ADDIS ABABA UNIVERSITY

FACULTY OF VETERINARY MEDICINE

PREVALENCE AND PUBLIC HEALTH SIGNIFICANCE OF BOVINE FASCIOLIASIS IN SELECTED SITES OF NORTH GONDAR BORDERING LAKE TANA

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GETACHEW JEMBER BIZUNEH

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DEBRE ZEIT, ETHIOPIA

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

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Board of Examiners	Signature
1. Prof. Ph. Dorchies	______________________
2. Dr. Mohammed Abdella	______________________
3. Dr. Andy Catley	______________________

Academic Advisor	
1. Dr. Getachew Tilahun	______________________

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ABSTRACT

One thousand one hundred and seven local Zebu cattle in two Peasant Associations of Dembia wereda, North Gondar Administrative Zone bordering Lake Tana were examined from September, 2004 to February, 2005. The primary objective was to determine fascioliasis prevalence, characterize hepatic lesions and compare its relationship to parasite burden and faecal egg output, to see the seasonal fluctuation in snail density and magnitude of cerceriae shedding ones. In addition, retrospective data of hospital records were also studied to have a picture of the disease on its public health point of view. Prevalence was determined through coprological examination. For hepatic lesion studies in the abattoir and malacological studies on snails, standard procedures described in texts were implemented.

Prevalence was 79.1%. Among the potential risk factors considered, season and age were found to affect the occurrence of fascioliasis significantly ($p < 0.05$). The monthly prevalence of the disease was peak in the late rainy season (82%). Similarly, egg output (EPG) was significantly ($p<0.05$) influenced by season and age group where highest burden in the late rainy season and in animals aging between 1.5-5 years old.

Out of 200 total livers examined, 53(26.5%) were lightly, 71(35.5%) moderately and 76 (38%) severely affected. Low fluke count (60) was observed in severely affected livers than in moderately (96) affected ones. There is no significant association between fluke burden and intensity of hepatic lesion($r=0.020$, $p>0.05$) and also an inverse correlation between liver lesion and faecal egg output ($r=-0.04$, $p>0.05$).

All (100%) of the collected snails were *Lymnaea truncatula* 76% of the snails were found infected. The highest snail density and cerceriae shedding prevalence were obtained in the month of September. Comparison of density and infection proportion with the existing bionomic factors showed that there exists a strong functional relationship between them.

Retrospective data study of hospital records showed the lack of awareness about the disease but clinical symptoms and the epidemiology of the disease revealed its high public health significance in the study area. The results of the study are discussed in comparison with findings of workers in Ethiopia and abroad.

Key Words: **Bovine/ Prevalence/ Fascioliasis / Public Health/ Epidemiology/ North Gondar/ Dembia.**

1. INTRODUCTION

Ethiopia is one of the nations with highest population of livestock but production is far less than the potential. The rich potential from the livestock sector is not efficiently exploited due to several constraints, including malnutrition, traditional management and disease (Graber, 1978).

One of the main factors affecting the full exploitation of this resource is parasitic disease. Fascioliasis is known to be one of the most important parasitic diseases in Ethiopia that lowers production in ruminants, which are the natural hosts for Fasciola. Infestation is highest in cattle and sheep (Urquhart *et al*, 1996). Hence, the disease has significant impact in cattle and sheep causing lower milk yields and work power, poor growth rate and increased susceptibility to other diseases.

Fascioliasis is a parasitic disease caused by the genus Fasciola, which migrate through the hepatic parenchyma, establish and develop to the adult stage in the bile ducts. The two most important species are *F.hepatica* found in the temperateareas and cooler areas of high altitude in the tropics and subtropics and *F. gigantica,* which predominates in the tropical areas (Urquhart, *et al*, 1994). Snails of the genus Lymnae are mainly involved as intermidiate hosts in the life cycle of fascioliasis.

Fascioliasis occurs commonly as a chronic disease in cattle and the severity often depends on the nutritional status of the host (Graber, 1975). It is responsible for the wide spread morbidity and mortality especially in cattle and sheep characterized by weight loss, anemia and hypoproteinemia. It is also expressed in terms of liver condemnation at slaughterhouses, infertility, reduction in traction power and low weight at birth has been reported (Njau and Scholtens, 1991; Ngategize *et al*., 1993). Accurate assessment of the effects of Fasciola infestation is difficult due to concomitant nematode infestations in the field situation. It has been recognized that fascioliasis remains the largest single cause of condemnation of organs and carcasses followed by tuberculosis whereas in some parts of Africa such as Nigeria condemnation due to fascioliasis amounted to 94 % (Grev, 1993). Estimation of the economic losses due to fascioliasis at a national or regional level is, however, hampered by lack of accurate information of the disease prevalence, complexity in disaggregating and quantifying the direct and indirect effects of the disease and lack of a common methodology for assessing the economic loss (Ogunrinade, 1980). The annual loss due to endoparasitism including fasciolosis in Ethiopia is estimated at 700 million Birr and decreased productivity alone due to bovine fasciolosis is estimated at 300 million Birr (Ngategize *et al*., 1993).

A survey on bovine fascioliasis made by Bahru and Ephrem, (1979) in eight administrative regions of Ethiopia revealed a mean infestation rate of 61% with the highest incidence in Kaffa (86%) and the lowest in Sidamo (42%). They also estimated annual economic loss of 360 million Ethiopian birr due to decreased milk yield and reduced weight gain.

The prevalence of the disease is known to be relatively high (Bahru and Ephrem, (1979) causing considerable economic losses in livestock production. However, few attempts have been made to study the epidemiology of this parasitic problem in various section of the country with the specific aim of determining the parasitic burden, particularly in relation to seasons of the year, rainfall, humidity, temperature and other related factors with particular emphasison the emerging concern regarding its public health importance.

Limited studies in North Gondar administrative zone, the region which encompasses the current study area, showed a prevalence of 83% by Yehenew, 1985, 34% Roman, 1987 and 40% by Mesfin, 1999.

It is presumed that the climate of the study area with the vast plain land of North Gondar bordering Lake Tana is the most affected and endemic areas of fascioliasis in the region, farmers and veterinary professionals complain of considerable annual losses from the disease. Study on the prevalence, epidemiology and its potential zonootic hazard is of great economic and public health relevance as in many tropical countries where economic realities often determine the type and scope of control measures envisaged.

The primary objective of this work was to study the significance of fascioliasis

The specific objectives of the study were therefore:

1. To assess the prevalence of bovine fascioliasis in areas of North Gondar bordering Lake Tana.

2. To study on the factors associated with the distribution of the parasite

3. To study the species and epidemiological distribution of the snail intermediate hosts.

4. To evaluate the association between the intensity of liver lesion, fluke burden and feacal egg output in the abattoir.

5. To have base line information on its public health importance.

2. LITERATURE REVIEW

2.1 Etiology

Organisms that cause hepatic fascioliasis are classified taxonomically as Phylum *Platy-helminthes*, Class *Trematoda*, subclass *Digenea*, suborder *Distomata*, *F*amily *Fasciolidea,* Genus *Fasciola,* Species *Fasciola hepatica* and *Fasciola gigantica.*

Members of the genus Fasciola cause fascioliasis. The two most important species are *Fasciola hepatica* and *Fasciola gigantica.*

Description: The adult parasites are fairly large hermaphrodite worms with leaf-shaped body and spiny cuticle (Fig.1). *Fasciola gigantica* is exclusively tropical and measures 25-75 mm X 3-12 mm. *Fasciola hepatica* is found in temperate areas (high altitude regions in east Africa) and measures about 20-30mm x 10mm. These are large eggs with thin shell, containing morullar mass formed of cells surrounding the zygote. The eggs are operculate at one pole and yellowish in colour (Fig. 2).

Figure 1.Structure of ***Fasciola hepatica***

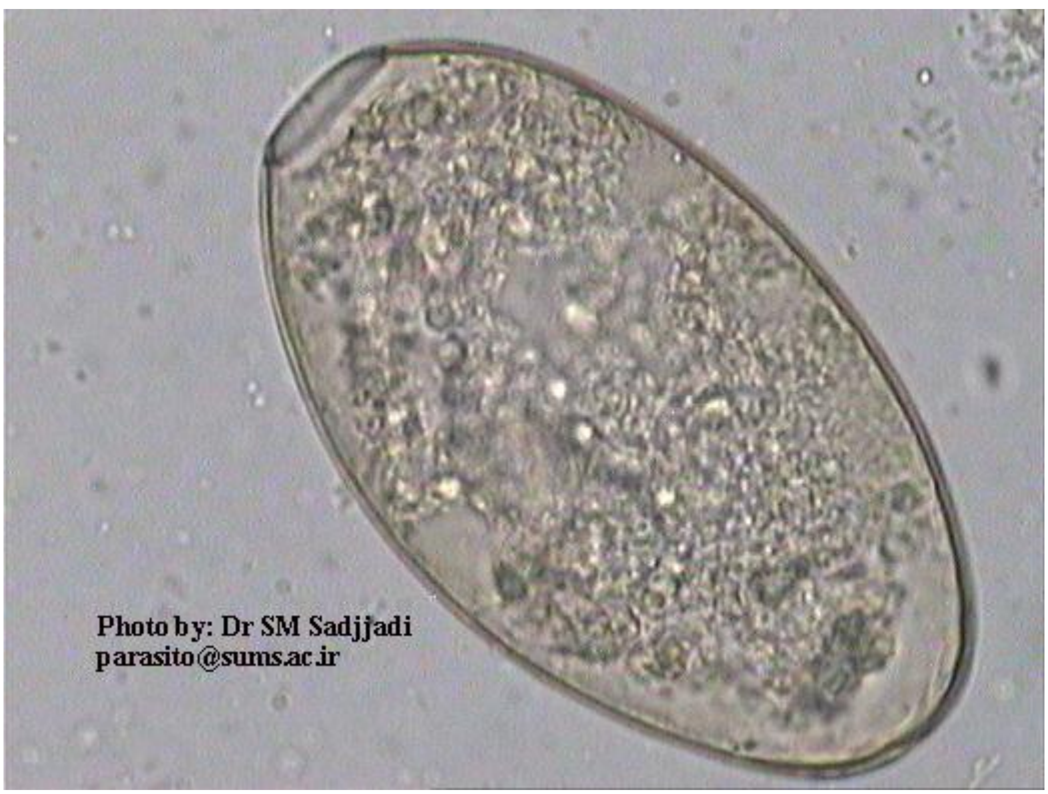

Figure 2. Morphology of *Fasciola hepatica* egg

Distribution: Fascioliasis is a cosmopolitan disease that occurs only in a sufficiently wet area (Urquhart *et a1*. 1996). In Ethiopia, the distribution of *F. hepatica* is reported to extend in areas situated over 1800- 2000m above sea level while that of *F. gigantica* is up to 1200 above sea level. Mixed infections with both species are encountered between 1200-1800 meters above sea level (Graber, 1978).

2.2 Intermediate Hosts

Snails of the genus Lymnaea are the most widely recognized intermediate hosts of the parasite. All require water especially for breeding but spend much of their time out of it (Urquhart *et al*., 1996). Lymnaea prefer a slightly acidic pH and requires a temperature of about 26 ^{O}C for development.

