THESIS

EPIDEMIOLOGICAL SURVEY AND ASSESSMENT OF OPTIONAL THERAPY FOR OVINE FASCIOLIASIS AROUND MIDDLE AWASH RIVER BASIN, AFAR, ETHIOPIA

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The Epidemiological survey was conducted in three districts (Gewane, Bure Modaytu and Amibera) of Middle Awash River Basin located in Afar Regional State, Ethiopia. A total number of 3,697 fecal samples (3,467 from Afar and 230 from Blackhead sheep breeds) were collected and performed by ethyl acetate centrifugation procedure to identify eggs of the parasites. Two experiments were also conducted at Gewane Agricultural Technical and Vocational Education Training College, Gewane district, Ethiopia, in order to study natural fasciola infections in local breeds of sheep and optional treatment of ovine fascioliasis in Afar and Blackhead local breeds of sheep. The survey result indicated that overall prevalence of ovine fascioliasis was 13.23%. Amibera district had the highest number (17.42%) of infections among the 3 districts. No significant differences of prevalence were associated with sex and breed. Infection rates for the different age groups were found significantly differences ($P<0.001$). As the age of the animals increased, the prevalence of the diseases also increased. Infection rates for the sheep with poor body condition were significantly higher ($P<0.001$) than that for the sheep with good body condition. With regard to the seasonal factors, the high infection rate was observed during the cool season (October to February) and a lower infection rate was recorded during the main rainy season (July to September). In addition mixed parasitism infection was higher than single infection. It was concluded that to control the disease the appropriate preventive controlling strategies have to be urgently designed to reduce the impact of the disease on sheep production. Results of the study on the overall prevalence of natural fasciola infections in 24 Afar and Blackhead local breeds of sheep were 54%. Infection rates for breeds were 37.5% and 33.3% for Blackhead and Afar breeds, respectively. The prevalence within sex groups was 61.5% and 38.5% for female and male sex groups, respectively. Infection rates for the different breeds and sex groups were found not to be significant ($p>0.05$).The result for healthy status also revealed a general reduction in body weight, red cell counts (RBC), packed cell volume (PCV), total protein (TP) and hemoglobin (Hb), which obviously indicated to be more severe in the infected sheep. The severity of the infection started around fourteen weeks after exposure and continued up to the end of the experiment. Breeds resistant potency occurred at sixteen weeks for all infected breeds; with the latter shedding the recovery rates of the Blackhead breed was higher than the Afar breed. On the basis of fecal egg count (FEC) and clinicopathology, the Blackhead breed was considered slightly more susceptible to *Fasciola spp*. infections than the Afar breed. The Afar breed may be better adapted to the study area as its percentage of PCV and other blood parameters were higher than the Blackhead breed, but no significant difference within treatments between two breeds and sex was observed.

Throughout 90 days study period for the optional treatment study of ovine fascioliasis, a total of 72 Afar and Blackhead local breeds of sheep naturally infected by fascioliasis were randomly allotted into 3 groups, two nutrition levels (supplementationt and no supplementation) and three section treatments. One treatment group received 10mg/kg of Tricalbendazole drenching orally on the 1, 21, and 42 days during the study period. The second group received 100 gm of crushed or chopped chili solution drenching orally on the 1, 21, and 42 days during the experimental period and the control group received no treatment. Tricalbendazole and chili treated groups of animals had the efficacy of 100% and 84%, respectively. Animals treated by Tricalbendazole and chill in addition supplementation had high reduction of fecal egg count (FEC) compared to the treatment groups with no supplementation ($p<0.05$). No significant difference within treatments between two breeds was observed. The result indicated that chili could be an alternative treatment for subclinical ovine fascioliasis to alleviate the production loss in sheep herds.

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Student's signature Thesis Advisor's signature

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percent
<	Less than
>	Greater than
µm	Micrometer
AFAP	Afar Forestry Action Plan
APAPB	Afar Pastoral and Agro Pastoral Bureau
AST	Aspartate Aminotransferase
dl	Deciliter
DM	Dry Matter
EARO	Ethiopian Agricultural Research Organization
ELISA	Enzyme Linked Immuno-Sorbent Assay
FAO	Food and Agricultural Organization
g	Gram
ha	Hectare
Hb	Hemoglobin
kg	Kilogram
km	Kilometer
m.a.s.l	Meter Above Sea Level
mm	Millimeter
^{o}C	Degree Centigrade
PCV	Packed Cell Volume
pp.	Pages
RBC	Red Blood Cell
SAS	Statistical Analysis System
SE	Standard Error
spp.	Species
TP	Total Protein
WHO	World Health Organization

EPIDEMIOLOGICAL SURVEY AND ASSESSMENT OF OPTIONAL THERAPY FOR OVINE FASCIOLIASIS AROUND MIDDLE AWASH RIVER BASIN, AFAR, ETHIOPIA

INTRODUCTION

The Afar Regional State has the total land area of 120,000 km^2. According to the aerial survey conducted in the Afar regional state, there are 4,044,323 heads of livestock in the region, out of this number there are goats 2,014,629, sheep 1,007,303, cattle 703,602, camel 301,815 and donkeys 16,974 which accounts for 49.8%, 24.9%, 17.4%, 7.5% and 0.42%, respectively (AFAP, 1998). The number of horse and poultry are negligible. Almost all these species are indigenous to the region, and the number of improved (exotic) species is insignificant.

Production of sheep for meat, milk, wool, hair, skin, and manure is an attractive agricultural enterprise for Ethiopian farmers because of the relatively low cost of breeding stock and the high productive rate of sheep. Sheep is also the source of cash income. Sheep requires minimal inputs and maintenance costs to live in a wide variety of environment from desert to humid rainforest (Gatenby, 1991). In Ethiopia sheep is the dominant livestock sector providing up to 63% of the cash income and 23% of the food subsistence value obtained from livestock production (Zelalem and Flecher, 1993).

However, there are different constraints such as inadequate veterinary services, poor nutrition, and no record of breeding and poor management systems; therefore, productivity and revenues derived from this sector are not satisfactory (Tembely, 1998; Degefe and Nega, 2000; Desta *et al*., 2000). The annual per capita mutton production in Ethiopia is 9.9 kg per head, but the mutton production in Kenya and the Sudan are 12.2 and 16 kg, respectively (ILRI, 2000).

Nematodes, trematodes and cestodes are known to be prevalence in parts of Ethiopia (Bahiru and Ephraim, 1979; Bekele *et al.*, 1981 and 1982; Brook *et al.*, 1985). As reported by Bergeon (1968), Graber (1975), Scott and Goll (1977), Getachew (1987), Mulugeta (1993) and Daniel (1995), fascioliasis is one of the major parasitic diseases that cause immense economic losses in livestock productivity.

Fascioliasis is one of the most important parasitic diseases of domestic ruminants caused by *Fasciola hepatica* and *F. gigantica* that result a significant economic loss in animal production in tropics. The disease can also infect humans, and there are reports of increasing incidences worldwide (Maurice, 1994). The prevalence of infections on animals is as high as 30-90% in Africa (Fabiyi, 1987). The geographical distribution of *F. hepatica* and *F. gigantica* is considered to be determined mainly by the distribution patterns of snails as the intermediate hosts (Pantalouris, 1965; Boray, 1982a; Over, 1982). In Ethiopia, the presence of both *L.truncatula* and *L.natalensis* has been reported (Bergeon, 1968; Graber, 1975). Erich (1983) reported that the prevalence and distribution of fascioliasis varied from 11% in the Rift Valley to 100% in the central highlands of Ethiopia.

Reduction of pasture contamination is accomplished through the use of anthelmintics, management regimes, molluscicides and biological methods of snail control as a component of an integrated control program. Constraints to the use of anthelminthics by poor farmers due to the high cost are reported to be a problem in Indonesia (Dorny *et al.*, 1996), India (Cheah and Rajamanickam, 1997) and Kenya, (Onieke, 1999). Current control of these infections is mainly achieved by a combination of prophylactic use of antiparasitics and grazing management. Though potentially effective, the applicability of these control measures among the traditional poor farmers and the moving pastoralists is limited, because of the expenses, irregular availability and the current practiced communal grazing system and labour necessary to restrict grazing likely to be in short supply. The use of alternative medicinal plant will be beneficial for farmers not to rely on modern drugs.

Tobacco (*Nicotiana tabacum L.*) is used in Zaire, Tanzania and central Ethiopia for treatment of the internal parasitism including fascioliasis. In Zaire, leaves are pounded and put into half a glass of milk, and after 24 hours the mixture is filtered and given to the sick animals (Byavu *et al*., 2000). Chili is used worldwide for treatment of the internal parasites. Chili (*Capsicum annuum*) is also to treat liver fluke. Chili has been used as an anthelmintic in Southern America and Mexico. In Mexico, tzotzil sheepherders give 13 chili peppers blended with water to sheep with fascioliasis (McCorkle *et al.*, 1995). The powdered drug kamala derived from the fruit of *Mallouts philippinesis* has been used as anthelmintics of the Indian subcontinent and significant reductions in cestode egg in sheep have been demonstrated (Akhtar and Ahmed, 1992).

Sheep have been demonstrated a variation of parasitic resistance and to create relatively resistant animals. Indonesian Thin-Tailed sheep have high resistance to *F.gigantica* (Wiedosari and Copeman, 1990). On the other hand, the interaction between the level of nutrition and the ability of animals to cope with internal parasites has long been recognized (Mukasa *et al*., 1991). Protein supplementation has been shown to help improve the resistance of lambs to parasitic infections (Knox and Steel, 1996).

The Awash River basin is mostly located in the arid lowlands of Afar Region in the north eastern part of Ethiopia, covers a distance of some 1,200 km from its headwaters, North-West of Addis Ababa to its final destination in the Lake Abe in the Afar Region. It frequently floods in August/ September following heavy rains in the eastern highland and escarpment areas, The Awash River has 14 tributary rivers draining the high lands eastwards which can increase the water level of the Awash River in a short period of time and cause flooding and forms swamps because of these wet and warm climatic conditions, and the micro-climates of many localities, it is suitable for the growth and multiplication of disease causing agents and disease vectors that lead to outbreaks of diseases in animals, many of which are economically important (Appendix 3).

In Awash River Basin, ovine fascioliasis is the most important disease. The main reason is that the Awash River and its tributaries create a favorable environment for the growth and the multiplication of the intermediate host (snail) by providing moisture from flooding during the rainy season and from the irrigation schemes during the dry season. Unfortunately, the data of the prevalence and the distribution of fascioliasis in sheep and other ruminant species are fragmented or not well documented in the basin and most of stockowners of sheep cannot afford the increasing cost of anthelminthic drugs. In addition the problem of parasitic resistance created a big problem for the farmers. Therefore, the prevalence of *Fasciola spp*. of sheep breed resistance to fasciola, and the anthelminthic efficacy of the chili and nutritional supplementation on ovine fascioliasis were designed. To determine the prevalence of the disease and to compare the prevalence on the basis of breed, sex, age, season, body condition and to alleviate the production loss in the area were carried out on sheep herd in the Middle Awash River Basin areas.

OBJECTIVES

General Objectives:

The overall objectives of this study were to assess prevalence and an optional therapy of ovine fascioliasis around Middle Awash River Basin of Afar Region, Ethiopia. Surveys for prevalence of *Fasciola spp*. infections and experiments were conducted with the following specific objectives:

Specific Objectives:

1. To determine the prevalence and intensity of *Fasciola spp*. infections and other parasites in sheep in Middle Awash River Basin of Afar Region in relation to the geographical area, season, breed, sex, and age of host.

2. To identify the practical optional treatments for ovine fascioliasis in the area.

3. To identify a breed resistance in the area for ovine fascioliasis.

4. To determine the factors influencing resistance by using nutritional supplements with high protein and energy diet for health fitness.

LITERATURE REVIEW

Fascioliasis

1 Description of the parasite

Fascioliasis is a parasitic disease in ruminants throughout the world. It causes significant morbidity and mortality (WHO, 1995; Okewole *et al*., 2000). It is also increasingly being recognized as an important disease of human. According to Dunn (1987) and Soulsby (1982), *Fasciola spp*. classified into class Trematoda, Order: Digenea, Family: Fascioloidae. The genera *Fasciolidae trematodes* of the geneus *Fasciola*. The two most important of *Fasciola spp*. are *F. hepatica*, found in the temperate areas and cooler areas of high altitude in the tropics and subtropics, and *F.gigantica*, which predominates in the tropical areas (Urquhart *et al*., 1994).

F. hepatica and *F.gigantica* are polymorphic with many factors affecting the morphology including the age of the fluke, host species and intensity of the infection (higher intensity, smaller flukes). Moreover, fixation of the specimen can have profound effect not only on the absolute size of the fluke, but also on the relative size of the various parts of the body used for identification (Kendall, 1965; Ternopol'skaya, 1984).

F. hepatica, when it is fully mature, with the dorsoventrally flattened, adult flukes are measure between 18 to 32 mm long and 7 to 14 mm wide (Boray, 1982b). The adults have leaf- shaped and a narrow cephalic cone at the anterior end. Two suckers are present, an oral sucker at the tip of the cephalic cone and a ventral sucker located at the level of the "shoulders". The ventral sucker is functioned as an organ of attachment, while the oral sucker is opening to the pharynx and the digestive tract. The digestive tract is consisted of a pair of highly branched intestinal ceca that extends to the posterior end of the body. A single branched ovary lies to the right side of the midline and slightly posterior to the ventral sucker with coils of the uterus situated between the ovarian branches. Two extensively branched testes, one anterior to the other, lie posterior to the ovary occupying a considerable portion of the

remaining body. Numerous vitelline glands extend along the sides of the body from the area of the shoulders to the end of the body where they are confluent behind the testes (Olsen, 1974).

F.gigantica is a parasite very similar to *F. hepatica*, but much larger, reaching from 25 to 75 mm long and 15 mm wide (Soulsby, 1982). The anterior conical structure is similar, but the widening of the body is not as distinct as *F.hepatica* (Figure 1 and 2). In addition, it requires warmer temperature and is more associated with permanent areas of water. The egg is also very similar to that of *F. hepatica*, but it is about twice the size of the trichostrongyle egg. The life cycle is similar to *F.hepatica.*

Figure 1 Adult *Fasciola hepatica*

Source: - Wikipedia, the free encyclopedia (2007)

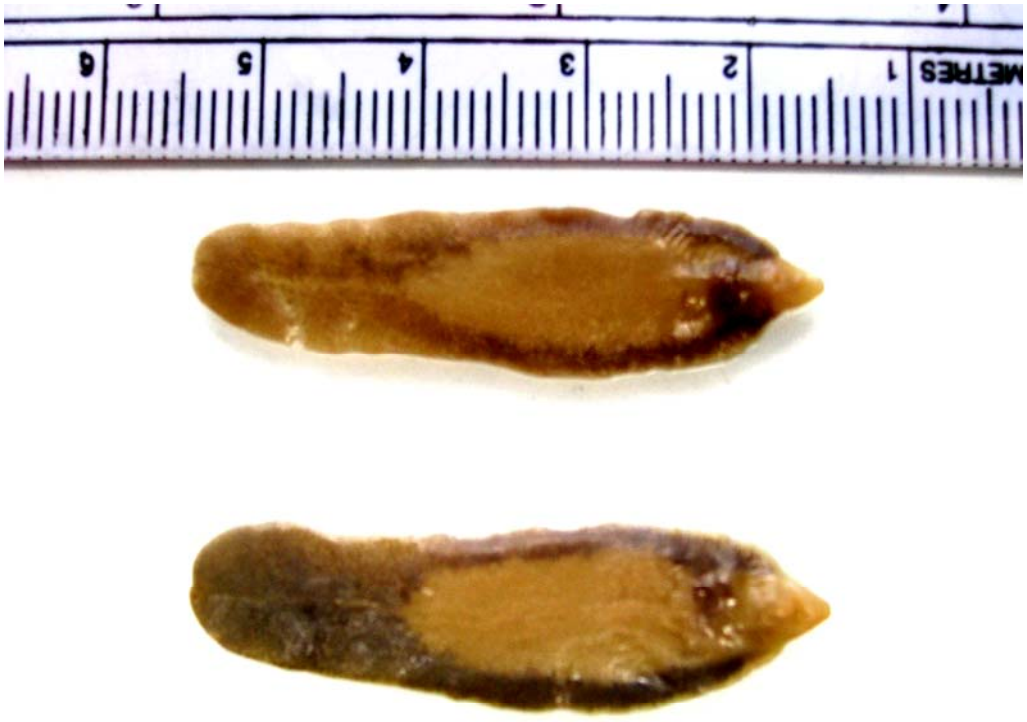

Figure 2 Adult *Fasciola gigantica*

2 Life cycle of *Fasciola hepatica*

Fasciola spp. pass their life cycle into two different hosts, the intermediate host (snail) and the final host, cattle, sheep, buffalo are the most important species of farm livestock although goats, horse, pigs, deer and many other species of herbivore are the primary definitive hosts (Soulsby, 1982; Urquhart *et al*., 1996). A human is also a suitable host and in some areas of the world, the human fascioliasis is an important cause of ill health.

F. hepatica exhibits a typical life cycle of trematodes and it involves two distinct stages within two different hosts. One is sexual in the adult stages of the

parasite and the other is asexual in the intermediate stages. Briefly, the life cycle of the flukes is consisted of seven stages; 1) development to the adult in the definitive host; 2) passage of the eggs from the definitive host; 3) embryonation of the eggs in the environment; 4) hatching of the miricidia in water and its search for a snail intermediate host; 5) development of larval stages (sporocyst, rediae and cercariae) in the snail; 6) emergence of cercariae from the snail and successful encystment of the metacercariae; and 7) ingestion of the metacercariae by the definitive host (Figure 5)

Starting with the adult parasite in the biliary system of the liver of the definitive host, the adults lay on average between 8,000 and 25,000 eggs per day. While an individual fluke is hermaphroditic, cross-fertilization between two adult flukes is believed to be the most common form of sexual reproduction (Chen and Mott, 1990)

As the adult fluke is found in the bile ducts of the liver of the host its eggs are laid into the bile and pass with this into the host's small intestine. From this they reach the exterior in the hosts' droppings. The eggs consist of the fertilized ovum surrounded by a large number of yolk granules. They are yellowish brown in color, oval in shape, 130-140 μm long by 70-90 μm wide and the eggs are passed in the faces and then develop into miracidia in 2-4 weeks depending on various optimal conditions.

The development of eggs was influenced by the temperature and the moisture (Thomas, 1883). A temperature of at least 10^0 C is necessary for embryonation (Ross and Mckay, 1929). The effect of moisture is likely to become critical when conditions dictate slow development of the egg and thus long exposure to other factors in the environment may not be favorable. Under such conditions, maintenance of the surface films of the moisture around the egg for at least 3 weeks is essential. Eggs on soil, however will develop without the presence of free surface water, provided that the soil is saturated (Ollerenshaw, 1959).

