

**ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY**

**Infection prevalence of ovine fasciolosis in irrigation schemes along the
Upper Awash River Basin and effects of strategic anthelmintic treatment
in selected upstream areas**

By

MICHAEL ASRAT

In the partial fulfillment of the requirement for the attainment
of the degree of Master of Science in Biology
(Biomedical Science)

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SCHOOL OF GRADUATE STUDIES**

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**By
Michael Asrat**

*A Thesis Presented to the School of Graduate Studies of the Addis Ababa
University in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Biology*

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The image shows seven handwritten signatures in blue ink, each written on a horizontal line. The signatures are: 1. A stylized signature for Dr. Abebe Wonssen. 2. A signature for Ato Berhanu Erko. 3. A signature for Dr. Beyene Petros. 4. A signature for Dr. Yilma Jobre. 5. A signature for Dr. Don Peden. 6. A signature for Dr. Yoseph Shiferaw. 7. A signature for Dr. Girma Tadese. The signature for Dr. Dawit Abate is not clearly visible as it appears to be the same as the one above it.

December, 2004

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Acronyms

AAU	Addis Ababa University
CSA	Central Statistics Authority
DZARC	Debre Zeit Agricultural Research Center
EARO	Ethiopian Agricultural Research Organization
ELISA	Enzyme Linked Immuno-Sorbent Assay
FAO	Food and Agricultural Organization of the United Nations
GSH	Glutathione
ILCA	International Livestock Center for Africa
ILRI	International Livestock Research Institute
IWMI	International Water Management Institute
Km	Kilometer
MT	Metric Tonne
SGS	School of Graduate Studies
TCBZ	Triclabendazole
WHO	World Health Organization

Abstract

An attempt was made to determine the infection prevalence of ovine fasciolosis in small-scale irrigation schemes in three agro-ecologic zones in the Upper Awash River Basin from November 2003 to October 2004. In addition, a survey was conducted to determine snail fauna and habitat (water temperature and pH) in the same locations. Furthermore, the effects of a one- and two-strategic triclabendazole (anthelmintic) treatments on some indicator parameters and associated economic returns were evaluated. Statistical comparisons, using Chi-square and ANOVA statistics, were made to determine the presence of difference in prevalence rates between irrigated and non-irrigated areas, agro-ecologic zones, seasons, age and sex.

Feecal samples of 1296 sheep were examined and 729 (56.3%) were found positive for fasciolosis. The infection prevalence in the highlands (62.9%) was significantly higher ($p < 0.05$) than both mid-altitude (51%) and lowlands (52%). No statistically significant difference ($p > 0.05$) was depicted between fasciolosis infection prevalence in mid-altitude and lowland study sites, on the one hand, and between sexes as well as age categories, on the other hand. The overall wet season prevalence (59.2%) was significantly higher ($p < 0.05$) than the dry season prevalence (53.6%). Similarly, prevalence in irrigated sites (60.8%) was significantly higher ($p < 0.05$) than non-irrigated lands (50.4%). Closer analysis of results, however, indicated that there was no statistically significant difference ($p > 0.05$) in prevalence rates between irrigated and non-irrigated study sites; as well as between the dry and wet seasons in the highland sites. On the other hand, irrigation has significantly increased ($p < 0.05$) the prevalence of ovine fasciolosis in mid-altitude during the dry season and in lowlands both during the dry and wet seasons. In the present study, small-scale irrigation schemes were shown to heavily influence fasciolosis prevalence in mid-altitude and lowland areas. These findings warrants that special schemes need to be instituted for fasciolosis and other water-borne animal, and human diseases in agricultural development efforts involving irrigation agricultural in these environments.

Of the two recognized snails serving as intermediate hosts for *Fasciola spp.*, *L. truncatula* was found in all agro-ecological sites, while *L. natalensis* was only encountered in the lowland areas. No marked difference was observed in water pH (7.03-8.58) and temperature (17.2°C – 26.8 °C) in all sampled snail habitats across the study areas. This indicated that snail inhabited within the tolerable biotic ranges despite their population size difference along with the agro-ecologic zones where they were found.

The trial on strategic anthelmintic treatment revealed that twice triclofenazole treatments are effective in markedly decreasing fecal egg output (0.03 ± 1.32), improving packed cell volume (6.50 ± 1.28), body weight gain (4.10 ± 0.76) and body condition scores (0.57 ± 0.09) than a one-time treatment 0.35 ± 1.55 , 3.56 ± 1.16 , 0.90 ± 0.73 and 0.17 ± 0.08 , fecal egg output, packed cell volume, body weight gain and body condition scores, respectively.

The economic return from one and twice strategic triclofenazole treatments was 5.24 and 26.16 birr/animal/year. Untreated control animals showed negative weight gains, hence, no monetary return over the study year. The non-monetary benefits that smallholder farmers obtain from strategic anthelmintic treatment interventions were discussed. Future studies to investigate the long-term effects of such strategic treatments intervention in the highlands and replication of similar trials in mid-altitude and lowland sites are recommended.

Keywords: - Fasciolosis, Infection Prevalence, Sheep, small-scale irrigation, strategic treatment, Upper Awash River Basin, Ethiopia.

I. Introduction

It is evident that water resources can play a significant role in improving food security and household income (Encarta encyclopedia, 2001). Agricultural production that depends on rain is mostly aimed at self-provision and this kind of production system is severely affected by climatic irregularities. An effective method to reduce vulnerability of climatic irregularities is to use irrigation for the agricultural production (Rahmato, 1999). Wrongly planned irrigation, however, impedes production and results in wasted effort by favoring the incidence and spread of common water-borne animal diseases such as fasciolosis, which are known to cause significant economic losses (Traoer, 1989; Encarta encyclopedia, 2001). On the other hand, a reliable water supply suitable for irrigation, coupled with the necessary input, can boost agricultural production and ensure self-sufficiency. Globally, many countries are dependent on irrigated agriculture to produce food for consumption and cash crops to enhance the food security of their people and to generate income. Also, irrigation is the most common means of ensuring sustainable agriculture and coping with periods of inadequate rainfall and drought (Rahmato, 1999; Tesfaye, *et. al.*, 2000; Mekonen and Tarekegne, 2001).

One of the problems associated with irrigation is its potential to facilitate transmission of water-borne human and animal diseases. Aquatic or amphibian intermediate hosts transmit diseases such as malaria, fasciolosis and schistosomiasis. In fact, the shift from a rain-fed to an irrigation agriculture system favors the development and propagation of water-borne infections to both humans and livestock.

In the highlands of Ethiopia, agriculture is the pillar of the economy and is basically a subsistence crop-livestock mixed farming system with considerable dependence on natural rain. The current trend towards food self-sufficiency is through the use of irrigation as a means to increase food production to cope with the rapidly increasing population of the country. Thus, implementation of

irrigation projects will be expected to bring about changes in land use patterns, and intensification of labor (Rahmato, 1999). The increasing number of dams and irrigation canals built to boost energy and food production will also increase the number of potential snail habitats and with them the risk and incidence of fasciolosis (Traoer, 1989).

Fasciolosis, caused by *Fasciola hepatica* and *F. gigantica*, is one of the most prevalent helminth infections of ruminants in different parts of the world. It causes significant morbidity and mortality (Okewole, *et al.*, 2000; WHO, 1995).

The economic significance of fasciolosis in the highlands of Ethiopia has been reported by several workers (Yilma, 1985; Yadeta, 1994; Mezgebu, 1995; Wassie, 1995). Although the lowland areas of the country have been known to use some irrigation, information on the impact of fasciolosis in such environments is scanty. In recent years, expansions of small-scale traditional irrigation schemes are practiced in many parts of Ethiopia. It is predicted that these recent moves towards irrigated agriculture will influence the life cycle progression and increase the occurrence of fasciolosis.

1. General accounts and descriptions

1.1 The parasite

Fasciolosis is a disease of sheep, goat, cattle (Andrews, 1999) and occasionally affects humans, thus considered as a zoonotic infection (Okewole, *et al.*, 2000; WHO, 1995). According to Dunn (1978) and Soulsby (1982), the taxonomic classification of the organisms that cause fasciolosis is presented as follows:

Phylum: - Platyhelminthes,

Class: - Trematoda,

Sub- class: -Digenea,

Super Family: -Fasciolidea,

Genus: -Fasciola,

Species: - *Fasciola hepatica* Linnaeus, 1758 and

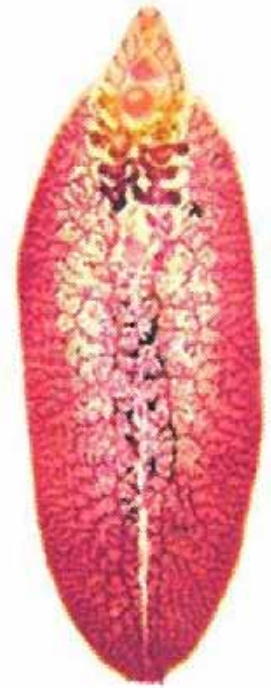
Fasciola gigantica Cobbold, 1885

Morphology

The adult parasite *F. hepatica* has a flat leaf-like body (Fig. 1a), typical of flukes, and measures 20 to 30 mm long by 8 to 15 mm wide (Dunn, 1978). It has an anterior elongation (a cephalic cone) on which the oral and ventral suckers, which are approximately of equal size, are located. The intestine of the adult parasite is highly branched, with numerous diverticulae extending from the anterior to the posterior of the body. The pair of testes, also highly branched, is located in the posterior half of the body. The relative compact ovary is located just above the testes and is linked to a short convoluted uterus opening to a genital pore above the ventral sucker. The vitellaria are highly diffuse and branched in the lateral and posterior region of the body. *F. gigantica* is a parasite very similar to *F. hepatica*, Its length may vary 25 to 75 mm long by 15 mm wide (Soulsby, 1982) (Fig. 1b). In addition, the cephalic cone is proportionally shorter than that of *F. hepatica*, and its body even more leaf like in shape (Soulsby, 1982).



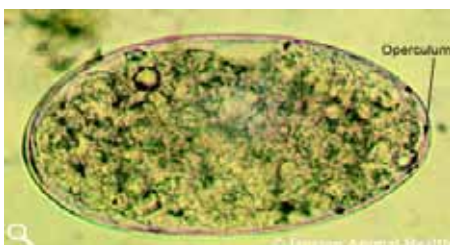
a) *Fasciola hepatica*



b) *Fasciola gigantica*

Figure 1. Adult stage of *Fasciola spp.*

The egg of *F. hepatica* measures 150µm by 90µm in size and also very similar in shape to that of *F. gigantica* (Soulsby, 1982). The egg of the latter is larger in size (200µm x 100 µm) (Dunn, 1978). *Fasciola* eggs should be distinguished from the eggs of other flukes, especially from the large eggs of *Paramphistome*. *Fasciola* eggs has a yellowish brown shell with an indistinct operculum and embryonic cells whereas *Paramphistome* egg has transparent shell, distinct operculum with embryonic clear cells, and possess a small knob at their posterior end (Soulsby, 1982).



a) *Paramphistomum spp.* egg



b) *Fasciola spp.* egg

Figure 2. Rumen and liver fluke egg

1.2 Intermediate hosts

Snails of the genus *Lymnaea* are the intermediate hosts for genus *Fasciola*. The epidemiology of fasciolosis is dependent on the ecology of the snail intermediate hosts. *Lymnaea* species, most important in the transmission of *F. hepatica*, include: *Lymnaea truncatula*, widespread in Europe, Asia, Africa and North America; *L. bulimoides* in North America; *L. tomentosa* in Australia. Other species, which have been incriminated in the transmission of *F. hepatica* include *L. viator* and *L. diaphena* (South America), *L. columnella* (USA, Australia, Central America and New Zealand) and *L. humilis* (North America) (Soulsby, 1982; Dunn, 1978).

L. truncatula (Fig. 3) is the most common intermediate host for *F. hepatica* in different part of the world (Njau *et. al.*, 1989) and in Ethiopia (Graber, 1974). It is an amphibious or mud-dwelling snail which prefers moist temperature conditions (15-22⁰C) though it appears that variants found in the tropics have adaptation to higher temperature mostly in the lowlands areas and can breed and survive at 26⁰C with sufficient moisture.

The most important intermediate hosts of *F. gigantica* are *L. natalensis* and *L. auricularia* (Urquhart, 1996; Dunn, 1978; Soulsby, 1982). *L. natalensis* (Fig. 3) is the recognized intermediate host for *F. gigantica* (Yilma and Malone, 1998) Other species serving as secondarily hosts to this species are *L. rufescens* and *L. acuminata* (Indo-Pakistan) and *L. rubiginosa* (Malaysia).

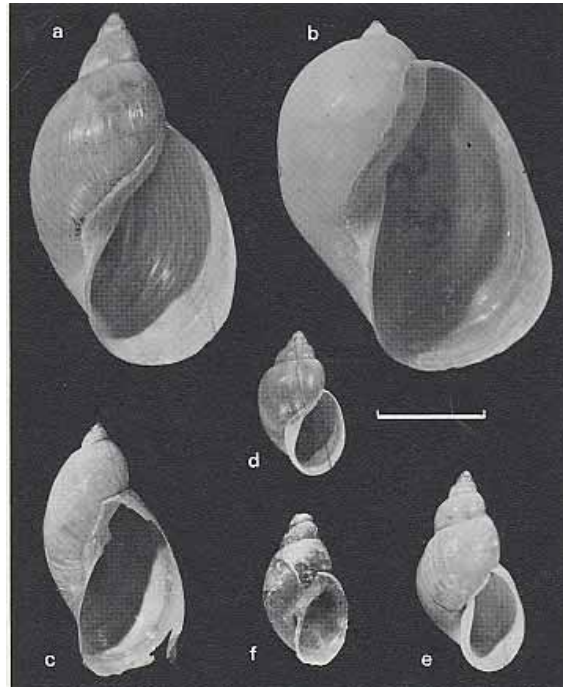


Figure 3. *Lymnaea* spp. a) and b) *L. natalensis*; c) *L. columnella*; d), e) and f) *L. truncatula*

L. natalensis is a strictly aquatic snail often found in Africa. It serves as the intermediate host for *F. gigantica* (Urquhart, 1996) and requires well-oxygenated non-polluted water bodies and can aestivate during dry periods. Optimal temperature requirement for the completion of parasite developmental stages within the snails is 22-26°C. However, in irrigated areas snail breeding is less circumscribed and will continue all year around, except for periods extreme temperature levels.

1.3 Life cycle

The life cycle of *Fasciola spp.* is a typical of Digenetic trematodes. Eggs laid by the adult parasite in the bile ducts of their hosts pass into the duodenum with the bile. The eggs then leave the host through the faeces. At this stage, eggs are still not embryonated, further development to maturation taking approximately two weeks. The eggs then hatch to release the motile miracidium, which will then locates and penetrates the intermediate snail host. The need to find a suitable host to penetrate is an urgent one, for those miracidia failing to do so generally die within 24 hours.

After penetrating the snail, the miracidium loses its cilia and becomes a sporocyst. The sporocyst dividing and forming redia (forum with sucker and primitive gut), and a fully mature redia showing redia and cercaria stages. The cercaria of *Fasciola spp.* have a rounded body measuring between 0.25 and 0.35mm long, with a long thin unbranched tail measuring approximately 0.5mm long. The mobile cercaria snail generally leaves the snail 4-7 weeks after infection by migrating through the tissues of snails. This is during moist conditions when a critical temperature of 10°C is exceeded. On emerging from the snail the cercaria attaches to submerged blades of grass or other vegetation like watercress; the tail falls away and the cercarial body secretes a four-layered cyst covering from cystogenous glands located on the lateral regions of the body. The formation of the cyst wall may take up to two days. The metacercaria (encysted, resistant cercariae) is the infective form to the definitive host. Generally, metacercaria are infective to ruminants such as cattle and sheep, but also to other mammals including human beings. One miracidium hatching from a fluke egg can produce up to 4,000 infective cysts (metacercariae) due to the vegetative multiplication at the sporocyst and redai stages.. The metacercarial cyst is only moderately resistant, not being able to survive dry conditions. If however they are maintained in conditions of high humidity and cool temperatures, they may survive for up to a year (Andrews, 1999; Soulsby, 1982; Dunn, 1978), infection through hay as a vehicle of infection in non-endemic areas.