L*ymnaea natalensis*: It is the intermediate host for *F. gigantica* and is primarily aquatic found in streams, irrigation channels and marshy swamps. The snail has a comparatively high requirement for oxygen and usually is intolerant of desiccation. It is found up to 2, 200 meters above sea level *(*Burch, 1989*)*. Morphologically the spire is generally much less high than the aperture. The surface may have spiral rows of small transverse grooves, but always lacks strong spiral ridges of periostracum. Two forms with exceptionally low spires were described from Ethiopia,*L.exserta* Martens *Erithrea*) and *L. gravieri* Bourgugnat (lower Awash Valley, Brown, 1980). The name L. *caillaudi* Bourguignat (Lake Tana, Ethiopia) has frequently been applied to snails from Eastern Africa in the belief that they belong to a narrower form than typical *L.natalensis (*Brown, 1980).

Lymnaea truncatula; is the most widespread and important snail species involved in the transmission of *F. hepatica* in temperate regions. Comparatively small, with the spire about as high as the aperture and strongly convex whorls, Columella straighter and broadly reflected than in *L. natalensis (*Brown, 1980). The snails are amphibious and spend hours in shallow water and periodically emerge onto surrounding mud. They are capable of withstanding summer drought or winter freezing for several months by respectively aestivating or hibernating deep in the mud. Optimal conditions include a slightly acidic pH environment and slowly moving water medium to carry away waste products. They feed on algae and the optimum temperature range for development is 15-22^{O} C. One snail is capable of producing up to 100,000 descendants over three months (Mira and Ralph, 1989).

2.3 Host range

Though wild ruminants are susceptible, infestation is highest and most frequent in sheep and cattle. Pigs are very rarely infected. Man may also contract the disease and even if there are few parasites, the disease is always severe (Mira and Ralph, 1989).

2.4 Life cycle

The eggs laid in the bile ducts pass down in to the intestine and out in the feaces. They develop and hatch releasing motile ciliated miracidia. This takes about nine days at optimal temperatures of 22-26 ^{O}C and no development occurs below 10 ^{O}C (Urquhart *et al.*, 1996).

The liberated miracidium has a short life span and must locate a suitable snail within 3 hours for successful penetration. In infected snails, development proceeds through sporocyst and redial stages to the final stages in the intermediate host, the cercariae. The cercariae are shed from the snail as motile forms, which attach themselves to firm surfaces such as grass blades and encyst there to form the infective stage metacercariae. It takes a minimum of 6-7 weeks for completion of development from miracidium to metacercaria; although under unfavorable circumstances a period of several months is required. Infection of a snail with one miracidium can produce over 600 metacercariae (Georgi, 1985). Metacercariae ingested by the final host excyst in the small intestine, migrate through the gut wall, cross the peritoneum and penetrate the liver capsule. The young flukes tunnel through the parenchyma for 6-8 weeks then enter the bile ducts where they migrate to the larger ducts.

The entire cycle of the liver fluke in the snails takes 2 - 3 months under favorable conditions in the field. If ingested by sheep, cattle or other hosts, including man, the metacercariae excyst in the small intestine and the released immature flukes penetrate the intestinal wall and enter the abdominal cavity. The young fluke penetrate the liver capsule and migrate through the liver tissue for 6 to 7 weeks before entering the bile ducts to become adult fluke. The fluke reach sexual maturity and commence egg production 8 to 10 weeks after infection Fig.3.

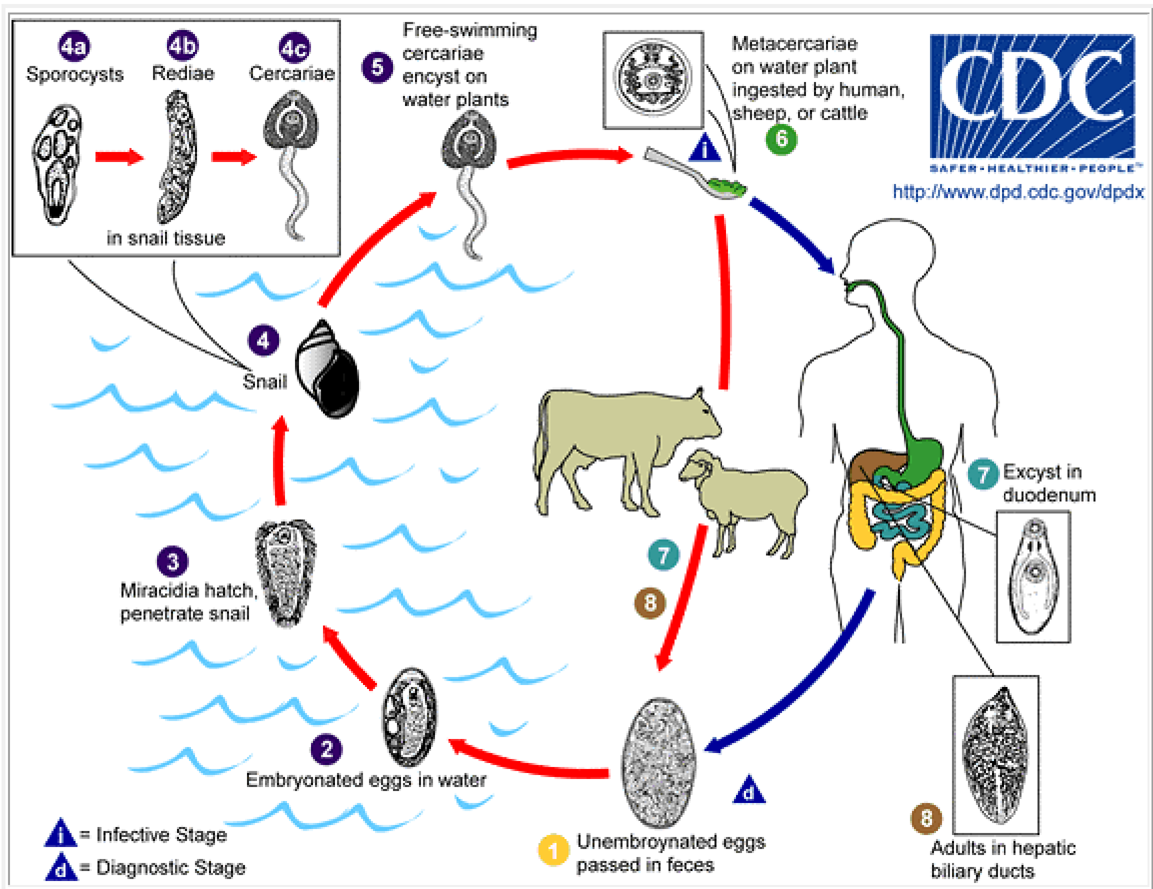

Figure 3. Life cycle of Fasciola

Life cycle of the intermediate snail host

The important *Lymnaea* species of snails involved in the transmission of fascioliasis vary in their geographical distribution in the world. The habitat requirements of the intermediate hosts of the two most important liver flukes differ slightly. The intermediate hosts for *F. hepatica* are amphibious snails that live close to the edge of slow moving or stagnant water bodies whereas those transmitting *F. gigantica* live in deeper water and are close to being true aquatic snails in their behavior. They can, however, adapt to an amphibious existence in adverse conditions. The optimum temperature range for development of the snail is 15-26 °C, when rapid production of snail egg masses occurs. These eggs hatch within two weeks and the resulting snails mature a month later. Thus one snail can produce several thousand descendants within a period of 10-12 weeks (Kaplan *et al*, 1997). No development and no reproductive activity takes place at temperatures below 10 °C but snails may survive adverse conditions for months buried in the mud.

2.5 Epidemiology

There are three main factors influencing the production of the large number of metacercariae necessary for outbreaks of fascioliasis.

1. Availability of suitable snail habitats, altitude and soil type: In general *F. hepatica* is found at an altitude above 2000 meters and *F. gigantica* at an altitude below 1200 meters and both species are found between 1200-2000 meters above sea level (Radostits *et. al*, 1994). Media with slightly acidic pH are considered optimal for the development of lymnaeidae. In Ethiopia, the general pattern is that the low-lying areas in the highlands have poor drainage and the soil seems to be acidic. This condition favors the development of fascioliasis. The low incidences of fascioliasis in goats are related to there grazing behavior. Goats naturally are browsers and usually prefer to feed on higher plants rather than grazing grasses (Georgi, 1985).

2. Temperature: Only when the temperature rises to 15 ^{O}C and is maintained above that level, that a significant multiplication of snails and fluke larval stages ensues.

3. Moisture: The ideal moisture conditions for snail breeding and the development of *F. hepatica* within snails are provided when rainfall exceeds transpiration and field saturation is attained. Such conditions are also essential for the development of fluke eggs, miracidia searching for snails and the dispersal of cercariae being shed from the snails.

The eggs produced by adult fluke pass out in the host faeces into wet areas. They hatch when temperatures increase above 10° C (typically from mid-September to May). The miracidia invade a suitable snail (Lymnaea spp) and develop and multiply. One miracidium hatching from a fluke egg can produce up to 4,000 metacercariae. These cysts attach to grass and other vegetation and the host is infected when this vegetation is ingested.

Adult flukes can survive for many years in the livers of infected sheep; the adult fluke lays between 20 000 and 50 000 eggs per day over a long period. The rate of egg production is responsible for the degree of pasture contamination and thus greatly influences the epidemiology of the disease.

The epidemiology of the disease is also influenced by the grazing habits of the animals. Animals grazing in wet marshy areas, favored by the fluke snail, are more likely to become infected. Typically, long, wet seasons are associated with a higher rate of infection. However, sheep are more likely to ingest large numbers of cysts during dry periods following a wet season. This is due to a reduction in available pasture, forcing the animals to graze in swampy areas or in areas where the water has receded, thus exposing them to vegetation heavily infected with metacercariae.

Animals are infected by *F. gigantica* at water holes. Infection depends on several factors related to the vectors, biology of the parasite and management of the flocks and herds. In Europe, the mollusc population reaches maximum density between November and March and start decreasing after April. During the rainy season, from June to October only few snails remain to ensure survival of the species (Mira and Ralph, 1989).

Table 1 Results of coprological examinations in different regions of Ethiopia.

Regions	No of areas surveyed	Total samples collected	No (%)Positive
Arsi	14	1181	774(68)
Gondar	4	749	498(65)
Kaffa	6	707	625(86)
Illubabor	12	754	547(70)
Shewa	23	1475	697(48)
Sidamo	7	1286	355(42)
Wellega	9	1032	486(47)
Wollo	15	1319	718(62)

Source: Bahru and Ephrem (1979).

Table 2 Prevalence studies conducted in different parts of Ethiopia

Study area	Species affected	Prevalence
Awassa	Bovine	30.4
Debre birhan	Bovine	81
Addis Ababa	Caprine	63.8
Dembi Dollo	Bovine	77.8
Asella	Ovine	32.7
Assela	Caprine	13.9
Wolaita	Bovine	47%
Nekemtie	Bovine	22.7
Nekemtie	Ovine	23
Kombolcha	Bovine	53.5%
E, Gojjam	Bovine	50.56%
Tigray	Bovine	26.13

Source: Moges, E., 2003

2.6 Pathogenesis

Variations in the pathogenesis of fascioliasis are mainly dependent on the phase of parasitic development in the liver and the species of the host involved. Phases of the pathogenesis are one that occurs during migration in the liver parenchyma and is associated with when the parasite is in the bile ducts and results from the hemorrhagic activity of the adult flukes and from damage to the mucosa by their cuticular spines (Urquhart *et al.,* 1996).