No development of the eggs will take place when they present in a concentrated fecal suspension, although they will survive for more than twice as long in aerobic conditions than in anaerobic conditions. Eggs kept in cultures without feces show little variation in mortality, but those in aerobic conditions hatch in one fifth of the time taken by those at a lower oxygen tension (Rowcliffe and Ollerenshaw, 1960).

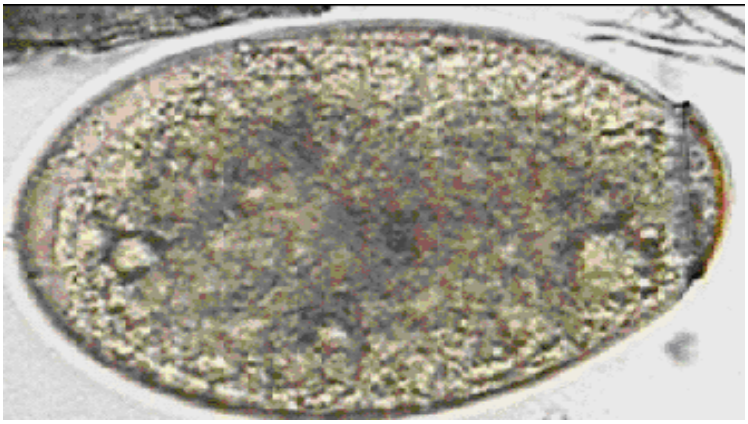

Figure 3 Fasciola egg
Source: - Ohio State University (2000)

Eggs incubated at 27^0 C will develop and hatch within a pH range of 4.2 to 9.0, but above pH 8.0 developments is prolonged (Rowcliffe and Ollerenshaw 1960). The optimum pH for embryonation appears to be 7.0 (Al-Habbib, 1974).

Under optimal conditions the egg will hatch, releasing the larval or the miracidia. The miracidium is about 130 μm. in length, broad interiorly and tapering posterior to a blunt end. The cuticle is ciliated and there is an anterior papilla form protrusion and a pair of darkly staining eyespots visible nears the anterior end of the body. Once hatched from the egg, miracidium becomes active, immediately starting to swim at a great speed on average, 1mm/ sec (Wilson and Denison, 1970).

The characteristics of swimming movements have been used as a mean of determining the infectivity of miracidia. The miracidia invade the lymneaid within

three hours if successful penetration is to occur in snails and they develop as sporocyst, rediae and cercariae. The penetration process involves a mechanical boring action by miracidial anterior papilla and also likely to be facilitated by the secretion of proteolytic enzymes (Smyth and Halton, 1983). Tissue at the point of penetration generally near the bronchial aperture is observed to be degraded (Wilson *et al*., 1971).

Fascioliasis is absent in areas where conditions are unsuitable for the development of suitable intermediate host snails. Suitable snails belong to the *Phylum Mollusca* and *Class Gastropoda* and the species of interest fall into the subclass *Euthyneurra* or *Pulmonata*, depending on the system of classification (Wright, 1971).

Lymnaea natalensis, aquatic snail is an important host of *F.gigantica* in Africa. *Lymnaea truncatula* with a wide distribution throughout the world, is the most common intermediate host of *F. hepatica* (Soulsby,1982; Urquhart, *et al.*,1994).They are mud living and amphibious, living in environment niches which are subject to flooding and desiccation (Over,1982). They are more likely to be found in habitats that are intermittently wet (flush habitat) than in permanently wet sites and in water that is generally slightly acid (Villegas, 1984) and move at 15- 20 cm s-1 (Boray, 1964). Distribution is not uniform because, within each habitat, the snail may be concentrated in small, very wet areas such as ditches and seepages (Kendall *et. al*., 1975). Snails can travel large distances by drifting in water (Ollerenshaw,1971) reported that in Australia large, permanent water areas harboring a few snails are thought to be important in recolonization of temporary water courses (Boray, 1964). Thirty percent of snails survive a 12- month artificial drought by aestivation (Soulsby, 1982) and even newly hatched snails can survive 2 months of aestivation (Kendall, 1949). Once water returns, however, snails are able to breed very rapidly.

Figure 4 Type of snail serves as intermediate host of *Fasciola spp*. in the study area

The cercariae leave the snail about 5 to 8 weeks after the original infection in the snail and each has a tail, which is shed on encystement to form a metacercaria. Some of the metacercariae may float around on the surface of the water, although most encyst on vegetation. When the infected grasses or water are ingested by sheep, the larvae excyst in the small intestine, and pass through the intestinal wall into the body cavity and penetrate the liver. They eventually reach the biliary passages; develop to maturity in about 3 months. Some flukes reach the liver through the blood stream or crawl up to the bile ducts. Under favorable conditions it requires about 5 months for the liver fluke to complete its life cycle (Morgan and Hawkins, 1951). The minimum prepatent period of fasciola is about 8 weeks (Dunn, 1987), and the mature fasciola can live in the final host of the animal as long as 11 years (Read, 1960).

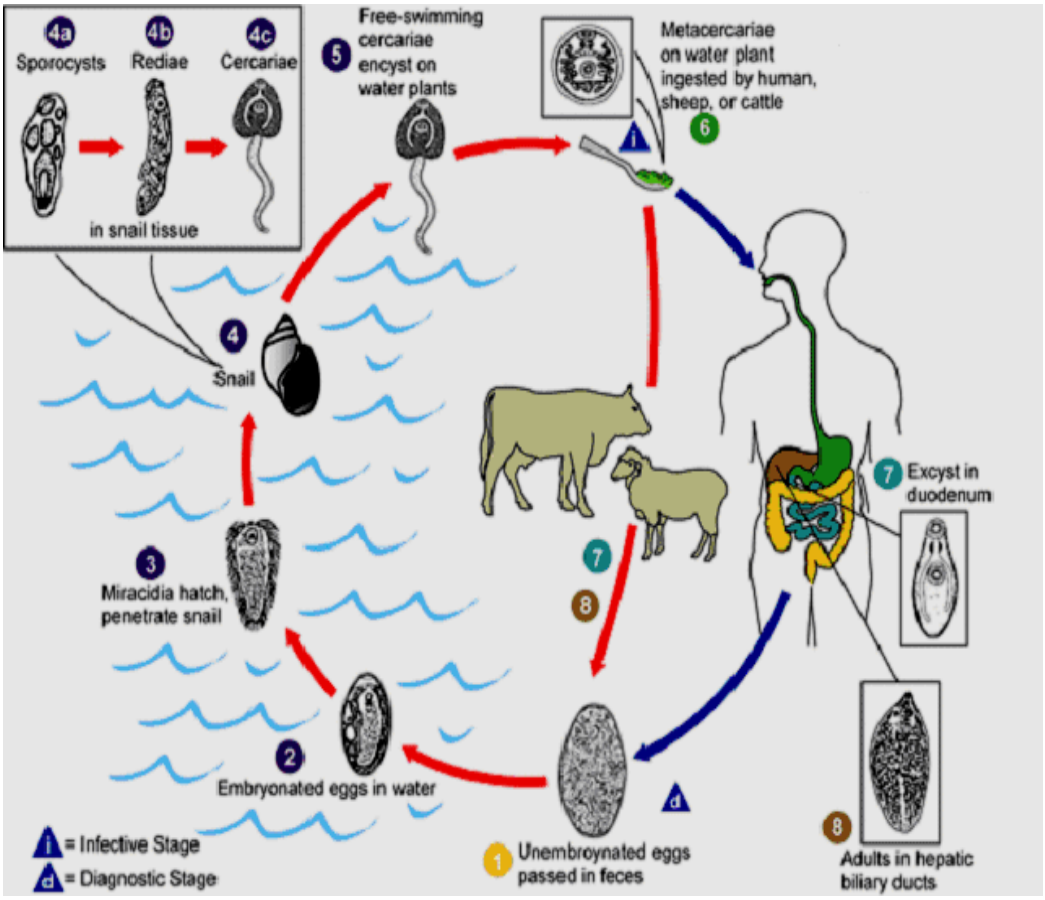

Figure 5 The life cycle of Fasciola spp.

Source: - DPD (Division of Parasitic Diseases), 2004.

3 Clinical aspects and pathology

Clinical fascioliasis in sheep can usually be classified into acute, sub-acute and chronic, according to the number and the stage of development of flukes in the liver, but since any such classification is always arbitrary, there will be considerable overlap between these categories.

In sheep acute fascioliasis occurs seasonally and is manifested by distended, painful abdomen; anemia; and sudden death. Deaths can occur within 6 weeks of infection. The acute syndrome must be differentiated from “black disease.” In sub

acute disease, survivals are longer (7-10 weeks) even in cases with great damage to livers, but deaths occur due to hemorrhage and anemia. Chronic fascioliasis is associated with the presence of adult fluke in the bile ducts and is characterized by gradual loss of condition, progressive weakness, anemia and hypoproteinemia with development of edematous subcutaneous swellings, especially in the intermandibular space (Figure 6) and over the abdomen (Bowman, 1995). Heavy chronic infection is fatal in sheep (Susan, 1998).

Figure 6 Sheep with chronic fascioliasis

During the migratory phase in the mammalian liver, parenchymal destruction is caused by the direct activity of the migrating flukes. The inflammatory response of the host also results in cellular infiltration of the tissue in the wake of the tunneling fluke which in heavy infections terminates in a wide spread fibrosis. Migration of flukes into any of the larger hepatic blood vessels can cause hyperproteinemia, hyperglobulinemia and hypoalbuminemia (Reid, 1973). The major pathological findings in chronic fasiolosis are the development of hepatic fibrosis and thickening of the bile ducts. During migration flukes may carry on to penetrate organs such as the diaphragm and lung. In sheep there appears to be little disease associated with the penetration of organs apart from the liver. Lung lesions are found in many animals

(Boray, 1969). Adult flukes in the bile ducts are active blood suckers and, if present in sufficient numbers (greater than 200), can cause severe anemia (George, 2002).

4 Diagnoses

Diagnosis is most accurately based on a good post-mortem examination confirmed where possible by clinical examination of the survivors (clinical sign), history of the flock grazing, seasonal occurrence, fecal and blood sample tested by the laboratory.

Post mortem examination of fresh carcasses is the best method of diagnosis if fascioliasis is suspected as untreated animals provided the most accurate indication of the level of challenge. A post mortem examination will also identify any lesions resulting from concurrent disease or parasitic gastroenteritis. Confirmation is by postmortem examination, when small flukes can be expressed from the liver parenchyma (Blood *et al*., 1983). In live animals, laboratory diagnosis can be used in a variety of tests on feces and /or blood samples. Elevation of liver enzyme levels (aspartate aminotransferase and glutamate dehydrogenase) can be useful for the diagnosis of acute fascioliasis as early as two to three weeks post infection, while raised L-gamma glutamyl transferase levels can indicate chronic disease once adult flukes are present in the biliary tree (George, 2002).

Over the past 15 years the vast majority of investigators have used an ELISA based assay or variant, with counter electrophoresis or haemagglutination as close second, for the detection of antibodies. The ELISA, especially the FAST ELISA is an excellent screen test, followed by the western immuno-blot for the confirmatory test (Hillyer and Soler de Galanes, 1988).

5 Control and treatments

Reduction of pasture contamination may be accomplished through the use of anthelmintics, management regimes, molluscicides and biological methods of snail

control, a component of an integrated control program. In addition, the use of resistant animals to reduce the impact of infection may have potential, especially where treatment costs are relatively high (Roberts and Suhardono, 1996).

Efficient control of fascioliasis requires a well planned and executed, integrated control program designed for each farm, area, country or region. The available strategies, which can be used individually or in combination, are: strategic application of anthelminthics, eliminating the parasites from the host at the most appropriate time for effective prevention of pasture contamination; reduction in the number of intermediate host snails by chemical or biological control; reduction in the number of snails by drainage, fencing and other management practices and reduction in the risk of infection by the planned grazing management.

5.1 Anthelmintic treatments

Anthelmintic drugs have been widely used against helminthes parasites. An effective control program should be based on a sound understanding of the ecology and life cycle of the parasites prevalent in the area. Lack of information on the epidemiology and pathogenesis of gastrointestinal nematodes is recognized by Lonka *et al.* (1993) to be a constraint to the development of effective control measured in India. The timing of treatment is important in order to achieve the greatest efficacy. For example, it appeared that clinical manifestations of helminthiasis in sheep and goats at the start of the wet season in Kenya emerged too quickly to be accounted for by the acquisition of infection from the pasture (Gatongi *et al*., 1997)

Seasonal strategic application of effective anthelmintics specific for trematodes, as well timed prophylactic and curative treatments, play an important role in the control of liver fluke infections. Strategic treatments have been developed for several regions of the world based on meteorological data. However, it is advisable to supplement meteorological data with sound epidemiological information in order to improve the timing, and thereby the efficiency, of treatments. The basic principles of strategic anthelminthic application (treatment/prophylaxis) are:

(a) Prophylactic treatment of ruminants towards the end of a period of ecologically reduced activity of the parasites and the intermediate hosts. One treatment is therefore recommended towards the end of a period when larval development in the fluke eggs or in the snails has been retarded, and when the reproductive rate of snails is low or their activity is impaired (such as during a prolonged dry season, or extreme cold). At that time, a prophylactic effect can be achieved by reducing the pasture contamination of eggs before favorable climatic conditions for larval development and snail activity resume.

(b) Curative treatment at about one to two months after the expected peak infection of the hosts. A curative effect can be achieved by one treatment to remove the residual fluke burden acquired from metacercariae which have survived on the herbage.

(c) Additional treatment in highly contaminated areas where seasonal variations do not significantly affect the life cycle of the flukes. These additional treatments may be required occasionally, when the seasonal climatic conditions are favorable for parasite and snail development, or in areas where high metacercariae intake often occurs as a result of the restricted grazing of wet areas during dry seasons. The most important prerequisite for efficient chemotherapy and chemoprophylaxis is a prior knowledge of the epidemiology of the disease based mainly on meteorological data and seasonal surveys in hosts.

The economics of chemotherapy should be evaluated for each farm, area and country, including assessments of the availability of anthelminthics, their price and the economics of the livestock production system in which they are to be used. More treatments are necessary if the drugs available (or selected on the basis of cost) are those that are only effective against mature flukes. Efficient control programs can be developed based on less frequent treatments with drugs effective against early immature and immature flukes. However, the price of these drugs is considerably higher than those effective only against older flukes and their use may therefore be restricted to the more intensive livestock production systems. If animals are grazing

communal areas, it is important to achieve a synchronized reduction in pasture contamination of eggs, if possible. Ideally all animals in the area should receive treatment within a short period of time.

Malone and Yilma (1999) noted that any strategies for the treatment and prophylaxis of fasciola infections were based on epidemiological data. Drugs which only work against adult flukes, such as albendazole, oxy-clozanide and clorsulon at the low dose rate combined with ivermectin, are unsuitable for effective chemoprophylaxis.

Suppression of *F. hepatica* infections has been attempted using treatments every 6 weeks with rafoxanide from spring to autumn for two years resulting in 90% reduction of infection and reduction of the infection rate in snails (Armour, *et al.*, 1973).

In a field experiment, sheep suffering from heavy acute, sub acute and chronic infections were treated with triclabendazole (10 mg/kg), with an efficacy of 99.8%. Subsequently, all sheep, cattle and horses were treated on the property every 8 to 11 weeks for a period of 14 months. No patent infection could be detected and the contamination of the pastures was reduced to a negligible level for a period of 12 months after the last treatments. It is the effective treatment during the preptent period for an extended duration that could eliminate fasciola infection or reduce contamination to a very low level, requiring less frequent treatments for a considerable time (Boray, 1986). Frequent treatments of sheep were carried out in the field with triclabendazole in a strategic pattern, between June and January, for a period of 5 years and reduced the prevalence of infection from 49 to 1% (Fawcett, 1990).

Eight-week treatments with triclabendazole between April and October did not reduce infection in the first year but achieved a 70-75 % reduction when the treatment commenced in February and were carried out four times a year for years (Taylor *et al.*, 1994). Paul and Claxton (1999) mentioned that the less frequent strategic

treatments with a possible yearly rotation of anthelmintics or anthelmintic combinations, which are effective against both immature and adult flukes, will provide the best method of successful control of fascioliasis.

Table 1 Anthelmintics for the treatment of ovine fascioliasis

Generic name	Administration route[1]	Dose rate (mg/kg)	Minimum age of fluke in weeks efficiency > 90% *
Hexachlorophene*	O	15	12
Hexachloroethane	O	25-300	12
Tribromsalan	O	20	12
Bithionol*	O	75	>12
Hexachloroparaxylene	O	150	12
Bromophenophos	O	16	12
Clioxanide*	O	20	12
Oxyclozanide*	O	15	12
Niclofolan*	O	4	12
Nitroxynil	SC	10	8
Brotianide*	O	5.6	12
Rafoxanide*	O	7.5	6
Closantel	O	7.5-10	8-6
Diamphenetide	O	80-120	1 day - 6 weeks
Albendazole	O	4.75	>12
Triclabendazole	O	10	1 day -7 weeks

* = Also effective against paramphistomes.

[1] O = orally administered, SC= Subcutaneous administered.

Source: - Hansen and Brain (1994)

Emerging evidence of drug resistance against some products (Boray, 1990) may limit treatment options. Anthelmintic resistance is by far the greatest problem in the small ruminant industries throughout the world, especially so in the warmer, more humid environments such as the tropics (Waller, 2003a; 2003b). Resistance of *F. hepatica* to triclabendazole has been recorded in Australia (Overend and Bowen, 1995) and Ireland (Mulcahy and Dalton, 1998) and fluke have shown a degree of resistance to salicylanides (Miller *et al.*, 1994).

5.1.1 Local remedies

Local remedies to control fluke may also be available, though with unproven efficacy. Extracts of Artichoke leaves or of an unclassified plant 'Jaya-shipata' in Peru have been used to control fasciola infection in sheep, where they reduce the number of adult parasites (Arevalo and Bazalar, 1989). Chili is used worldwide for the treatment of internal parasites. In Mexico, 13 chili peppers blended with water, administrated orally to treat ovine fascioliasis, chili (*Capsicum annuum*) were used by 19% of the respondents of the Rake community to cure liver fluke diseases (McCorkle *et al.*, 1995).