The metacercarial cysts, when ingested along with the contaminated vegetation by the definitive host enter into the small intestine, releasing the young parasite which penetrates the gut wall, entering the peritoneal cavity. From there, it migrates directly to the liver over a period of approximately seven days, directly to the liver. The juvenile fluke (also referred to as adeloscaria) then penetrates the liver tissues, through which it migrates, feeding mainly on blood, for about six weeks. After this period, the fluke enters the bile ducts, maturing in to a fully adult parasite after about 3 months from initial infection. Egg production then commences and completing the life cycle (Fig. 4).

Adult flukes can survive for many years in the livers of infected hosts and lay between 20,000 and 50,000 eggs/day. The rate of egg production is responsible for the degree of pasture contamination and thus greatly influences the epidemiology of the disease. The epidemiology of the disease is also influenced by the grazing habits of the animals. Animals grazing in wet marshy areas, favored by the intermediate host, are more likely to become infected. Typically, long and wet seasons are associated with a higher rate of infection. However, sheep are more likely to ingest large numbers of cysts during dry periods following a wet season. This is due to a reduction in available pasture, forcing the animals to graze in swampy areas or in areas where the water has receded, thus exposing them to vegetation heavily infected with metacercariae (Richter, *et. al.*, 1999).

In the past, human fasciolosis was limited to populations within well-defined watershed boundaries; however, recent environmental changes and modifications in human behavior are defining new geographical limits and increasing the populations at risk (WHO, 1999).

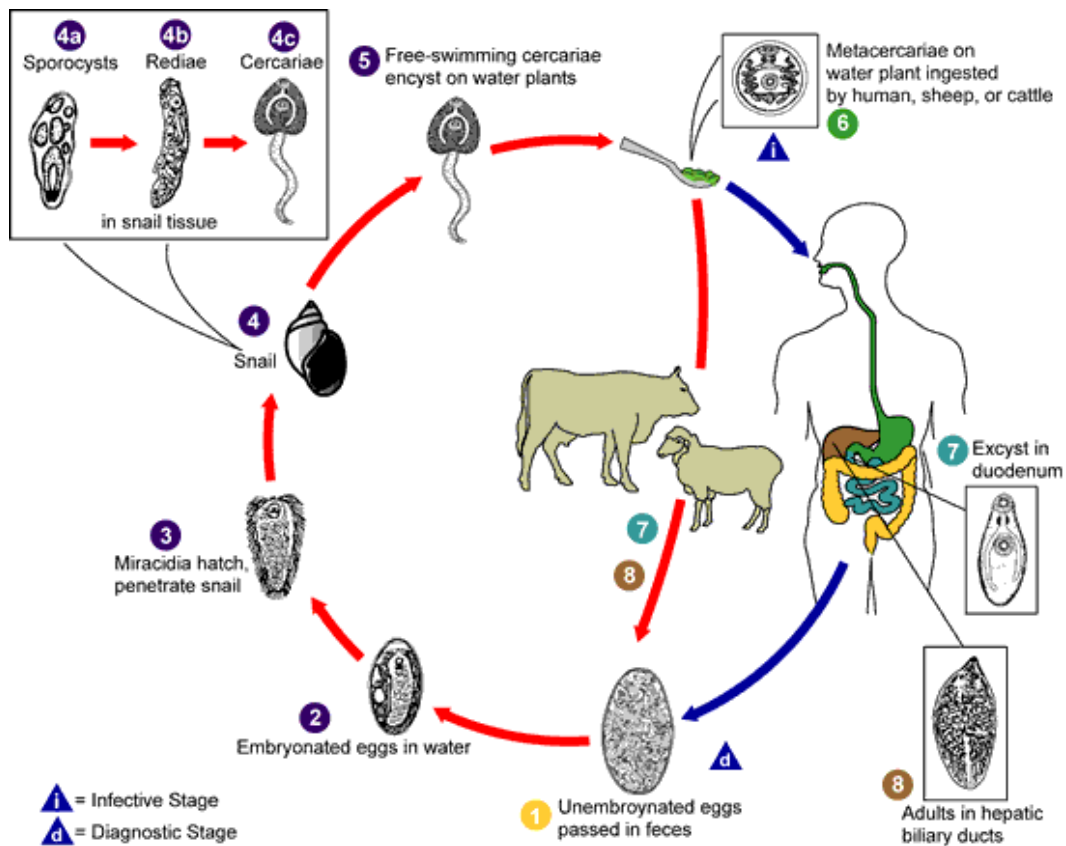


Figure 4. Life cycle of *Fasciola*

2. Epidemiology

Fasciolosis is considered an important limiting factor for ovine and bovine production. In general, infection of domestic ruminants with *F. hepatica* and *F. gigantica* causes significant economic loss estimated at over US\$ 200 million per annum to the agricultural sector worldwide, with over 600 million animals infected (Ramajo, *et. al.*, 2001). In developed countries, the incidence of *F. hepatica* ranges up to 77% (Spithill *et. al.*, 1998). Evidence suggests that sheep and cattle may be considered the main reservoir host species, pigs and donkeys being secondary (Mas-Coma *et. al.*, 1999). In tropical regions, fasciolosis is considered the single most important helminth infection of cattle with prevalence rates of 30-90% in Africa, 25-100% in India and 25-90% in Indonesia (Spithill, *et. al.*, 1999).

F. hepatica is a temperate species and it is found in Southern America, Northern America, Europe and Australia and Africa, but found in the highlands of Ethiopia and Kenya (Yilma and Malone, 1998). It is the major cause of liver fluke disease in Ethiopia. Its tropical counterpart, *F. gigantica*, on the other hand is widely distributed in tropical countries, in Africa and Asia, parasitizing domestic ruminants and other herbivores in almost every continent. In Ethiopia, *F. gigantica* is found at altitudes below 1800 m.a.s.l. while *F. hepatica* is found at altitude between 1200-2560 m.a.s.l. (Yilma and Malone, 1998). Mixed infections by the two species can be encountered at 1200-1800 m.a.s.l.

The annual loss due to endo-parasite in Ethiopia is estimated at 700 million Ethiopian birr/annum (Mulugeta, *et. al.*, 1989). Particularly financial loss due to ovine fasciolosis alone is estimated at 48.8 million Ethiopian birr/annum of which 46.5%, 48.8% and 4.7% were due to mortality productivity (weight loss) and liver condemnation, respectively (Ngategize, *et. al.*, 1993).

The epidemiology of fasciolosis depends on the grazing habitat preference of the animal. Njau and Scholtens (1991) reported that metacercaria can survive up to 3 months after harvesting in hay from endemic highland areas that are consumed by the ruminants in arid and lowland areas,

particularly during the dry season when suitable grazing pastures are scarce; local crowding of animals along the banks of streams and ponds during the dry season. When nutritional conditions are generally compromised also provides an important dynamics for infection transmission. Irrigation would have major effects on transmission (Yilma and Malone, 1998).

3. Clinical signs

The clinical features of fasciolosis can have acute, sub-acute and chronic forms.

Acute fasciolosis occurs as disease outbreak following a massive, but relatively short-term, intake of metacercariae (Urquhart *et. al.*, 1989). The high fluke intake is often the result of certain seasonal and climatic conditions combined with a lack of appropriate fluke control measures. It typically occurs when stocks are forced to graze in heavily contaminated wet areas as a result of overstocking and/or drought. Animals suffering from acute fasciolosis especially sheep and goat, may display no clinical signs prior to death; while some may display abdominal pain and discomfort and may develop jaundice (Soulsby, 1982; Urquhart *et. al.*, 1989). In some cases, the liver capsule may rupture and fluid may leak into the peritoneal cavity causing death due to peritonitis. More commonly, on ingestion of fewer metacercaria, fever and eosinophilia is seen (Soulsby, 1982). Death usually results from blood loss due to hemorrhage and tissue destruction caused by the migratory juvenile flukes in the liver resulting in traumatic hepatitis. This is more commonly seen in sheep than in other hosts.

Sub-acute fasciolosis is caused by ingestion of a moderate number of metacercaria and is characterized by anemia, jaundice and ill-thrift. The migrating fluke causes extensive tissue damage, hemorrhage and in particular liver damage. The result is severe anemia, liver failure and death in 8-10 weeks (Urquhart *et. al.*, 1989).

Chronic fasciolosis is the most common clinical syndrome in sheep and cattle. It occurs when the parasite reaches the hepatic bile duct. The principal effects are bile duct obstruction, destruction of

liver tissue, hepatic fibrosis and anemia. The onset of clinical signs is slow. Animals become gradually anemic and anorectic, as the adult fluke becomes active within the bile duct and signs may include dependent oedema or swelling under the jaw ('bottle jaw'). Affected animals are reluctant to travel. Death eventually occurs when anemia becomes severe. Additional stress upon anemic animals, such as droving, may lead to collapse and death. Cattle typically present with signs of weight loss, anemia and chronic diarrhea (Mitchell, 2001).

In addition to these, a condition known as '*black disease*' is a complication, which usually is fatal. Here, a secondary infection due to the bacterium *Clostridium novyi* Type B, proliferating in necrotic lesions produced by the young larvae migrating in the liver is responsible for the fatal outcome (Radostits, *et. al.*, 1994). Chronic fasciolosis provides the right environment in the liver for the germination of the spores of the bacterium. This form of the disease is much more common particularly in man. In humans the presence of the flukes causes a number of non-specific symptoms including malaise, an intermittent fever, mild jaundice, anemia, eosinophilia and frequently pain under the right costal margin. Furthermore, *Fasciola spp.* do not appear to be fully adapted to using man as a definitive host, as the flukes may often give rise to ectopic infections, particularly in the lungs and subcutaneous tissues, where they may be found encysted.

4. Pathology and pathophysiology

Pathogenesis of fasciolosis varies according to the parasitic development phases: parenchymal and biliary phases. The parenchymal phase occurs during migration of flukes through the liver parenchyma and is associated with liver damage and hemorrhage. The biliary phase coincides with parasite residence in the bile ducts and results from the haematophagic activity of the adult flukes and from the damage to the bile duct mucosa by their cuticular spines (Urquhart, *et. al.*, 1989). In the bile ducts of some permissive hosts, such as the sheep, rabbit, rat and mouse, the biliary stage

of the disease is common. In others, such as cattle and humans, few flukes survive beyond the migratory phase and biliary disease is relatively rare (Behm and Sangster, 1999).

Light infections due to *Fasciola hepatica* may be asymptomatic. However, they may produce hepatic colic with coughing and vomiting; generalized abdominal rigidity, headache and sweating, irregular fever, diarrhea and anemia (Behm and Sangster, 1999). In domestic ruminants, an adverse effect of acute or chronic fasciolosis includes decreased weigh gain and milk production, decreased female fertility, work power and mortality. Hepatic pathology, even when only limited areas of the liver are damaged, results in significant disturbances in mitochondrial bioenergetic metabolism of carbohydrates, proteins, lipids and steroids, as well as bile flow and bile composition (Calléja, *et. al.*, 2000).

Sheep and goat are very susceptible to acute fasciolosis and the damage results from the immature flukes tunneling through the liver parenchyma with extensive tissue damage and hemorrhage that culminate in severe clinical disease and high mortality in the grazing sheep in Africa (Okewleo, 2000). During the movement of the immature stages of *Fasciola hepatica*, which may continue for months, symptoms may include abdominal pain, an enlarged live, fever, and diarrhea. Mitchell (2001) indicated that the pathology associated with diseases are caused by the inflammation of the bile ducts which causes thickening of the lining and eventually leads to fibrosis that results in reduced flow of the bile and back pressure builds leading to atrophy of the liver parenchyma and cirrhosis.

Occasionally the worms penetrate the bile duct wall into the liver parenchyma causing liver abscesses. The complexity arises from several sources. Maturation of flukes involves development and growth for over 12-16 weeks during which time the fluke travels between and within organs. Because an individual fluke may pass the same part of the liver twice (or more) during these peregrinations, fresh and resolving lesions caused by the sequential insults may be found in the

same section of tissue; as the migratory fluke grows the size of its track through the liver increases as does the damage and the inflammatory response. Calves are susceptible to fasciolosis but in excess of 1000 metacercariae are usually required to cause clinical fasciolosis (Behm and Sangster, 1999).

The disease is characterized in calves by weight loss, anemia, and hypoproteinemia after infection with 10,000 metacercaria (Behm and Sangster, 1999). Resistance develops with age so that adult cattle are quite resistant to infection.

Even though, the rate of development of human fasciolosis is similar to that in sheep, as an unnatural host, only few flukes develop sufficiently to reach the bile duct.

Fasciolosis has a major effect on blood components (plasma proteins). Hypoalbuminemia and Hyperglobulinemia commonly occur in liver fluke infections in all host species. During the parenchymal stage of the infection, liver damage caused by the migrating flukes compromise liver function, which in sheep and calves is reflected in a decline in plasma albumin concentrations, attributed partly to reduced rate of synthesis and partly to an expansion of the plasma volume (Behm and Sangster, 1999; Urquhart, *et al.*, 1989). Nevertheless, during biliary stage of the infection loss of blood from haematophagia and into the intestines is so extensive, causing severe anaemia, that synthetic capacity of the liver is insufficient to replace the lost albumin (small molecular size) that oozes through the hyperplastic bile ducts (Challengitis). Thus, a progressive loss of plasma albumin occurs in all infected host species, starting from around the time the fluke commences blood feeding. This results in disturbance in intravascular and extravascular oncotic pressure leading to the development of oedema, often markedly visible at the mandibular region of ruminants ('bottle jaw').

Liver trauma is the abrasion caused by cuticular spines and the prehensile action of the suckers and appears to account for the majority of the damage caused in the liver. Death of the host is a

consequence of the hemorrhage induced by this damage. The oral sucker is the route by which liver flukes obtain most of their nutrition. It appears to cause considerable damage to liver tissue and macerated hepatic cells have been observed inside the sucker and pharynx. The oral sucker extends during migration and feeding from the earliest stages is capable of disrupting cells. The muscular pharynx assists in this process and oral sucker is a major organ involved in tissue disrupting (Behm and Sangster, 1999).

Although the inflammatory process has an important role in protecting the host against severe consequences of liver damage by the flukes, perhaps by retarding the growth of the parasite and contributing to hepatic healing process, there is accumulated evidence, in rats, that the response also contributes to hepatic dysfunction. There is evidence also that the infected rat liver is under oxidative stress during the parenchymal stage of the infection.

The liver plays a central role in the physiology of the body, being responsible for a large proportion of the body's amino acid metabolism, for carbohydrate and lipid balance, urea synthesis, detoxification metabolism, ketogenesis, albumin and glutathione synthesis as well as aspects of homeostasis. Therefore, it is to be expected that many systemic changes will be induced by liver fluke infections that ultimately cause reduced productivity in livestock. Both anorexia (inappetance) and the quality of the diet of infected sheep contribute to hypoalbuminaemia during the infection (Behm and Sangster, 1999).

5. Diagnosis

Diagnosis of fasciolosis may consist of tentative and confirmatory procedures. A tentative diagnosis of fasciolosis may be established based on prior knowledge of the epidemiology of the disease in a given environment; observations of clinical signs, information on grazing history and seasonal occurrence. Confirmatory diagnosis, however, is based on demonstration of *Fasciola* eggs through standard examination of feces in the laboratory; postmortem examination of infected animals and

demonstration of immature and mature flukes in the liver. The latter is helpful in deciding the intensity of infection. There are other laboratory tests (enzymatic and/or serological procedures used to qualify the infection mainly for research purposes.

Serological assays are often used to detect infections due to immature forms where fecal egg output is often nil. Such tests allow the detection of substance like cathepsin L proteases, excretory-secretory products, detection of Ag in milk, and ELISA detection of antibodies against the flukes plasma concentration of Gamma-glutamyltransferase(GGT), which are increased with in the bile duct damage.(Cornelissen, *et. al.*, 2001; Soulsby, 1982; Urquhart *et.al.*, 1989) for example, Oxidative stress would be one of the consequences of the activity of inflammatory cells such as neutrophils, macrophages and eosinophils in producing oxygen-derived free radicals, nitric oxide and their products. A useful indicator of oxidative stress is the concentration of reduced glutathione (GSH) in cells. For chronic fasciolosis, confirmatory diagnosis could easily carried out by coproscopic examination employing sedimentation technique. *Fasciola* eggs have high specific gravity and sedimentation is preferred to floatation. When the latter is employed, floating medium such as ZnSo₄ should be used. As *Fasciola* eggs may be confused with *Paramphistomum* eggs, addition of methylene blue in the fecal suspension will facilitate ease identification by providing a blue and contrasting microscopic field.