Acute fascioliasis:

Acute fascioliasis occurs as an outbreak of disease following a massive but relatively short-term intake of metacercariae. This high intake is often the result of certain seasonal and climatic conditions combined with a lack of appropriate fluke control measures. It typically occurs when stock are forced to graze in heavily contaminated wet areas as a result of over-stocking and/or drought. Animals suffering from acute fascioliasis may display no clinical signs prior to death; some may display abdominal pain and discomfort and may develop jaundice.

Acute hepatic fascioliasis occurs 5-6 weeks after ingestion of large number of metacercariae and is due to the sudden invasion of the liver by masses of young flukes. Sufficient parenchyma may be destroyed to cause acute hepatic insufficiency and to this may be added the effect of hemorrhage into the peritoneal cavity by the movement of the young flukes in the parenchyma. There is hypoalbuminemia due to reduced albumin synthesis and plasma volume expansion caused by liver damage (Radostits *et al*., 1994).

Sub-acute fascioliasis:

Sub-acute fascioliasis is characterised by anaemia, jaundice and ill thrift. The migrating fluke cause extensive tissue damage, haemorrhage and, in particular, liver damage. The result is severe anaemia, liver failure and death in 8-10 weeks.

Chronic fascioliasis:

Chronic fascioliasis is the most common clinical syndrome associated with liver fluke infection in sheep and cattle. It occurs when the parasites reach the hepatic bile ducts.

The principal effects are bile duct obstruction, destruction of liver tissue, hepatic fibrosis (scarring) and anemia.

The onset of clinical signs is slow. Animals become gradually anemic and in appetent, as the adult fluke become active within the bile ducts; signs may include dependent edema or swelling under the jaw (˜bottle jaw’). Affected animals are reluctant to travel. Death eventually occurs when anemia becomes severe. Additional stress upon anemic animals, such as droving, may lead to collapse and death. Cattle typically present with signs of weight loss, anemia and chronic diarrhea.

Chronic fascioliasis provides the right environment in the liver for the germination of the spores of the bacterium, Clostridium novyi Type B, which cause Black disease. Vaccines against this bacterial organism are available and should be given to all animals, particularly those at risk of fascioliasis.

Chronic fascioliasis develops slowly and is due to the activity of the adult flukes in the bile ducts. This cause cholangitis, biliary obstruction, destruction of hepatic tissue, fibrosis and anemia.

The cause of anemia in chronic fascioliasis is the blood sucking activity of the adult flukes and continuous drain of iron reserve that this imposes. But recent work suggests that a substance produced by the worms, possibly proline, may contribute to the development of anemia (Urquhart *et al.,* 1996). Hypoalbuminemia is more marked in chronic case whose liver is damaged and is due mainly to the increased protein plasma leakage into the gut. It is more severe in sheep with anorexia or those on a low plane of nutrition. Chronic infection has been shown to limit growth rate and feed conversion in growing heifers and to reduce growth rate in beef cattle. Fascioliasis also increases the susceptibility of the animal to other diseases and reduced fertility.

Death usually results from blood loss due to hemorrhage in the liver caused by the migration of the immature fluke through the liver.

2.7 Clinical Signs

2.7.1 Ovine Fascioliasis

In sheep, fascioliasis may be acute, sub acute or chronic. The acute disease occurs 2-6 weeks after the ingestion of large number of metacercaria, usually over 2000 and is due to severe hemorrhage, which results when the young flukes, migrating in the liver parenchyma, rupture blood vessels. Damage to liver parenchyma is also severe (Radostitis *et. al.,* 1994). Outbreaks of acute fascioliasis are generally presented as sudden deaths during autumn and early winter. On examination of the remainder of the flocks, one may find some sheep, which are weak, with pale mucous membrane, dyspnoeic and in some instances, have palpable enlarged livers associated with abdominal pain and ascites. Sometimes these outbreaks are complicated by concurrent infections with *Clostridium novyi* resulting in Black Disease (Mira and Ralph, 1989).

A subacute fascioliasis results from ingestion of large number of metacercariae, usually 500-1500 over long period. The major clinical signs are weight loss and pallor of the mucous membrane. It is presented as a rapid and severe hemorrhagic anemia with hypoalbuminemia and if untreated can result in a high mortality rate. An enlarged and palpable liver intermandibular space or facial edema and ascites may be present (Wilford, 1974).

Chronic fascioliasis, which is seen mainly in late winter, is the most common form of the disease, it occurs 4-5 months after ingestion of moderate number of 200-500 of metacercariae. Clinically chronic fascioliasis is characterized by a progressive loss of condition and the development of anemia and hypoalbuminemia, which can result in emaciation, pale mucous edema of the intermandibular space and ascites (Radostitis *et al.,* 1994).

2.7.2 Bovine Fascioliasis

Although acute and subacute disease may occur occasionally under conditions of heavy challenge, especially in young calves, the chronic form of the disease is far more common in bovine fascioliasis.

The pathogenesis is similar to that in sheep but has the added features of calcification of the bile ducts and enlargement of the gall bladder. Cattle also lose weight especially when there is the added drain of lactation, milk production falls and chronic diarrhea develops (Urquhart *et al*., 1996). Aberrant migration of the flukes is more common in cattle and encapsulated parasites are often seen in the lungs. On reinfection of adult cows, migration to the fetus has been recorded resulting in prenatal infection (Dineen *et. al*., 1978). There is some experimental evidence that fascioliasis increased the susceptibility of cattle to infection with *Salmonella dublin* (Urquhart *et. al*., 1996).

In heavy infections, where anemia and hypoalbuminaemia are severe, sub-mandibular edema frequently occurs. With smaller fluke burdens, the clinical effect is minimal and the loss of productivity is difficult to differentiate from inadequate nutrition. It must be emphasized that diarrhea is not a feature of bovine fascioliasis unless it is complicated (Dunn, 1978). Fasciola infections may cause loss of production in milking cows during winter. Clinically, these are difficult to detect since the fluke burdens are usually low and anemia is not apparent. The main effects are a reduction in milk yield and quality, particularly the solid-not-fat part.

2.8 Diagnosis

A provisional diagnosis may be based on the clinical signs, grazing history and season. Acute fascioliasis should be suspected in cases of sudden death in sheep and goats exposed to an environment where infection is possible. Confirmation is by postmortem examination when the small flukes can be expressed from the liver parenchyma. In chronic fascioliasis, the characteristic eggs are found in the faeces but counts are often very low in cattle, especially in longer standing cases (Radostitis *et. al*., 1994).

Enzyme Linked Immunosorbent Assay (ELISA), Immunodiffusion tests and PCR are also important diagnostic tools. In India PCR is used to detect *F. gigantica* infection in the snail intermediate host. This technique is highly specific and sensitive and possesses good prospects of its utility as an epidemiological tool for ascertaining the infectivity status of ubiquitous snail populations (Velusamy *et al*, 2004). Isolates of fasciola from different host species and geographical locations in Mainland China are characterized genetically by using PCR (Huang *et al*., 2004).

2.9 Treatment

A number of agents may be employed. Carbon tetrachloride and other old drugs have been used for more than 50 years. It remains in use and, in some areas is the only drug available for the treatment of *F. hepatica* in sheep since it is of low cost. In sheep, a routine dose of 1 ml is satisfactory for strategic control and this may be increased to 5 ml for the control of outbreaks of the disease. In general, sheep tolerate carbon tetrachloride well (Soulsby, 1982).

However, efficient drugs are now available and the ones of choice are diamphenethide and triclabendazole, which removes all developing stages over one week old. Two other drugs are rafoxanide and nitroxynil, which at increased dosage rates will remove flukes over four weeks old.

A single dose of diamphenethide accompanied with movement of cattle to fluke free or well-drained pasture, recently cultivated field should be adequate treatment. However, with rafoxanide or nitroxynil a second treatment may be needed 2-3 weeks later (Mira and Ralph, 1989). Outbreaks of chronic fascioliasis can be successfully treated with a single dose of any of a range of drugs (rafoxanide, nitroxynil, closantel oxyclozanide and triclabendazole) and following treatment; the anemia usually regresses within 2-3 weeks. The roundworm anthelmintics, albendazole is also effective against adult flukes (Urquhart *et al*., 1996).

For bovine fascioliasis, there is only one drug, namely triclabendazole, which will remove the early parenchymal stages. Albendazole is also effective at an increased dosage rate. In lactating cows, where the milk is used for human consumption, the above drugs are either banned or have extended withdrawal periods in most countries. An exception is oxyclozanide, which is licensed for use in lactating animals in many countries and has milk-withholding time of up to 3 days (Urquhart *et al*., 1996).

2.10 Control

Control of fascioliasis may be approached in two ways; by reducing population of the intermediate snail host or by using anthelmintics (Urquhart *et al.*, 1996).

1. Reduction of snail population: A survey of the area for snail habitats should be made to determine whether these are localized or widespread before any scheme of snail control is undertaken. The best long-term method of reducing mud snail populations such as *L.trancatula* is drainage, since it ensures permanent destruction of snail habitats. However, farmers are often hesitant to undertake expensive drainage schemes. When the snail habitat is limited, a simple method of control is to fence off this area or treat annually with a molluscicide.

2. Use of anthelminthics: The prophylactic use of fluke anthelmintics is aimed at reducing pasture contamination by fluke eggs at a time most suitable for their development removing fluke populations at a time of heavy burdens or at a period of nutritional and pregnancy stress to the animal.

Immunity: Natural differences in host susceptibility to infection with *F. hepatica* are seen. Cattle and Pigs have a high or at least moderate degree of natural resistance to fascioliasis, others such as sheep are highly susceptible. Similarly, sheep do not normally develop a protective immune response to re-infection. The ability of cattle to develop immunity against re infection with *F. hepatica* suggested that immunization might be feasible (Soulsby, 1982). Nansen (1975) was able to immunize calves with three doses of irradiated metacerceriae, and after grazing infected pastures, there was a marked decrease in faecal egg count and a 71 percent reduction in the number of flukes recorded from immunized calves as compared to un immunized animals.

2.11. Economic and public health significance of the disease

2.11.1 Economic Significance

Parasitism nowadays represents a major drawback to development in the tropics. Fasciola parasites damage the liver, which is an important organ in the body.

Losses in infected livestock often take the form of reduced milk production in dairy cows, poor weight gain in beef cattle and sheep, condemnation of infected livers at meat inspection, mortalities, reduced fertility rate, and reduction in wool production (Urquhart *et a1*, 1988).

Liver condemnation is a well-known source of economic loss due to fascioliasis. Fascioliasis is also associated with poor carcass conformation and predisposes the animal to other infections principally Clostridia, Salmonella and possibly blood parasites (Allonby, 1976).

It is also possible that facioliasis plays some role in condition leading to the condemnation of the whole carcass particularly when there is jaundice or severe emaciation in cattle since *F. gigantica* is a common cause of jaundice and emaciation (Allonby, 1976).

Experimental evidence on the effects of fascioliasis on weight gain in infected animals is inconclusive as the effect probably depends on parasite load and other factors (Cowdry, 1984). However, Ross (1970) estimated a weight loss of 1.61b in infected and untreated cattle over a 3-week period. Cowdry (1984) also estimated a loss of body weight between 8-12% in groups of animals exposed to light and heavy fasciola worm burden in a six months period. German workers also mentioned a 10% weight loss estimate due to fascioliasis (FAO, 1994). In sheep wool, production can be reduced by 20-30% due to sub fatal fascioliasis (Cowdry, 1984).