There are many plants, which have some anthelminthics effect and justify continued investigation: *Artemisa maritina, Caesalpina crista, Milia ozedrach, Mallotus philippensis, Chrysanthemum spp., Matteucca orientalis, Carica papaya, Heracleum spp., Hedysarum coronarium, Aloe barteri, Termininalia avicennioides and Diospyros mollis* (Hammond *et al*., 1997). Tobacco (*Nicotiana tabacum L.*) is used in Zaire, Tanzania and central Ethiopia for the treatment of the internal parasitism including fascioliasis, In Zaire the leaves are pounded and put in half a glass of milk, after 24 hours the mixture is filtered and given to the sick animals (Byavu *et al*., 2000).

Chili or hot peppers (*Capsicum spp.*) belongs to the Solananaceae or nightshade family. Chili has 90 genera and some 2000 species (Heiser and Pickersgills,1969) and has a propensity for establishing itself wild or domesticated so

that it can be found throughout the tropical parts of North or South America (Andrews, 1984). It is believed that chili is originated from Bolivia and Peru. From here the various domesticated forms developed and the secondary center with *Capsicum annuum* can be located the Middle America (Mexico). Capsicum belongs to the plant kingdom and classified is as follows;

Division: *Magnoliophyta* (Angiosperms)

Class: *Magnoliopsida* (Dicotyledons)

Order: *Solanales*

Family: *Solanaceae*

Genus: *Capsicum* L.

Domesticated species include: *C. annuum*, *C. baccatum*, *C. chinense* and *C. pubescens*

Chili was introduced to Europe (Spain) by Columbus and to India and other parts of Asia by the Portuguese merchants and thus, spread throughout the world (Nonneck, 1989). The most widely cultivated are: *C. annuum*, *C. baccatum*, *C. chinense* and *C. pubescens*. In 81 countries of the world, *C. annuum* is important (Tay, 1988). Capsicum species are classified into two groups, sweet pepper and hot pepper depending on the amount of active ingredients of pungency called capsicinoid (Table 3). Sweet pepper is not pungent whereas hot pepper is highly pungent (Jamtsho, 1997).

Table 2 The amount of active ingredients of capsaicinoid (%) in chili

Item	Content (%)
Capsaicin	46-47
Dihydrocapsaicin	21-40
Nondihydrocapsaicin	2-11
Homocapsaicin	0.6-2
Homodihydrocapsaicin	1-2

Source: - Heiser and Pickersgills (1969).

Chili is used for pickles, relish, sauces and dry powder. Chili is a good sources of vitamins A, B, C, and E; potassium, phosphorus and calcium (Andrews, 1984). They are also known to have good medicinal value for black vomit, various tropical fevers, gout, and paralysis. Chili extracts can also be used as insect repellents.

In Ethiopia, hot pepper and chili (Mitmita) are the leading vegetable and spices grown in the country, grown in different ecological areas in which hot pepper not predominates. It is mainly produced in the peasant sector. But there is a very great potential for production of hot pepper in the country. The crop is grown in state farms, producers' cooperatives and both by rural and urban private garden growers. Hot pepper (*Capsicum annuum*) is one of the most promising vegetable crops in Ethiopia, featuring in major daily dishes. There is a general belief among Ethiopians that hot pepper or chili is uses to treat various diseases.

5.2 Snail control

5.2.1 Molluscicides

Molluscicides have been used both successfully and cost effectively to control snail populations (Urquhart *et al*., 1970). However, this approach has not achieved widespread acceptance. Risk of environmental contamination may be unacceptable, particularly when Molluscicides kill utility species such as fish and crabs. Furthermore, certain parts of the pasture can be difficult to access with a sufficient amount of the chemical. As a result of the snails' high biotic potential, pasture can quickly become reinfested, making repeated applications necessary, thus adding to the expense and increasing the risk of contamination.

The most important compounds, which can be applied to control snails, are niclosamide, sodium pentachlorophenate and N-tritylmorpholine (Boray, 1982a). At present niclosamide, copper sulfate is used in different parts of African countries (Brown, 1980). Niclosamide is highly toxic to snails and eggs at 0.1-0.2 ppm and has a low toxicity for mammals. It is applied at 1-3 ppm for aquatic snails, and higher

concentrations (Anon, 1970). For controlling amphibious snails, a ground application of 0.2g m^{-2} on snail habitats may be used. Sodium pentachlorophenate is also effective when applied at 0.4-10g m^{-2} for amphibious snails and 2-5 ppm in areas containing free water. Like niclosamide, it is highly toxic to fish. N-tritymorpholine is effective against snails but not their eggs at concentrations of 0.15- 0.5 ppm. Slow release formulations, when combined with snail attractants, may reduce some of the potential risks to the environment (Pfister *et al*., 1994)

5.2.2 Managemental and biological control

Reducing snail abundance through improved pasture, either by draining or fencing-off wet areas, prevents infestation of grazing areas. However, this approach is expensive and often not cost effective (Wilson *et al*., 1982) and may not be feasible in many situations. Lymnaeid snails are vulnerable to predators such as arthropods, amphibians, reptiles, birds and rodents. Generally snails exist in equilibrium with their predator species, although intensive duck and goose husbandry has been shown to eliminate lymnaeid snails and effectively control fasciolosis (Levine, 1970). Certain sciomyzid fly larvae have been shown to predate lymnaeid snails (Berg, 1953) and this has been put forward as a method for the biological control of snails.

Brown (1980) reported that different molluscicides properties have been demonstrated in extracts from different plants. Endod or Lemma toxins derived from the fruits of shrubs *Phytolacca dodecandra* (Lemma, 1970 cited in Brown 1980). Endod (*Phytolacca dodecandra*) is very promising in the control of snail borne diseases because of its high molluscicides potency and low mammalian toxicity.

5.3 Selecting resistance animals

It is now well documented that indigenous livestock that have evolved over the centuries in the diverse, often stressful tropical environments, have a range of unique adaptive traits e.g. disease resistance, heat resistance, water resistance, ability to cope with poor quality feed, etc. which are enable them to survive and be

productive in these environments (Fitzhugh and Bradford 1983, Devendra 1987, Baker and Rege 1994).

Infection with fasciola may result in a degree of acquired resistance, which varies depending on the host species. In addition, some animals show degree of innate resistance: horses are less susceptible than ruminants (Nansen *et. al.,* 1975); pigs are only significantly susceptible when under 8 weeks old (Nansen *et. al*., 1972). Although it is well documented that sheep can mount an effective immune response (self cure) to nematode parasites, it has been demonstrated that they are unable to acquire resistance to liver flukes (Haroun and Hillyer 1986; Boyce *et al*., 1987). Boyce *et al*. (1987) found significant breed differences in fecal egg counts and fluke counts after live breeds of sheep were experimentally infected with *F. hepatica.* Barbados Blackbelly sheep were the most susceptible to infection while St.Croix and Florida Native sheep were the most resistant. While none of the breeds demonstrated an ability to resist reinfection with *F.hepatica,* clear breed differences were detected in response to the primary infection. Wiedosari and Copeman (1990) reported relatively high resistance to *F.gigantica* in Javanese Thin Tail sheep, although there was no contemporaneous breed comparison. Roberts *et al*. (1997) compared the resistance to *F.gigantica* of Indonesian Thin Tail sheep sampled from Java and Sumatra with St. Croix sheep and F2 and F3 crosses between these breeds. They concluded that the Indonesian Thin Tail sheep were more resistant than St. Croix sheep and that resistance may be controlled by a major gene with incomplete dominance. In contrast, the Indonesian Thin Tail sheep were as susceptible to *F.hepatica* as the Merino sheep that they were compared with (Roberts *et al*., 1997).

A study showed that under natural pasture challenge there was no difference in resistance to endoparasites between the indigenous Menz and Horro sheep evaluated in the highlands of Ethiopia (Tembely *et al*., 1998, Rege *et al*., 2002). However, under artificial challenge there was some evidence that the Monz may be somewhat more resistant than Horrro lambs (Haile *et al*., 2002). Although there is some evidence for parasitic resistance among typically adapted breeds of goats, this seems to be not as marked as for sheep.

Breeding animals with increased genetic resistance is another possible alternative to the use of anthelmintics. Livestock that have lived for a long time in an area infested by parasites will often have evolved greater levels of resistance. Thai native goats showed higher levels of resistance to *H. contortus* than did 50% Thai native x 50% Anglo-Nubian crosses. Live weight gains were, however, greater among the 50% Thai native x 50% Anglo Nubian crosses (Pralomkarn *et al*., 1997). Crossing the small indigenous wool sheep breeds of Sumatra with the exotic breeds St. Croix or Barbados Blackberry, produced sheep that were both more resistant to nematodes and larger than the indigenous breed (Romjali *et al*., 1997). The Red Masaai sheep has been shown to be more resistant than Blackhead Somali or Dorper sheep to *H. contortus* (Mugambi *et al*., 1997; Wanyangu *et. al.,* 1997). Considerable variability in the level of resistance to H. contortus within goat breeds and crosses in Thailand was observed by Pralomkarn *et al.* (1997). This suggests the possibility of selective breeding for resistance and of selective treatment of these animals if they can be identified.

5.4 Vaccination

At present there are no commercially available vaccines against fasciolosis, although glutathione S-transferase and proteinases secreted by *F.hepatica* are possible candidate antigens (Wijffels *et al*., 1994). Attempts are being made to develop vaccines against *Fasciola gigantica,* based on antigens derived from adult flukes. Low but significant reductions in fluke burdens have been demonstrated (Estuningsih *et al*., 1997). It may not be possible- or necessary- for a vaccine to be 100% effective to control fluke in the field, but vaccines will need to be cost effective in comparison with anthelmintic treatments. A vaccine, which increased herd immunity sufficiently to significantly reduce fluke transmission, might effectively control the parasite over a period of time and result in a positive cost-benefit return.

6. Economic effects of fascioliasis

6.1 Veterinary importance

Fascioliasis often causes mortality and significant loss of blood and metabolizable energy, and impaired appetite and nitrogen retention, thus reducing weight gain and milk production (Hope Cawdery 1984; Paul and Claxton 1999). All these lead to noteworthy negative economic effects.

In Ethiopia, the annual losses due to ovine fascioliasis were estimated at 48.4 million Ethiopian Birr (1.00 US$ = 2.07 Ethiopian Birr) per year of which 46.5, 48.8 and 4.7% were due to mortality, productivity (weight loss and reproductive wastage) and liver condemnations, respectively (Ngategize *et al*., 1993). In the United States direct and indirect losses in excess $ 30,000,000 are incurred annually by cattle and sheep producers due to fascioliasis. In England losses estimated due to condemnation bovine livers total $ 1.7 million (Haseeb *et al*., 2002).

The effect of fascioliasis on the live weight gain of the animals has been demonstrated by many workers. Most of these reports concern *F. hepatica* in cattle and sheep. However, Sewell (1966) reported that there was a depression in weight gain of cattle infected with *F. gigantica* and that reduction was related to parasite numbers that is each *F. gigantica* reduces the annual live weight gain by 200 g/fluke per annum. Sinclair (1962) reported a 70% reduction in weight gain in sheep with a mean burden of 200 flukes. Coop and Sykes (1977) demonstrated that the depression of live weight gain in-groups of sheep with a mean of 87, 157 and 233 fluke was 26%, 22% and 33%, respectively.

Wool growth and quality have been shown to be depressed in fasciola infections with as few as 30 adult flukes (Dargie, 1986). Hawkins and Morris (1978) demonstrated that the number of flukes present can have a significant impact on wool quality. They determined that an infection of 45 flukes decreased wool quality by 14%, 117 flukes, 19%, and 230 flukes, 33%. Berry and Dargie (1978) reported that

the nutritional status of an animal, in particular the intake of iron and protein, played an important role in how severely it would be affected by fascioliasis.

The reduction in the milk yield may be dependent on the magnitude of the parasite burden and animals can, to a certain extent, compensate by increased appetite (Hope Cawdery and Conway, 1972). Hope Cawdery (1984) reported that the reduction in milk yield can vary between 6 and 30% depending upon fluke burden. Oakley *et al.* (1979) and Hope Cawdery (1984) suggested lower fertility rates in infected or inadequately treated cattle, while fewer lambs are born to infected ewes (Hope Cawdery, 1976).

6.2 Human fascioliasis

Fasciola is a well known parasite of herbivorous animals. It has a worldwide distribution in the animal reservoir, host such as sheep, goats, cattle, buffalo, horses and rabbits show infection rates that may reach 90% in some areas. Human can become infected with this fluke via the consumption of watercress, water lettuce and chestnuts, or other plants that are contaminated with parasitic cysts. The numbers of cases of human fascioliasis caused by *F. hepatica* has increased significantly since 1980. Esteban *et al.* (1998) compiled a total of 7071 human cases reported from 51 countries over the last 25 years and thus he believed that the true number of human cases is much greater than that reported. There are areas with true endemic human fascioliasis, ranging from low to very high prevalence and intensity. The total number of fascioliasis cases in the world has been estimated to be between 2.4 million (Rim, 1994) and even up to 17 million people (Hopkins, 1992). In Ethiopia human fascioliasis 100-1000 cases are documented (Mas-Coma *et al.*, 1999).

The first record of human fascioliasis is that of Pallas in 1760 in a female patient found infected upon autopsy in Berlin (Grove, 1990) Infection of the human host was very sporadic until the last two decades when clinical cases and outbreaks were reported. It has now become an important emerging food borne trematode infection of increasing concern (Chen and Mott, 1990).

The disease in human clinically, occurs in acute and chronic phases with complications, particularly in small children (Abou, 1989). And also it may be associated with other parasites in the same order of their prevalence in the community. Association of fasciola with *Schistosoma mansoni* is common, but studies have proved the absence of mutual influence (immunological, pathological or epidemiological). Both *F. hepatica* and *F.gigantica* prevail in the human host and infection with both species is not uncommon (Farag, 1979).

Some believe that man is not a suitable host because most migrating flukes become trapped in the liver parenchyma and die without reaching the bile ducts (Acosta-Frreira *et al*., 1979). In contrast, Mas-Coma and Bargues (1997) suggested that at least in the hyperendemic areas the parasite is better adapted to the human host than the animal host. Compared with *F.hepatica*, *F.gigantica* appears to be less infective and less adapted to the human host. *F. gigantica* is known to give rise to ectopic lesions much more commonly than *F. hepatica* (Boray, 1966; Sewell, 1966; Hammond, 1974).

A particular syndrome known as halzoun or parasitic pharyngitis has been attributed to *Fasciola* in which immature flukes attach to the pharyngeal mucosa after the ingestion of raw liver, primarily from sheep or goats, frequently causing irritation and edema of the throat (Kerim, 1956). Recently, this condition has been attributed to the pentastomid *Linguatula serrata*, so some controversy as to the true etiology of this unique disease still exits (Schacher *et al*., 1969; Drabick, 1987; Saleha, 1991).

Drugs used for treatment are triclabendazole with a single dose of 10 mg/kg body weight, and bithinol, 50 mg/kg body weight every other day for 10 days (Poltera and Rouan, 1990). A clinical trial for treatment of human fascioliasis was undertaken in Egypt on triclabendazole, the fasciolicide drug of choice for animal infection (WHO, 1995). Triclabendazole are proved to be safe, well tolerated and very effective (the cure rate reached 90% in chronic infections).

Health education and orientation towards proper washing of salad vegetables before consumption should be adopted using either 6% vinegar (100 ml/l) or potassium permanganate (24 mg/l) for 5 to 10 minutes (El-Sayad, 1992). In endemic areas, all efforts should be directed towards control of this problem. Sound programs for prevention and control should be planned and applied.

MATERIALS AND METHODS

The study was divided into three experiments. The first experiment is an Epidemiological survey on the prevalence of *Fasciola spp*. infections of sheep in Middle Awash River Basin of Afar Regional State and the other two experiments are with the objectives of investigating optional treatment of *Fasciola spp.* of infections in different local breeds of sheep and natural *Fasciola spp*. infections to local breeds. The title of each experiment, materials and methodologies employed to execute each experiment are presented as follows:-

Experiment 1: Epidemiological Survey on Prevalence of *Fasciola spp*. Infections of Sheep in Middle Awash River Basin, Afar, Ethiopia

1. Description of the study area

Epidemiological disease survey was carried out from January to December 2004 in Middle Awash River Basin, Afar National Regional State, Ethiopia (Figure 7). Middle Awash River Basin is the area include between Awash Station and Mile River located between latitude 8° to 10° N and longitude 38° to 42°E and situated at lower altitudes ranging from 850 meters at sea level at Melka Werer area to 550 meters in the Gewane area. The areas are 270 to 365 kms away from Addis Ababa, the capital of Ethiopia. Gewane, Bure Mudaytu and Amibera were selected based on their being wet and low situated along the irrigated and flooding areas of Awash on ecology suitable for the transmission of ovine fascioliasis. The sheep population was estimated at around 24,308, 22,823, and 44,290 heads in Amibera, Gewane and Bure Mudaytu districts, respectively (AFAP, 1998).

Livestock are important component in providing food and generating income to the pastoral households. Furthermore, they play an important role in saving in the form of livestock, social security and cultural taboos (sacrifices, borrowing, gift etc). Their distribution depends on vegetation cover and other conditions.

The plant composition and range productivity decreases from west to east as the result of decreasing precipitation and rising temperatures. The major vegetation types of the rangelands are riparian woodland, bushland/ scrubland, grassland, wetland and bareland. The vegetation cover and the range conditions vary in the different areas of the region.

In the study areas, riparian, bushland/ scrubland, grassland wetland and bareland are the major vegetation cover types (Appendix 7, 8, 9). The better rangelands are found in Amibara, Buremudaitu and Gewane districts. In Amibara district, in addition to grazing land being converted into farmland causing conflict with the lssa tribe, lack of water and the invasion of the grazing land by *Prosopis juliflora* are problems encountered by pastoralists. The Buremudaitu district range is dominated by wetland. The conflict with the lssas tribe and the flood water has restricted the movement of the pastoralists in Buremunaitu to a narrow fringe of moist grassland surrounding the swamps; on the other hand, the Gewane district has the Awash River flood plain and Gewane plain as the major ecological zones. The former consists of bush grassland, swamps and marshy spots, where as the latter is reasonably well covered with grass vegetation. The major part of Gewane plain is not accessible to the Afar community due to the conflict with the lssa tribe.

The majority of the population is working in search of better grazing and watering site for their livestock (Figure 9). The climate of the area is normally hot and dry. The rainfall is between July and September and a short rain is during March and April. The total annual rainfall of the areas ranges from 663.7 to 687.8 mm. The mean maximum and minimum temperature ranges between 33.2°C to 42.8°C, and 19.6°C to 26.7^{0}C, respectively and the average humidity is around 50.4 % to 52%.