6. Control and prevention

Several control methods against ruminant fasciolosis are available and can either be used independently and as a combination of two or more of them. These methods involve reduction in the number of intermediate snail hosts by chemical or biological means, strategic application of anthelmintics, reduction in the number of snails by drainage, fencing and other management practices and reduction in the risk of infection by planned grazing management.

6.1 Snail Control

Control of parasitic diseases is crucial to improve the productivity of the animals. In most fasciolosis endemic areas, the control of the intermediate snail host population offers a good opportunity for the reduction of transmission and is generally effective when combined with one or more other methods such as chemotherapy or environmental sanitation.

Although eradication of the snail hosts is the most effective method of total fluke controls this, however, is often very difficult in low-lying, wet areas with a mild climate. Snails multiply extremely rapidly and hence eradication is almost impossible in irrigation areas. There are different types of snail poison available that are safe for stock but need care and precision in their application. Other useful methods of fluke control include biological control of the intermediate host, fencing the waterlogged area and so on (Hansen and Perry, 1994; Soulsby, 1982; Mitchell, 2001).

The use of molluscicides for the control of snail intermediate hosts is a potential tool for the control of fluke infections. Before considering chemical control of snails, it should be noted that many habitats are topographically unsuitable for the use of molluscicides and it is often very difficult to apply them effectively. They are toxic to the environment, cooperation between neighboring properties is required for effective cover and regular (at least yearly) application is required because rapid repopulation of snails may occur. whereas, they are not species-specific, may destroy edible snails highly valued as food in some communities and expensive (Hansen and Perry, 1994).

A great number of chemicals have been used as molluscicides in the past, but at present Niclosamide (Bayluscide or mollotor) and copper sulfate are used in different part of African Countries (Brown, 1980).

Brown (1980) indicated that molluscicidal properties have been demonstrated in extracts from a variety of plants. A substance 'Endod' or Lemma toxins derived from the fruits of shrubs *Phytolacca*

dodecandra (Lemma, 1970 cited in Brown, 1980). Substance such as 'Endod' might provide means of snail control less costly to developing countries than synthesized by molluscides but the production naturally molluscides on a commercial scale has yet to be achieved. Tadesse and Getachew, (2002) from their findings they also indicated that 'Endod' used for the control of fasciola transmitting snails particularly *L. truncatula* and *L. natalensis*.

6.2 Chemotherapy

Effective control of most trematode infections is based on strategically applied chemotherapy (Hansen and Perry, 1994). Combination of chemotherapy, intermediate host control, sanitation and environmental manipulation are believed to be more efficient but very expensive.

A flukicidal drug of choice must fulfill the following

- It must act against both immature and mature flukes
- It must not be toxic to the recipient animal
- It must be cheap and available

Chemotherapy with drugs remains the most cost-effective way of treating parasitic diseases, and is usually at the heart of any major control campaign. Compared to environmental engineering, drug treatment is very cheap (Gaasenbeek, *et. al.*, 2001). The drugs to be used against flukes should ideally destroy the migrating immature flukes as well as adults in the bile ducts. Several drugs are now available for the treatment of fasciolosis, which are against the adult flukes, and the parenchymal stages. These include Rafoxanide, Nitroxynil, Brotanide, Closantel and Albendazole. Diamphentide kills all immature flukes even a day old once and the Triclabendazole (TCBZ) is highly effective against all stages of fluke (Table 1). It is one of the widely used drugs worldwide for the control of fasciolosis (Spithill and Dalton, 1998; Gaasenbeek, *et. al.*, 2001). Chemotherapy normally reduces the prevalence and intensity of infection as measured by faecal egg counts (Hansen, *et. al.*, 1999).

Table 1. Anthelmintic for the treatment of liver flukes

No.	Common name	Generic name	Admin. Route*	Dose rate (mg/kg)		Minimum age of fluke in weeks efficiency	
				Sheep	Cattle	Sheep	Cattle
1	Halogenated Phenols	Hexachlorophene	O	15	20	12	>20
		Hexachloroethane	O	250-300	300	12	12
		Bithionol	O	75	30	>12	>12
		Hexachloroparaxylene	O	150	130	12	12
		Bromophenophols	O	16	12	12	>12
		Niclofolan	O	4	3	12	>12
			SC	NR	0.8	NR	<12
	Nitoxynil	SC	10	10	8	10	
2	Salicylanilides	Oxyclozanide	O	15	13-16	12	>14
		Brotianide	O	5.6	NR	12	NR
		Rafoxanide	O	7.5	7.5	6	12
			SC	NR	3	NR	12
		Closantel	O	7.5-10	NR	6-Aug	NR
SC	NR		3	NR	>12		
3	Phenoxyalkanes	Diamphenetide	O	80-120	100	1day -6weeks	1day-7weeks
4	Benzimidazoles	Albendazole	O	4.75	10	>12	>12
		Triclabendazole	O	10	12	1	1
5	Sulphonamides	Clorsulon	O	-	7	-	8
			SC	-	2	-	>12

Source: Hansen and Perry, 1994; Fairweather and Boray, 1999; Soulsby, 1982

* O= orally administered, SC=Subcutaneous administered and NR=Not recommended

6.3 Environmental sanitation and manipulation

Draining swamps, building sewage systems and providing clean water supplies are used to control water-borne /including snail borne/ helminths but it is very expensive compare to chemotherapy (Hansen and Perry, 1994; Gaasenbeek, *et. al.*, 2001).

Strategies for the treatment and prophylaxis of infections with *Fasciola* are developed based on epidemiological data. Effective treatment during the prepatent period for an extended duration could eliminate *Fasciola* infection or reduce contamination of pasture to a very low level, requiring less frequent treatments for a considerable time (Hansen and Perry, 1994; Yilma and Malone, 1998. Retardation of immature flukes, which survive treatment, appears to be applicable to all anthelmintics and the degree of retardation depends on the efficacy of the drugs against the immature stages. This phenomenon has a great advantage in strategic control by reducing early pasture contamination with eggs. Less frequent strategic treatments with a possible yearly rotation of anthelmintics or anthelmintic combinations that are effective against both immature and adult flukes has been reported to provide the best method of successful control of fasciolosis (Parr and Gray, 2000). Other control methods includes Rotational grazing (i.e. grazing animals in divided paddocks; grazing equines, then sheep etc) and also avoiding missed grazing of animals of different age groups (NB: Young animals are generally susceptible to helminthes infections).

7. Importance of ovine fasciolosis

7.1. Economic

FAO (2002) estimated that Africa has 241 million Sheep and 209 million goats representing approximately 23% and 29% the world total population, respectively. The latest animal population census (CSA, 2004) shows that Ethiopia has 23.62 million sheep and 23.33 million goats. Despite the huge population size productivity of small ruminants in Ethiopia is very low because of prevalent

diseases, sub-optimal nutrition, and poor management (Scott and Goll, 1977). Animal diseases are widely distributed and one of the major causes of livestock mortality, ill thrift and sub-optimal productivity in all agro-ecological zones of the country (EARO, 2000) is diminishing the benefit of their high reproductive performance. Mulugeta *et al.*, (1989) and Goll and Scott (1978) showed that productivity losses attributed to helminth parasite in Ethiopian highland sheep are considerable, and fasciolosis is a major factor in this respect. Direct losses due to fasciolosis are host mortality and liver condemnations whereas losses indirect losses may occur in a form of losses in body weight and decreased weight of lambs from infected ewes and decrease wool production (Malek, 1985; Scott and Goll, 1977; Behm and Sangester, 1999).

7.2 Public health

Fasciolosis has recently been shown to be an important public health problem with human cases reported from countries in five continents, the level of endemicity ranging from hypo- to hyper-endemic (Dela-Valero *et al.*, 2001). Human fasciolosis has also been reported in Europe, including Belgium, France, United Kingdom, Ireland, Switzerland and Spain (Ramajo, *et al.*, 2001).

The perception of human fasciolosis, caused by *Fasciola hepatica* or *Fasciola gigantica*, as an sporadic disease of low economic importance, is no longer tenable as the estimate of global prevalence is between 2.4 and 17 million human infections world-wide (Slifko, *et al.*, 2000) and a further 180 million at risk of infection (Ramajo, *et al.*, 2001). As a result, WHO (1995) has recognized fasciolosis as an emerging disease of humans. The distribution of the disease is predominantly rural, being associated with cattle and sheep breeding, although high prevalence in humans are not necessarily associated with areas where fasciolosis is a significant veterinary problem. A few studies showed that the incidence appears to be concentrated within families, as they are all likely to consume the same contaminated product.

The most common transmission route is the ingestion of watercress contaminated with encysted metacercariae, although, depending upon the geographical location, and a variety of edible aquatic plants can be vehicles of transmission. Water containing floating metacercariae has also been implicated in disease transmission, as have salads contaminated with metacercaria-contaminated irrigation water. Among the risk factors are included the use of animal manure as fertilizers and wastewater effluent for irrigating aquatic or semi-aquatic vegetables (Slifko *et. al.*, 2000). High prevalence of human fasciolosis is recorded from Peru and Bolivia. Very few human fasciolosis reports exist in Ethiopia (Yilma Jobre (2004), personal communication). The anticipated common means of transmission is the habitual use of grass as a toothpick in this environment.

7. 3 The situation in Ethiopia

Fasciolosis is a serious problem in Ethiopia where sheep raising is of major importance to local economy (Njau *et. al.*, 1989). Studies so far conducted on fasciolosis in Ethiopia were mostly based on coprological examinations and abattoir surveys. Brook, 1983 and Brook, *et. al.*, (1985) carried out an investigation on the epidemiology of ovine helminthosis in four ecological regions and found that fasciolosis was highly prevalent in Debre Berhan area. ILCA (1992) Annual Report showed it to be the main cause of loss of weight and death in small ruminants. Senior students from the Faculty of Veterinary Medicine, Addis Ababa University, have conducted several studies on prevalence and economic importance of fasciolosis in Ethiopia. These studies indicated that infection prevalence of fasciolosis varied from region to region, and reported prevalence rates of 49 % in Holeta (Yilma, 1985), 30.2 % around Ziway (Adem 1994), 70.4 % in Western Shoa and Nekemte (Yadeta, 1994; Mezgebu, 1995; Wassie, 1995).

Mamo (1980) indicated that fasciolosis is the most prevalent parasitic disease of sheep in the highlands of Ethiopia and reported the existence of the intermediate host, *Lymnae truncatula* in marshy areas.

Graber and Danes (1974) reported the existence of this intermediate host in Illubabor, Shoa, Jima, Sidamo, Harrar, Wello and Bale.

In Ethiopia, the highlands contain pockets of waterlogged marshy areas. These provide suitable habitats year round for the snail intermediate hosts (Argaw, 1998). The prevalence of fasciolosis in arid and semi-arid areas is very low. In the presence of irrigation in semiarid and arid areas, the prevalence of fasciolosis is increasing.

However, none of the previous works had indicated the importance of irrigation as a risk factor for the high prevalence of an intermediate host snail (*Lymnaea truncatula* and *L. natalensis*) and hence assessment of the magnitude of the problem will necessary for institution of a rational fasciolosis control strategy suitable to the irrigated areas. Therefore, this study focuses on determining the infection prevalence of fasciolosis in sheep owned by the farmers inhabiting around small-scale traditional irrigation schemes in the highlands and, mid-altitude and lowland areas of the upper Awash River basin; and assessing the potential impact of community-based irrigation on the spread of fasciolosis in the study areas; and evaluation of the effect of a strategic anthelmintic treatment on some productivity parameters in a selected upstream areas.

II. Objective

1. General:

To assess the potential impacts of community based irrigation on the spread of fasciolosis in the Upper Awash River Basin and suggest strategies to the control of the ovine fasciolosis.

2. Specific:

- To determine the seasonal variations in infection prevalence of ovine fasciolosis in community-based irrigation schemes in different agro-ecological zones of the Upper Awash River Basin.
- To assess the fauna of intermediate snail hosts in grazing and watering points.
- To evaluate the effect of strategic anthelmintic (flukicidal) drug treatments on some indicative parameters in an upstream (Wolemera) locality.

III. Materials and Methods

1. The study areas

The Awash River starts from the Ginchi highlands of Ethiopia and flows towards the east of the country covering a total length of 1,200 km length and an area of 110,000 km². The Awash Basin has been divided into five major zones based on physical and socio-economic factors as outlined below by Halcrow in 1989 (Fig. 5). These are:

1. Uplands – all lands in the basin above 1500m that area of the Basin with the mean annual rainfall in excess of 800 mm.
2. Upper Valley – That area of the Basin between Koka reservoir and Awash Station which lies between 1500 m and 1000 m altitude.
3. Middle Valley – That area of the basin between Awash Station and Mile River lying between 1000m and 500 m with a mean annual rainfall variation from about 600 mm to 200 mm.
4. Lower Plains – These are the deltaic alluvial plains in the Tendaho, Asayita and Dit Bahari areas as well as the terminal lake environs.
5. Eastern Catchments – this area is of some 47000-km² extent and ranges from the Wabi Shebelle watershed at about 2500 m altitudes down to the desert plains, which range from 1000 m down to 300 m.

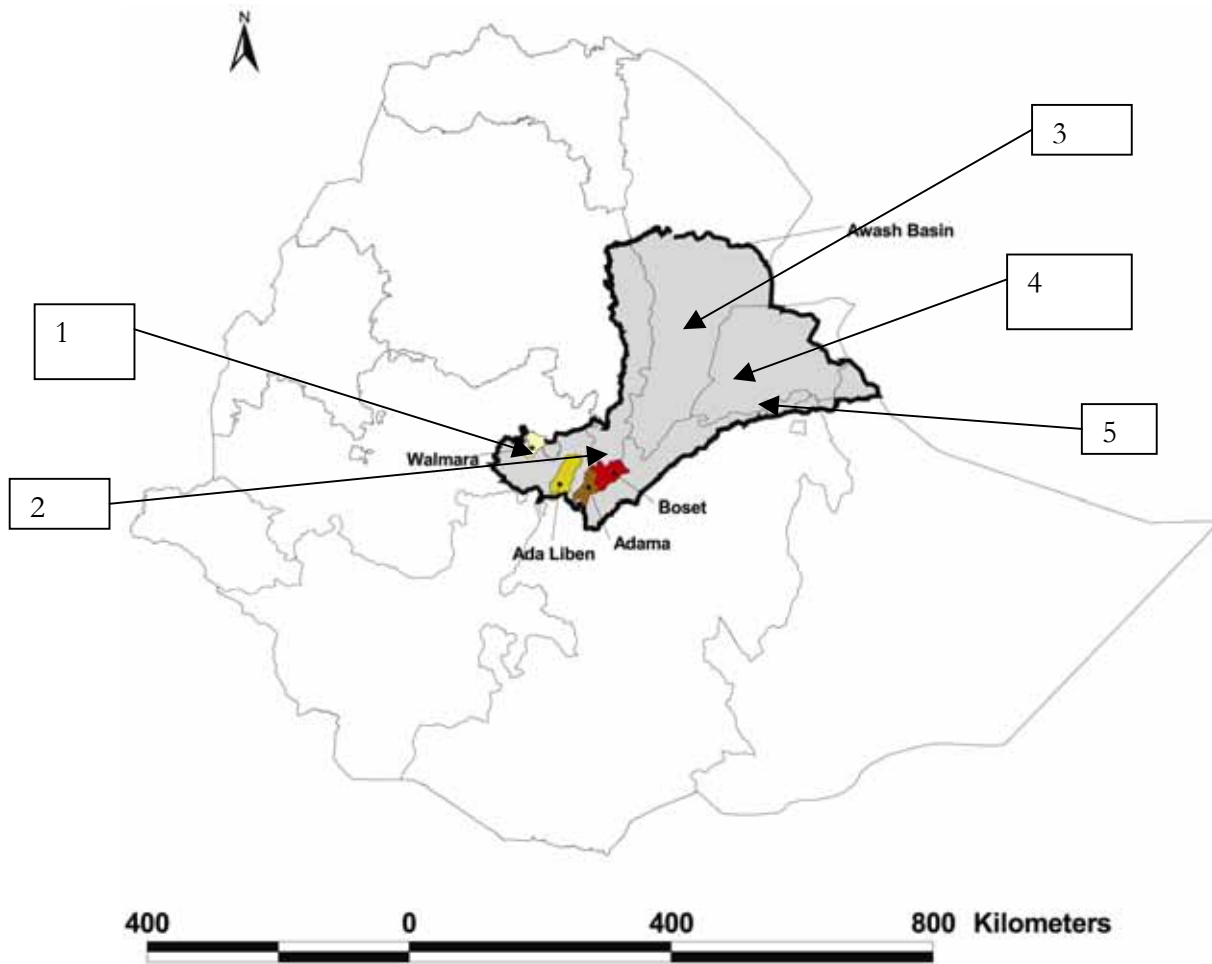


Figure 5. Map of Ethiopia showing the Awash River Basin

From the total irrigated land of Ethiopia, about 161,125 hectares (over 43 %) is found within the Awash River Basin. Out of this, 26.5% are under traditional and modern small-scale irrigation. The remaining potential for irrigated agriculture using Awash River is estimated at 136,220 hectares (Mekonen and Tarekegne, 2001).