A rough estimate of economic loss due to decreased production has been estimated to be over 300 million birr per year (Getachew, 1984). The same author has reported a prevalence rate of 77% in cattle in Addis Ababa Abattoir and loss due to liver condemnation alone was estimated to reach 631, 320 birr per year. Loses due fascioliasis were estimated at 48.4 million Ethiopian Birr per year of which 46.5, 48.8 and 4.75 percents were due to mortality, productivity (weight loss and reproductive wastage) and liver condemnation, respectively. (Ngategize *et al.*, 1993).

The cost of drugs used to treat infected animals will bring economic loss to the nation. Although most of the newer drugs have high efficiency against adult and immature flukes, they are very expensive.

2.11.2 Public Health Importance

Fascioliasis, a zoonotic disease of domestic herbivorous animals such as sheep, cattle and goats, which are the definitive hosts, is caused by infection with the liver fluke, *F. hepatica*. Humans become infected by eating uncooked, and usually unwashed, aquatic vegetables on which larval parasites are encysted.

Although animals can support enormous worm burdens without developing serious disease, Fasciola spp. can cause severe, even fatal disease in humans. In the past, fascioliasis was limited to populations within well-defined watershed boundaries; however, recent environmental changes and modifications in human behaviour are defining new geographical limits and increasing the populations at risk (Saviolli, *et al*, 1999).

Sporadic human cases of fascioliasis occur throughout the world. It has been reported from a number of countries such as Argentina, Cuba, France, Germany, Russia, Uruguay (Goll and Scott, 1979).

In Europe and other areas, these cases are associated primarily with the eating of watercress contaminated with metacercariae. Human cases are common after wet summers when watercress or other vegetable beds may be overrun with water drainage from wet swampy annual pastures. In man, the presence of adult *F. hepatica* in the bile ducts causes a variety of symptoms such as malaise, intermittent fever, weight loss, pain under the right costal margin and often purities with eosinophilia. Urticaria, jaundice and anemia may also occur. Adult *F.*

hepatica may be found in aberrant sites such as in the lungs and subcutaneously. Here the parasites are found in the cysts (Dittmar and Teegen, 2003).

Treatment of human fascioliasis has not always been optimal. Praziquantel, the drug of choice for treating all human foodborne trematode infections, is ineffective against fascioliasis. Bithionol, although not fully satisfactory, has long been recommended as a treatment for fascioliasis, at a dosage of 30-mg/kg-body weight per day for 5 days (Saviolli, *et al*, 1999).

The drug of choice to treat human cases of fascioliasis is now triclabendazole, a benzimidazole compound, which has been used in veterinary practice since 1983 to treat the disease. The first instance of its use in humans was during the 1989 epidemic of fascioliasis in northern Islamic Republic of Iran near the Caspian Sea, when the health authorities approved the use of veterinary formulations to address the problem. Triclabendazole, which is given as asingle dose of 10-mg/kg body weight, is available as scored 250-mg tablets, and is effective against both adult and immature flukes, which migrate through the liver parenchyma. The process of registering triclabendazole for human use is currently under way in other endemic countries.

The availability now of an effective, single-dose, safe treatment for fascioliasis using a formulation specifically designed for human use creates new opportunities to implement a strategy to control the disease in areas of high risk. It is envisioned that, in those areas where there is significant morbidity due to fascioliasis and intense transmission is taking place, control programmes will be undertaken using chemotherapy as an important operational component (Saviolli, *et. al*, 1999).

Priority setting for disease control at the country level is based not only on mortality and morbidity indicators, but also on the availability of effective, safe, cheap and simple tools (Saviolli, *et. al*, 1999).

With the development of triclabendazole for human use, fascioliasis can now move up the list of priorities to be addressed urgently in endemic areas. WHO has spearheaded and recommended community-based chemotherapy for the control of helminthic infections in endemic communities. The control of schistosomiasis and intestinal nematodes employs single-dose chemotherapy as one of the major tools for morbidity control in endemic areas. It may be premature, however, at this stage, to propose large-scale, community-based, chemotherapy for the control of fascioliasis since epidemiological tools have not been developed to stratify endemic areas according to the prevalence and intensity of infection.

3. MATERIALS AND METHODS

3.1. Study Area

The area under study, North Gondar bordering Lake Tana, is located in the North Western part of Ethiopia, at latitude12.4^0, longitude of 27.25^0 and stands at an altitude range of 1802-2200 meters above sea level. Roughly, the soil type in this area falls into three categories: Heavy black clay soil, loam brown and red soil and sandy loam soil. The soil in the immediate vicinity of Lake Tana has sandy clay characteristics. The dominating species concerning vegetation of the swampy area are the Graminae (example papyrus species).

The plains, which lie adjacent to the lake such as Dembia, around Gorgora, have heavy black clay soil and become waterlogged in the rainy season and pockets of swampy areas remain throughout the year giving a favorable condition for the existence of snail vectors and metacerceriae. It is also a usual practice for farmers to water their animals at the lake, slow moving small rivers originating in most cases from swampy areas, and stagnant water from swampy areas originating from tides of Lake Tana.

The average maximum and minimum temperatures of the area vary between 22-30.7 ^{0} C and 12.3-17.1^{0}C respectively. The region receives a bimodal rainfall, the average annual precipitation rate being 1000mm. The short rains occur during the months of March, April and May while the long rains extend from June through September (Ministry of Agriculture, 1987).

Number and distribution of livestock: North Gondar administrative zone is one of the regions highly livestock populated area and the recent census shows as follows.

Table 3 The livestock population of North Gondar administrative zone

Animals	Number
Cattle	1936543
Sheep	524087
Equine	273826
Camel	666
Chicken	3165069

Source: CSC, (2003)

The livestock population of Dembia, where the specific study sites are included, is as follows;

Table 4 The livestock population of Dembia wereda

Animals	Number
Cattle	166046
Sheep	8886
Goats	5427
Equine	11009
Chicken	236466

Source: CSC, (2003)

Descriptive parameters

Predominant species: Bovine are abundant but ovine as well as caprine are existing in significant numbers.

Livestock management (Husbandry) system: Mainly extensive type, and mixed farming (crop and livestock) is the dominant farming practice in the area.

Status of disease under investigation: Fascioliasis is still one of the major problems in the study area. Yehenew, (1984), and Mulualem, (1998) in weredas of the zone around Lake Tana have recorded 83% and 83.08% prevalence respectively while Mesfin, (1999) about 40% prevalence of the disease at zonal level.

3.2. Study Population

According to the recent census the total cattle population of Amhara Regional State is estimated to be 10,512,777(Central Statistics Commission, 2003). Out of this population, the male constitute about 50.2% (5,273,390); the remaining 49.8% (5,239,386) are female. The bulk of cattle population is found in rural areas, while a very small proportion is accounted for in urban areas (1.5%). Except for a few hybrid (0.4%) and pure exotic (0.1%) animals, the majority (99.5%) of cattle population in the region is local breeds.

Study population: Cattle of different age (<1.5yrs., 1.5-5yrs and above 5yrs.age) and sex categories managed under extensive system were considered.

3.3. Study Design

The study was cross sectional type conducted from a period of September 2004 up to April 2005.

3.3.1. Questionnaire Survey

Questionnaire was compiled to evaluate the impact of potential risk factors associated with the disease.

Data on each sampled animal were collected using a pre-tested format (Annex 1). These include age, breed, sex, previous history of fascioliasis, and other relevant information related to fascioliasis. Information was also collected on the marketing, housing system; feeding and other management practices related to fascioliasis for possible explanation on the proportion and distribution of the disease. In addition, drug usage practices in the study area was collected to evaluate its positive and negative contribution to the control of fascioliasis.

3.3.2. Prevalence Study

Prevalence of fascioliasis was determined cross sectionally from September 2004 to February 2005 in selected sites bordering Lake Tana based on coprological examinations. Prevalence was calculated according to the formula given in Thrusfeild (1995).

$$\text{Prevalence} = \frac{\text{No. of animals with the disease}}{\text{No. of animals at risk}}$$

3.3.2.1. Sample size and sampling method

The sampling strategy was cluster-sampling method (Martin, *et al.*, 1987) and herds were considered as clusters. The sample size was determined at 95% confidence interval, 5% precision and from previous studies in the study area (Mesfin, 1999), with an expected prevalence of 40%. As a result a total of 369 animals were needed to be sampled (Thrusfield, 1995). However in the case of cluster sampling where the study subjects were not independent and hence larger sample size was required. Therefore double or above the number of animals required for simple random sample are needed for cluster sampling (Martin, *et al.*, 1987). So the optimum sample for this study was 1107 animals, and actually 552 during the dry and 555 during the rainy seasons respectively. One stage clustering was considered on herds in the study area. Herd is defined as a group of cattle owned by peoples living together in a village where their animals share the same barn at night as well as same grazing area and watering points. About 150 herds were estimated in the 2 peasant associations (PAs.) of the study area out of which 25 herds (552) during the dry season 25 herds (555) during the rainy seasons were considered.

3.3.2.2. Coprological examination

Feacal sample collection and processing was according to the procedures described by MAFF, 1979 (Annex 2) Feacal samples were collected from 1107 cattle. The samples were directly taken from the rectum and were transported to the laboratory in universal bottles preserved with 5% formalin
and examined.

Two laboratory techniques were employed;

Direct Smear Method:

A small quantity of faeces were taken and placed on a slide and was mixed with a drop of water, covered with a cover slip and examined under low power of the microscope.
Three such slides were examined for each sample.

Sedimentation Technique:

This technique was conducted according to the procedures described by MAFF, 1979, where the detail is presented on Annex 2.
Body Condition Evaluation: Body condition scoring of each sampled animal was made in cattle living under the same environmental conditions to assess the possible effect of fascioliais using the method described by Radostitis *et al.*, (1994) on Annex 6.

3.3.3. Abattoir study

Post mortem examination was conducted in both the municipal and industrial abattoirs of Gondar. The livers of the slaughtered animals were examined by thorough inspection, palpation and systematic incision to recover fasciola species. Those livers condemned as unfit for human consumption due to fascioliasis during post mortem examination were registered daily and representative samples were taken to the laboratory to count the fluke burden per animal and to study the relationship between the fluke burden and the intensity of pathological lesions.

Fluke recovery and count was done following the method described by Hammond and Swell (1974), (Annex 3). Pathological categorization of affected livers was determined by using an approach forwarded by Ogunrinade (1982) and the detail is indicated on Annex 4.

3.3.4. Malacological study

For the collection of snails, the methods described by González-Lanza, *et al*., (1989*)* and Manga *et al*., (1991) were used, which was the quadrant method of snail collection and study was employed (Annex 5). Hence, during the six months study period, a total of 60 quadrants were thrown (10 quadrants per month).

So apart from coprological and abattoir survey efforts were made to find out the foci of snails and to identify the species, the seasonal dynamics of snails and stages of snails shedding cerceriae. Further details of the procedure are discussed on Annex 5.

3.3.5. Public Health Significance

In addition to the above studies, efforts have been made to have a base line information and future public health hazard of fascioliasis, which have become a growing concern now a days. In this case, retrospective studies of hospital records have been made and besides to this, physicians were subjected to an interview of the magnitude and knowledge of the problem.

3.4. Statistical Analyses

Descriptive statistics were computed using the Excel spread sheet program of the computer and that of prevalence determination and testing the different associated factors was calculated with Stata 7.0 software.