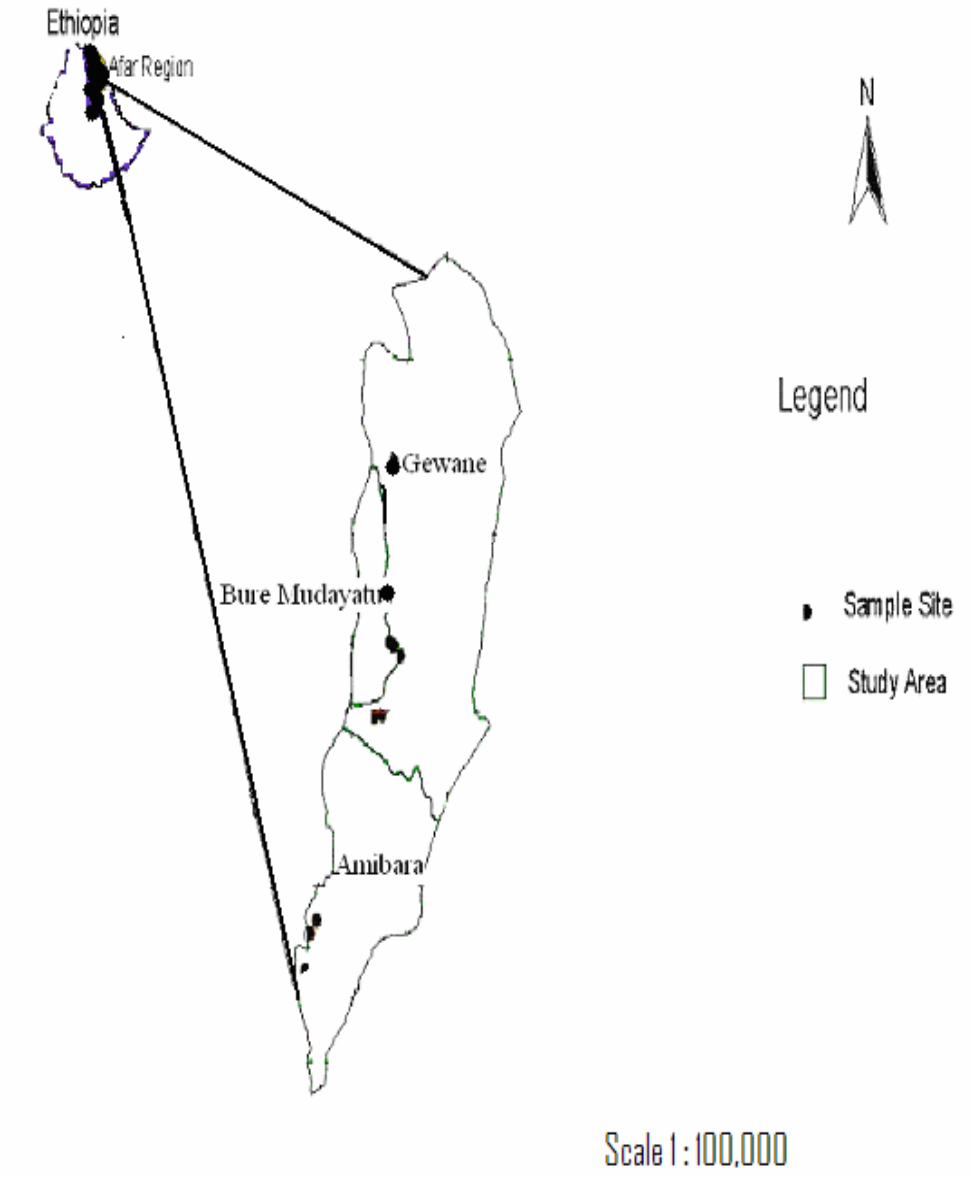

Figure 7 Map of survey areas of Middle Awash River Basin, Afar Regional State, Ethiopia

2. Study animals and sampling collection

A total of 3,697 fecal samples were taken from 9 selected sites of the three districts: 3,465 from Afar and 232 from Blackhead local sheep breeds. Two thousand

eight hundred and forty five samples were taken from females and 852 were taken from males. Age groups were classified as adult, young and lamb.

Using a plastic glove, five grams of fresh fecal samples were collected from the rectum or during defecation. The samples were kept in plastic bags, well labeled, and placed in an icebox, until used. Coproscopic examinations were performed to detect *Fasciola* eggs using the standard sedimentation method according to Garcia (1997) and Tiber (1999). The age of the animals was recorded by interviewing stockowners and using dental formula (Gatenby, 1991) for analysis. The study sites were randomly selected and the distribution of samples was the proportion of one herd per one study site and all sheep in the herd were sampled. Samples were collected based on the seasonal basis.

Figure 8 Fecal sample collection activities in Gewane district site

The seasons are traditional classified as: Kerma (July-September) as the long rainy season, Sugum (March-April) as the short rainy season, Hagai (May-June) as the hot dry spell and Gilal (October-February) as the cool season. Gilal is sometimes interrupted by raining in January and February and is known as Dedda. Totally in the region the livestock movement depends on seasonal pattern (Figure 9).

Body condition scoring of animals was conducted during sample collection according to the method described by Thompson and Meyer (1994).

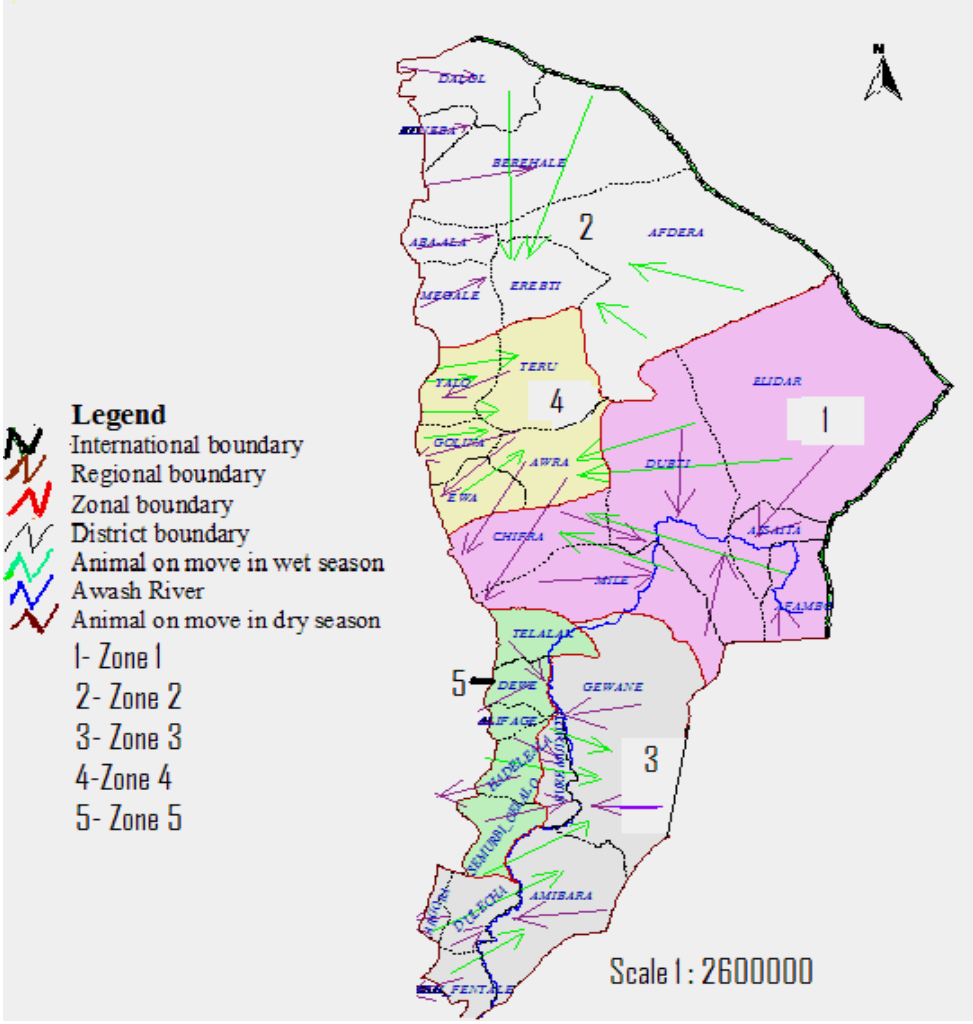

Figure 9 The livestock movement of Afar Region in dry and wet seasons

Source: - APEDCB, 2005

3. Design and data analysis

The nested design was employed to determine factors affecting the infection rate. The Chi- square was used to test the goodness of fit for the infection rates among different locations under study. Infection rates based on age and the seasonal variations on the prevalence of fascioliasis were analyzed by the Pearson's correlation coefficient (Putt *et. al.*, 1988).

Experiment 2: Natural *Fasciola spp*. Infections in Local Breeds of Sheep in Middle Awash River Basin, Afar, Ethiopia

1. Description of the study area

This work was carried out from March to July 2005 in Gewane Agricultural Technical and Vocational Education Training College, Gewane district, Ethiopia. Gewane district is an endemic area for ovine fascioliasis with a hot and semi-aired climate. The location of the study area is 10°9´59 N latitude and 40° 8´43 E longitude. The college is 344 kms away from Addis Ababa. The altitude of the area is 560 meters above the sea level.

The majority of the populations in the area lead a pastoral way of life searching for better grazing and watering sites for their livestock. During the rainy season the animals are grazing freely on the open range and when the dry season starts, the animals are moved to swampy and low-laying areas near the Awash River path.

2. A brief description sheep breeds used in the experiment

2.1. The Afar Sheep

The sheep in the study area are of indigenous type namely Afar and Blackhead. The Afar sheep are fat tailed with a fat broad base. The gestation period of ewes is about 5 months. The age at the first lambing varies from 8- 12 months and the lambing interval ranges from 6- 12 months. The Afar type of sheep is considered to be a good producer of meat and milk. The average daily milk yield of a ewe is about 770 milliliters. The milk is used for household consumption and the skin is used as grain sack, sleeping mats, cushion pads protecting the animal's backs, and women's knees while grinding corn. Sheep are also sources of cash income and mutton is needed for religious ceremonies, circumcision, burial, visitors, famine, and women giving birth (Zeleke, 1997).

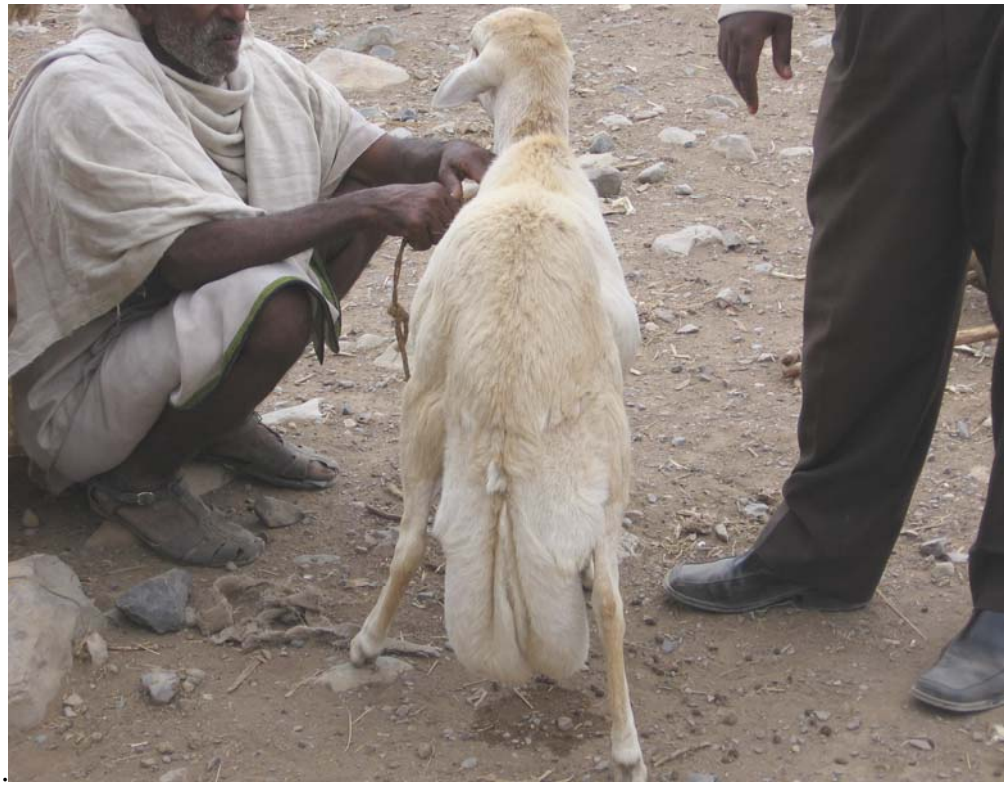

Figure 10 Afar sheep

2.2. The Blackhead sheep

The fat rumped, Blackhead sheep is indigenous to Somali and Northern Kenya. The average weight is of 30 kg. It has black and white in color with the head being black, fat deposit in the rump and dewlap and it is seldom horned. The important features of these animals are that they can withstand harsh environmental conditions, but are poor milk producers. They are sometimes milked (Pratt and Gwynne, 1977). A survey result in the Ogaden plateau also indicated that lamb crop is once per year and most of the ewes (96%) give birth to a single lamb (Girma, 1999). Other reports also showed that the twinning rate in the Blackhead sheep varies from 1 to 10% but the rate most frequently quoted is 4 to 5%. The age at first lambing varies from 16-18 months of age.

Figure 11 Blackhead sheep

3. Animal management

The study was carried out on two local breeds of sheep (Afar and Blackhead) and sex response naturally acquired infected with fascioliasis. Twelve each of Afar and Blackhead sheep, aged between 5-6 months were selected, and each breed was divided into two sex groups of 6 (Table 3). Prior to the treatment they were housed for 15 acclimation days. The acclimation period of time was given for the adaptation to weather conditions. During this period body weights were measured, fecal samples and blood samples were also examined to confirm that they were free from ovine fascioliasis. All animals were ear tagged, and housed separately in pens at night. The sheep were released to graze in infected pasture with fascioliasis for 5 months between March to July 2005 when the sheep started to use intensively previously flooded areas of pasture that are considered to be highly risk for fascioliasis. All the

sheep were allowed to graze on native communal grazing land for 8 hours (8:00 AM up to 4: 00 PM) per day. Water was freely provided from natural sources.

Figure 12 Experimental animals grazing near Awash River in Gewane site

The sheep were vaccinated against the common infectious diseases of the area like Anthrax and Pasteurellosis with the vaccine obtained from the National Veterinary Institute, Debre Zeit, Ethiopia. Spraying of sheep of acaricides against ectoparasites was performed throughout the study period regularly every month with Diazinol 60% E.C. (Kafr El Zayat Pesticides and Chemical Co., Egypt). The susceptibility to infections with F*asciola spp.* was evaluated based on pre and post treatments fecal and blood parameters examination.

4. Meteorological data

The meteorological data of Gewane district were obtained from the Ethiopian Metrological Services Agency. The climate of the area is normally hot and dry. The rainfall is between July and September and a short rain is during March and April. The mean maximum annual temperature reaches 40 ^{o}C and the mean minimum temperature is 19.4 ^{o}C (Figure 13) and the total annual rainfall is 663.7 mm (Figure 14). Mean average relative humidity is 52% (Figure 15).