The present study was conducted in four selected areas belonging to three different ecological zones in the Upper Awash River Basin areas: Wolemera District (Highland), Ad'aa District (Mid-altitude), Batu Degaga (Sodere area) and Boset (Doni area) Districts, both the Lowland (Fig. 6).

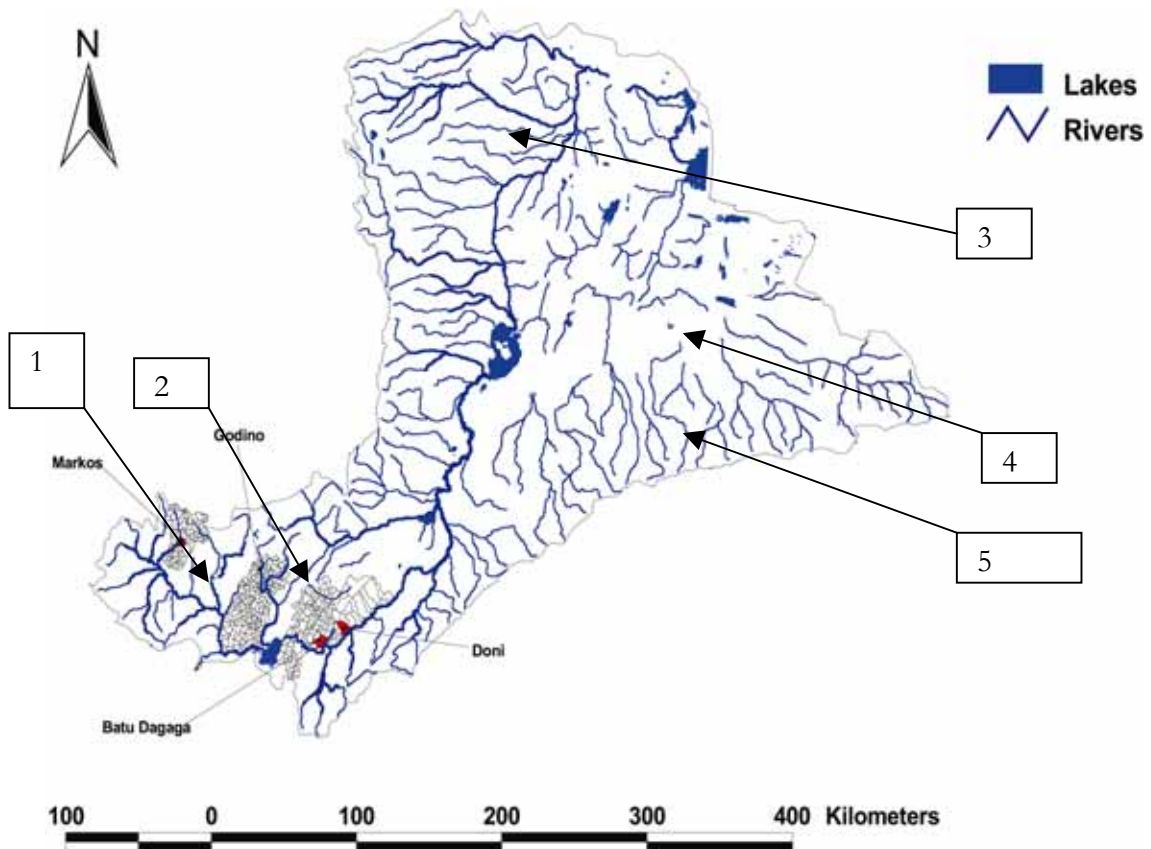


Figure 6. Map showing the location of the study areas

1.1 Agro-ecologic zones description

1.1.1 Highland - Wolemera District

Wolemera district is located in western Shoa Zone some 40km west of Addis Ababa, at 09°02N latitude and 038° 34E longitude and an altitude of 2390 m.a.s.l. The area receives an annual rainfall of above 1060 mm and has a bimodal rainfall pattern, the short rains occurring from March to April, and the long rains from July through October (Fig. 7). Farmers in the area are engaged in crop-livestock mixed agriculture. Small ruminant are raised in traditional extensive system and are herded by village children during the cropping season, and during the dry season are allowed to browse and graze over cropping fields, along the stream and irrigation canals. The total population reaches 3880 sheep and 1050 goats (Wolemera District Agricultural Bureau). The irrigation scheme is located in Wolemera

District in Berfeta Lemefa peasant association and its about 42 km from Addis Ababa on the way to Ambo and is accessible throughout the year. Member of peasant association diverted the Guntuta River using earth dam.

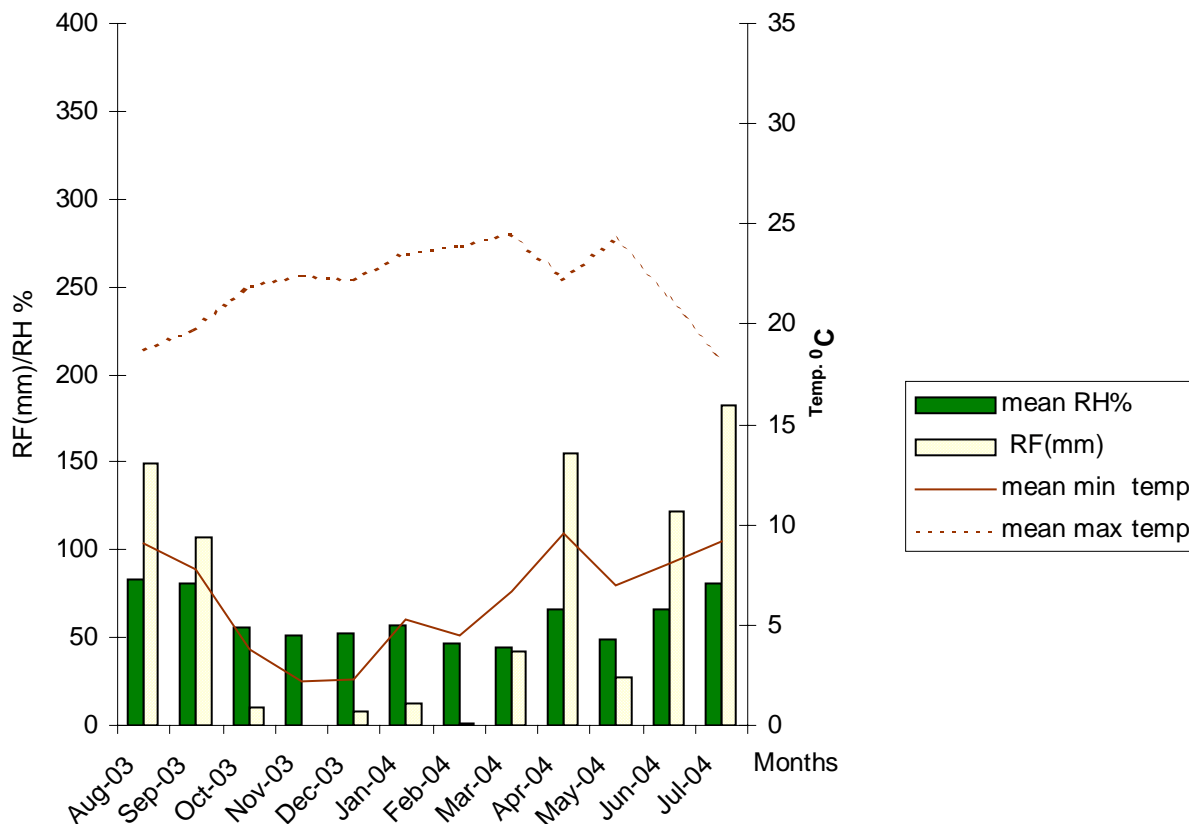


Figure 7. Monthly rainfall (mm), mean relative humidity, mean maximum and minimum monthly air temperatures (°C) of Holeta area for the period from August 2003 to July 2004.

1.1.2 Mid-altitude- Ad'aa District

Ad'aa District is located in the upper catchments of the Awash River Basin along the road from Debre Zeit to Chefie Donsa. The area lies at latitude 08°44' N and longitude 38°58' E with an altitude of

about 2000 m.a.s.l. The annual rainfall reaches about 1000 mm and is bimodal; the short rains occurring from February to May, and the long rains from June through September. The mean annual temperature is 18°C. The hottest season is May and the mean annual relative humidity is 56 (DZARC, EARO) (Fig 8).

The irrigated scheme is located at about 63km from Nazareth and 15km North of Debre Zeit. Wedecha and Belbela are the two micro-dams constructed by using Wedecha River. The water for irrigation scheme is the Wedecha and Belbela Dams. The main sources of water for the dams are the run-off of the surrounding catchments supplying the Wedecha and Belbela streams. The agricultural activity is typically mixed crop-livestock farming dominated by crop production. Small ruminants are important stocks and are traditionally managed. They are grazed on cropping farms, along the streams and irrigated canals.

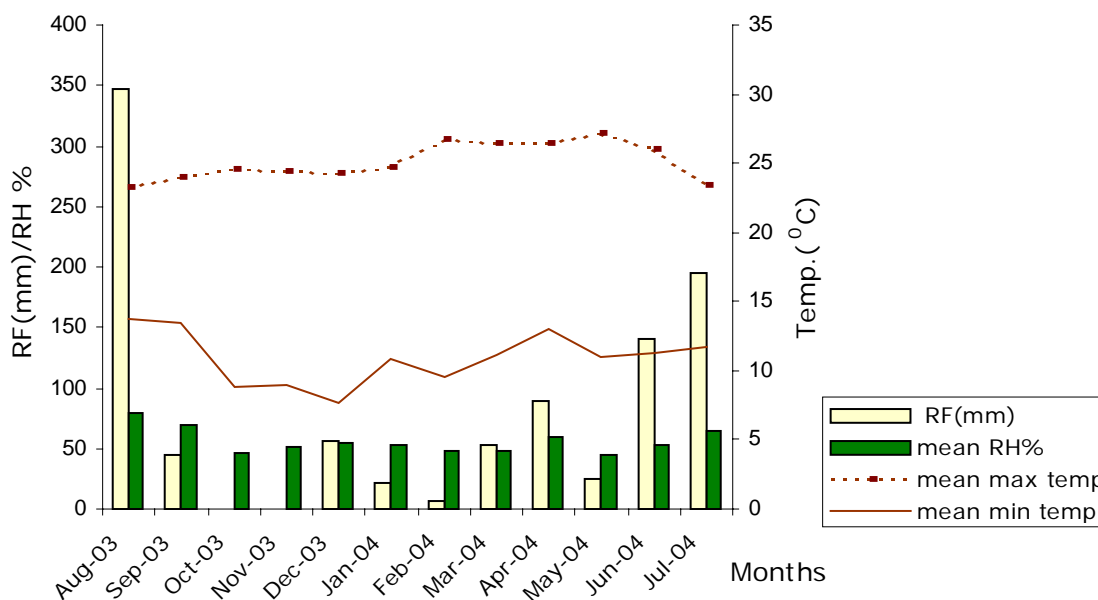


Figure 8. Monthly rainfall (mm), mean relative humidity, mean maximum and minimum monthly air temperatures (°C) of Debre Zeit area for the period from August 2003 to July 2004.

1.1.3 Lowland - Batu Degaga and Boset Districts

A) Batu Degaga District (Around Sodere)

This area is located at latitude 08°25'N and longitude 39°25' E in eastern Shoa and 7 km away, to the east of Sodere resort center, on the road that leads to Tibila. The area is semi-arid draught prone. In this irrigation project, water is diverted from Awash River. The elevation of the project area is 1350-1372 m.a.s.l. The agriculture is dominated by livestock production. The mean annual rainfall is between 700 to 760 mm mainly received from June to September followed by a distinct dry spell up to January. The mean monthly minimum temperature is 10 °C while the maximum is 34 °C. The pattern and magnitude of rainfall at Melkas Station are as shown Fig. 9.

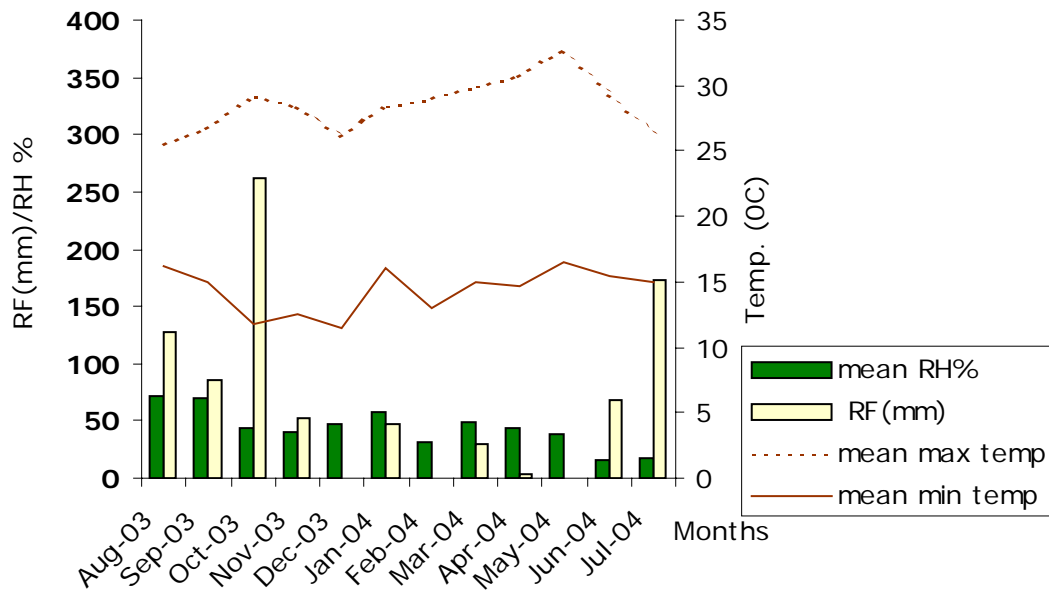


Figure 9. Monthly rainfall (mm), mean relative humidity, mean maximum and minimum monthly air temperatures (°C) of Melkasa area for the period from August 2003 to July 2004.

B) Boset (Doni Area) District

Doni area is located on the left hand side of the rural road from Awash to Melkasa to Nura Era state farm. The area elevation varies from 1240m-1280 m.a.s.l. and geographically the area is located at latitude 8°30.202'N longitude 39°33.225'E. The source of water for the Doni irrigation scheme is the Awash River. It is located down stream of wonji sugar state farm. The area is situated at an undulating alluvial plain with open vegetation with dominant natural grazing lands. The diversion work is about 1.3 Km away from the road. The main canal crosses the road and the distribution canal ends about 7.3 km north east of Doni village.

The climate is arid (dry hot). The rainfall is erratic and crop failure as a result of insufficient rainfall is very common. According to Nura era state farm the highest rainfall is 216 mm, the highest amount received in July and August. The mean monthly minimum temperature is 13 °C while the mean monthly maximum temperature is 34 °C as shown in Fig. 10. Agro-Pastoralism is the dominant production system and the livelihood of the households in the study area is mainly based on livestock production.