Factors that influence the prevalence of fascioliasis included in this study were age, body condition, season of the year and sex. In addition to aforementioned factors, the effect of age and season with respect to egg count per gram of feaces (EPG) was also evaluated. In case of prevalence of fascioliasis, factors of age, sex, body condition, study sites and rainy and dry seasons of the year were tested using the Pearson's chi-square. Effect of age and effect of sex within a season was also tested using chi- square. Arithmetic mean was applied to calculate mean EPG for dry and late rainy seasons and t-test to evaluate whether significant difference occurs between these two seasons. For analysis of EPG among the different age groups, Analysis Of Variance (ANNOVA) test was used.

For EPG statistical test was carried out on Log_{10} transformed EPG to fulfill assumption of normality. The analysis was run with Stata 7 software.

For abattoir data testing correlation of liver lesions to fluke count and to mean EPG, the correlation coefficient was used.

For the malacological data to test the association between the dry and wet seasons to number of snails and number of cerceriae shading snails, the chi –square test was used.

In data entry, positive results were coded as 1 and negative result as 0. Sex was coded as female-1 and male-2 and, season of late rainy–1 and dry–2, age was categorized in to three levels and coded 1 to 3, representing age groups <1.5, 1.5_5, >5 respectively. Body condition was also denoted as good-1 and poor-2. Status of liver lesion was coded as 1, 2, and 3 for light, moderate and severe lesions respectively. Study site codes were Chuahit as 1 and Gorgora as 2.

4. RESULTS

4.1. Questionnaire Survey

Data obtained from the questionnaire survey showed that cattle are the predominant livestock of the area accounting about 30% of the total population of livestock and 99% of the livestock management was extensive system and crop/livestock farming was the dominant type of farming practice in the area. The herd composition of the area was mainly animals with age group of between 1.5-5years of age (42%) followed by younger animals ranging less than years of ages (38%) the rest being old animals aging above 5 years old.

About 85% of the respondents indicated the presence of a disease with signs of bottle jaw, emaciation and diarhoea. 95% responded its presence by this time and 83% its presence till a decade with a decreasing trend of progress. 65% believed the presence of the disease throughout the year and only 35% reported its seasonality particularly during the wet seasons of the year. 97% reported that the disease affects all age groups. 100% responded that it is a wasting disease known locally as Gechita affecting milk production, weight gain and draught power of their animals.

95% of them responded that they use different antihelminthics mainly Albendazole. 65% of the source of the drugs was smugglers and only 35% from veterinary clinics. Administration of the drugs was done mainly by livestock owners, which accounts to about 65% and only 35% by veterinary personnel. Only 42% of the respondents delivered full doses of the drugs.

4.2. Prevalence and Risk factors

4.2.1. Prevalence

A total of 1107 cattle managed extensively in the vast plains of the two selected sites, namely Gorgora and Chuahit bordering Lake Tana were investigated cross sectionally from September 2004 to February 2005 to determine the magnitude of fascioliasis. Out of 1107 feacal samples examined from indigenous zebu cattle, 876 (79%) were positive for fascioliasis. (Table 5).

4.2.2. *Risk factors affecting prevalence of fascioliasis*

Five factors were considered as potential risks for the occurrence of fascioliasis in this study. These were epidemiological area, sex, age, season and body condition. In addition, effect of season on sex and age with respect to prevalence of fascioliasis and effect of season and age on feacal egg output (EPG) was also studied. In the followings, we will be looking the effects of these different risk factors on the prevalence of fascioliasis with their respective statistical significance.

Table 5 Prevalence of bovine fascioliasis in selected sites bordering Lake Tana

Study Site	**Total examined**	**No. (%) Positives**	**95% confidence interval**
Chuahit	553	435(78.7)	(75.3 ----- 82.1)
Gorgora	554	441(79.6)	(76.24 ----- 82.96)
Total	1107	876(79.1)	(76.71------ 81.49)

Chi=0.148, df=1, P>0.05

The prevalence of fascioliasis showed higher in Gorgora (79.6%), an area closer to the Lake, than Chuahit (78.7%) though statistically not significant (p>0.05) (Table 5).

Table 6 Prevalence of bovine fascioliasis by sex

Sex	**Total examined**	**No. (%) Positives**	**95% confidence interval**
F	541	419(77.4)	(73.88---- 80.92)
M	566	457(80.7)	(79.05---- 82.35)
Total	1107	876(79.1)	(76.71---- 81.49)

Chi= 1.816, df.= 1, p>0.05

Rates of infection between male and female animals were compared. The statistical analysis 80.7% and 77.4% respectively showed no significant difference (chi= 1.81, df. =1, p>0.05)

Table 7 Prevalence of bovine fascioliasis by age

Age group	**Total examined**	**No. (%) Positives**	**95% confidence interval**
<1.5	186	145(78)	(72.05--- 83.95)
1.5-5	361	341(94.4)	(91.89---- 96.91)
>5	560	390(69.6)	(65.8- -- 73.40)
Total	1107	876(79.1)	(76.71----- 81.49)

Chi=82.05, df.= 2, P<0.05

Analysis of the prevalence rates based on age showed that there was statistically significant difference (Chi=82.05, df.= 2, P<0.05), with higher rates in cattle of age group 1.5-5 years old (94.4%) followed by those of<1.5 years (78%) and least in older aged animals above 5 years of age (69.6%) (Table7).

Table 8 Prevalence of bovine fascioliasis by season

Season	**Total examined**	**No. (%) Positives**	**95% confidence interval**
Late rainy	555	455(82)	(78.81 --- 85.19)
Dry	552	421(76.3)	(72.75 --- 79.84)
Total	1107	876(79.1)	(76.71-----81.49)

Chi= 5.47, df.= 1, p<0.05

The seasonal prevalence rate of bovine fascioliasis in the study area revealed high rates during the months of September to November that is during the wet season of the study period. Prevalence was 82% in the wet season and 76.3% in the dry season (Table 8) and the difference was statistically significant ($p<0.05$).

To see the effect of season on both sexes, analysis of prevalence was also done between male and female within a season but found statistically insignificant difference (chi=0.88, $p>0.05$) in the rainy season and (Chi=0.85, $p>0.05$) in the dry season. Similarly, analysis was also done on age basis within a season and there was a significant effect of season on the prevalence of fascioliasis among age groups (Chi=54.73, $p<0.05$) in the rainy and (Chi=30.84, $p<0.05$) in the dry seasons.

Analysis of EPG during the late rainy and the dry seasons were also made using the t-test and the results showed that there was a statistically significant difference ($p<0.05$) in the egg count per gram of feaces, (Table 9, Annex 8).

Table 9 Comparison of EPG in the late rainy season and dry season

Season	**EPG**	**Mean EPG**	**Mean** %
Late rainy	132000	290	50.3
Dry	119613	286	49.7
Total	252213	576	100

P (t) <0.05, df.=1105,

Mean EPG of age groups were also analyzed by ANNOVA and there was statistically significant difference ($p<0.05$) in egg count among age groups other than the first and the last ones (Table10, Annex 8).

Table 10 Comparison of EPG among age groups

Age in yrs.	**EPG**	**Mean EPG**	**Mean %**
<1.5	30400	163	23.9
1.5-5	128900	357	52.4
>5	90000	161	23.6
Total	249300	681	100

df=2, F=78.2, $p<0.05$

Results of egg count per gram of feaces showed that the majority 344 (40%) of the animals had an egg count interval of between 1-100epg (Fig.4) followed by counts of intervals >400 EPG which were 220 (25%) animals.

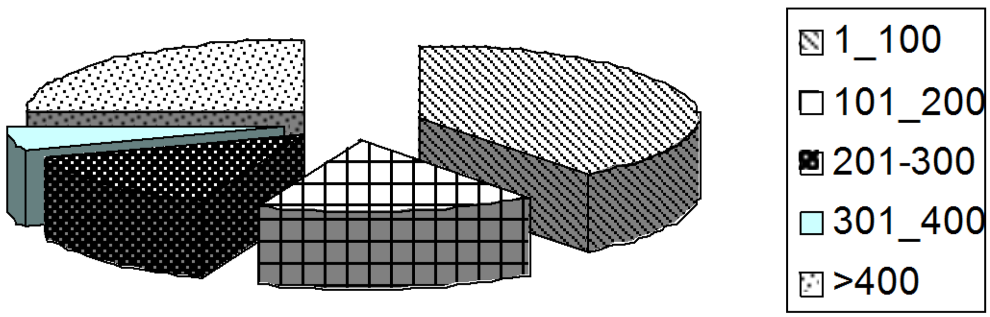

Figure 4. Results of egg count interval per gram of feaces

Body Condition Evaluation

Body condition scoring of each sampled animal was made on subjective basis to see the effect of fascioliasis in emaciated infected animals based on a standard body condition-scoring matrix adopted from Radostitis *et al*, (1994) (Annex 6). Statistically the effect of body condition on prevalence of fascioliasis was not significant (Table11).

Table 11 Prevalence of fascioliasis by body condition

Body condition	**Total examined**	**No. (%) Positives**	**95% confidence interval**
Good	691	546(79.0)	(75.97---- 82.03)
Poor	416	330(79.3)	(75.41---- 83.19)
Total	1107	876(79.1)	(76.71---- 81.49)

Chi=0.015, df. =1, P>0.05

4.3. Abattoir Study

Of 200 randomly selected fasciola positive livers, 53 (26.5%) were lightly, 71 (35.5%) moderately and 76 (38%) severely affected (Fig.5).

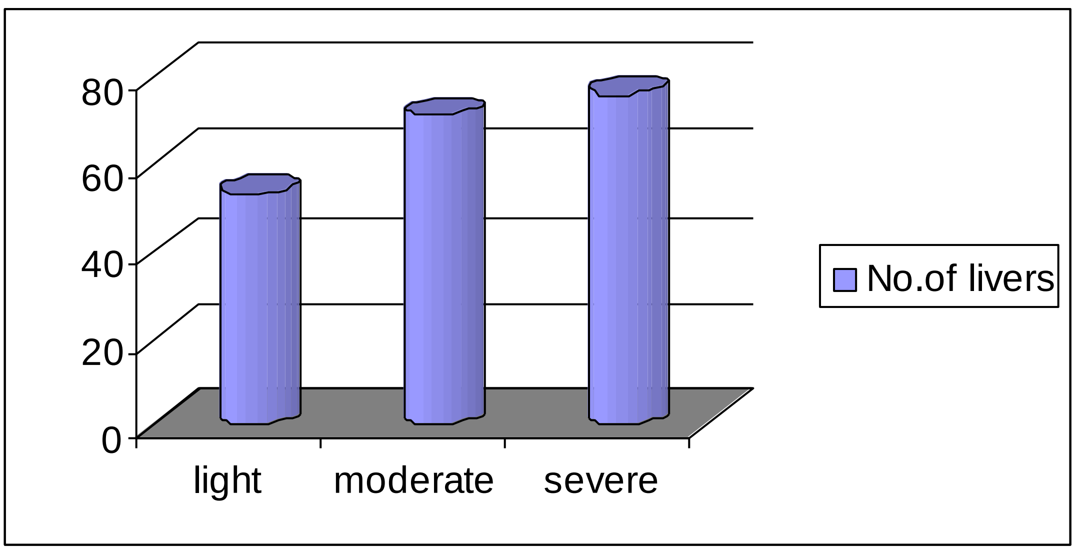

Figure5. Results of liver damage in infected animals

Animals with severely affected livers were found to show the least fluke count and faecal egg output. There is no significant association between fluke burden and intensity of hepatic lesion (r=0.020, p>0.05), and liver damage to that of faecal egg count showed inverse correlation existed (r =-0.04, p>0.05) (Fig.6).