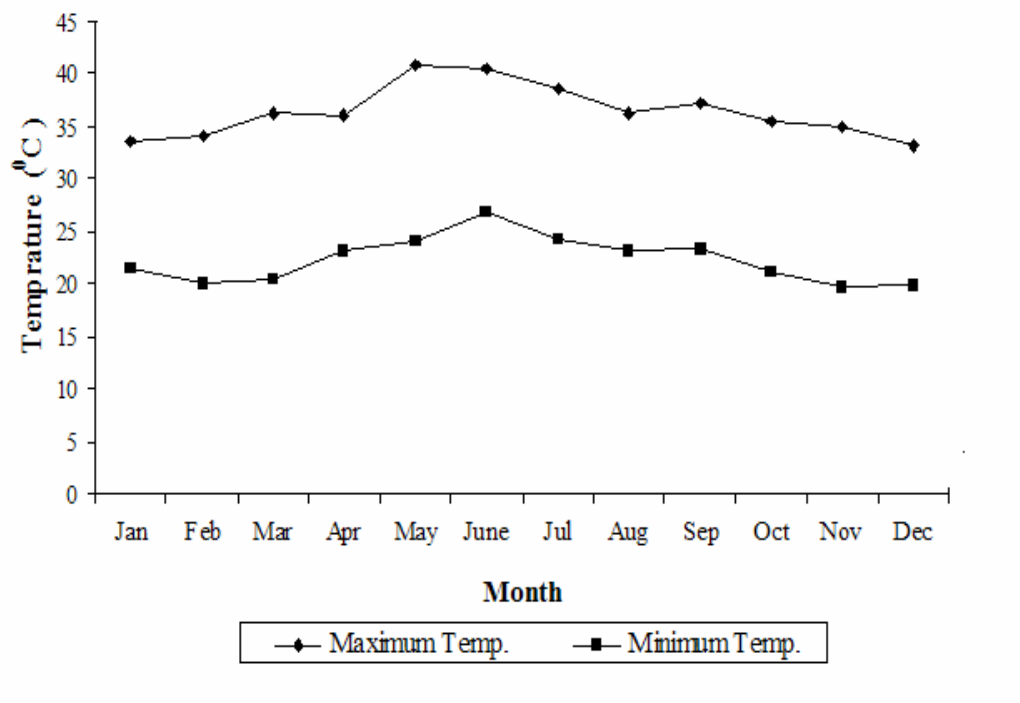

Figure 13 Total monthly mean temperature at Gewane district

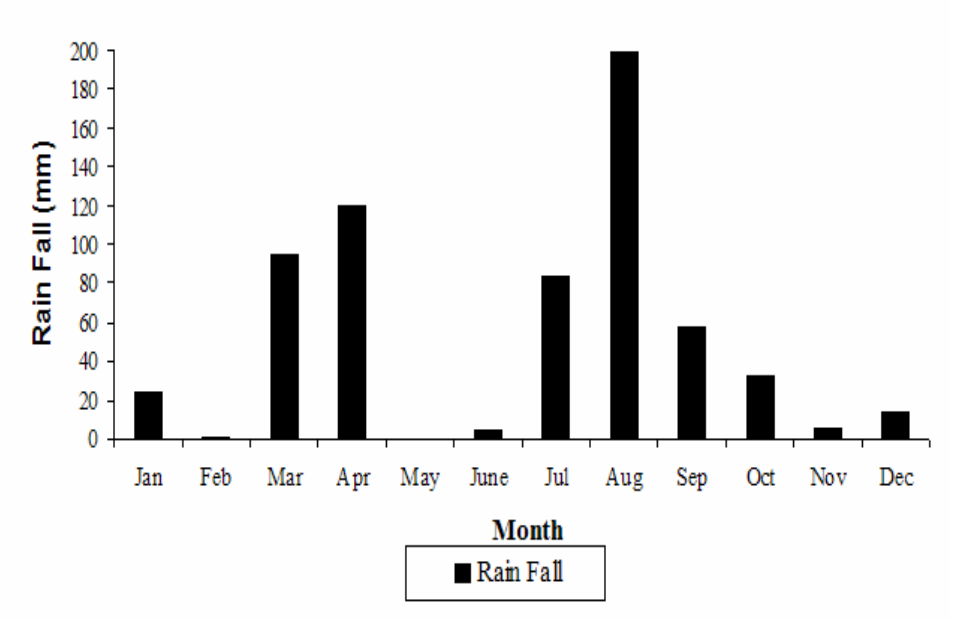

Figure 14 Total monthly mean rainfall at Gewane district

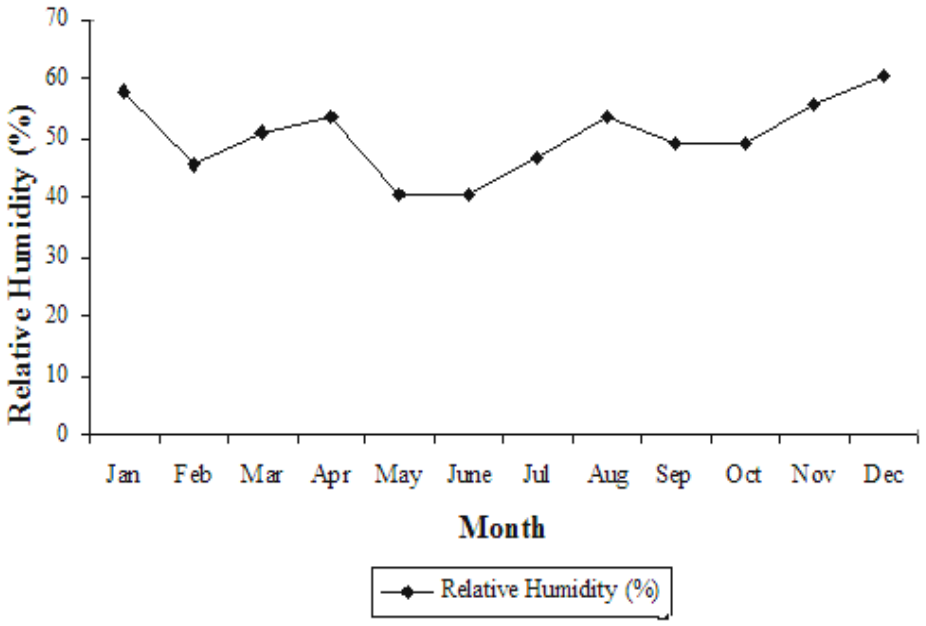

Figure 15 Total mean average relative humidity (%) at Gewane district

5. Detection of fasciola egg in feces

Fecal samples were taken directly from the rectum and also during defecation every two weeks. Fecal Egg counts (FEC) were performed using standard sedimentation methods (Garcia, 1997; Tiber, 1999). For the detection of fecal egg count per gram of feces, was determined according to the following equations:

$$\text{FEC} = \text{Amount of egg counted} \times \text{Consistency correlation factor} \times 100$$

Where consistency value was given for a form of stool, (1= normal stool and hard; 2 = soft, 3 = moisture, 4 = diarrhea and 5 = watery)

6. Blood sampling and hematological analyses

The information gained from the blood parameters would substantiate the physical examination and when coupled with medical history, it would provide an excellent basis for medical judgment (Schalm *et al.,* 1975).

Blood samples which were collected from each animal were taken into 10 ml test tubes containing ethylene diamine tetraacetic acid (EDTA) by jugular vein puncture at two week intervals. The packed cell volume (PCV) was determined by microhematocrit method Jain (1993) and blood was further centrifuged at 12,000 rpm for 10 minutes to remove the erythrocytes and the plasma was collected. The following parameters namely, RBC count was determined using haemocytometer (Benjamin, 1961) and also the hemoglobin (Hb) concentration was determined in the form of cyanmethaemoglobin (Kraus and Ganther, 1980).Total protein (TP) concentration in plasma was determined (Josephson *et al*., 1957) by using commercial kits supplied from Life Science Dynamic Division, Arnaparn Co.Ltd. Nonthaburi, Thailand.

7. Weight gain

Animals' weight gain performance in the study was aimed to assess the effect of treatments on monthly live weight. Each animal was weighed in the morning every week, in order to minimize mass fluctuation due to eating or drinking, and before they were released to graze. Animals were weighed using a spring scale, and each sheep was hanged on a strap in attached to the hook of a spring scale and then weighed.

Table 3 Experimental layout with 2 treatment combinations on field trials

Type of breeds	Type of managements	Sex groups		Number of animals
		Male	Female	
Afar	Expose animals to contaminated pasture	6	6	12
Blackhead	Expose animals to contaminated pasture	6	6	12

8. Data analysis

The differences between treatment groups for different parameters were tested using SAS procedures for the following statistical model. A 2x2 (two breeds and two sexes) factorial experiment in complete randomized design (CRD) with 6 heads was used for each sub group. The model to be used was:

Model $Y_{ijk} = \mu + B_j + S_i + (BxS)_{ij} + e_{ijk}$

Where as, Y_{ijk} = Response variable

μ = Overall mean

B_j = Effect of j_{th} breed (j=1-2)

S_i = Effect of i_{th} sex (i =1-2)

$(BxS)_{ij}$= Effect of i_{th} sex of j_{th} breed, and

e_{ijk} = Error term ~ NID (o,σ_e^2)

Experiment 3: Anthelminthic Efficacy of Chili (*Capsicum annuum longum*) and Nutritional Supplementation on Ovine Fascioliasis in Middle Awash River Basin, Afar, Ethiopia

1. Description of the study area

The study was conducted from January to March 2006 in Gewane Agricultural Technical and Vocational Education Training College, Gewane district, Ethiopia. Gewane district is an endemic area for ovine fascioliasis with a hot and semi-aired climate. The location of the study area is $10^{o}9´59$N latitude and $40^{o}8´43$E longitude. The college is 344 km away from Addis Ababa. The altitude of the area is 560 meters above the sea level.

2. Animal management and supplementation

Seventy two local breeds of sheep (Afar and Blackhead) equally through naturally infected with fascioliasis, were collected from different sites with age above two years and weights between 19 and 30 kg. Prior to the treatment they were housed for 15 acclimation days. During this period, animals were weighed, fecal and blood samples were examined. During the experimental period all animals were housed separately in the pens at night while at feeding time; they were kept together on the same pasture. Water, from natural sources was provided *ad libitum*. The presence of fasciola was confirmed by pre-examination and all sheep were vaccinated against the common infectious diseases of the area lsuch as Anthrax, Pasteurellosis and Sheep Pox with the vaccine obtained from the National Veterinary Institute, Debre Zeit, Ethiopia. Spraying of acaricides against ectoparasites was applied regularly every month with Diazinol 60% E.C. (Kafr El Zayat Pesticides and Chemical Co., Egypt) throughout the study period.

The concentrate supplementation was provided for daily dry matter intake (DMI), crude protein (CP) (g) and metabolizable energy (ME) (MJ/kgDM) requirements of the lambs per head estimated according to (McDonald *et al.,* 1988) of

615g, 113g and 6.25MJ, respectively. It was assumed that about 52% of the total DM (318g), CP (25g), and ME (2.8MJ) were obtained from grazing, while the rest were provided from mixed concentrate supplementation. Composition of the concentrate was wheat middling (50%), cotton seed cake (30%), corn (15%), lime (4%) and common salt (1%). The concentrate was analyzed to contain 20.2 % CP and 11.8 MJ/kgDM, metabolized energy. All sheep were allowed to graze on native grasses (Appendix 7) for 8 hours (8:00 AM up to 4:00 PM) per day. The efficacies of the treatments were evaluated based on pre and post treatments evaluation of fecal, hematological and biochemical examinations, as well as post mortem findings on slaughtered animals.

3. Fecal examination

Five grams of fecal samples were collected from the rectum using a plastic glove. The efficacies of the Tricalbendazole treatment and chili with or without supplement were evaluated by measuring egg shedding. Fecal samples were collected monthly. For the detection of fecal egg count (FEC) per gram, the sedimentation method according to Tiber (1999) and Garcia (1997) was used. FEC was determined according to the following equations:

FEC= Amount of egg counted $\times$ consistency correlation factor $\times 100$

Where consistency value was given for a form of stool, (1 = normal stool and hard; 2 = soft, 3 = moisture, 4 = diarrhea and 5 = watery)

4. Evaluation of the efficacy

The efficacy or the percentage or de-parasitism was extrapolated according to the method used by Carvier (1973). The formula was as follows.

$$\textbf{Efficacy}\ (\%) = \frac{(N-n)\times 100}{N}$$

Where N = average fecal egg count (FEC) in negative control animals

n = average FEC in treated animals

5. Percentage reduction in FEC

The percentage reduction in fecal helminthes egg output following treatment administration was calculated for the test group and control group. The formula was as follows.

$$\text{Reduction \%} = \frac{(Mean\ FEC\ day_0 - Mean\ FEC\ day_X) \times 100}{Mean\ FEC\ day_0}$$

Where; day $_0$ = before administrating treatments and Day $_X$ = the days post treatment administration.

6. Chili, preparation and administration

For the treatment of infected *Fasciola spp.* of experimental sheep, one hundred grams of crushed or chopped crude mixture of chili (*Capsicum annuum longum*) was mixed with 200 ml water so that the crude mixture of the solution could be delivered through a 300 ml plastic container and administrated orally on day 1,21, and 42.

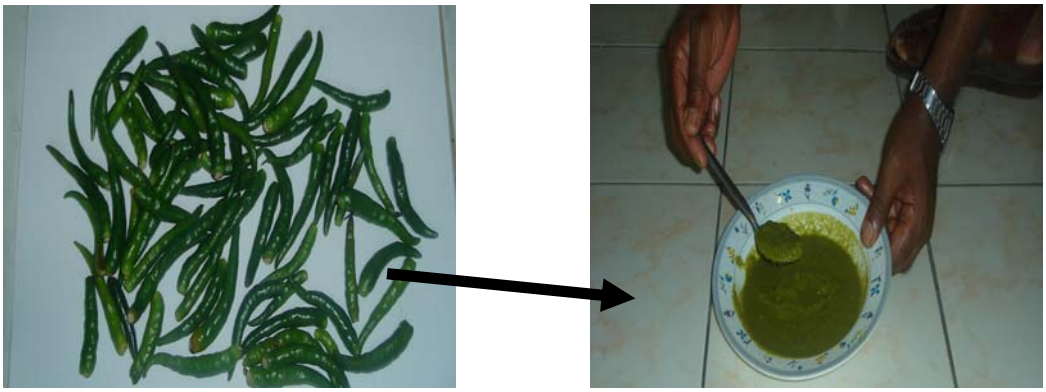

Figure 16 Chili preparations for orally drenching treatment of *Fasciola spp*. infection.

7. Blood sample collection

From the commencement of the experiment and monthly thereafter, 10 ml

blood samples were collected through the jugular vein into vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Red blood cell (RBC) count was determined using haemocytometer (Benjamin, 1961) and the Packed Cell Volume (PCV %) was determined by the micro-haematocrit centrifugation technique as described by Jain (1993). The hemoglobin concentration was measured in the form of cyanmethaemoglobin (Kraus and Ganther, 1980) and total protein (TP) in plasma was determined (Josephson *et al*., 1957) by using commercial kits supplied from Life Science Dynamic Division, Arnaparn, and Nonthaburi, Thailand.

8. Weight gain

The animals were weighed once a month in the morning before they were released to graze and water was offered. Each sheep was hanged on a strap in attached to the hook of a spring scale and then weighed.

9. Experimental protocol

Animals were allotted to 12 treatments with 6 animals each. The treatment combination were formed from 3 treatments (Tricalbendazole, chili (*Capsicum annuum longum*) and no treatment), 2 breeds (Afar and Blackhead), and 2 feeding systems (supplement and no supplement). The treatment combinations are as illustrated in Table 4.

T1: twelve sheep from two breeds were treated with a commercial preparation of Tricalbendazole (Fasinox-250, East African pharmaceuticals, Addis Ababa, Ethiopia) in an oral suspension form given at a dose of 10 mg/kg body weight on day 1, 21, and 42. This group was allowed to graze native grass only.

T2: twelve sheep from two breeds were treated with the same type and dose of Tricalbendazole. This group was allowed to graze native grass with nutritional supplementation.

T3: twelve sheep from two breeds were treated with traditional treatment by giving orally 100gm crushed or chopped of local chili (*Capsicum annuum longum*) mixed with 200 ml water for each sheep and this group was allowed to graze native grass only.

T4: twelve sheep from two breeds were treated in the same way with chili treatment dose and was allowed to graze native grass with nutritional supplementation.

T5: twelve sheep from two breeds were served as a control group with out any treatment and was allowed to graze native grass only.

T6: twelve sheep from two breeds were also a control group without any treatment but was allowed to graze native grass with nutritional supplementation.

Table 4 Experimental groups with 3 treatment combinations

Treatment	Feeding system	Breed	Number of animals	Total No. of animals
T(Tricalbendazole)	Grazing (G)	Afar (A)	6	12
		Blackhead(B)	6	
	Grazing (G) and Supplement (S)	Afar (A)	6	12
		Blackhead(B)	6	
C (Chili)	Grazing (G)	Afar (A)	6	12
		Blackhead(B)	6	
	Grazing (G) and Supplement (S)	Afar (A)	6	12
		Blackhead(B)	6	
N (No treatment or control)	Grazing (G)	Afar (A)	6	12
		Blackhead(B)	6	
	Grazing (G) and Supplement (S)	Afar (A)	6	12
		Blackhead(B)	6	

Note: Treatment (T =Tricalbendazole), C= Chili, N = No treatment), Feeding system S= Supplement, G = grazing only, breed A = Afar B = Blackhead).

10. Data analysis

The differences between treatment groups for different parameters were tested using a three way factorial analysis of variance (ANOVA). That is (3 x 2 x 2) factorial in complete randomized design (CRD). The complete model is given below. Differences between means were tested using Duncan's Multiple Range test (Steel and Torrie, 1980).

Model $Y_{ijkl} = \mu + T_i + B_k + F_j + (TxB)_{ik} (TxF)_{ij} + (BxF)_{kj} + (TxFxB)_{ijk} + e_{ijkl}$

Where, Yijkl = Response variable

μ = Overall mean

T_i = Effect of i_{th} treatment (i =1 to 3)

B_k = effect of K_{th} breed (k = 1 to 2)

F_j = Effect of j_{th} feeding system (j =1 to 2)

$(TxB)_{ik} (TxF)_{ij} + (BxF)_{kj} + (TxFxB)_{ijk}$ = interaction effects, and

e_{ijkl} = Error term ~ NID (o,σ_e^2)

11. Post mortem examination

Post mortem examination on animals was carried out soon after they were slaughtered at the end of experiment. A total of 36 sheep (three from each treatment group) were randomly selected for slaughtering and examination of all internal organs for abnormalities, with particular attention to the size, weight, color, and appearance of the livers were conducted to determine the effects of treatments. During the examination, the whole liver is cut with a sharp knife into slices approximately 1cm thick, and soaked in saline or water, and then each slices was squeezed or pressed until adult and juvenile *Fasciola spp*. was released. The gall bladder was also being opened, washed and any flukes were removed.

After soaking, the liver slices were again squeezed, then rinsed in clean water and discarded. Both washings were filtrated through a fine sieve (aperture 100 μm). The flukes were transferred to a petridish in a shallow layer of saline for closer inspection and counting. Counts were carried out microscopically.

RESULTS AND DISCUSSION

Experiment 1: Epidemiological Survey on Prevalence of *Fasciola spp.* Infections of Sheep in Middle Awash River Basin, Afar, Ethiopia

Comprehensive knowledge of parasite ecology is crucial to their sustainable control because parasites interact differently with hosts in specific climatic, management and production environments (Almeria and Uriate, 1999; Waller, 1999; Papadopoulos *et al.*, 2003). The result indicated that exposure of domestic sheep to *Fasciola spp.* infections in Middle Awash River Basin in Afar National Regional Sate was common with an overall prevalence of 13.2%. The results agreed with the report of Graber (1975) who noted that fascioliasis was rare in the Rift Valley areas, Ethiopia. Michael *et al. (*2005) have reported the prevalence rates of 56.3% for ovine fascioliasis in Upper Awash River Basin. The variations might be due to the different agro-ecological condition, the traditional pasture management, the pattern of movement of the animals from grazing near water-logged and agricultural irrigation practices during the rainy season.

Several factors were associated with a higher prevalence of liver fluke infection. Geographically, the *Fasciola spp.* prevalence in the three districts ranged from 11.3 % in Bure Mudaytu, which was lower than 17.4% in Amibera ($p<0.001$). The high incidence of *Fasciola spp.* infections was recorded at Awash Sheleko (18.8%) of Amibera, Debel (13.1 %) of Bure Mudaytu and Gebaya Bora (13.7%) of Gewane district (Table 5). The variation in prevalence among the different locations was likely to be due to the presence of the landscape such as swampy areas, the agricultural irrigation and management practices.

Table 5 Prevalence of ovine fascioliasis in 3 districts and sites of Middle Awash River Basin Afar Region

Factors	Category	Number of examined	Number of positive (%)	Prevalence (%)	P-Value
District	Gewane	1,712	223	(13.0)[b]	
	Bure Mudaytu	1,296	146	(11.3)[b]	P<0.001
	Amibera	689	120	(17.4)[a]	
Sites	Gewane				
	Egele	721	95	(13.2)	
	Gebaya Bora	482	66	(13.7)	P>0.05
	Galela Dora	509	62	(12.2)	
	Bure Mudaytu				
	Debel	659	86	(13.1)[a]	
	Beadafore	411	41	(9.9)[b]	P<0.001
	Gelalu	226	19	(8.4)[b]	
	Amibera				
	Aleysumalie	297	50	(16.8)	
	Ambash	174	29	(16.7)	P>0.05
	Awash Sheleko	218	41	(18.8)	

*Prevalence results within the same column and grouping followed by the same letters superscript are not different.