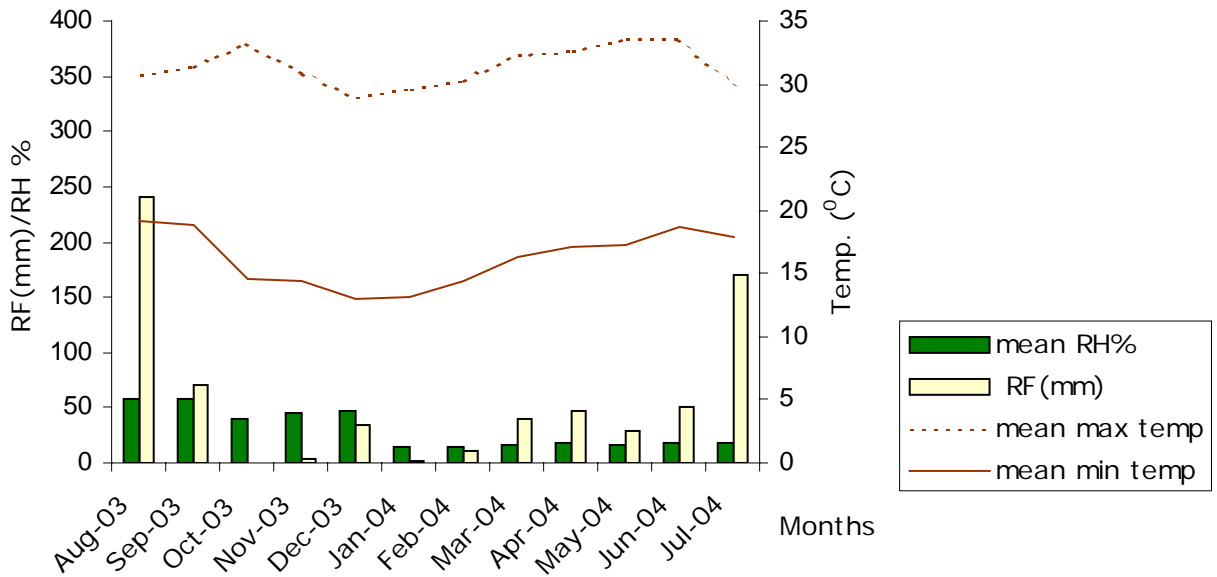


Figure 10. Monthly rainfall (mm), mean relative humidity, mean maximum and minimum monthly air temperatures (°C) of Nura Era area for the period from August 2003 to July 2004.

2. Animals

2.1 Infection prevalence study

A total of 1296 sheep (529 from highlands, 302 from mid-altitude and 465 from lowland sites) were examined to determine the infection prevalence of ovine fasciolosis. All these animals were privately owned by smallholder farmers. Study animals were managed under traditional extensive system and depend mostly on grazing and receive a minimum or no supplementary feed and health care.

Households whose animals were involved in the study both from irrigated and non-irrigated areas were randomly selected. Infection prevalence datasets obtained from previous works were considered in the determination of the sample size required for the purpose of this study (5% of confidence interval to historical/true prevalence was considered acceptable (Panse and Sukhatme, 1978).

2.2 Measuring effects of strategic anthelmintic treatments

This study was conducted only in the Wolemera woreda for convenience and, involved 125 head of privately owned sheep by smallholder farmers. The animals were identified with ear tags and randomly divided into 3 groups. The data were collected during November 2003 – October 2004.

3. Study Protocol

3.1 Infection prevalence

Feecal samples, from a total of 1296 subjects, were collected directly from the rectum into universal laboratory-sampling bottles and immediately transported to Holleta Agricultural Research Center (EARO) for detailed coproscopic examination. Samples that were not processed within 24 hours from collection were stored in a refrigerator at 4°C. During every feecal sampling, information on sex and approximate age individual animals were recorded. Based on their dental eruption formula (Annex I) animals were classified into two age categories: young (<2 years) and adult (≥2 years). Feecal samples were collected twice, one during the dry season (690 samples) and the other during the wet season (606 samples).

Table 2. Sample collection time with respect to agro-ecological zones, age and sex.

Seasons	Agro ecological Zone	Age and Sex				Sub total
		Male Young	Male Adult	Female Young	Female Adult	
Dry Season (November 2003 - February 2004)	Highland	54	8	128	102	292
	Mid-altitude	31	6	37	68	142
	Lowland	32	18	73	133	256
Wet Season (June 2004 - August 2004)	Highland	43	14	85	95	237
	Mid-altitude	33	15	49	63	160
	Lowland	42	10	58	99	209
Sub total		235	71	430	560	1296

McMaster fecal egg counting technique was used to determine fasciolosis infection prevalence and fecal egg outputs. In order to determine fecal egg outputs a total of 729 sheep positive for fasciolosis were taken from four different sites representing three agro-ecological zones during dry and wet seasons. A detail of this procedure (MAFF, 1986) is presented in Annex II. *Fasciola* eggs were identified using ova identification keys (Soulsby, 1982).

3.2 Snail Survey

Snail habitat survey was carried out for both the dry and wet seasons in all selected study sites described above. Three potential habitat sites in an irrigation channel was selected;

Site 1 = near the diversion point

Site 2 = between the diversion and farm

Site 3 = within the farming areas

In each of these sites, a length of about 20 meters was covered and 6 different locations within each site were surveyed. A 1m by 1m quadrant was thrown on each sampling site; all snails were collected (using forceps along the periphery of irrigation canal, by using scoops along the irrigation canals that are deep and full of vegetation). The number of snails collected, water pH and temperature were recorded in a standard format prepared for this purpose (Annex IV). Collected snail specimens were then transported to Institute of Pathobiology, Addis Ababa University for identification. The latter was carried out on the basis of the snail's shell morphology and classified into major categories as per the criteria (key) described by Hansen and Perry (1994) and Malek (1985).

3.3 Measuring the effects of strategic anthelmintic treatments

Trials to measure the effects of strategic anthelmintic treatments were conducted on some productivity parameter in naturally fluke-infected sheep at Wolemera District (Highland site). In this area sheep are grazed in both irrigated and non-irrigated lands. At trial initiation, experimental animals were

selected on the basis of the presence of fluke infection, which was confirmed by standard coproscopic procedures (McMaster egg counting technique, MAAF, 1986).

A total of 125 *Fasciola*-infected sheep were used in this trial. Experimental animals were ear-tagged for permanent identification and randomly divided into 3 groups:

Group I (n=35): Each animal received a single Triclabendazole, treatment at 10mg/kg, on day₀ (November 10, 2003).

Group II (n=36): Each sheep received two-times Triclabendazole treatment, at 10mg/kg each, on day₀ (November, 2003) and on day₁₅₀ (June, 2004).

Group III (n=54): No treatment was given and the group served as a positive control.

The first treatments were given at D₀ for both Groups I and II and the second treatment given to Group II animals on day 150 post trail initiation.

The effects of strategic anthelmintic treatment was estimated through analysis of measurements on *Fasciola* faecal egg output (epg), packed cell volume (PCV), body weight gains (Kg), body condition scores (BCS) and off take rates (sell). All the above indicated parameters were measured at D₀, D₂₂, D₁₅₀, D₁₇₂ and D₃₂₂. During the course of the study, 7 (2 from Group I and 5 from Group III) sheep died and 38 (15, 3 and 20 from Groups I, II, and III, respectively) were sold. Data from these animals was, thus, disregarded inform the subsequent analysis.

A) Faecal Egg output

Faecal samples were collected and examined as described earlier in (Section 3.1) using modified McMaster technique (Hansen and Perry 1994; MAAF, 1986) (Annex II).

B) Packed Cell Volume determination (PCV)

PCV (%) values for all experimental groups were determined by Microhaematocrit centrifugation technique (Hansen and Perry, (1994), (Annex V). The PCV findings from each experimental groups were analyzed independently before being compared with other measured parameters.

C) Body condition scores (BCS)

BCS was determined following the key set by Thompson and Meyer, (1994). This measurement was based on physical inspection of individual animals and by taking the estimates on the level of musculature and fat deposition over and around the vertebrae in the loin region. In addition to central spinal column, loin vertebrae had a vertical bone protrusion on each side (transverse process). Body condition score was judged as 1 up to 5 (for details Annex VI).

D) Lives weight gain

Body weights of all experimental sheep were taken regularly using a portable scale, Group body weight gain (BWG) at a particular sampling occasion was calculated as differences of the values at trial initiation: $BWG_t - BWG_x$, where 't' is referring to measurement at D_0 and 'x' denotes a specific sampling occasion after trail initiation.

E) Mortality and off-take rates

Deaths and sells of animals were regularly registered in order to determine mortality and off-take rates among experimental animals.

3.4 Estimation of economic benefits from strategic treatment

Economic benefits, in monetary terms, from the two- strategic treatment interventions were calculated based on the group body weight mean and by comparing it with the average weight estimates of sheep (22kg) often sold in markets in the study area. A sheep weighing 22kg is sold at 150 birr in a market at Holeta. The cost of treatment of an average-sized sheep (Triclabendazole 300mg/kg) is 0.90 birr/

treatment. Another element considered in the calculation of the economic benefits at day 322 in this trail. The calculated body weight gains difference between initial values at D0 and final values at D322 is as follows:

Group I = 0.9 kg/animal

Group II = 4.1 kg/animal

Group III = -0.27 kg/animal

The associated direct economic benefit from sell of an animal at the local markets is estimated as follows:

Group I = 0.9 kg/animal x 6.82 birr = 5.24 birr

Group II = 4.1 kg/animal x 6.82 birr = 26.162 birr

Group III = -0.27 kg/animal x 6.82 birr = -1.84 birr

N.B. The cost of the treatment for each animal was deducted (0.90 birr in Group I, 1.80 birr for Group II and 0 birr for Group III) in the computation of the net monetary return from the various interventions.

4. Data Management and statistical analysis

All raw data generated from this study were entered into Microsoft Excel database system and referenced with location (district and peasant association) and agro-ecological zones. Other attribute data imported into the database system are information on grazing lands, age, sex, EPG, PCV, BCS and live weight measurement results.

Chi Square (χ^2) test was used to determine the variation in infection prevalence between irrigated and non-irrigated grazing lands, agro-ecologic zones, seasons, age and sex.

Results on faecal egg outputs were log transformed ($\log_{10}^{(50+x)}$) before being subjected to Chi-square (χ^2) test. Fasciolosis prevalence data was analyzed using one-way ANOVA and General Liner Model (GLM) Procedure using SPSS 10.0, a computer-based statistical package for Windows. Variations in egg count, PCV, body condition score and live weight gain measurement between experimental groups were also analyzed using one-way ANOVA, General Liner Model (GLM) and Student's T-test.

VI. Results

1. Fasciolosis infection prevalence

1.1 Overall

Faecal samples of 1296 sheep were examined, of which 56.3% were found positive for fasciolosis (Annex VIII). The infection prevalence of ovine fasciolosis in the highlands, mid-altitude and lowland study sites were 62%, 51% and 52%, respectively. The findings suggest that the infection prevalence in the highlands is significantly higher ($p < 0.05$) than in both mid-altitude and lowland areas. Similarly, the infection prevalence during the wet season was significantly higher ($p < 0.05$) than the dry season. Furthermore, irrigated lands had also significantly higher ($p < 0.05$) infection prevalence rate as compared to the non-irrigated study sites. On the other hand, there was no discernible statistical difference ($p > 0.05$) in infection prevalence within the age and sex groups.

1.2 Variation between irrigated and non-irrigated grazing lands

The overall infection prevalence was higher in the irrigated study sites when compared with non-irrigated lands. The overall difference, however, was statistically significant ($p < 0.05$) only during the dry season. Closer analysis of prevalence findings in relation to agro-ecology revealed that no statistically significant difference ($p > 0.05$) the irrigated and non-irrigated study sites in the highlands both during the dry and wet seasons. However, the variation in infection prevalence rates between irrigated and

non-irrigated grazing lands depicted to be statistically significant ($p < 0.05$) during the dry season both in mid-altitude and lowland areas, and during wet season in lowland sites (Annex VIII).

1.3 Interactions

A. Infection prevalence versus season

The dry season infection prevalence of fasciolosis in irrigated and non-irrigated areas of highlands was 64.5% and 57.3%, respectively (Fig. 11). In addition, the wet season picture in the highlands was in the order of 64% for the irrigated and 63.5% for the non-irrigated lands. In both cases, the differences were not statistically significant ($p > 0.05$). On the other hand, in mid-altitude, the infection prevalence during the dry season in irrigated (57.3%) and non-irrigated (37.3%) areas was significantly different ($p < 0.05$). The wet season infection prevalence in mid-altitude sites revealed no significant difference ($p > 0.05$) between the different land use types. Fasciolosis in the lowland sites during dry season in irrigated areas (58.3%) was significantly higher ($p < 0.05$) than in the non-irrigated areas (32.1%). In addition, the wet season prevalence in the irrigated (61.5%) and non-irrigated (55%) areas also revealed the presence of significant difference ($p < 0.05$) (Annex VII).

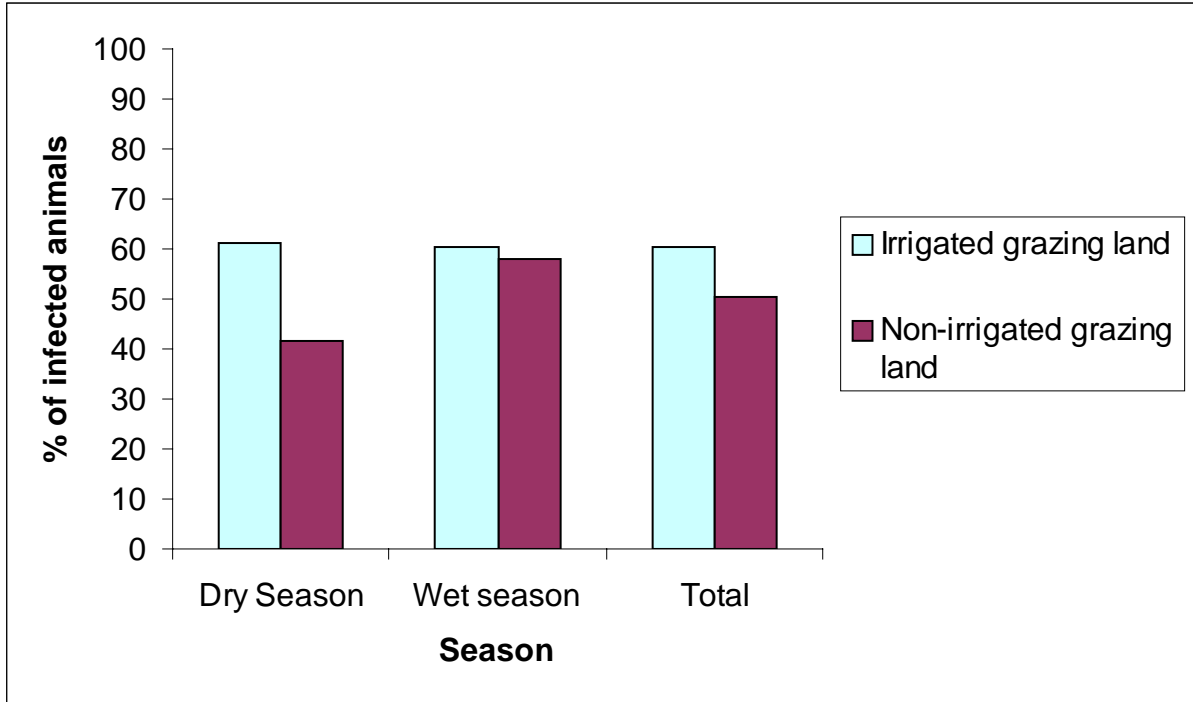


Figure 11. Distribution of ovine fasciolosis by grazing lands and seasons in the Awash River Basin (November 2003 – October 2004) .

The overall dry season infection prevalence of ovine fasciolosis in irrigated and non-irrigated areas were 61.1% and 41.8%, respectively (Fig. 11), and the difference between the two was statistically significant ($p < 0.05$). Although the wet season prevalence rate was higher in the irrigated (60.3%) areas than non-irrigated (58.1%) sites, no statistically significant difference ($p > 0.05$) were depicted (Fig. 12).