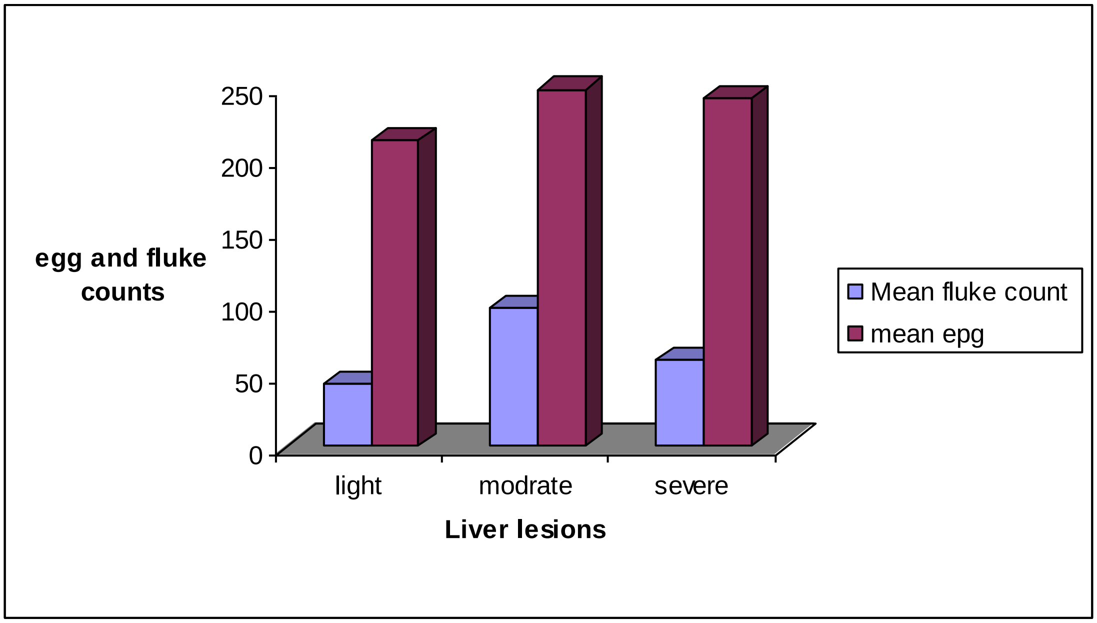

Figure 6. Relationship between liver lesion to fluke count and faecal egg count

4.4. Malacological Study

During the six months study period, a total of 60 quadrants were thrown and 90 snail intermediate hosts of fasciola which all were *L.truncatula* were collected. The monthly density gradually drops from 34 in September to 1 in February. In addition, 76% of the examined snail samples were found infected and there was a significant association between the number of snails and number of cerceriae shading snails (Chi=19.029, p<0.05).

As a pattern of variation, the numbers of snails shedding cerceriae were also decreasing on monthly basis from 31 in September to 0 in February. (Fig.7)

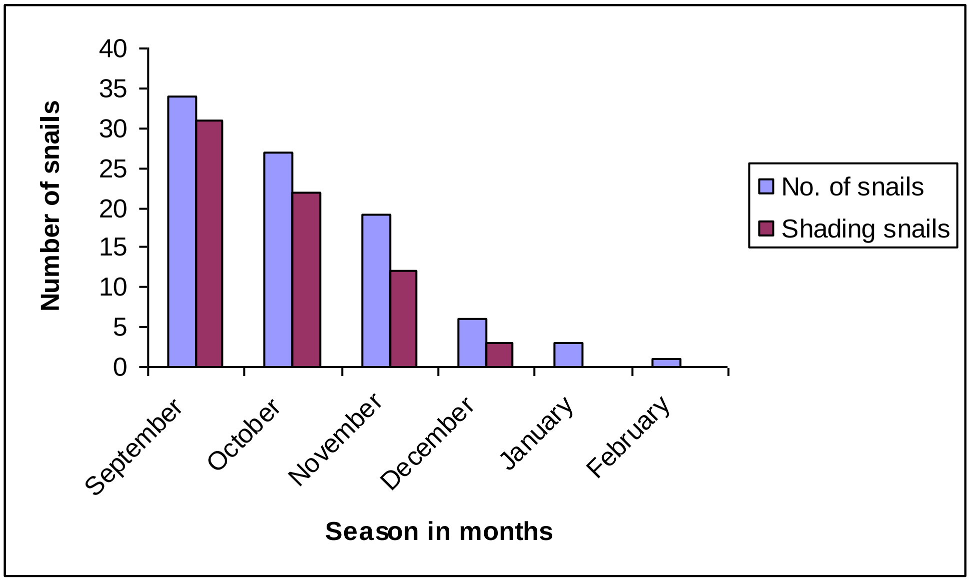

Figure 7 Seasonal snail densities and its seasonal cerceriae shedding status

The relationship between density of snails and proportion of cerceriae shedding snails with the bionomic factors, which are rainfall, relative humidity and air temperature were also evaluated and was found statistically significant (Chi=21.07, $p<0.05$) (Fig.8).

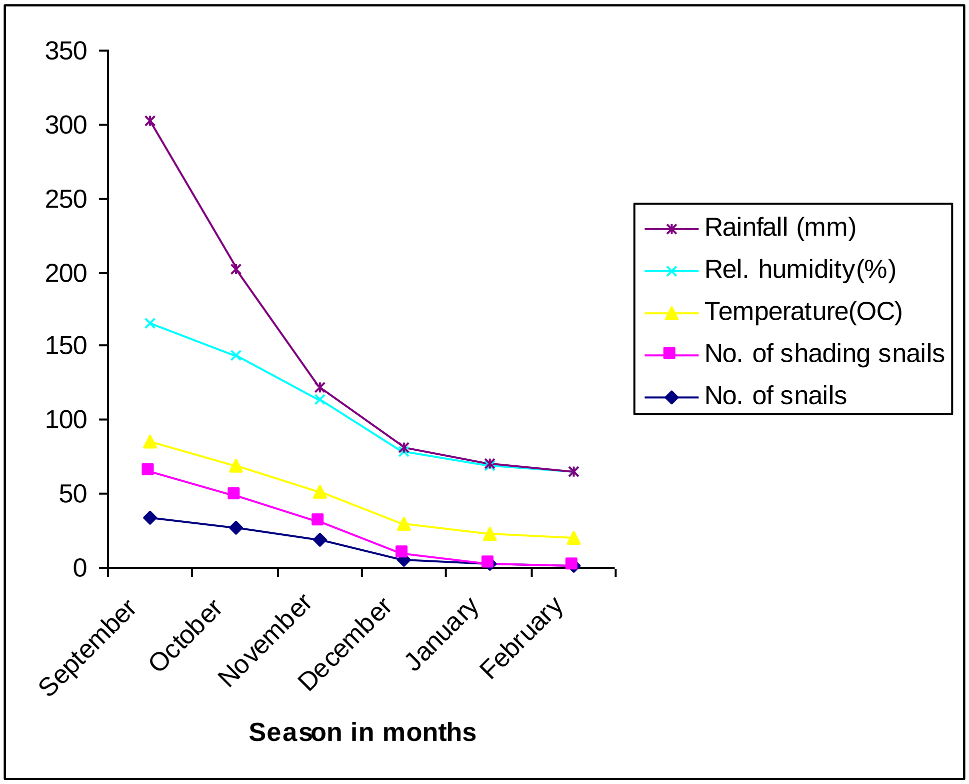

Figure 8 Number of snails and number of cerceriae shedding snails in relation to the bionomic factors

4.5. Public Health Study

Results of retrospective data analysis in Gondar Referral Hospital and clinics found in the study area, revealed no defined diagnosis of the disease fascioliasis. In addition, there was no concrete knowledge of the disease by the physicians. But after being briefed them about the symptoms, signs and epidemiology of the disease, they told me that there were increasingly enormous complains of such type with unconfirmed etiological origin especially in those people coming from villages around the lake and over flooded irrigation sites complaining of suffering after consuming raw vegetables.

5. DISCUSSIONS

5.1. Questionnaire Survey

Results of the questionnaire survey showed that cattle is the main component of livestock of the area accounting about 30% of the total population of livestock and 99% of the livestock management was extensive system and mixed farming is the dominant type of farming practice in the area. The fact that 99% of the livestock management is extensive system, together with the mixed farming practice in the area has its own contribution to the high prevalence of fascioliasis in this study (79.1%) that there is a high dissemination of the parasite from one stock to the other, further more it is difficult to implement integrated approach of treatment and control strategies such as fencing, systematic grazing etc.

The herd composition of the area is mainly (42%) animals with age groups of between 1.5-5years followed by younger animals (38%) ranging less than years of age, the rest being old animals aging above 5 years old. This composition of different age groups in a herd has impact on the distribution and dissemination of the disease in that one age group that is highly prone to infection (in this case the middle age group) will be a source of infection to the other age group at risk.

85% of the respondents indicate the presence of a disease with signs of bottle jaw, emaciation and diahorhiae indicated history of the disease and the full awareness by farmers also indicated how far the problem is in the area.

There is a trend that fascioliasis is decreasing from time to time, from year to year (83% of the respondent’s response) probably indicates the continual use of the drug anthehelmintics and better veterinary infrastructure developed nowadays has greatly reduced its incidence. In addition, it indicates the direction, which we should follow in the further control of the disease.

65% responded its presence throughout the year and only 35% told about its seasonality in particular to the wet seasons of the year. This could probably be due to the all year round marshy and flooding of the study area favoring suitable epidemiological situation for the parasite and its snail intermediate host.

100% responded that it is a wasting disease in milk production, weight gain and draught power of their animals and it is known locally as Gechita. This, besides to its diagnostic value, gives information on the impact of the disease on the livestock production system in the area.

95% of them responded that they use different antihelminthics mainly albendazole and its source is 65% from smugglers and only 35% from veterinary clinics. Drug delivery by livestock accounts about 65% and only 35% by veterinary personnel. Only 42% of the respondents deliver full doses of the drug. The above results of the questionnaire survey, which indicate the misuse of drugs against the disease fascioliasis, could be one of the main obstacles for the strategic control of the disease and also contribute its persistently high prevalence in the area.

5.2. Prevalence and Associated Risk Factors

The prevalence of fascioliasis in different parts of the world has been reviewed by different workers including Rangel-Ruiz *et al,* (1999), Santos, *et al*, (1999), and others. In Africa, many researchers reported prevalence of fascioliasis as El-Aziz *et al*, (2001) recorded the prevalence rate of 26.5% in Egypt, Grev, (1993) 94% in Nigeria.

