F	M	F	M	F				

Muscids = Moscas and *Stomoxys*;

Tabanids = (Tab. = *Tabanus*; Chri. = *Chriosops* and Haema. = *Haematopota*).

9. CURRICULUM VITAE

A. Biographical Data:

Name: Solomon Kebede kotu
Date of Birth: March 24, 1983
Place of Birth: Addis Ababa, Ethiopia
Sex: Male
Qualification: Doctor of Veterinary Medicine
Nationality: Ethiopian
Languages: Amharic, English
(Speaking, listening, reading and writing)
Address: P.O.Box: 27704, Addis Ababa, Ethiopia
Tele: +251-911-571321 or +251-116-456529
E-mail: solkik2010@yahoo.com

B. Educational Background

Year	School attended
1988 – 2000	Wondyrad Primary and Secondary School
2001 -2002	Addis Ababa University, Faculty of Natural Science
2003 – 2007	Addis Ababa University, Faculty of Veterinary Medicine

C. Professional Experience

2005 - 2007 Lecturer at Alage Agricultural Technical Vocational and Educational Training College), Department of Animal Health (Alagae, Ethiopia)

D. Research output/ Technical paper

The effect of mating system and herd size on reproductive performance of dairy cows in market oriented urban dairy farms in and around Addis Ababa. Alemayehu Lemma and **Solomon Kebede** (2011) *Revue Méd. Vét.*, **162(11)**: 526-530.

Arthropod vector saliva: its composition, host defence modulation and application, A paper presented for the course: seminar on current topics in tropical veterinary epidemiology (asc-702) by solomon kebede

E. Training

Computer skill (Application softwares: MS Dose, MS Word, MS Excel, MS Access)
Teaching Methodology (From Hawassa Teacher's Training College)

F. Membership to professional societies

Ethiopian Veterinary Association (EVA)

10. SIGNED DECLARATION SHEET

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

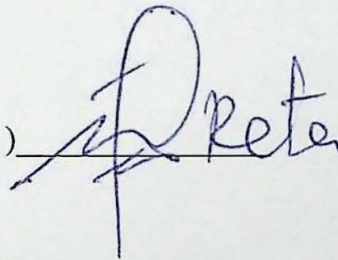
Name _____

Signature _____

Date of submission _____

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

Reta Duguma (DVM, MSc, Asist. Proffesor)

A handwritten signature in blue ink, appearing to read 'Reta', is written over a horizontal line.