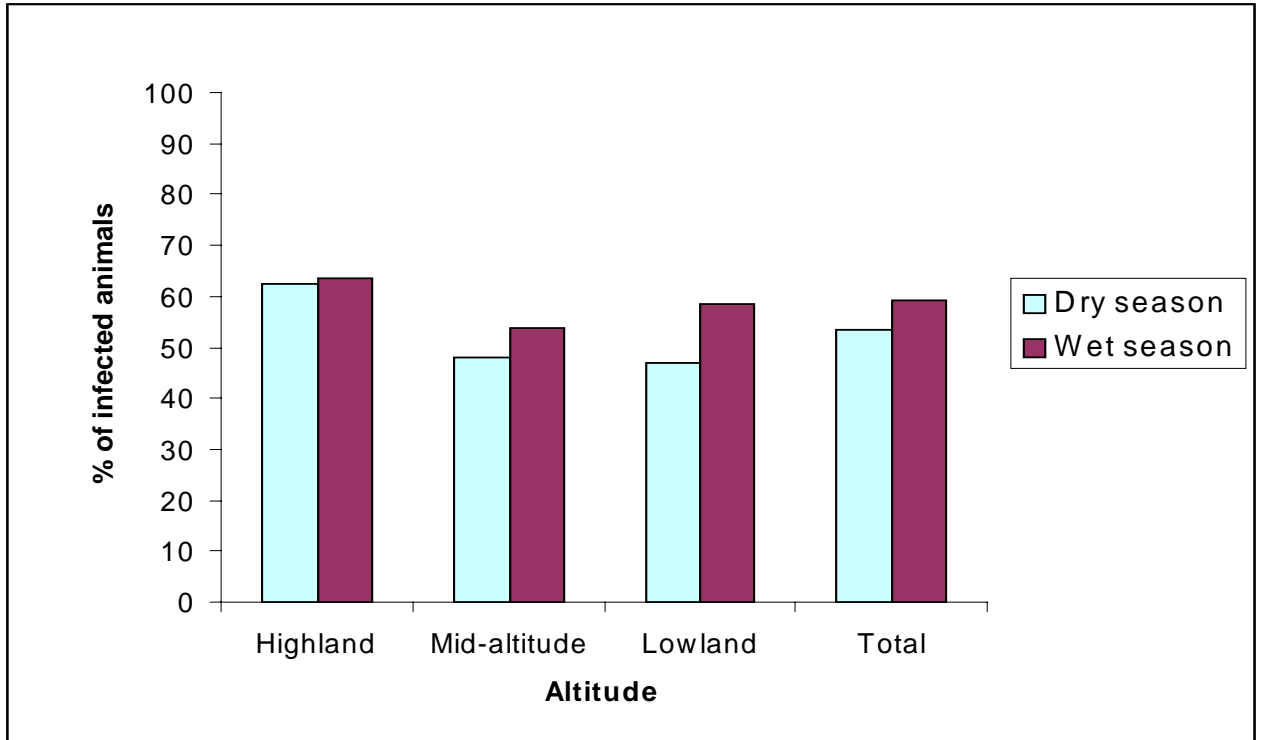


Figure 12. Distribution of ovine fasciolosis along different agro-ecological zones and seasons in the Awash River Basin (November 2003 – October 2004).

B. Infection prevalence versus Agro-ecology

The infection prevalence of fasciolosis in the irrigated areas of the highland, mid-altitude and lowland was 64.5%, 59.7% and 55.8%, respectively. The prevalence was highest in the highlands as compared to the lowland and mid-altitude study areas. The results showed that there is statistical difference ($p < 0.05$) in fasciolosis infection prevalence between highland and mid-altitude, as well as between highland and lowland sites.

For non-irrigated sites in the highland, mid-altitude, and lowland areas, the infection prevalence of fasciolosis was 60.9%, 45.3% and 42.9%, respectively (Fig. 13). With the exception of mid-altitude and lowland areas, all agro-ecologic comparison between prevalence rates showed significant differences ($p < 0.05$).

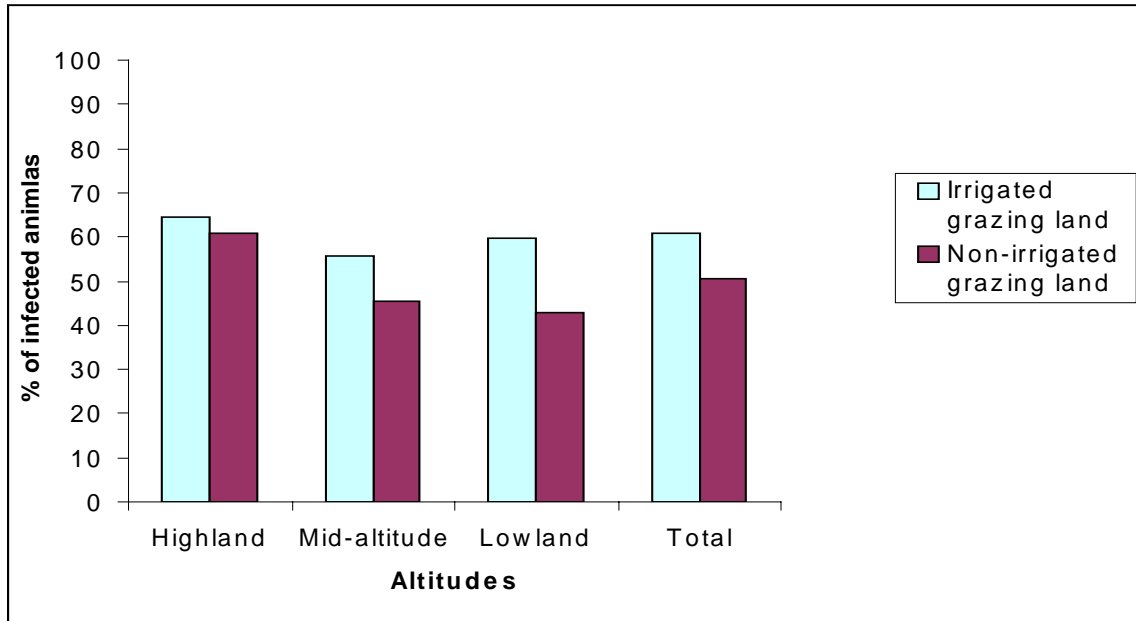


Figure 13. Distribution of ovine fasciolosis along different Agro-ecological zones and grazing lands in the Awash River Basin (November 2003 – October 2004).

The dry season infection prevalence of fasciolosis in irrigated and non-irrigated areas of highlands was 64.5% and 57.3%, respectively. The wet season prevalence picture in the highland was in the order of 64% and 63.5%, respectively, for irrigated and non-irrigated areas (Annex VIII). The results revealed that there is no significant difference ($p > 0.05$) between the two land use systems both in dry and wet seasons in the highlands.

The infection prevalence during the dry season in irrigated and non-irrigated areas of mid-altitude was 57.3% and 37.3%, respectively. This was significantly different ($p < 0.05$), unlike the wet season prevalence of 54.4% and 52.9% respectively, that showed no significant difference ($p > 0.05$).

The prevalence of the dry season in irrigated and non-irrigated areas of lowlands was 58.3% and 32.1% respectively, showing a highly significant difference ($p < 0.05$), whereas, the prevalence in the wet season was 61.5% and 55%, respectively with no significant difference ($p > 0.05$).

The overall infection prevalence in the dry season of the irrigated and non-irrigated areas of all altitudes was 61.1% and 41.8%, respectively, showing highly significant difference ($p < 0.05$). The findings for the wet season were 60.3% and 58.1%, respectively and showed no significant difference ($p > 0.05$). The dry season infection prevalence of fasciolosis in the irrigated areas of the highland, mid-altitude, and lowland was 64.5%, 57.3% and 58.3%, respectively. The prevalence for the wet season for the same were 64%, 54.4% and 61.5%, respectively and showed no significance difference ($P > 0.05$) in both cases.

The dry season infection prevalence of fasciolosis in the non-irrigated areas of the highland, mid-altitude and lowland was 57.3%, 37.3% and 32.1%, respectively with the highland prevalence showing significant difference ($p < 0.05$) from the rest. The prevalence of the wet season of the same were in the order of 63.5%, 52.9% and 55%, respectively with the highland prevalence showing significant difference ($p < 0.05$) from that of the mid-altitude and the lowland.

The infection prevalence of fasciolosis in irrigated areas of highland in dry and wet seasons was 64.5% and 64.0%, respectively. The result in non-irrigated areas was 57.3% in the dry and 63.5% in the wet, respectively, showing no significant difference ($p > 0.05$) in both cases. Similar analysis in irrigated areas of mid-altitude prevalence in dry and wet seasons revealed 57.3% and 54.4% respectively, showing no significant difference ($p > 0.05$). The findings in non-irrigated areas were 37.3% for the dry and 52.9% for the wet season, respectively and the difference was significant ($p < 0.05$). On the contrary, the prevalence of the irrigated areas of the lowland dry and wet period was not different whereas, the prevalence in the non-irrigated areas of the lowland was 32.1% (dry) and 55% (wet) the difference was significant ($p < 0.05$).

The overall infection prevalence of the irrigated areas of all altitudes in dry and wet seasons were 61.1% and 60.3%, respectively and showed no significant difference ($p>0.05$), whereas in the non-irrigated areas of all altitudes the prevalence was 41.8% (dry) and 58.1% (wet) with a significant difference ($p<0.05$).

2. Faecal egg output

The overall *Fasciola spp.* egg output (mean \pm S.E.) by altitude, season, grazing land, age and sex are shown in Table 3 and 4. EPG counts in the infected sheep ranged from 50 to 4,500. The overall mean faecal *Fasciola spp.* egg output (n=729) was significantly higher in the highlands (270 ± 1.06), as compared with the mid-altitude (188 ± 1.09) and the lowland (170 ± 1.07) areas (Table 3). On the other hand, there was no significant difference ($p>0.05$) between faecal egg output from sheep in mid-altitude and lowland areas. In addition, egg counts from animals grazing in irrigated land (237 ± 1.05) were significantly higher ($p<0.05$) than their counterparts in the non-irrigated grazing lands (178 ± 1.06). Similarly, faecal *Fasciola spp.* egg counts in the wet season (260 ± 1.06) were significantly higher ($p<0.05$) than the results obtained during the dry season (162 ± 1.06). It was also noted that, the mean *Fasciola spp.* egg output from young animals (<2 years of age) (251 ± 1.06) was significantly higher than adult subjects (182 ± 1.05) ($p<0.05$).

Table 3. Faecal egg output (mean±S.E.) by altitude, season, grazing land, age and sex in ovine fasciolosis (November 2003 – October 2004) (n=729).

Category	Number of positive cases	EPG (mean±S.E.)
1) Altitude		
Highland	333	270±1.06 ^a
Mid-altitude	154	188±1.09 ^b
Lowland	242	170±1.07 ^b
2) Season		
Dry	370	162±1.06 ^a
Wet	359	260±1.06 ^b
3) Grazing land		
Irrigated	445	237±1.05 ^a
Non-Irrigated	284	178±1.06 ^b
4) Age		
Young	368	251±1.06 ^a
Adult	361	182±1.05 ^b
5) Sex		
Male	183	218.77±1.09 ^a
Female	546	213.80±1.05 ^a

Different letter (a,b) along columns signify the presence of significant difference (p<0.05).

Table 4. Summary findings on fecal egg output EPG (mean±S.E.) by altitude, season and grazing land in the Upper Awash River Basin (November 2003 – October 2004).

EPG (Mean±S.E)	Altitude Zones							
	Highland		Mid-altitude		Lowland		Total	
Grazing Land	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season	Dry Season	Wet Season
Irrigated Land	212±1.09 ^a	393±1.12 ^a	173±1.16 ^a	325±1.15 ^a	139±1.11 ^a	269±1.11 ^a	172±1.07 ^a	327±1.08 ^a
Non-irrigated land	203±1.15 ^a	315±1.12 ^a	110±1.2 ^a	203±1.18 ^b	158±1.18 ^a	139±1.1 ^b	152±1.1 ^a	207±1.08 ^b
Total	208±1.09	352±1.08	138±1.1	257±1.1	148±1.1	196±1.09	162±1.06	260±1.06

Different letter (a,b) along columns signify the presence of significant difference ($p < 0.05$).

The dry season mean *Fasciola spp.* egg output in irrigated and non-irrigated areas of highland was 212 ± 1.09 and 203 ± 1.15 , respectively, and that of the wet season was in the order of 393 ± 1.12 and 315 ± 1.12 , respectively; the values in both cases showing no significant difference ($p > 0.05$).

The mean *Fasciola spp.* egg output of the dry season in irrigated and non-irrigated areas of mid-altitude was 173 ± 1.16 and 110 ± 1.2 , respectively, and the wet season values were 325 ± 1.15 and 203 ± 1.18 , respectively, with no significant difference ($p > 0.05$) in both cases. Furthermore, no significant difference ($p > 0.05$) were depicted during the dry season between irrigated (139 ± 1.11) and non-irrigated (158 ± 1.18) areas of lowlands. Significant difference ($p < 0.05$) was seen between the mean faecal egg outputs during the wet season in irrigated (269 ± 1.11) and non-irrigated (139 ± 1.1) areas. On the other hand, the dry season mean *Fasciola spp.* egg output of the irrigated areas of the highlands (212 ± 1.09) was the highest as compared to the mid-altitude (173 ± 1.16), and the lowland altitude (139 ± 1.11). The wet season highland, mid-altitude and lowland mean *Fasciola spp.* egg outputs were 393 ± 1.12 , 325 ± 1.15 and 269 ± 1.11 , respectively (Table 4).

The dry season mean *Fasciola spp.* egg output of the non-irrigated areas of the highland, mid-altitude and lowland was 203 ± 1.15 , 110 ± 1.2 and 158 ± 1.18 , respectively, and the difference was statistically significant ($p < 0.05$). On the other hand, the findings of the wet season of the same were in the order of 315 ± 1.12 , 203 ± 1.18 and 139 ± 1.1 , respectively and showed no significant difference ($p > 0.05$).

3. Snail survey

More than 1,000 snails belonging to different genera (*Lymnaea*, *Biomphalaria*, *Bulinus*, *Physa* and *Melanoides*) were collected. *L. truncatula* was the most abundant (688) snail species encountered (Table 5). With respect to their agro-ecological zone distribution, *L. truncatula* (329) were abundant in the highland while the rest were collected from the mid-altitude (193) and lowlands (166). Of the

two recognized snails serving as intermediate hosts for *Fasciola spp.*, *L. truncatula* was found in all agro-ecological sites, while *L. natalensis* was only encountered in the lowland areas (Table 5).

Table 5. Survey of snail fauna in the Upper Awash River Basin (November 2003 – October 2004).

Altitudes	snail spp.	No. specimens collected	Mean density (snail/m ²)
Highland	<i>Biompholaria spp.</i>	16	2
	<i>Bulinus spp.</i>	46	2
	<i>L. truncatula</i>	329	12
Mid-altitude	<i>Bulinus spp.</i>	32	3
	<i>L. truncatula</i>	193	8
Lowland	<i>Physa spp.</i>	25	3
	<i>L. truncatula</i>	166	7
	<i>Bulinus spp.</i>	29	2
	<i>L. natalensis</i>	65	3
	<i>Biompholaria spp.</i>	41	3
	Melanoides	13	2
	<i>B. forskalii</i>	5	1

In terms of habitat preference, the results of the present survey indicated that *Lymnaea spp.* were abundant in the irrigation channels and the adjacent vegetation. Analysis of obtained data also suggested that *Lymnaea truncatula* was the highest abundance was in the highlands (55), mid-altitude (31) and lowland (26) (Table 6).

Table 6. Habitat preference and density of snails (snail/m²) in different agro-ecological zones in the Upper Awash River Basin (November 2003 – October 2004).

<i>L. truncatula</i> / m ²		Highland	Mid-altitude	Lowlands
Site 1 (near the diversion point)	Dry	2	2	2
	Wet	6	4	2
	Sub total	8	6	4
Site 2 (between the diversion and farm)	Dry	10	3	3
	Wet	8	6	5
	Sub total	18	9	8
Site 3 (within the farming areas)	Dry	13	8	7
	Wet	16	8	7
	Sub total	29	16	14
Grand Total		55	31	26

The result obtained from different agro-ecological zones and sites, water temperature ranged between 17.2°C and 26.8 °C, while water pH ranged between 7.03 and 7.71 in the highlands, 7.57 and 8.24 in mid-altitude and 7.5 and 8.58 in the lowlands. Thus, pH of the water and the temperature did not show marked difference and were within the tolerable ranged by snails.

4. Effects of strategic anthelmintic treatment

Parameters monitored include changes in fecal egg output as eggs per gram (EPG) of the faeces, packed cell volume (PCV %), live weight gain measurement (Kg), body condition scores (BCS), mortality and rate of off-take through sells of sheep during the course of the study.

Table 7. Measurement of some indicator parameters of experimental animals subjected to strategic anthelmintic trial (November 2003- October 2004).