In Ethiopia, bovine fascioliasis exists in almost all regions. The result of the present study has revealed a relatively high prevalence of bovine fascioliasis (79.2 %), which is comparable to other workers as 77.8% in Dembi Dollo (Aberra, 1990), 85.4% in Western Shoa (Yadeta, 1994), and 83.08% in South Gondar (Mulualem, 1998). This insignificant difference in the prevalence of fascioliasis in different regions of the country may be due to the similarity of the climatic conditions and regions such as altitude, rain fall, temperature, humidity and management system of livestock. The high prevalence in the study area may be attributed to the presence of conducive ecological factors for the snail intermediate host and the parasite

(Fasciola) in the area, the feature of the land-escape being plain with poor drainage, heavy dark brown clay soil (with slightly acidic pH) which has high capacity of water retention, with annual over flooding during the rainy season leaving pockets of water bodies and is mostly marshy areas for long periods during the dry season. Lake Tana, slowly flowing rivers and swampy parts of the lake and the river border the area. Such ecological conditions are considered favorable for breeding and survival of the intermediate host snails and the parasite (Graber, 1975, Soulsby, 1982, Urquhart, *et al.,* 1996, Cruz- Mendoza *et al*., 2004).

Both of the study areas were having high prevalence of 80 %(Gorgora) and 79 %(Chuahit) with statistically no significant difference (p>0.05). This insignificance in difference might be due to the similarity in the epidemiology of the two areas and share a common influence of over flooding by Lake Tana and other rivers, which covers the vast plain lands of different peasant association (PAs.) and villages in the region and hence create a very favorable environment for both the snail intermediate host and the parasites.

There was no statistically significant difference (p> 0.05) in prevalence between females (77.5%) and males (80.8%). This shows that sex seems have no effect on the prevalence and both sexes are equally susceptible and exposed to the disease. Similar results have been reported by Yehenew, (1987), Fekadu, (1988), Dinka, (1996) and Mulualem, (1998). However, there are reports of higher prevalence in male than females and their explanation behind is attributed to the management system, with longer exposure of males outdoor while females kept indoor at the beginning of lactation period (Boyce and Courtney, 1990).

In this study, significant variation in prevalence in cattle of different age groups were observed (p <0.05) (Table7), a finding that agrees with the works of Dwinger, (1982). The increase in resistance (low prevalence) as age increases is most likely related to the high level of tissue reaction seen in bovine liver, severe fibrosis which impedes the passage of immature flukes, acquired resistance, thickening stenosis, and calcification of bile ducts, assumed unfavorable site for adult parasites and consequently fastens their expulsion. In older age group, the prevalence was low since the longer the period the animal is grazing in the fluke areas, the higher will be its repeated infection might be responsible for the development of resistance of the host to infection. The conclusion forwarded by Ross, (1977) on rabbits in this regard, was that the initial experience of infestation resulted in reduction of the number of flukes established and those established are retarded in their development. In Ethiopia, similar results indicating inverse relation of prevalence and age of cattle were reported by Fekadu, (1988), Aberra, (1990), Mulualem, (1998) and many other workers.

The seasonal variation in prevalence of bovine fascioliasis was studied in both the wet and dry season and the variation was statistically significant (Chi= 5.47, df.= 1, $p<0.05$ The highest prevalence was observed from September to November 2004 that is during the months when both rainfall and humidity were high. It was decreasing from December to February during the dry period**.** The seasonal pattern of fascioliasis is related to the seasonal activity of the intermediate host snails. Breeding of the Lymnaea snails and development of the larval stages of the flukes within the snail are optimum during the wet months of the year which provides sufficient moisture and moderate temperature favorable for breeding of the snails and development of the larval stage flukes within the snails as well as for the survival of the metacerceriae on the herbage. During the dry periods, breeding of the snails and development of the larval flukes slows down or stops completely and snails undergo a state of aestivation (Armour, 1975; Craig, 1978, Soulsby, 1982; Urquhart *et. al*, 1996; FAO, 1994).

Rise of its prevalence during the wet season of the study period from September to November is due to seasonal peak of snail activity in breeding and development of the larval stage flukes within the snails. The aestivating snails become active, breed and develop to maturity and produce further breed during wet time. The rain washes many of the eggs out of the feacal material and keeps them moist enough to hatch and develop so that there are plenty miracidia by this time and many snails become infected. After some time the infected snails with flukes release cerceriae, which become encysted on the herbage forming metacerceriae, which is ingested by the animals, thereby animals acquire a heavy infestation and consequently the incidence of fascioliasis increases.

Low prevalence observed during the periods from December to February could be attributed to the dry climatic conditions prevailing in these months. Active population of snails will dramatically decrease in the dry period due to scarce moisture and a relatively high temperature, which creates unfavorable condition for the breeding of the snails. In addition many of the eggs don not have the chance to hatch and develop as they are trapped by the feaces' mass' and also insufficient moisture for its hatching and development. Survival of metacerceriae on the herbage and the breeding of snails also depend on the availability of moisture. Because of the drying of temporary water habitats during the dry period, snails are forced to undergo aestivation deep in the mud and only those snails in permanent water sources have the opportunity to shed cerceriae (Radostits *et al*., 1994; Soulsby, 1982). Therefore there is minimum metacerceriae on herbage, which account for low infestation rates during the dry period.

Regardless of all the above facts, there is still a relatively high prevalence in the dry period (76%) in the study area. This could be explained in light of its association to earlier infection during peak time of snail activity with consequent release of many cerceriae during the wet season and existence of conducive ecological conditions like border of lakes (Lake Tana), slowly flowing rivers and low-lying marshy areas which provide favorable condition for breeding of the snails and development of the larval stage of the parasite during the dry period. High populations of cattle are also concentrated in these areas for grazing since these areas stay green during the dry periods.

Regarding the effort to examine the effect of season on male and female cattle, analysis of results obtained indicate that there were no statistically significant differences ($p>0.05$) in both the rainy and short rainy season of the year. This might indicate that season indifferently have no effect on sex in the prevalence of the disease. But on the contrary, season was found to have effect differently on different age groups within each of the season where the statistical analysis was significant ($p<0.05$). Lower prevalence rates in both seasons were found in older animals. The gradual build-up of resistance as animals get older (continued exposure) applies in both the dry and wet seasons of the year.

Analysis of EPG in the late rainy and the short rainy seasons made using the t-test showed that there was a statistically significant difference ($p<0.05$) in the egg count per gram of feaces, which were higher in the rainy season of the year (50.3%). This fact could be attributed to the very conducive nature of the climate in the early rainy season with its optimum moisture and temperature favoring the ample multiplication and development of both the intermediate host snail and the parasite which ensures the heavily intake of the metacerceriae by animals and the subsequent production and release of excess eggs in the late rainy season.

Analysis of feacal egg counts (EPG) using the ANNOVA statistical test among age groups showed a statistically significant difference ($p<0.05$) in egg count where high count in the middle, followed by the first and least counts in the older age groups were encountered. This fact could be justified like the age-prevalence association where the increase resistance (low EPG) as age increase is most likely related to the high level of tissue reaction seen in bovine liver, severe fibrosis which impedes the passage of immature flukes, acquired resistance, thickening, stenosis, and calcification of bile ducts, assumed unfavorable site for adult parasites and consequently fastens their expulsion (and subsequent reduction in egg production). In older age group, the EPG was low since the longer the period the animal is grazing in the fluke areas, the higher will be its repeated infection might be responsible for the development of resistance of the host to infection. The conclusion forwarded by Ross, (1966)in rabbit in this regard, was that the initial experience of infestation resulted in reduction of the number of flukes established and those established are retarded in their development (in total result in lowered EPG).

The prevalence of bovine fascioliasis was statistically analyzed on the basis of body condition to study the impact of the disease on debilitating animals. There was a higher prevalence in animals with' poor' body condition but was not statistically significant ($p>0.05$) in contrast to the studies of Mulualem, 1998 and some other workers. This might be attributed to the heavy infestation of the area by the parasite and its intermediate host due to the favorable conditions created by the lake, the slowly running rivers, and the vast marshy plain land of the study area thereby increasing the chance of infection for each grazing animal regardless of its body condition.

5.3. Abattoir Study

In the abattoir survey, the proportion of liver condemnation as it is indicated in Fig.5; there were only 53 livers from a total of 200 with light infestations, which were partially trimmed. This is due to mainly bovines are sacrificed at older age. In the present study, fluke counts in moderately affected livers by far exceeds than that observed in severely affected ones (Fig.6). Lossos (1986) have shown, in beef cattle that relatively less flukes were found in severely affected livers suggesting that severe fibrosis impedes the passage of immature flukes and acquired resistance resulted in the expulsion of flukes from the bile ducts. The same fact may explain the finding of a relatively higher EPG in moderate hepatic lesion than in severely affected cases. This could be associated with in severe fascioliasis, calcification and cirrhosis, which hinder the further passage of the young immature flukes. This limits the life span of the primary infection, slows the migration of secondary infection and eventually reduces the number of flukes established (Urquahrt *et al*, 1996). Lightly correlation between fluke count and faecal egg output estimated that the excretion of fluke eggs was slightly in accordance with the number of flukes recovered. Lossos, (1986) supported the idea that, it is some what difficult to correlate the number of eggs obtained per gram of faeces to the level of infection, since it is only up to7 to 10 weeks that the number of eggs passed in feaces increased, and there after counts cannot be associated with degree of infection and egg production may be low even in heavy fluke infestation. In addition, expulsion of eggs in case of liver fluke infection is associated with the peristaltic movement of the digestive system where the eggs are deposited in the gall bladder and pushed to the lumen of the intestine along with the pour of bile to the intestine.

The mean fluke burden per affected liver was found to be 66, implying high pathogenicity of flukes in the region. Soulsby (1982) demonstrated that the presence of more than 50 flukes per liver indicate high pathogenicity. Fekadu, (1988) reported a high pathogenicity with mean fluke burden of 67. The reason for the high fluke burden in this study may be due to the presence of persistent transmission sites.

5.4. Malacological Study

When monthly variation of density and proportion of cerceriae shedding snails considered (Figs.7), a gradual decrease in snail density and the proportion of infected snails were observed from September onwards to February. This may depend on the proliferation of snails in relation to moisture availability where highest in September (wet) and lowest in February (dry). A low population dynamics and infected snail proportion in January and February may have resulted from decline in the active population of snails, because of drying of temporary water habitats, snails are forced to under go aestivation deep into the mud rapidly enough to escape sampling and only those snails in permanent water source have the opportunity to shed the cerceriae. Therefore, there was only minimum proportion of infected snails, which accounts for a relatively low proportion during these months. Boray, (1969) found that at temperature above 20 0c, there was an increased mortality of infected snails. Therefore, ideal condition for the snail breeding and development is a temperature of 10-15 0c and the rainfall exceeds transpiration.

The graph on Fig.8 shows a functional relationship between density and the proportion of infected snails with bionomic factors.

5.5. Public Health Study

Results of retrospective data records in Gondar Referral Hospital and clinics found in the study area with unknown etiological origin, was lack of awareness about the disease fascioliasis. In addition, there was no concrete knowledge of the disease by the physicians. But after I briefed them about the symptoms, signs and epidemiology of the disease, they told me that there were increasingly enormous complains of such type with ‘unknown’ (undiagnosed) etiological origin especially in those people coming from villages around the lake and over flooded irrigation sites complaining of suffering after consuming raw vegetables.

So from these findings and the epidemiology of the disease, we can at least speculate the possible presence of the disease and how far it imposes a health problem in the region.

6. CONCLUSIONS AND RECOMMENDATIONS

Fascioliasis has been and is still one of the major obstacles to livestock productivity in Ethiopia by inflicting direct and indirect losses. Its occurrence is mainly associated with the presence of ecological and bionomic factors suitable for the development of the snail intermediate host.