There was an insignificantly higher prevalence of *Fasciola spp.* infections among Afar breed (13.5%) than Blackhead (9.1%) sheep (p> 0.05). Differences in *Fasciola spp.* infection between sheep breeds were similarly as the previous report (Pralomkarn *et al*., 1997). In Ethiopia, sheep are usually reared under non-intensive conditions whereby animals may be brought out to graze and wander. The prevalence of *Fasciola spp.* among male sheep (13.4 %) was higher than among females (13.2%), but this difference was not statistically significant (Table 6). Solomon (2005) has suggested that fascioliasis equally affected both sexes. However, in this study, higher prevalence of parasitic infection was not associated with sex (p>0.05) and, although

not statistically significant, males actually had a higher infection prevalence than females. This might be due to the fact that all the animals were also grazing similar pasture land.

Table 6 Prevalence of ovine fascioliasis on breed and sex basis

Factors	Category	Number of examined	Number of positive (%)	Prevalence (%)	P-Value
Breed	Afar	3,465	468	(13.5)	P>0.05
	Blackhead	232	21	(9.1)	
Sex	Male	852	114	(13.4)	P>0.05
	Female	2,845	375	(13.2)	

Higher *Fasciola spp.* infection prevalence (p< 0.001) was observed among sheep >2 year (14.9 %) than sheep 1-2 years (10.9 %) or < 1 years old (8.1 %). Young animals had a lower prevalence of *Fasciola spp.* infections in this study (Table 7). This finding was consistent with other reports, and it was not surprising because native kids had maternal immunity. A higher infection rate was recorded in adults than in other age groups (P<0.001). Based on this result, it may be concluded that the higher exposure risk of adults may be due to physiological differences such as stress, pregnancy, lambing, inadequate nutrition and infectious diseases. Similar results were reported by Ayalew (1994).

Table 7 Prevalence of ovine fascioliasis on age grouped basis

Factors	Category	Number of examined	Number of positive (%)	Prevalence (%)	P-Value
Age	<1 year	444	36	$(8.1)^{b}$	
	1-2 year	819	89	$(10.9)^{b}$	P<0.001
	>2 year	2,434	364	$(14.9)^{a}$	

*Prevalence results within the same column and grouping followed by the same letters superscript are not different.

Climate conditions, particularly rainfall, were frequently associated with differences in the prevalence of *Fasciola spp.* infection because this was suitable for intermediate hosts like snails to reproduce and enabled the eggs to survive longer under moist conditions. During the rainy season the volume of rainfall alone does not seem to play a decisive role in the study site. The flood of the Awash River creates favorable conditions and favors the development of the intermediate host (snail) and the transmission of the diseases. Similar findings and assertions were reported by Graber (1975), Michael *et al.* (2005) and Solomon (2005).

Seasonal prevalence of ovine fascioliasis in the study areas was observed during the cool season (6.9 %), followed by a short rainy period (4.1%). During the hot season, most animals were returned from wet grazing areas to the farms near the Awash River. Animals were possibly infected at this time. From October, the prevalence of the disease increased and clinical signs were observed. During the rainy season, animals moved from place to place in search of grazing. As a result of this, the prevalence was relatively low (Table 8).

The prevalence of ovine fascioliasis in animals of different body conditions demonstrated that animals with poor body condition were greater infected than animals with good body condition ($p<0.001$). Hunter (1953) observed that a well-fed animal was not in trouble with worms and usually a poor diet resulted in more helminthes infections. Furthermore, helminthes also led to a loss of appetite and poor utilization of food, resulting in loss of body weight. Hawkins and Morris (1978) demonstrated that weekly growth rates of wool and live weight decreased with the increasing fluke burdens in sheep.

Table 8 Prevalence of ovine fascioliasis on season and body condition basis

Factors	Category	Number of examined	Number of positive (%)	Prevalence (%)	P-Value
Season	Cool dry	951	257	(6.9)[a]	
	Short rainy	825	153	(4.1)[b]	P<0.001
	Hot dry	933	49	(1.3)[c]	
	Main rainy	988	30	(0.8)[c]	
Body condition	Poor	2,306	450	(19.5)[a]	P<0.001
	Good	1,391	39	(2.8)[b]	

*Prevalence results within the same column and grouping followed by the same letters superscript are not different.

The study indicated that sheep in Middle Awash River Basin were usually infected with *Fasciola spp.* parasites. Economic evaluations consistently show that major losses due to parasitism affect animal production rather than mortality and in Middle Awash River Basin parasitism could influence productivity, morbidity and mortality of these animals (Githigia *et al.*, 2001). Parasite-nutrient interactions are probably exacerbated by the effects of poor nutrition and management practices, leading to decreased efficiency in feed utilization. The intensity of infection is reportedly related to available intermediate hosts, thus better snail control and separate grazing for different age groups would likely reduce the infection rate and the prevalence of fascioliasis among sheep in Ethiopia.

During the survey it was observed that other endoparasites like nematode, cestodes and other trematodes were evident together with fascioliasis of which Strongyle, Trichris, Oesophagostomum, Monezia, Strongyloides, Paramphistomum and lungworm were the predominant ones. The existence of these parasites together with fascioliasis greatly affects the body condition of the animals. The intensity of infection for different breed, sex and age groups is presented.

In Table 9, 10 and 11 as indicated that mixed infection incidence was detected more than single infection and this indicated that helminthes represent a major ovine health problem for sheep in Middle Awash River basin. The incidence of mixed infections with fascioliasis was 57.3% and 42.9% in Afar and Blackhead breed sheep, respectively. The prevalence of mixed infections according to sex groups was 59.7 and 55.7% in male and female, respectively. In both sex groups mixed infection were higher than single infections. In 1- 2 year and above 2 years age groups were also higher than < 1 year age group. The incidence of mixed infection according to age group 50%, 58.4% and 57.1% for lamb, young and adult age grouped animals, respectively.

Table 9 Frequency distribution and incidence of *Fasciola spp*. infection associated with other parasites by breed recovered during fecal examination.

Name of parasite spp.	Breed			
	Afar		Blackhead	
	No.	Incidence (%)	No.	Incidence (%)
Total number of positive specimen	468		21	
Fasciola spp.	200	42.74	12	57.14
Total number of mixed infestation	268	57.26	9	42.86
Fasciola and Strongyles spp.	50	10.68	2	9.52
Fasciola and *Monezia*	40	8.55	1	4.76
Fasciola and *Teania spp*.	25	5.34	1	4.76
Fasciola and *Paramphistomum*	23	4.91	-	-
Fasciola and *Trichuris*	22	4.70	1	4.76
Fasciola, Strongyles and *Trichuris*	25	5.34	1	4.76
Fasciola,Paramphistomum and *Monezia*	14	2.99	1	4.76
Fasciola, Strongyles and *Monezia*	25	5.34	1	4.76
Fasciola,Trichuris and *Teania spp.*	25	5.34	-	-
Fasciola,Paramphistomum,Trichuris and Strongyles	4	0.85	-	-
Fasciola, Strongyles, *Teania spp.* and *Monezia*	5	1.07	-	-
Fasciola, Strongyles,*Teania spp*., and *Trichuris*	7	1.50	1	4.76
Fasciola, Strongyles, *Monezia, Paramphistomum* and *Trichuris*	2	0.43	-	-
Fasciola, Strongyles, *Teania spp*., *Monezia* and *Paramphistomum*	1	0.21	-	-

Table 10 Frequency distribution and incidence of *Fasciola spp*. infection associated with other parasites by sex groups recovered during fecal examination

Name of parasite spp.	Sex group			
	Female		Male	
	No.	Incidence (%)	No.	Incidence (%)
Total number of positive specimen	375		114	
Fasciola spp.	166	44.27	46	40.35
Total number of mixed infestation	209	55.74	68	59.65
Fasciola and Strongyles spp.	36	9.6	10	8.77
Fasciola and *Monezia*	29	7.73	9	7.89
Fasciola and *Teania spp*.	25	6.67	8	7.02
Fasciola and *Paramphistomum*	10	2.67	5	4.39
Fasciola and *Trichuris*	10	2.67	3	2.63
Fasciola, Strongyles and *Trichuris*	31	8.27	8	7.02
Fasciola,Paramphistomum and *Monezia*	21	5.6	9	7.89
Fasciola, Strongyles and *Monezia*	21	5.6	7	6.14
Fasciola,Trichuris and *Teania spp.*	10	2.67	5	4.39
Fasciola,Paramphistomum,Trichuris and Strongyles	4	1.07	1	0.88
Fasciola, Strongyles, *Teania spp.* and *Monezia*	5	1.33	2	1.75
Fasciola, Strongyles,*Teania spp*., and *Trichuris*	4	1.07	1	0.88
Fasciola, Strongyles, *Monezia, Paramphistomum* and *Trichuris*	1	0.27	-	-
Fasciola, Strongyles, *Teania spp*., *Monezia* and *Paramphistomum*	2	0.53	-	-

Table 11 Frequency distribution and incidence of *Fasciola spp*. infection associated with other parasites by age groups recovered during fecal examination

Name of parasite spp.	Age group					
	<1 year		1-2 years		>2 years	
	No.	Incidence (%)	No.	Incidence (%)	No.	Incidence (%)
Total number of positive specimen	36		89		364	
Fasciola spp.	18	50.00	37	41.57	157	43.13
Total number of mixed infestation	18	50.00	52	58.39	207	57.13
Fasciola and Strongyles spp.	2	5.56	7	7.87	41	11.26
Fasciola and *Monezia*	1	2.78	4	4.49	27	7.41
Fasciola and *Teania spp*.	3	8.33	6	6.74	21	5.76
Fasciola and *Paramphistomum*	2	5.56	5	5.61	19	5.22
Fasciola and *Trichuris*	2	5.56	6	6.74	20	5.49
Fasciola, Strongyles and *Trichuris*	2	5.56	5	5.61	21	5.76
Fasciola,Paramphistomum and *Monezia*	2	5.56	5	5.61	15	4.12
Fasciola, Strongyles and *Monezia*	1	2.78	6	6.74	14	3.85
Fasciola,Trichuris and *Teania spp.*	3	8.33	3	3.37	13	3.57
Fasciola,Paramphistomum,Trichuris and Strongyles	-	-	1	1.12	4	1.10
Fasciola, Strongyles, *Teania spp.* and *Monezia*	-	-	3	3.37	2	0.55
Fasciola, Strongyles,*Teania spp*., and *Trichuris*	-	-	1	1.12	6	1.65
Fasciola, Strongyles, *Monezia*, *Paramphistomum* and *Trichuris*	-	-	-	-	3	0.82
Fasciola, Strongyles, *Teania spp*., *Monezia* and *Paramphistomum*	-	-	-	-	1	0.27

Experiment 2: Natural Fasciola Infections in Local Breeds of Sheep in Middle Awash River Basin, Afar, Ethiopia

The success of parasitic infection depends on the host and parasite interaction, and various factors may influence the relationship (Waruiru *et al.*, 2000). The most important aspects of the host that may affect it are breed, age and sex. Estimation of the prevalence of fascioliasis has classically been done by coprological analysis.

During the rainy season the volume of rainfall alone does not seem to play a decisive role in the study site. The flood of the Awash River creates favorable conditions and favors the development of the intermediate host (snail) and the transmission of the diseases. Similar findings and assertions were reported by Graber (1975), Michael *et al.* (2005) and Solomon (2005). Irrigation based agricultural practice and swampy areas were very important ecologies for the continuity of the life cycle of fascioliasis.

Fasciola spp. infections overall prevalence were detected at 54.2%. The prevalence within breeds was 37.5% and 33.3% for Blackhead breed and Afar breeds, respectively. The prevalence of sex groups were 61.5% and 38.5% for female and male sex groups, respectively. Nevertheless the prevalence of *Fasciola spp*. infection was not significantly influenced by breed and sex.

The means of different parameters are presented in Table 12. The lower result in fecal egg count (FEC) per gram was obtained in the Afar breed sheep and male sex group (Figure 17 and 18). The blood parameters which were analyzed were PCV, RBC, TP and Hb concentration. The effect of both breeds was not significant ($p>0.05$) and breed traits were analyzed, but for some animals after infection the result indicated that they were significantly lower ($p<0.05$) in all parameters. There was not a significant interaction of breeds by sex.

The overall estimates of PCV, RBC, TP, Hb, FEC and daily weight gain were for Afar breed slightly higher than Blackhead breed sheep after the exposure of the

animals to the contaminated pasture. The results indicated that the Afar breed in the study areas had slightly higher resistance to *Fasciola spp*. infections than Blackhead breed sheep. This result might be due to the better adaptation to the area by the Afar breed sheep than the Blackhead sheep breed. The result coincides with Asegde (1990) that he compared four breeds of indigenous Ethiopian sheep for their resistance to endoparasites (predominantly *Haemonchus contortus)* at Awassa in Southern Ethiopia. The breeds evaluated were Afar and Blackhead originating from semi-arid and lowland regions of Ethiopia, Horro and Arsi from the humid Highlands. The result indicated that the Blackhead Somali breed were the most susceptible to endoparasites while Arsi breed were the most resistant.

There were no significant ($p>0.05$) sex differences for FEC, PCV, RBC, TP and Hb but difference in weight, in the female sex group was significantly different ($p<0.05$) lower than the male sex group. The values of blood parameters were not significantly ($P>0.05$) but higher in females than males (Table 12). These findings were consistent with Schalm *et al.*, 1975; Jain, 1993 and Coles, 1986.The higher results of FEC were also recorded in the female group after infection compared to the male sheep. In weight gains although Afar sheep had higher gains than Blackhead but it was lower in female sheep after infection ($p<0.05$).

At the end of the experiment, Afar breed maintained a significantly higher ($p<0.05$) PCV than Blackhead sheep; however, the PCV response in the two breeds was significantly ($p<0.05$) different in the infected sheep compared to the non infected sheep at the end of the experiment. It has been suggested that the higher PCV in Afar VS Blackhead sheep may be due to the fact that Afar sheep were better adapted to the area of this location. Similar results were reported in comparing Menz and Horo sheep breeds in Debere Brhan station (Rege *et al.*, 1996). The findings have demonstrated the preliminary results of combining the two diagnostic methods of the FEC and the blood parameter examination in establishing the influence of breed and sex naturally acquired on ovine fascioliasis. Any apparent influence was not found, so further studies would be very important in this area.

Table 12 Effects of *Fasciola spp*. infections to breed and sex on different parameters

Factors	Wt.gain(g)	FEC (g)	RBC ($x10^6$µl)	PCV (%)	TP (g %)	Hb (g/dl)
Afar male	20.3 ± 2.6[a]	66.7 ± 81.5[a]	7.3 ± 1.1[a]	26.8 ± 0.9[a]	63.0 ±12.2[a]	9.3 ± 4.1[a]
Afar female	15.6 ± 2.4[a]	66.8 ± 103.3[a]	7.8 ± 2.1[a]	28.2 ± 1.0[a]	65.7±12.4[a]	9.7 ± 5.6[a]
Blackhead male	17.6 ± 2.7[a]	116.7 ± 160.2[a]	7.1 ± 1.0[a]	23.0 ± 0.9[a]	58.1 ±14.8[a]	8.7 ± 4.7[a]
Blackhead female	13.8 ± 3.7[b]	200 ± 126.5[a]	7.4 ± 1.2[a]	25.2 ± 0.9[a]	58.2 ± 9.7[a]	7.5 ± 4.2[a]

*Means in the same column and grouping followed by different superscripts are statistically different ($p<0.05$).

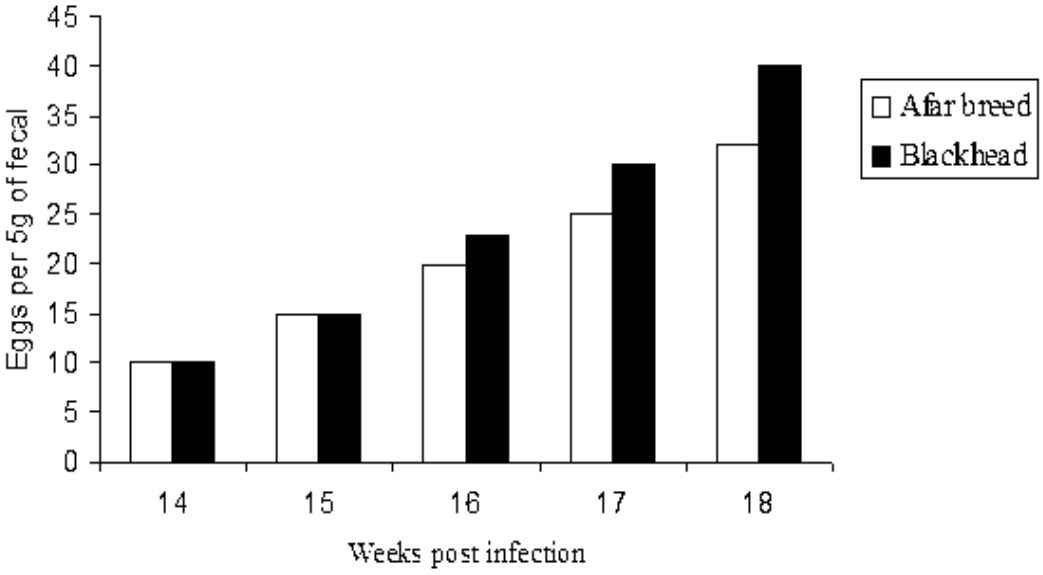

Figure 17 Mean values of FEC between Afar and Blackhead breeds

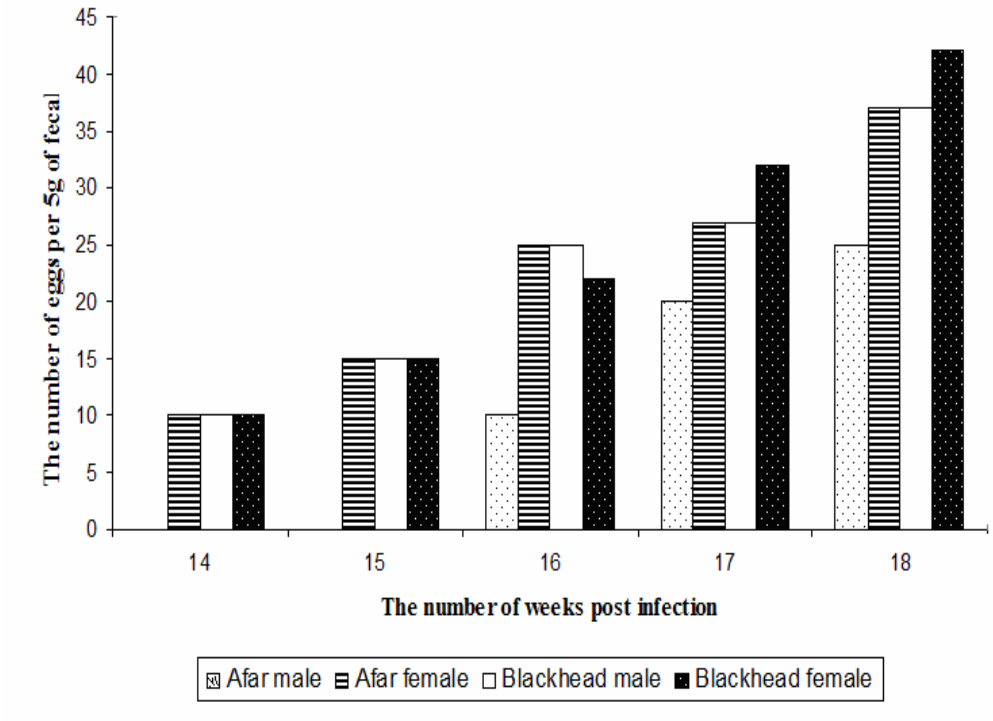

Figure 18 Mean values of FEC between Afar, Blackhead breeds and sex group

Experiment 3: Anthelminthic Efficacy of Chili (*Capsicum annuum longum*) and Nutritional Supplementation on Ovine Fascioliasis in Middle Awash River Basin, Afar, Ethiopia

The average live weight gain by anthelmintic treated groups were the highest followed by chili treated animals. The control groups (not treated) were the groups with the lowest live weight gains. The results have demonstrated that there was significant difference in weight gain of animals treated with anthelmintic, and this is supported by previous reported research (Scott *et al.,* 1974). The weights of the animals were compared to prior treatment had shown significant improvements during the experiment: the animals were well managed and the treatment did not affect the health of the experimental animals. The weight changes in infected animals were found to depend upon two variables: the fluke burden and the quality of the diet available to the host. Njau *et.al* (1990) also reported that nutrition played a key role in efficient parasite control.