Treatment group	No. Examined	Measured parameters (Mean±S.E.)			
		Faecal egg count (EPG)	PCV (%)	Body weight (kg)	BCS
Group I	23	0.35±1.55 ^b	3.56±1.16 ^b	0.90±0.73 ^a	0.17±0.08 ^a
Group II	28	0.03±1.32 ^c	6.50±1.28 ^b	4.10±0.76 ^b	0.57±0.09 ^b
Group III	29	3.09±1.26 ^a	-2.03±0.91 ^a	-0.27±0.90 ^a	-0.02±0.05 ^a
Total	80	0.32±18.20	2.56±0.76	2.30±0.49	0.24±0.05

Different letter (a, b and c) along columns signify the presence of significant difference ($p < 0.05$).

The analysis of data treatment variance showed that strategic anthelmintic treatment significantly reduced fecal egg output, increase the PCV, live weight gain and BCS (Table 7).

A) Faecal egg output

Significant reduction in the *Fasciola spp.* faecal epg counts were obtained both in animals following one-time (0.35 ± 1.55), two-times (0.03 ± 1.32) treated and the control group (3.09 ± 1.26), was statistically ($p < 0.05$) different (Fig. 14).

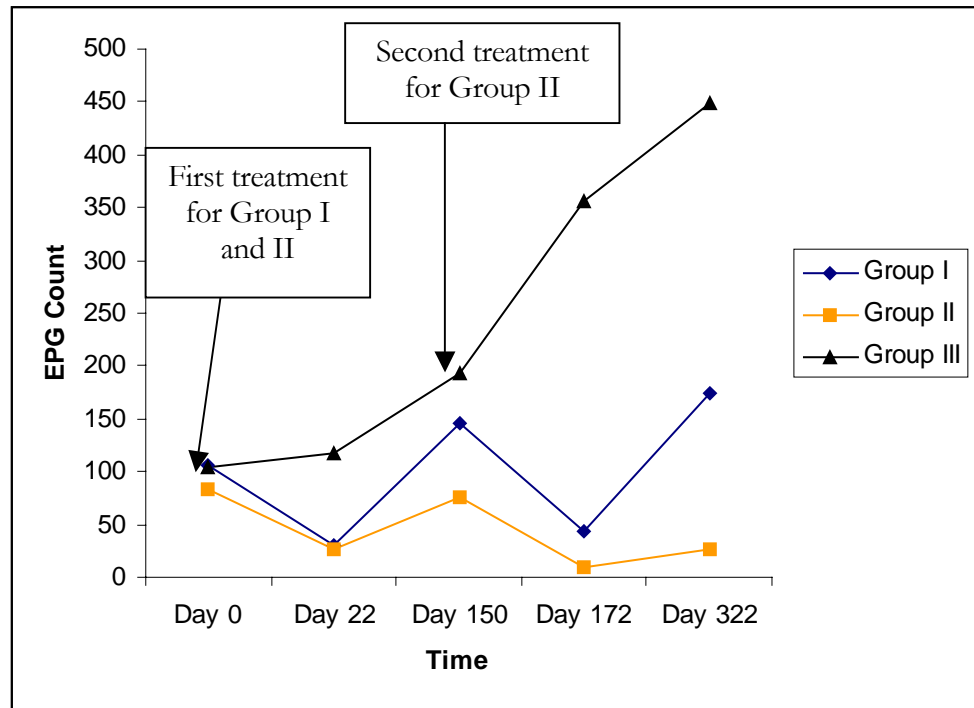


Figure 14. Mean faecal egg count of one time and two times anthelmintic treated and control groups of experimental sheep in Wolemera area (November 2003- October 2004).

B) Packed Cell Volume determination (PCV)

The Group II (two-time treatment) showed the highest mean increase in PCV values (6.50 ± 1.28) followed by Group I (one-time treatment) (3.56 ± 1.16) as opposed to Group III (Positive control) group (-2.03 ± 0.91). The improvements in PCV values in Group II and I were significantly ($p < 0.05$) different when compared with to the control group (Fig. 15).

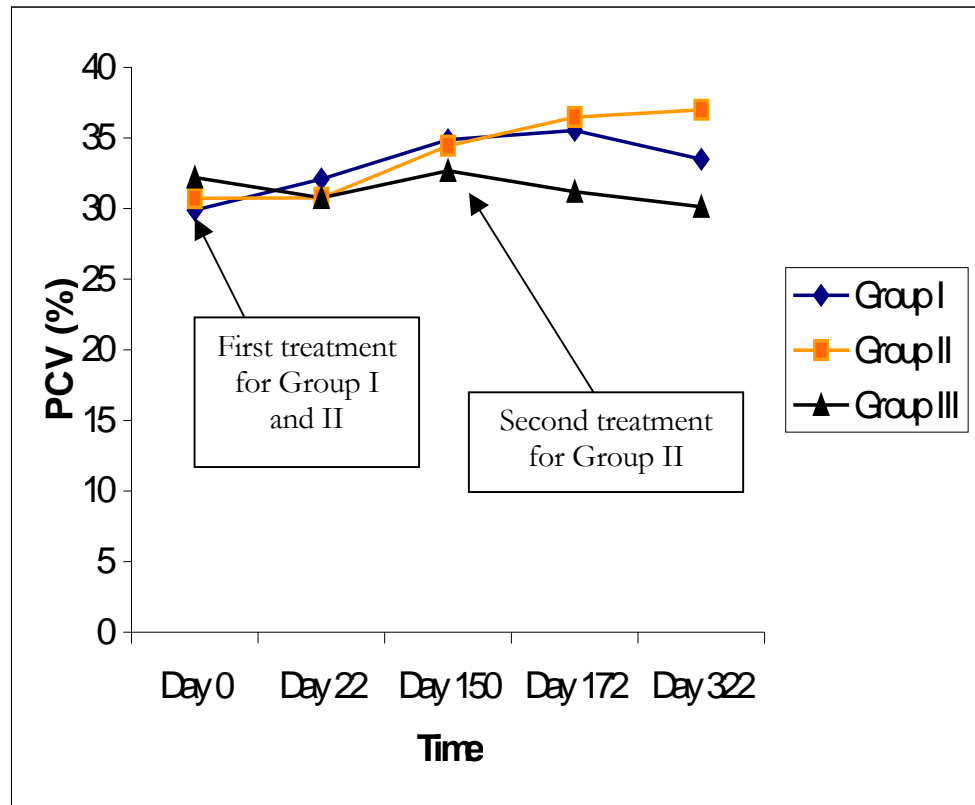


Figure 15. Mean PCV of sheep following one and two time anthelmintic treated and control group of experimental sheep in Wolemera area (November 2003- October 2004).

C) Body condition scores (BCS) and body weight gain (kg)

Group I and Group II (0.57 ± 0.09) showed significant improvements ($p < 0.05$) in body condition scores as compared with Group III (0.02 ± 0.05) (Fig. 16). However, there was no difference ($p > 0.05$) between Group I (0.17 ± 0.08) treated and Group III group.

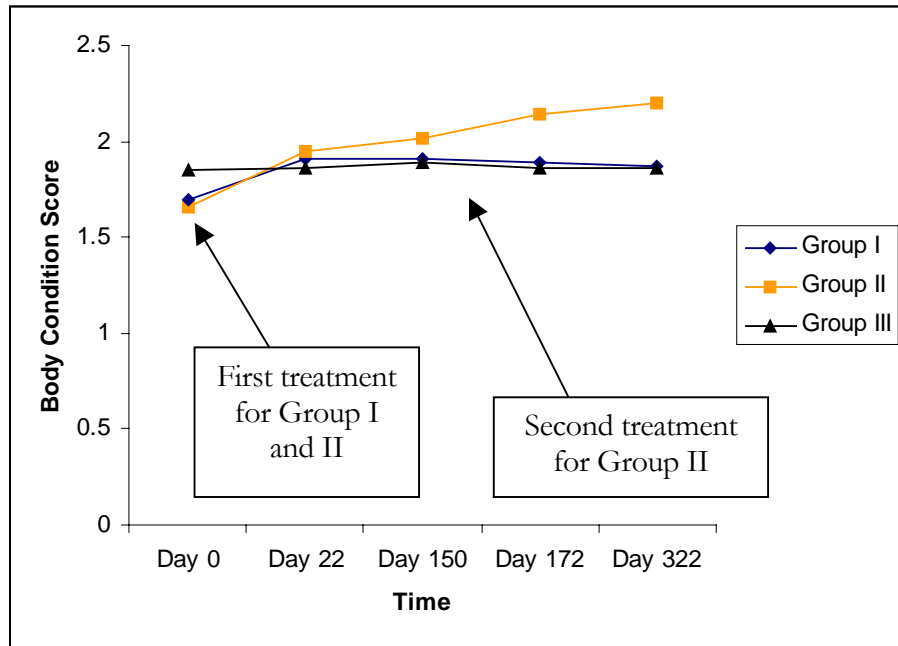


Figure 16. Mean body condition score of one-time and two-times treatment anthelmintic treated and control groups of sheep in Wolemera area (November 2003- October 2004).

The mean difference between the live weight measurements of Group I (0.90 ± 0.73), Group II (4.10 ± 0.76) and Group III (-0.27 ± 0.90) were statistically significant ($p < 0.05$) (Fig. 17).

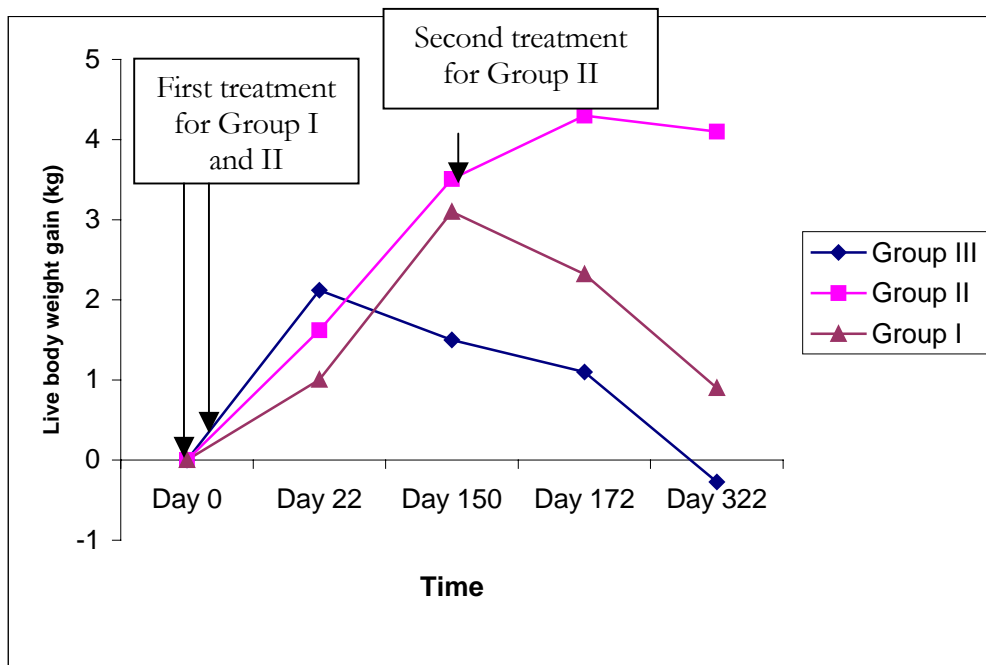


Figure 17. Difference in body weight gain between animals subjected to different strategic anthelmintic treatment regimes.

D) Mortality and off-take rates

Table 8 describes mortalities and off take rates of animals during the course of this study. All data collected from these animals were not considered in the analysis process.

Table 8. Description of and cause for exclusion of some animals from the experiment

Description	Group I (One treatment)	Group II (twice treatment)	Group III (Positive Control)
Dead	2	-	5
Sold	15	3	20

5. Economic benefits of strategic anthelmintic treatment

Average weight estimate of a sheep ready to be sold in the study area is 22kg. The market price for such an animal in the study area is 150 Eth. birr. The cost of treatment is 0.90 birr/sheep (Triclabendazole 300mg/kg). The approximate price of 1kg live weight is thus 6.82 Eth. Birr (Table 9). The analysis was made on the mean difference in body weight gains.

The mean weight gain in Group II animals (4.10kg/animal) was markedly superior than in Group I animals (0.90 kg/animal) and Group III animals (-0.27 kg/animal). The approximate market price of 22 kg sheep was 150 Eth. Birr, Table 9 shows the monetary net profit obtained from different treatment intervention.

Table 9. Economics benefits of strategic treatments calculation based on mean live weight gain difference (November 2003- October 2004)*.

Group	Body weight gain (BWG) (Kg)	Approximate unit price of BWG (Kg)	Gross value Birr	Net value
Group I	0.90	6.82	6.14	5.24
Group II	4.10	6.82	27.96	26.16
Group III	-0.27	6.82	-1.84	-1.84

* Price of TCBZ during the period of investigation was birr 0.90/300mg

V. Discussion

Fasciolosis is widespread ruminant health problems and causes significant economic losses to the livestock industry in Ethiopia. As reported by (Brook *et. al.*, 1985; Heinonen *et. al.*, 1995) water logged and poorly drained areas with acidic soils in the highlands are often endemic areas for fasciolosis. In the present study, irrespective of the seasons and irrigation status of the grazing lands, the highest infection prevalence of fasciolosis was recorded in the highlands. The findings, therefore, strongly suggest that the climatic factors in the highland areas are more favorable for the propagation and activity of the snail intermediate hosts and progression of the parasite life cycle for most part of the year, as compared with the scenario in mid-altitude and lowland areas. The overall infection prevalence was found to be significantly higher ($p<0.05$) in irrigated grazing lands than non-irrigated study sites. The difference in prevalence rate between irrigated and non-irrigated sites is more pronounced during the dry season; and in the mid-altitude (dry season) and in the lowland (both dry and wet season) areas. This indicated the irrigation help to maintain optimal wetness required for the development of both the snail intermediate host and intra-molluscan parasite phases within the snail. Thus the availability of water, which is the most important limiting, was responsible for the increased seasonal prevalence of fasciolosis in mid-altitude and lowland areas. Similar observations and assertions were previously made by Richter, *et. al.*, (1999).

The existence of permanent surface water, which is more frequently seen in the highlands, associated with high infection fasciolosis prevalence. Several scholars (Scott and Goll, 1977; Brook, *et. al.*, 1985 and Asegde, 1990) have previously reported similar observations. The increase in prevalence in the mid-altitude and lowlands associated with permanent surface water, contributes to the endemicity of fasciolosis in these areas. The presences of adequate moisture from irrigated channels in these areas created favorable condition needed for the progression of the lifecycle, survival and multiplication of the snail intermediate hosts and consequently attributed to the increased fasciolosis prevalence. It is generally recognized that, during the dry season, when

biomass for animal feed are generally scarce, animals tend to graze along the banks of rivers and sides of irrigation channels. This provides an ideal environment for fasciolosis infection to take place. The apparent increases in infection prevalence in irrigated sites in mid-altitude and lowland areas could be explained by the above assertion. Yilma and Malone (1998) indicated that losses from dry season fasciolosis in the tropics are relatively severe during dry season as the pathologic consequences are further aggravated due to sub-optimal nutrition during this period.

The present study showed that faecal egg counts from highlands and irrigated areas increased during the wet season. Similar reports were made by Mamo, 1980; Asegde 1981 and Brook, *et. al.*, 1985. In general the highland areas, where there are a number of water pockets, marshy and water-lodged areas are found associated with more faecal egg output during the dry and wet periods as compared with mid-altitude and lowland areas. Such topography is well suited for the proliferation of the snail intermediate hosts that would contaminate the grazing lands.

The predominant factor determining the marked seasonal patterns of *Fasciola* egg excretion was; in the wet season the grazing land which favorable for the development of the number of parasites and intermediate host and produce a number eggs and another major factor this parasite related with moisture and temperature.

Results of faecal egg counts different ages of the animals revealed that young infected animals excreted more number of eggs as compared with the adult animals indicates that repeated exposure to fluke infection would have led to development of resistance in the adult animals, thus preventing heavy parasite build up. Such drop in faecal egg output in adult sheep has been reported by Mamo (1980); Asegde (1981) and Asegde (1990). The relatively higher egg output detected from the irrigated areas as compared to the non-irrigated is a reflection of higher exposure to infection under an environment that favors parasite abundance. The absence of sex related differences in the faecal egg output, seen in the present work, has also been reported by Asegde (1980).