Observation from the present work suggest that fascioliasis is a disease of prime concern in North Gondar bordering Lake Tana and due emphasis must be given in any strata of livestock disease control program to be envisaged in the region. Because of the biological complexity associated with the disease, the process of choosing the best control strategy for particular circumstances would be difficult, since all relevant factors may not be early addressed. However, institutionalizing a workable control scheme leading to the disruption of the life cycle of the parasite is undoubtly beneficial. Strategic anthelmintic treatment preceded by detailed study on the seasonal fluctuation of infection in different climatic zones is the most suitable measure at least in the short term. This will undoubtly reduce the level of parasitic contamination.

Control of the snail which has been the major facet of fluke control for many years, need careful ecological studies, but the tremendous reproductive potential of the snails and the high cost needed for their control makes the snail control program often unsuccessful. A combination of control measures, including drainage, grazing management; fencing and molluscicides have to be used to ensure a satisfactory degree of control in the long run.

Further study on the epidemiology of the disease, the biology and ecology of the snail intermediate host are useful in planning and programming control strategies.

The last but not least is the issue that it imposes on the public health point of view. There is a growing concern in its human health hazard especially in countries having the habit of eating raw vegetables and living in riverbanks and areas where flooding is common (an area similar to this study site). In Ethiopia, the problem is yet not adequately addressed hence serious attention should be given towards the understanding of the problem among stakeholders and professionals to seek alternative isolations.

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8. ANNEXES

Annex 1 Questionnaire survey format:

Respondent name----------------------------

Code-------------------

Village----------------------------

Date-----------------------

Status: Worker---------

Owner---------

Location: Region----------------

District-------------------

Village-------------------- -

Herd structure: upto1.5yrs. -------------- 1.5-5yrs. -------------Above5yrs---------

Production system: Nomadic---------------Sedentary-------------------Mixed--------------

How do you manage cattle: Free grazing---------Tether---------Stall-feeding-----------?

Presence of a disease with: Bottle jaw------------

Diahorria and emaciation----------

Is it present now? -----------------

What is its local name? ---------------------------------

Last time of its occurrence: Month---------------Year---------------Season------------------

How frequently does it occur? Every year--------Always there-------------Every-------year

When does it occur normally? In winter ----------summer-----------------rainy----------

Does it affect the entire herd? -------------Proportion--------------

Which age group is highly affected? -------------

Does it cause death? --------------------

Number of deaths in cattle upto1.5yrs. ----------1.5-5yrs. -----------above5yrs------------age

Effect on milk production and weight gain: ----------------

Is it necessary to give a vaccine? --------------

What do you do when your animals get sick? Treat at home ---------

Vet. Clinic--------------

Others (specify) -------------

What is the local name for treatment? ---------------------------

Who is applying the treatment? You yourself---------Vets. -------------Drug smugglers----------

Which antihelmintics are you commonly using to treat your animals? --------------------

----

What quantity of antihelmintics do you use to treat your cattle?

How much money do you pay to get a single mature oxen treated?

How many times did each animal get vet. Treatment against Fascioliasis since last year?

One time only----------------

Two times----------------------

Three times----------------------

More than three times----------

Do you have antihelminthics now in stock? ----------

If yes, can you show please?

How do you use it?

Thank you

Annex 2 Sedimentation Technique

From collected samples for each case 3 grams of faeces were measured and put into a mortar. Then 42 ml. of $ZnSO_4$(Zinc sulphate) solution as a floating medium were added and crushed thoroughly with a pistle. The suspension then was allowed to pass through a mesh sieve into a beaker and the one left on the mesh was discarded. After gentle shaking, the suspension was poured into a conical centrifuge tube and was centrifuged at 1500 RPM for 3 minutes. After decanting the supernatant, the sediment was agitated till thick homogenous fluid was obtained at the bottom of the tube and then was filled equal amount of water to the previous level. The content of the tube was mixed thoroughly with thumb over the open end of the tube. And then, using a Pasteur pipette, a 0.15ml. fluid was taken from the suspension and placed on a microscope slide covered with a cover slip. Then was examined under low power objective. Finally number of eggs observed were registered and multiplied by 100 to get the total number of eggs per gram of faeces.

Annex 3 Post mortem fluke recovery and count

The gall bladder was removed and washed to screen out the mature flukes. The liver was cut to slices of about 1 cm. thick and was placed in metal trough of warm water to allow escape of mature flukes lodged in smaller bile ducts and then the heads of the flukes were counted.

Annex 4 Pathological categorization of affected livers

According to Ogunrinade, 1982 they were classified into 3 groups;

Lightly affected –when a quarter of the liver was affected or if one bile duct was prominently enlarged on the ventral surface of the liver.

Moderately affected – if half of the organ was affected or if two or three bile ducts were hyper plastic.

Severely affected – if the entire organ was involved or if the liver was cirrhotic and triangular in out line or the right lobe atrophied.

Annex 5 Malacological studies

The method of González-Lanza (1989) and Manga *et al* (1991) was used, which was the quadrant method of snail collection and study, implemented by random throwing of 1 m*1 m quadrant in a given snail habitat site and counting of all snails in this delimitation zones. Ten quadrants were thrown at any sampling occasion and all the snails in the delimitation zone were counted and the procedure was repeated once per month for 6 consecutive months.

The samples were transported to Parasitology Laboratory. The snails were placed on trays and classified according to previously established criteria (McCraw 1957); Burch and Cruz-Reyes1987*;* Burch 1989*)*. Living snails were individually placed into plastic bags with 60 ml aerated water so that those infected could release their *F. hepatica cercariae.* The snails were subjected immediately to abrupt changes in temperature, placing them in the refrigerator for 15-20 min and then being left for 24 h under an electric light bulb of 100 W located 1 m away, at room temperature. Released cercariae were counted by examining the plastic bag under a stereoscopic microscope.

Sites expected to be favorable for snail breeding were surveyed. These sites were low-lying swampy areas, water lodged areas, drainage ditches, slow flowing streams and moist vegetation of which most of them were found around Lake Tana.

In the collection, snails visible to the naked eye and were floating on the surface of water and those on the moist mud were picked up by hand wearing gloves, water sites which were deep and full of vegetation were searched by using palm-leaf trap and sieve made from cloth that could pass water through and which was tied to a long stick.

Identification of the snail species was made by studying the morphological features of the shell based on given traits for the major snail categories in literature. Personnel from parasitology department were participated in the identification of the species of snails. The collected snail samples were brought from field preserved in 5% formalin solution.

Annex 6 Body Condition Scoring

The body condition of study animals was measured by visual inspection and palpation of the loin area and tail head of the animal. The degree of fatness over these areas is assessed, and a score of poor and good was given. The method of scoring is adapted to that described by Radostits and Blood, (1985) assuming or modifying the degree of fatness scored from 0 to 1 as POOR and degree of fatness scored from 2 to 4 as GOOD. The description of each score is as follows:

0 No fatty tissue is felt, shape of transverse processes clearly visible, deep cavity under tail and around tail head. Animal appears emaciated.

1 Deep depression in loin, ends of transverse process sharp to touch and upper surfaces can be felt easily, cavity present around tail head.

2 Some fatty tissue felt under the skin, pelvis felt easily, ends of transverse processes feel rounded and upper surfaces felt only with pressure, visible depression in the loin.

3 Fatty tissue easily felt over the whole area, skin appears smooth, slight depression visible in loin; end of transverse processes can be felt with pressure, but tick layers of tissue on top.

4 Transverse processes can not be felt even with firm pressure, no depression visible in loin between back bone and hip bones, patches of fat apparent under the skin, pelvis felt only with firm pressure.

Annex 7 Data of prevalence and associated risk factors

Annex 8 Stata software outputs of prevalence and associated risk factors

use "C:\stata\update.dta", clear

. tab fasciola season,chi row

season

Fasciola 1 2 Total

0 100 131 231

43.29 56.71 100.00

1 455 421 876

51.94 48.06 100.00

Total 555 552 1107

50.14 49.86 100.00

Pearson chi2(1) = 5.4717 Pr = 0.019

. tab fasciola agegrp, chi row

agegrp

Fasciola 1 2 3 Total

0 41 20 170 231

17.75 8.66 73.59 100.00

1 145 341 390 876

16.55 38.93 44.52 100.00

Total 186 361 560 1107

16.80 32.61 50.59 100.00

Pearson chi2(2) = 82.0549 Pr = 0.000

. tab fasciola sex, chi row

	sex		
Fasciola	1	2	Total
0	122	109	231
	52.81	47.19	100.00
1	419	457	876
	47.83	52.17	100.00
Total	541	566	1107
	48.87	51.13	100.00

Pearson chi2(1) = 1.8163 Pr = 0.178

. anova epglog agegrp

Number of obs = 1107 R-squared = 0.1241

Root MSE = .947502 Adj R-squared = 0.1225

Source	Partial SS	df	MS	F	Prob > F
Model	140.40162	2	70.2008102	78.20	0.0000

agegrp 140.40162 2 70.2008102 78.20 0.0000

Residual 991.12638 1104 .897759402
-----------+--
Total 1131.528 1106 1.02308137

. ttest epglog,by(season)

Two-sample t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
1	555	1.895586	.0408648	.9627119	1.815317	1.975855
2	552	1.762174	.044899	1.054889	1.67398	1.850368
combined	1107	1.829061	.0304005	1.011475	1.769411	1.88871
diff		.1334117	.0606963		.0143187	.2525046

Degrees of freedom: 1105

Ho: mean(1) - mean(2) = diff = 0

Ha: diff < 0	Ha: diff ~= 0	Ha: diff > 0
t = 2.1980	t = 2.1980	t = 2.1980
P < t = 0.9859	P > t = 0.0282	P > t = 0.0141

P < t = 0.9859 P > t = 0.0282 P > t = 0.0141

dit

- preserve

. tab fasciola bodycondition,chi

Body Condition

Fasciola	1	2	Total
0	145	86	231
1	546	330	876
Total	691	416	1107

Pearson chi2(1) = 0.0152 Pr = 0.902

. tab fasciola villages,chi

Villages

Fasciola	1	2	Total
0	118	113	231
1	435	441	876
Total	553	554	1107

Pearson chi2(1) = 0.1484 Pr = 0.700

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9. CURRICULUM VITAE

Name: Getachew Jember Bizuneh

Sex: Male

Date of birth: July3, 1961Eth. cal

Place of birth: Chilga, Gondar, Ethiopia

Nationality: Ethiopian

Marital status: Single

Profession : Veterinarian

Education

1966-1973Eth. Cal. Seraba Junior Secondary and Senior Secondary School

1974- 1977Eth. Cal Aykel secondary high school,

1978-1983 Eth. Cal Faculty of Veterinary Medicine, Addis Ababa University

Work Experience

1984-1992 District veterinarian, Bureau of Agriculture of the Amhara National Regional State

1993-1994 Zone Veterinarian, Bureau of Agriculture of the Amhara National Regional State

1995 District Head of Animal production and Health Desk, Bureau of Agriculture of the Amhara National Regional State

Publication

Getachew Jember (1993): Prevalence of Hydatidosis in Awassa Abbatoir. DVM Thesis.

10. SIGNED DECLARATION SHEET

I, the under signed, declare that the thesis is my original work and has not been presented for a degree in any university

Name ___________________________________

Signature ___________________________________

Date of Submission ___________________________

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

Dr Getachew Tilahun _____________________