Chili treatment could be used to treat infected sheep under subclinical infection of ovine fascioliasis. This was consistent with supplements and had a significant effect on treatment. Implementing the control of helminthes without adequate management of host nutrition caused weight loss or morbidity and masked the advantages of the treatment.

The overall result of the present study demonstrated that animals received treatments of T (tricalbendazole) and chili were found to have reduced fecal egg count per gram, increases in PCV, RBC, Hb, TP and live weight gain (LWT) measurement as compared with those animals that received no treatments ($P<0.05$). No differences were observed in LWT, FEC, RBC, TP and Hb ($P>0.05$) while there were significantly differences among breeds in PCV ($P<0.05$). These results are in agreement with the result of Jain (1993) who reported that several factors such as age, sex, breed, health, physiological status, nutrition and other factors may influence the normal blood values of various species.

Results in regard to the hematological and biochemical evaluation effect of the different levels of treatments on blood parameters are shown in Table 13. There were significant differences among treated animals in PCV ($P<0.05$) the values which were contrary to what was reported by Davis *et al.* (1999) that in which he obtained no difference in treated goats. The RBC, PCV, Hb and TP concentration revealed significant differences among supplementation and treated levels ($P<0.05$). The TP result among the supplement group were higher than the non supplement group but were not significantly different ($P>0.05$). The PCV was highest (36.9 %) in sheep with supplement and treatment and was significantly different from those of non-supplement in non-treated animals. Oyedipe *et al.* (1984) observed that animals fed with good quality rations had higher values of RBC, PCV, TP and Hb concentration, which was in line with the current study.

Differences in those treated with supplement in infected animals were observed between the two breeds ($P<0.05$). The Afar breed had slightly higher PCV value ($p>0.05$) than The Blackhead breed. PCV was used as an indicator of anemia; the more anemic the animal, the lower the PCV. Animals with PCV less than 24% were considered to be anemic (Kelly, 1974). The PCV of Afar and Blackhead sheep were 36.1 and 34.2 %, respectively.

At the end of the experiment, no important clinical sign was observed in treated animals. However, in the control group, 4 sheep had easily observed visible anemic mucus membranes, progressive emaciation, and two animals revealed mandibular edema from day 60 to 90. No mortality was found during the experimental period.

Table 13 Effects of breed, supplement and different treatment options on body daily weight gain, fecal and blood parameters

Factors	Daily weight gain (g)	FEC (g)	RBC ($x10^6$µl)	PCV (%)	TP (g %)	Hb (g/dl)
Breed						
Afar	36.7 ± 4.2[a]	219.6±27.5 [a]	8.7±0.1[a]	36.1± 0.4[a]	59.7±1.2[a]	7.1± 0.2[a]
Blackhead	43.9 ± 4.2[a]	222.6±27.5[a]	8.7±0.1[a]	34.2± 0.4[b]	58.8±1.2[a]	7.6± 0.2[a]
Feeding						
Supplement	59.3± 4.6[a]	146.7±27.5[b]	9.1±0.1[a]	36.9± 0.4[a]	60.6±1.2[a]	8.6± 0.2[a]
No supplement	21.4±4.6[b]	295.5±27.5[a]	8.3±0.1[b]	33.4± 0.4[b]	57.9±1.2[a]	6.1± 0.2[b]
Treatment						
Fasinox	56.2 ± 5.7[a]	0.2 ±33.7[a]	9.1±0.2[a]	38.3± 0.5[a]	66.4±1.4[a]	8.9± 0.3[a]
Chili	43.0± 5.7[a]	183.3±33.7[b]	8.8±0.2[a]	34.9± 0.5[b]	60.5±1.4[b]	7.2± 0.3[b]
No treatment	21.7± 5.7 [b]	479.8±33.7[c]	8.9±0.2[b]	32.3± 0.5[c]	50.8±1.4[c]	5.9± 0.3[c]

[abc] means in the same column within the same group with different superscripts are different ($p<0.05$).

The result of the efficacy of treatment and the percentage reduction in fecal helminthes egg compared to negative control animals shown in tables 14 and 15, respectively. Tricalbendazole with the supplement treated group of animals recorded a fecal egg count of zero. This result indicated that this treatment was more efficacious than other treatment groups ($P<0.05$). The highest treatment efficiency was observed on days 60 and 90 in Tricalbendazole with the supplement treated group of animals. This was due to the treatment by Tricalbendazole in the fields using a frequent dose of treatment. It was found that all of them had 100% efficacy. Results for this study were concur with the finding of Richards *et al.,* (1990) who obtained 99.0 - 100% efficacy of anthelminthic treatment of the experimental animals.

The chili with supplement treated group result of efficacy of treatment and percentage reduction in fecal helminthes egg was 33-40% at 30 days post treatment. However, during 60 and 90 days post treatment results observed 69.2 and 83.8 %, respectively: the reduction of *Fasciola* eggs were 42-43%, 71-72 and 85-86% at 30,

60 and 90 days post treatment, respectively. Chili treated animals without supplement were 16-26.5% efficacious after 30 days, and then rose to 31-33 and 46.2 %, respectively after 60 and 90 days. The reductions of fecal eggs count were 33.3 % after 30 days, but increased to 38-42 and 56%, respectively, after 60 and 90 days post treatment. The results of control animals are also shown in Table 15.

Table 14 The efficacy (%) of treatment tricalbendazole, chili and nutritional supplement in experimental animals

Factor combination	Pre-treatment FEC (g)	Days post treatment		
		30	60	90
TSA	740	40 (93.3)	0 (100)	0 (100)
TSB	750	20 (97.1)	0 (100)	0 (100)
TGA	700	100(83.3)	50 (92.3)	0 (100)
TGB	700	100(85.3)	40 (94.1)	0 (100)
CSA	700	400(33.3)	200 (69.2)	100 (83.8)
CSB	720	410(39.7)	200 (70.6)	100 (83.9)
CGA	750	500(16.7)	433.3 (33.3)	333.3 (46.2)
CGB	750	500(26.5)	463.3 (31.9)	333.3 (46.2)
NSA	700	500(16.7)	350 (46.2)	330 (46.2)
NSB	750	500(26.5)	380 (44.1)	350 (43.6)

Note: Treatment (T = Tricalbendazole, C = Chili, N = No treatment); Feeding system (S = Supplement, G = grazing only); Breed (A = Afar, B = Blackhead)

* For calculation use the control groups of treatment (NGA and NGB) results in Table 4.

Table 15 Result of fecal egg count reduction (%) in each treatment group

Factor combination	Pre treatment FEC (g)	Days post treatment		
		30	60	90
TSA	740	40 (94.6)	0 (100)	0 (100)
TSB	750	20 (97.3)	0 (100)	0 (100)
TGA	700	100(85.7)	50 (92.9)	0 (100)
TGB	700	100(85.7)	40 (94.3)	0 (100)
CSA	700	400(42.9)	200 (71.4)	100 (85.7)
CSB	720	410(43.1)	200 (72.2)	100 (86.1)
CGA	750	500(33.3)	433.3(42.2)	333.3(55.6)
CGB	750	500(33.3)	463.3(38.2)	333.3(55.6)
NSA	700	500(28.6)	350 (50.0)	330 (52.9)
NSB	750	500(33.3)	380 (49.3)	350 (53.3)
NGA	750	600(20.0)	650 (13.3)	620 (17.3)
NGB	740	680 (8.2)	680 (8.2)	620 (16.2)

Note: Treatment (T = Tricalbendazole, C = Chili, N = No treatment); Feeding system S = Supplemented, G = grazing only); Breed (A = Afar, B = Blackhead)

During post mortem examination, different parasites spp. were observed. The most common and easily detectable parasite species like *D. filaria*, *Paramphistomum spp*. *Heamonchus contrortus*, T*richuris spp.* and *Moniezia spp*. were involved. The relative abundance of the whole parasite species involved was not determined but the adult fluke of *Fasciola spp.* had been determined and recorded. In the Tricalbendazole treated group, no liver flukes were recovered but the highest number of flukes was recovered in the control groups (Figure 19). Liver fluke data recovered from the slaughtered animals are presented in Table 16.

Table 16 Numbers of recovered *Fasciola spp*. total number in each experimental group by post mortem examination

Group of treatments	Fluke recovered			
	Small	Large	Total	Mean + SD
TSA	0	0	0	0.00 ± 0.00^{a}
TSB	0	0	0	0.00 ± 0.00^{a}
TGA	0	0	0	0.00 ± 0.00^{a}
TGB	0	0	0	0.00 ± 0.00^{a}
CSA	2	1	3	1.00 ± 1.00^{c}
CSB	1	1	2	0.67 ± 0.58^{b}
CGA	4	3	7	2.33 ± 0.58^{e}
CGB	4	2	6	2.00 ± 0.00^{d}
NSA	6	5	11	3.67 ± 1.15^{g}
NSB	5	4	9	3.00 ± 1.00^{f}
NGA	16	9	25	8.33 ± 0.58^{h}
NGB	20	9	29	9.67 ± 0.58^{i}

^{abcdefghi} means in the same column with different superscripts are different ($p<0.05$).

Figure 19 Post mortem finding of sub acute fascioliasis case with massive damage liver tissue in sheep

CONCLUSIONS AND RECOMMENDATIONS

In the survey study, all infected sheep harbored mixed helminthes of *Nematodes, Cestodes and Trematodes spp*. This result indicated that helminthes are affecting the health and productivity of sheep in the Middle Awash River Basin. The study revealed that the three districts are in the Middle Awash River Basin and are mostly endemic area for ovine fascioliasis with an overall prevalence of 13.23% recorded. Differences in the prevalence of parasitic infections among district sites were probably due to differences in management systems. A strategic control program must be launched to prevent the pile up of parasites in the environment and to avoid heavy contamination of the pasture by fecal eggs. Side by side strategic anthelmintics treatment with appropriate flukicidal drugs should be performed two times a year and should be done after the end of the rainy season (October) and at the end of during short rainy season (April). Traditional Pasture management (e.g. on restriction of animals early grazing during the starting rainy season around the Awash River) should be encouraged. Awareness must be encouraged among stockowners regarding fasioliasis. Moreover, it is recommended that further investigation should be undertaken regarding the epidemiology of fascioliasis, the biology and the ecology of the intermediate host (snail).

Study on natural infection of *Fasciola spp*. is being undertaken, and current efforts targeted at the identification and future exploitation of local sheep breeds' resistance to fascioliasis should help to minimize the reliance on frequent treatment with anthelmintics and the cost of investment. The findings have demonstrated the preliminary results of combining the two diagnostic methods of the FEC and the blood parameter examination revealed that the prevalence of *Fasciola spp*. infection was not significantly influenced by breed and sex. Further studies on epidemiology and between breed variations in susceptibility to infections are necessary because such variations in body resistance acting as immuno-competence may be of fundamental importance in epidemiology and also the management of the various breeds of sheep**.**

The study, anthelminthic efficacy of chili *(Capsicum annuum longum*) on ovine fascioliasis, has clearly demonstrated that chili with supplementation might be an appropriate alternative drug such as medicinal plant in the treatment of fascioliasis for animal owners due to its affordable cost and availability. This may be of particular importance to endemic areas of fascioliasis in general in Ethiopian lowlands, where there is an available growing chili and where other treatment costs are relatively high. While these studies are being undertaken, current efforts targeted at the investigation of an optional treatment of fascioliasis should help minimize the reliance on frequent treatment with anthelmintics. Anthelmintic drugs remain the most widely used parasitic control method. However parasite resistance is a serious problem in many areas so it would be necessary to minimize the emergent resistance and to look for alternative approaches to parasite control. Further study should be conducted on the anthelmintic efficacy of different varieties of chili spp. and compared with other traditional anthelmintics treatments for treatments of ovine fascioliasis.

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APPENDIX

Appendix 1 ANOVA tables for natural *Fasciola spp*. infection experiment

1.1 ANOVA table for weight gain.

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	31.0537	31.0537	3.64	0.0709
B = Sex	1	108.8004	108.8004	12.75	0.0019
Ax B	1	1.2604	1.2604	0.15	0.7048
Error	20	170.6816	8.5340		
Corrected total	23	311.7962			

R^2 = 0.4525 CV =17.3500

1.2 ANOVA table for fecal egg count.

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	50416.6666	50416.6666	3.42	0.0793
B = Sex	1	10416.6667	10416.6667	0.71	0.4106
Ax B	1	10416.6667	10416.6667	0.71	0.4106
Error	20	295000.0000	14750.0000		
Corrected total	23	366250.0000			

R^2 = 0.1945 CV = 107.9552

1.3 ANOVA table for Red Blood Cell Count (RBC).

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	0.6337	0.6337	0.32	0.5769
B = Sex	1	0.9204	0.9204	0.47	0.5021
Ax B	1	0.0504	0.0504	0.03	0.8745
Error	20	39.3916	1.9695		
Corrected total	23	40.9962			

R^2 = 0.0391 CV =19.0617

1.4 ANOVA table for Packed Cell Volume (PCV).

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	68.3437	68.3437	2.24	0.1499
B = Sex	1	17.5104	17.5104	0.57	0.4574
Ax B	1	0.8437	0.8437	0.03	0.8695
Error	20	609.7083	30.4854		
Corrected total	23	696.4062			

R^2 = 0.1244 CV = 21.3902

1.5 ANOVA table for Total Protein (TP).

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	231.5709	231.5709	1.51	0.2338
B = Sex	1	12.3697	12.3697	0.08	0.7795
Ax B	1	9.9459	9.9459	0.06	0.8018
Error	20	3072.9814	153.6490		
Corrected total	23	3326.8679			

R^2 = 0.0763 CV = 20.2358

1.6 ANOVA table for Hemoglobin (Hb).

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	12.0842	12.0842	0.55	0.4656
B = Sex	1	0.9322	0.9322	0.04	0.8384
Ax B	1	4.2757	4.2757	0.20	0.6629
Error	20	436.7113	21.8355		
Corrected total	23	454.0034			

R^2 = 0.0380 CV = 53.0378

Appendix 2 ANOVA tables for experimental optional treatment of ovine fascioliasis.

2.1 ANOVA table for fecal egg count.

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	161.441	161.441	0.01	0.9389
B = Treatment	2	2809462.690	1404731.345	51.52	<.0001
C= Feed	1	398155.428	398155.428	14.60	0.0003
Ax B	2	371.612	185.806	0.01	0.9932
AxC	1	151.120	151.120	0.01	0.9409
BxC	2	232755.375	116377.687	4.27	0.0186
AxBxC	2	349.448	174.724	0.01	0.9936
Error	59	1608679.906	27265.761		
Corrected total	71	5064711.111			

R^2 = 0.6823 CV = 74.6789

2.2 ANOVA table for average daily weight gain.

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	937.4450	937.4450	1.22	0.2729
B = Treatment	2	14615.4268	7307.7134	9.55	0.0003
C= Feed	1	25881.9168	25881.9168	33.81	<.0001
Ax B	2	640.2265	320.1132	0.42	0.6601
AxC	1	4.1184	4.1184	0.01	0.9418
BxC	2	1237.6158	618.8079	0.81	0.4504
AxBxC	2	832.0590	416.0295	0.54	0.5836
Error	59	45928.8889	765.4814		
Corrected total	71	90077.6973			

R^2 = 0.4901 CV = 68.6354

2.3 ANOVA table for Red Blood Cell Count (RBC).

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	0.0035	0.0035	0.01	0.9359
B = Treatment	2	7.9577	3.9788	7.23	0.0016
C= Feed	1	10.4439	10.4439	18.97	<.0001
Ax B	2	3.2935	1.6467	2.99	0.0579
AxC	1	0.1902	0.1902	0.35	0.5588
BxC	2	2.6793	1.3396	2.43	0.0965
AxBxC	2	1.7093	0.8546	1.55	0.2202
Error	59	32.4755	0.5504		
Corrected total	71	58.8927			

R^2 = 0.4485 CV = 8.5087

2.4 ANOVA table for Packed Cell Volume (PCV).

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	71.0122	71.0122	13.07	0.0006
B = Treatment	2	447.9694	223.9847	41.22	<.0001
C= Feed	1	219.8118	219.8118	40.45	<.0001
Ax B	2	56.9833	28.4916	5.24	0.0080
AxC	1	6.9993	6.9993	1.29	0.2610
BxC	2	26.1441	13.0720	2.41	0.0990
AxBxC	2	16.1542	8.0771	1.49	0.2345
Error	59	320.5820	5.4335		
Corrected total	71	1167.0165			

$R^2 = 0.7252$ CV = 6.6329

2.5 ANOVA table for Total Protein (TP).

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	13.7817	13.7817	0.28	0.5968
B = Treatment	2	2977.3989	1488.6994	30.57	<.0001
C= Feed	1	124.7984	124.7984	2.56	0.1148
Ax B	2	56.8172	28.4086	0.58	0.5613
AxC	1	3.2120	3.2120	0.07	0.7982
BxC	2	150.0319	75.0159	1.54	0.2228
AxBxC	2	33.0389	16.5194	0.34	0.7137
Error	59	2873.6467	48.7058		
Corrected total	71	6249.5801			

$R^2 = 0.5401$ CV = 11.7857

2. 6 ANOVA table for Hemoglobin (Hb).