Different species of snails were identified in the study sites. *Lymnaea spp.* was the predominant snail species encountered in this study. The optimum habitats for *Lymnaea* snails were permanent water, muddy and marshy area (dry season), clear water, presence of small number of aquatic weeds and an abundance of algae. The irrigation canals, in which almost all of the above conditions are available, provide ideal habitats for *Lymnaea* snails. The high density of snail intermediate hosts in the highlands (Brown, 1980) and the irrigated areas was additional evidence for a higher infection rate in these localities making animal feeds obtained from the irrigation canals a potent source of infections larva to the animals. Water temperature and pH is the most important factor for cercarial infectivity. The findings shown that the abundance of *Lymnaea spp.* was higher in the irrigation channels because the water temperature, water pH and the vegetation found in irrigation channels favors the development of the snails and the parasite. The water pH and water temperature values recorded during the study period didn't show marked difference and snails are within the tolerable range. Therefore, feed resource obtained from these irrigation channels could be a potent source for fasciola infection.

The results shown that, snails were the most abundant in the highland as compared with mid-altitude and lowland areas. Similarly Yilma, 1985 the population of snail highly distributed in the highland areas because of water temperature, soil type, moisture content and water pH. Reasons for small density of snails in the mid-altitude and lowland areas may be scarcity of vegetation, agro-ecological zones, moisture stress, soil types as a result accumulation of silt load in Irrigation canal. The prevalence of ovine fasciolosis was higher in wet season than dry season and also the snail population larger as compared with in the wet season this results in agreement with Brown, 1980.

Fasciolosis is one of the major parasitic diseases contributing to loss in productivity (Scott and Goll, 1977). Control of fasciolosis is based on good grazing land management practices that destroy the intermediate hosts, the snails, which is not always possible. Strategic anthelmintic treatment helps to reduce grazing land contamination with fluke eggs and increase productivity (Yilma and Malone,

1998; FAO, 1994; Bekele *et. al.*, 1992 and Hansen and Perry, 1994). In addition to avoiding pasture contamination and improving productivity of animals, two strategic anthelmintic treatments are affordable limits to smallholder farmers in the Ethiopia context. Among different flukicidal drug TCBZ is one of the most widely used, this drug administrated orally at the dose rate of 10mg/kg (Hansen and Perry, 1994; Ramisz, *et. al.*, 1990). It has been shown to be highly effective at removing all stage of liver fluke in sheep (Hansen and Perry, 1994; Fawcett, 1990).

Periodic anthelmintic treatment is the most commonly used means to control the devise effects of fasciolosis in ruminants. Yilma and Malone (1998); FAO (1994) and Hansen and Perry (1994) recommend that two time treatment under the Ethiopian condition. The first treatment has been given during the dry season to eliminate the adult parasite; such treatment enables the animals to survive the effects of the dry season, in addition, when nutritional condition are generally compressed avoids contamination with flukes eggs of water holes and irrigation channels. On the other hand, Chiejina and Emehelu 1986 suggested that late December might be a more appropriate month to administrated the treatment to sheep, where the rainy season sometimes extends into October, since animals treated before mid December are liable to significant re-infection. The second treatment has been given early wet season when the immature flukes migrate through the hepatic parenchyma.

The overall result of the present strategic anthelmintic trial showed that animals received two treatments were found reduced fecal egg output count, PCV, increased live weight measurement and body condition scores as compared with those animals received one and no treatments.

The finding of the present study is in agreement with observation of other works [Asegde (1990); Ramisz *et. al.* (1997); Brook *et. al.* (1985) and Scott and Goll (1977)] that indicates the mean difference in epg between animals received two treatment is almost zero while the difference between those received one treatment significant different than no treatment group.

Animals received two-treatment (Group II) showed better weight gains than those received one (Group I) and no treatments. The difference between groups III and I was not significant ($p > 0.05$). This could be as a result of contamination snail activity and re-infection favored by the presence of irrigation canals in the study. The concomitant effects of favored climatic for development parasite may have continued exerting its effect on infected animals after the first treated. The results show in agreement with Yilma and Malone (1998); Asegde, (1990), Brook *et al.*, (1985).

All the changes observed (epg, PCV, live weight gain and BCS) are all related with the effects of strategic treatments. TCBZ highly effective both the immature and mature parasite and it reduced the fecal egg output, the animals increase weight gain and shows good body condition scores. In this study from a total of 125 sheep 7 were dead. Of the case of deaths in Group III (the first 2 dead during dry season and the rest 3 were dead during wet season) and Group I (both sheep dead during wet season). All the animals showed high degree of emaciation, lack of appetite, slow moving of the animals and swollen bellies before death. Similarly clinical signs of chronic fasciolosis have been indicated by different scholars (Soulsby, 1982; Dunn, 1978 and Boray, 2003). A high number off-take rate observed in the study area and this might be to cover the cost of fertilizer

A number of authors (Soulsby, 1982; Dunn, 1978 and Boray, 2003) have proposed various mechanisms by which flukes may influence the productivity and physiological status of parasites animals. It has been shown by various workers that flukes consume liver tissues and suck blood, and it has also been suggested that they may produce toxic substance. It is therefore possible that the effect of liver flukes on productivity may be due to a number of independent or interacting causes.

The economic returns that were obtained from treatment with Triclabendazole on a cost benefit analysis of the proposed strategic programme indicated that treatment would be beneficial to the farmers. The estimated monetary benefits of the programme were above (Table 9) those of the

treated and untreated by approximately –1.84 birr Group III (positive control), 5.24 birr Group I (one-time treatment) and birr 26.16 Group II (two-times treatment). It can be conclude that flukicidal control strategies based on the use TCBZ could provide effective control of ovine fasciolosis in the study area.

VI. Conclusions

The inferences drawn from the present study are summarized as follows:

- Significantly higher prevalence of ovine fasciolosis was obtained in the highlands, irrespective of the season and land use status (irrigated and non-irrigated), than both the mid-altitude and lowland areas. This shows that, as compared with mid-altitude and lowland areas, the highlands in the Upper Awash River Basin are more favorable for the propagation and activity of the snail vectors and progression of the *Fasciola* life cycle for most months of the year.
- One of the remarkable findings of the present study is that fasciolosis prevalence significantly increased at irrigated sites in mid-altitude (dry season only) and lowland areas, (both during wet and dry seasons) when compared with the parallel scenario in non-irrigated study sites. This finding warrants that special schemes for fasciolosis must be instituted while undertaking development efforts such as expansion of irrigated agriculture.
- Snails were most abundant in the highland (329) as compared with mid-altitude (193) and lowland (166) areas. No significant variation was recorded in water pH (7.03-8.58) and temperature (17.2°C – 26.8 °C) between the study sites, a result suggestive of the fact that snails inhabit within tolerable biometric threshold ranges irrespective of locations.
- From the study meant to assess the effects of strategic anthelmintic trial (Triclabendazole 300mg/kg BW), the following core results were obtained:
 - A significant decrease ($p < 0.05$) in mean faecal egg output was seen in both Group I (received a one time treatment) and II (received twice treatment) as compared with untreated controls (Group III). In addition, similar significant trends were depicted in improvements of PCV values between the different groups. Further more, during the experiment periods, animals received two-treatment (4.1kg/animal) showed better weight gains than those received one-

time treatment (0.91kg/animal) as compared with the negative weight gain (-0.27kg/animal) for untreated controls. The difference between groups I and III, however, was not statistically significant ($p>0.05$). Accordingly, a considerable net return of birr 26.16/animal/year was obtained from twice strategic triclabendazole treatments. One time triclabendazole treatment conferred birr 5.24/animal/year while negative return (a net loss of 1.84 birr) was recorded from *Fasciola*-infected and untreated sheep. In addition, farmers have expressed some valuations on non-monetary benefits from strategic treatments such as avoidance of repeated journeys to markets because of the ease of selling and ability to bargain on prices of well-conditioned animals while in comparison with attempts to sell poor condition and untreated animals. This is paramount in terms of getting quick money to be able to solve urgent household needs, effective planning and in view of maximizing time utilization at farm levels.

Recommendation

Animal diseases continue to constrain, in a variety of different ways, livestock productivity, agricultural development, human well-being and poverty alleviation in many regions of the developing world. The most important and readily measurable direct effects of disease are often losses in productivity. These include the effects due to death, illness leading to condemnation, poor weight gain and poor feed conversion. Disease of livestock have many additional direct and indirect impacts on human nutrition, community development and socio-cultural and also in reduction in farm income, contributing to food insecurity and poor nutrition.

In the present study, small-scale irrigation schemes were shown to heavily influence fasciolosis prevalence in mid-altitude and lowland areas. These findings, therefore, warrant that special schemes need to be instituted for the control and prevention of fasciolosis and other water-borne human and animal disease in agricultural development efforts involving irrigation agriculture in these environments.

Strategic treatments must be implemented at appropriate timing and with the aim of reducing worm burden from infected animals and preclude pasture contamination. Integrated approaches in reducing the population and activity of snail intermediate hosts will enable maximization of long-term returns from such intervention and in sustainable livestock farming in endemic areas. Previous study also confirms that twice flukicidal strategic treatment is the most economical approach recommended in the context of smallholder mixed crop-livestock system in the highlands of Ethiopia (Yilma and Malone, 1998). A net return of more than 26 birr/animal is very considerable. However, the benefits from strategic treatments could be further investigated in terms of its application in terms of assessing the most suitable timing with respect to agro-ecological zones and long-term effects associated with reduction in pasture contamination will require further in depth investigations. Based on the findings from the present study, the feasibility of fasciolosis control either by treatment interventions made by individual farmers or through community involvement is anticipated to increase farm level productivity and household income. In the Ethiopian context, community involvement in fasciolosis control is most efficient and practical solution since communal grazing is often the tradition especially during the dry season.

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Annex

Annex I Age estimation

Age	Age Categories
Less than 2 years	Young
Greater or equal to 2 years	Adult

Adapted from Cringoli, *et. al.*, 2002

Annex II: - Modified McMaster Method

- 3g of faeces were put in the jar and added to 42ml of water and thoroughly broken up using a mortar pestil.
- The mixture was poured through a wire mesh screen with an aperture of 0.15mm and strained fluid caught in a bowl. The debris left on the screen was discarded
- the strained fluid was stirred and a sample of it was poured into a centrifuge tube to within 10mm of the top.
- the tube was centrifuged for 2 minutes at 1500r.p.m. and the supernatant was poured off and discarded.
- The tube agitated until the sediment was loosened and forms a homogeneous sludge at the bottom of the tube.

- The tube was filled with $ZnSO_4$ (Annex 3) to the same level as before the contents of the tube were thoroughly mixed by inverting it five or six times with the thumb over the end and sufficient of the fluid is immediately withdrawn with pasture pipette and carefully allowed to run into one chamber of the McMaster slide.
- After further mixing a second sample was withdrawn and run into the other chamber.
- All the eggs under the two-separate grids were counted. The numbers of eggs per gram of faeces was obtained by multiplying the total number of eggs in the two grids by 50. (3g of faeces yielded 45ml of suspension and 0.3ml were examined).

Annex III: - Preparation of floatation fluid, zinc sulfate ($ZnSO_4$)

- 331-385 grams granular zinc sulfate ($ZnSO_4$) dissolved in 1,000 ml of distilled water and then filtered.

Annex IV Snail survey field recorded***

- 1) Date_____ 2) Weather: Fine_____Overcast___Rainy____Temp. _____
- 3) Collector(s)_____
- 4) Locality River___ Lake_____; Altitude_____
- 5) Enviroment: Lotic____, Lentic____; temporary____, permanent____; artificial____,Natural____; water level fluctuation great____Moderate____small____ Cause of fluctuation_____
- 6) Size of habitat
- 7) Kind of substrata: mud___, snad___,gravel___losse stone___firm bedrock___decaying matter_____

8) Kind of margin: shore regular____, intende: water shallow__deep_;has aquatic veg.

9) Character of water: flow rate____ turbidity due to mud____ algae____ color____ human pollution
____PH____ water temperature_____

10) Aquatic vegetation: none__varied____uniform__abundance____Submerged____ Submerged
with floating leaves__emergent_____

11) Shore vegetation

12) Aqatic animals and abundance other than snails

13) Water use human frequent____,rare__no____;Animals,frequent__rare__no__

14) Miscellaneous data_____

15)Molluscus

*B.pfeifferi*_____

*ferrissia*_____

*Bulinus spp.*_____

*Ancylus*_____

*B.forskalii*_____

*Burnupia*_____

*L.natalensis*_____

*Valvata*_____

*L.truncatula*_____

*Melanoides*_____

*Physa*_____

*Bivalve*_____

*Anisus*_____

*Segmentorbis*_____

*Gyraultus*_____

*Lentorbis*_____ Others

***Adapted from PAHO/WHO sci. Pub. No. 168(1968)

Annex V :- Packed Cell Volume Determination

- Blood sample were taken from ear vein using capillary tube
- Draw the well-mixed blood up a 75 x 1.5 mm capillary tube for $\frac{3}{4}$ of its length.
- Sealed one end with sealant.
- Placed in the micro-haematocrit centrifuge, the sealant was at the outer end.
- Closed the centrifuge lid.
- Centrifuged the tubes at 12,000 rpm for 4 minutes.
- Placed the tubes in the reader and note the reading.
- Express the reading as a percentage of packed red cells in the total volume of whole blood.

Annex VI Body Condition Scoring Of Sheep***

Rank	Condition Score type	Description
Condition Score 1	Very thin	Spine prominent and Sharp
Condition Score 2	Thin	Spine prominent and smooth
Condition Score 3	Average	Spine smooth and rounded
Condition Score 4	Fat	Spine only detected as a line
Condition Score 5	Very fat	Spine not detectable; fat dimple over spine

*** Adapted from Thompson and Meyer 1994

Annex VII Infection prevalence of ovine fasciolosis in selected sites within the Upper Awash River Basin (November 2003 – October 2004) (n=1296).

Category	Number of examined sheep	Number of positive cases	Infection prevalence (%)
Altitude			
Highland	529	333	62 ^a
Mid-altitude	302	154	51 ^b
Lowland	465	242	52 ^b
Total	1296	729	56.25
Season			
Dry	690	370	53.6 ^a
Wet	606	359	59.2 ^b
Total	1296	729	56.25
Grazing land			
Irrigated	732	445	60.8 ^a
Non-Irrigated	564	284	50.4 ^b
Total	1296	729	56.25
Age			
Young	665	368	55.3 ^a
Adult	631	361	57.2 ^a
Total	1296	729	56.25
Sex			
Male	306	183	59.8 ^a
Female	990	546	55.2 ^a
Total	1296	729	56.25

Different letter (a, b) along columns signify the presence of significant difference ($p < 0.05$).

Annex VIII Infection prevalence of ovine fasciolosis by grazing land, season and altitude along the Upper Awash River Basin (November 2003 – October 2004) (n=1296).

Season	Grazing Land	Agro-ecological Zones											
		Highland			Mid-altitude			Lowland			Total		
		No. examined	No. Positive	Prevalence (%)	No. examined	No. Positive	Prevalence (%)	No. examined	No. Positive	Prevalence (%)	No. examined	No. Positive	Prevalence (%)
Dry Season	Irrigated land	203	131	64.5 ^a	75	43	57.3 ^a	144	84	58.3 ^a	422	258	61.1 ^a
	Non-irrigated land	89	51	57.3 ^a	67	25	37.3 ^b	112	36	32.1 ^b	268	112	41.8 ^b
	Total	292	182	62.3	142	68	47.9	256	120	46.9	690	370	53.6
Wet Season	Irrigated land	111	71	64.0 ^a	90	49	54.4 ^a	109	67	61.5 ^a	310	187	60.3 ^a
	Non-irrigated land	126	80	63.5 ^a	70	37	52.9 ^a	100	55	55.0 ^b	296	172	58.1 ^a
	Total	237	151	63.7	160	86	53.8	209	122	58.4	606	359	59.2
Overall	Irrigated land	314	202	64.3 ^a	165	92	55.8 ^a	253	151	59.7 ^a	732	445	60.8 ^a
	Non-irrigated land	215	131	60.9 ^a	137	62	45.3 ^b	212	91	42.9 ^b	564	284	50.4 ^b
	Grand Total	529	333	62.9	302	154	51.0	465	242	52.0	1296	729	56.3

Different letter (a,b) along columns signify the presence of significant difference ($p < 0.05$).

DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for any degree in any university and all the sources of materials used for the thesis have been duly acknowledged.

Name: Michael Asrat

Signature: 