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	5.5289	5.5289	3.27	0.0759
B = Treatment	2	103.2525	51.6263	30.49	<.0001
C= Feed	1	104.0853	104.0853	61.47	<.0001
Ax B	2	2.5889	1.2944	0.76	0.4702
AxC	1	10.2903	10.2903	6.08	0.0166
BxC	2	29.6406	14.8203	8.75	0.0005
AxBxC	2	0.1831	0.0915	0.05	0.9474
Error	59	99.9085	1.6933		
Corrected total	71	365.8572			

R^2 = 0.7269 CV = 17.7220

2.7 ANOVA table for post mortem examination.

Source	DF	SS	MS	F Value	Pr>F
A = Breed	1	0.0000	0.0000	0.00	1.0000
B = Treatment	2	248.2222	124.1111	319.14	<.0001
C= Feed	1	49.0000	49.0000	126.00	<.0001
Ax B	2	0.6666	0.3333	0.86	0.4370
AxC	1	1.0000	1.0000	2.57	0.1219
BxC	2	52.6666	26.3333	67.71	<.0001
AxBxC	2	2.0000	1.0000	2.57	0.0973
Error	24	9.3333	0.3888		
Corrected total	35	362.8888			

R^2 = 0.9742 CV = 24.4021

Appendix 3 The major animal diseases which are suspected to occur in the Afar National Region are listed below.

Type of diseases origen	Type of the diseases
Virus	Peste des petits ruminants (PPR), Sheep Pox, Rabies
Bacteria	Anthrax, Pasteurellosis, Brucellosis, and Streptothricosis
Protozoa	Babesiosis and Anaplasmosis
Helminthes	Nematodes, Cestodes, Trematodes and Lungworms
External parasites	Mangemites, Ticks, Myiasis and Biting flies

Source: Zeleke (1997)

Appendix 4 The composition of the mixed concentrate (Supplementation)

Ingredient	Composition (%)	Crude protein (CP) (g).	Dry matter metabolizable energy (ME) (MJ/kgDM),
Wheat middling	50	8.85	1230
Cotton seed cake	30	10	798
Corn	15	1.34	549
Lime	4	-	-
Common salt	1	-	-
Total	100	20.19	2577

Source: - Kaliti Feed Processing Enterprise, 2005.

Appendix 5 Body condition scoring (BSC) of sheep

Rank	Condition score type	Description of the condition	Grouping
Condition score 1	Emaciated	Spinous processes are sharp and prominent. Loin eye muscle is shallow with no fat cover.	Poor
Condition score 2	Thin	Spinous processes are sharp and prominent. Loin eye muscle has little fat cover but is full.	
Condition score 3	Average	Spinous processes are smooth and rounded and one can feel individual processes only with pressure.	Good
Condition score 4	Fat	Spinous processes can be detected only with pressure as a hard line.	Very good
Condition score 5	Very fat	Spinous processes can not be detected. Fat cover dense.	

Source: - Thompson and Meyer, 1994.

Appendix 6 Record sheet for survey study

District---------- Survey site----------

Owner of the sheep ----------Sample No.----------

Sample unit No.---------- Examined date----------

Owner of the herd	Breed	Sex	Age group	Body condition	Diagnosis

Appendix 7 Types of vegetation in Gewane district

Scientific Name	Afar-Vernacular Name
GRAMINEAE	---------
Aristida funiculata Trin& Rupr	Halbaito
Brachiaria eruciformis (J.E. sm,)	Mussa
Chrysopogon plumulosus Hochst	Durfu
Cenchrus ciliarris L.	Hantadi
Cynodon dactylon pers.	Irareyta
Cymbopogon pospischilii (K. schum)	Issusu
Dactyloctenium aegyptium beauv	Afar-amule
Eragrostis sp.	Denhito
Erarosstis cylindriflora Hochst.	Denhito
Lintonia nutans Stamf.	Afar-anole
Panicum coioratum (L.)	Randa
Pennisetum stramineum peter	Aiso
Sporobolus ioclados Trin.	Aitadoita
Tetrapogon cenchriformis A.rich	-----
CYPERACEAE	Godeyta
Cyperus obtusiflorus Vahl	-----
LEGUMINOSAE	Merkato
Acacia millifera (vahl)	Keselto
Acacia nilotia (L,) Del.	Gerento
Acacia oerfota/nubica Benth	Adado
Acacia senegal (L.) Willd	Eibeto
Acacia tortilis (Forsk.)	Eibeto
Acacia horrida L Willd	Elauto
Indigofera articulata Gouan	Eibeto
Prosopis Juliflora L	Weyane
Senna alexanderina Gouan Vahl	Sanu
ACANTHACEA	----

Appendix 7 (Continued)

Scientific Name	Afar-Vernacular Name
BALANITACEAE	----
Blepharis persica (Burm .fil)	Yamarukta
Balanites aegyptiaca L.Del.	Uddaito
CAPPARACEAE	----
Cadaba farinosa Forsk.	Dunibia
MALVACEAE	---
Abutilon fruticosum Goill & perr	Humbukto
RHAMNACEAE	---
Ziziphus spina christi	Kurta/Kusraito
SALVADORACEAE	---
Salvadora persica	Adaito
TILIACEAE	---
Grewia ferruginea Hochst	Hidaito
Grewia bicolor Juss	Adepto
TAMARICACEAE	---
Tamarix aphylla L.	Segento
TYPHACEAE	---
Typha sp.	Godeyta
ZYGOPHYLLAACEAE	--
Tribulus terrestris L.	Bunketo

SOURCE: - MCE (Metaferia Consulting Engineers), 2000

Appendix 8 Types of vegetation in Amibara district

Scientific Name	Afar – Vernacular Name
GRAMINEAE	---
Brachiaria eruciformis (J.E.sm)	Aiso
Botheochloa radicans Lehm.	Asaiso
Chrysopogon plumulosus Hochst	Durfu
Cynodon dactyion pers	Iraeyta
Cymbopogon pospischilii (k.schum)	Issusu
Cynodon plectosachyus	Sardoita
Dactyloctctenium aegyptium Beauv	Afar-amule
Enteropogon somalensis	Koref/subati
Eragrostis sp.	Denhito
Echinocloa haploclada stampf	Aiso
Hyparrhenia hirta (L.) Stampf	Denbehu
Lintonia nutans Stampt	Aiso
Sporobolus ioclados Trin	Hamilto
CYPERACEAE	----
Cyperus sp.	Godeyta
LEGUMINOSAE	----
Acacia etbaica Schweinf	---
Acacia seyal Del	Mekani /Hadgento
Acacia tortilis Forsk	Eibeto
Acacia nilotica (L.) Del	Keselto
Acacia senegal L. Willd.	Adado
Acacia oerfota/nubica Benth	Germoita/Gerento
Acacia mellifera Vahl	Merkato
Prosopis juliflora L	Weyane
BALANITACEAE	----
Balanites aegyptiaca L.Del	Uddaito
BORAGINACEAE	-----

Appendix 8 (Continued)

Scientific Name	Afar – Vernacular Name
Cordia sinensis Lam	Mederto
Salvadra persica L	Adaito
CAPPARACEAE	-----
Cadaba rotundifolia Forssk	Adangalita
Cadaba farinosa Forsk.	Adangalita
MALVACEAE	----
Abutilon fruticosum Gull&perr	Humbukto
RHAMNACEAE	----
Ziziphus spina christi (L.)Willd	Kurta/kusraito
SALVADORACEAE	----
Dobera glabra Forsk	Gorsa/Alaito
Grewia velutina Forsk	Oiea-yito
TAMARICACEAE	----
Tamarix aphylla (L.)	Segento
TLTLACEAE	----
Grewia ferres Hochst	Hidaito
ZYGOPHYLLACEAE	----
Tribulus terrestris (L,)	Bunketo

SOURCE: - MCE (Metaferia Consulting Engineers), 2000

Appendix 9 Types of vegetation in BuriMudayitu district

Scientific Name	Afar- Vernacular Name
GRAMINEAE	----
Cynodon dactylon pers.	Irareyta
Chryssopogon Plumulosus Hochst.	Durfu
Sporobolus iocldaos Trin	Hamilto
CYPERACEAE	----
Cyperus rotundus (L.)	Godeyta
LEGUMINOSAE	----
Acacia nilotica (L.) Del.	Keselto
Acacia tortilis (Forsk)	Hehebto
Prosopis juliflora	Weyane
Trifolium Spp.	Aculto
ASCLEPIADACEAE	-----
Calotropis procera Ait	Geleato
BURSERACEAE	-----
Commiphora africana	Mukkal
CAPPARACEAE	-----
Cadaba rotundifolia Forssk.	Adangalita / Angalita
MALVACEAE	-----
Abutilon fruticosum Guill & Perr	Humbukt**o**
SALVADORACEAE	-----
Salvadoro Persica	Adait**o**
TAMARICACEAE	-----
Tamaarix aphylla L.Kersten	Segento
ZYGOPHYLLACEAE	-----
Tribulus terrestris A. Rich.	Bunketa

SOURCE: - MCE (Metaferia Consulting Engineers), 2000

Appendix 10 Chemicals and reagents used during survey and experiments

Item	Expired date	Company
Ethyl acetate	April, 2007	Antibioticos S.P. A. Division CARLO ERBA reagent. Strada Rivoltana KM 6/7. Rodano. Italian
Formaldehyde 40% w/v	September, 2003	»
Sodium hydroxide	-	Merck KGaA, 84 271 Darmstadt, Germany.
Hemasol	December, 2006	Life Science Dynamic Division, Arnaparrn Co.Ltd. Nothaburi, Thailand.
Total color reagent	October, 2005	Life Science Dynamic Division, Arnaparrn Co.Ltd. Nonthaburi, Thailand.

Appendix 11 Drugs and vaccines used during survey and experiment

Item (drug or vaccine)	Route of administration	Dose	Expiry date	Country manufacturing
Fasionox - 250 (Tricalbendazole)	Oral	10mg/kg	April, 2005	East African Pharmaceuticals, PVT. Addis Ababa, Ethiopia.
Ashialben-300 (Benzimidazole)	Oral	7.5kg/kg	July, 2009	Ashish Life Science, PVT. Mumbai, India.
Anacomycin - 200 LA. (Oxyteteracycline base 200 mg/ml)	IM	1ml/10 kg/ body wt.	May, 2007	Anglian Nutrition products Co. Lady Lane, Hadlieigh, England.
Pendistrep 20/20 (Penicillin and Streptomycin)	IM	1ml/25 kg/body wt.	September, 2007	KELA N.V. Hoogstraten/ Belgium.
Ivermectin 1%	SC	1ml/ 50 kg/body wt.	July, 2008	Forguimica, Santiago Chili.
Diazinol 60% E.C.	Spray	0.1% (1ml/ live wt.	January, 2007	KAFR EL ZAYAT Pesticides and chemicals Co. Egypt.
Anthrax vaccine	SC	0.5 ml/ sheep	December, 2005	Debreziet, Ethiopia
Ovine Pasteurellosis vaccine	SC	1ml/sheep	December, 2005	Debreziet, Ethiopia

Appendix 12 Procedure - formalin-ethyl acetate method sedimentation technique

1. Assemble equipment and materials
2. Wash hands and put on gloves
3. Take a teaspoonful or 5 g of feces to the beaker.
4. Add 20 ml of normal saline and mix.
5. Strain the mixture through a wire sieve or two layers of cheesecloth.
6. Transfer 12 ml strained mixture into centrifuge tube.
7. Centrifuge for 5 min at 1,500- 2,000 rpm.
8. Discard the supernatant.
9. Add 10 ml of formalin and mix with sediment.
10. Add 2 ml of ethyl acetate to the mixture, screw tightly, and shake vertically.
11. Remove the stopper and centrifuge for 5 min at 1,500- 2000 rpm.
12. Ring around the plug and discharge all supernatant.
13. Mix the exist content; pipette 1-2 drops of content to glass slide.
12. Cover with cover slip and microscopic examine.

Appendix 13 Procedure- Stoll dilution-counting method

1. Assemble equipment and materials
2. Wash hands and put on gloves
3. Fill 56 ml of 0.1 N NaOH into Stoll flask and slowly fill the stool until solution shift to 60 ml Level.
4. Add 20 beads pellet, stopped the flask and shake vertically (every 10 min shaking).
5. After at least 1 hr, pipette 0.15 ml of the mixture and left to glass slide.
6. Place cover slip and microscopic examine (zig zag line examine).
7. By using the formula calculate the egg count estimation.

Egg per gram = Amount of egg counted X consistency correlation factor X 100

Where consistency value was given for a form of stool, (1= normal stool and hard; 2= soft, 3= moisture, 4= diarrhea and 5= watery)

Appendix 14 Procedure packed cell volume determination (PCV, haematocrit)

1. Take 5ml blood sample from ear vein into a test tube or vacationer containing anticoagulant (such as EDTA).
2. Mix the blood sample well but gently for 2 min
3. Draw the well-mixed blood up 2 capillary tubes for ¾ of its length.
4. Seal one end with sealant.
5. Place in the micro-haematocrit centrifuge, ensuring that the sealant is at the outer end.
6. Close the centrifuge lid.
7. Centrifuge the tubes at 12,000 rpm for 4 min
8. Place the tubes in the reader and note the reading.
9. Express the reading as a percentage of packed red cells in the total volume of whole blood.

Appendix 15 Procedure red blood cell counts

1. Assemble equipment and materials.
2. Place a clean hemacytometer coverglass over a clean hemacytometer.
3. Wash hands and put on gloves.
4. Collect 5ml blood from ear vein using vacutainer tube.
5. Mix the blood sample thoroughly before use.
6. Draw the blood to exactly the 0.5 mark (for routine counts)
7. Wipe the tip of the pipette clean of blood with a piece of cotton, gauze or filter paper without touching the opening of the capillary fluid.
8. Draw the diluting fluid steadily into the pipette by gentle mouth sanction to exactly the 101 mark past the bulb of the pipette, rotating the pipette on its long axis to ensure thorough mixing.
9. Immediately mix the contents of the pipette thoroughly by placing the thumb over one end and the first finger over the other end and shaking for minute.
10. Discard the first 3 or 4 drops of diluted blood from the pipette.
11. Fill both sides of the hemacytometer, avoid air bubbles and allow the mixture to settle in the chamber.
12. Place the hemacytometer on the microscope stage carefully and secure.

13. Use the power (10 x) objective to bring the ruled area into focus.
14. Locate the large central square
15. Rotate the high power (45x) objective into position carefully and focus with the fine adjustment knob until lines are clear. Adjust the light and / or condenser so that red blood cells are visible.
16. Count the cells in the four corner squares and one Center Square within the larger center squares of the counting area, using the left-to right, right-to-left counting pattern.
17. Record the results for each of the five squares (Four Corners and one center).
18. Repeat the count using the other side of the hemacytometer.
19. Use then worksheet to calculate the RBC count, the calculation
    RBC = Number cells X Number squares X Chamber depth X Dilution.
20. Record the result.

Appendix 16 Procedure hemoglobin determination

1. Assemble equipment and materials.
3. Turn on spectrophotometer.
4. Set wavelength at 540 nm
5. Label test tubes: blank, standard, and unknown (sample).
6. Dispense 5.0 ml of Hemasol reagent into blank and unknown tubes; dispense 5.0 ml hemoglobin standard into standard tube.
7. Mix the fresh blood sample for at least two minutes by hand or using mechanical mixer.
8. Draw up 0.02 ml (20 µl) of blood with micropipette.
9. Wipe excess blood from exterior of pipette with tissue.
10. Dispense blood sample into the unknown (sample) tube.
11. Mix contents of tube thoroughly and let stand for at least ten mins. (tubes can be mixed by inverting after placing laboratory stretch film over the top of the tube.
12. Transfer contents of the tubes to cuvettes. Place “blank” cuvette in the well of spectrophotometer; set absorbance to zero following manufacturers’ instructions.
13. Place “standard” cuvette in the well of spectrophotometer; read the absorbance and record results.

14. Place “sample” cuvette in the well of spectrophotometer; read the absorbance and record results.
15. By using the formula calculate hemoglobin concentration and record results.

$$Ct = Cs \times At / As$$

Where; Ct = Concentration of test (Unknown) g/100ml

Cs = Concentration of standard (14.36 g/100ml)

At = Absorbance of test (Unknown)

As = Absorbance of standard

16. Procedure for calibration curve with 3 points

step	Reagent	Point 1	Point 2	Point 3
1	Cyanmethemoglobin standard	2 ml	2 ml	4 ml
2	Hemasol	4 ml	2 ml	0 ml
	Hemoglobin concentration	4.79 g/100ml	7.18 g/100ml	14.36 g/100ml
	Absorbance measuring	As1	As 2	As 3

17. Mix well stands at room temperature 10 min before measuring at 600 nm against Hemasol as a reagent blank.
18. Prepare a calibration were by plotting absorbance and its corresponding concentration on a plain graph paper.
19. The calibration curve is linear up to 20 g/100 ml

Appendix 17 Test procedure for total protein determination

1. Q-TROL Level 1 and Q- TROL Level 2 after reconstituted each with deionized water exact 5 ml
2. Mix well by gently inverting till lyophilized serum complete dissolve.
3. Stand away from light at room temperature 10 min before use.
4. Mix equal volume of Q- TROL Level 1 and Q-TROL Level 2 in clean test tube. Both are ready for use as point 1 and point 2.
5. The concentration of the mixture is calculated by formula;

$$Concentration\ of\ the\ mixture\ (Po\text{int}\,2) = (Cs1 + Cs2) \times 1/2\ g/100\ ml$$

Where; Cs1= Protein concentration of Q_TROL Level 1

Cs2= Protein concentration of Q_TROL Level 2

6. Procedure calibration curve.

Step	Reagent	Point 1	Point 2	Point 3
1	TP COLOR REAGENT	4 ml	4 ml	4 ml
2	Q-TROL Level 1	0.1 ml		
3	Mixture of Level 1		0.1 ml	
4	Q-TROL Level 2			0.1 ml
Mix well. Stand for 10 minutes before measuring absorbance at 540 nm. Against TP COLOR REAGENT.				
4	Protein concentration	5.17 g/dl	6.11 g/dl	7.01 g/dl
5	Absorbance	As 1	As 2	As 3

7. Made calibration curve by plotting each absorbance and its corresponding concentration in a plain graph paper.
8. The linearity is up to 15 g/dl (Depending on type, condition and efficiency of colorimeter or spectrophotometer.

CURRICULUM VITAE

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4. Education

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