Review on the Biology of Fasciola Parasites and the Epidemiology on Small Ruminants

Mihretu Ayele*
College of Veterinary Medicine, Haramaya University, P. O. Box 138 Dire dawa, Ethiopia

Adem Hiko
College of Veterinary Medicine, Haramaya University, P. O. Box 138 Dire dawa, Ethiopa

Summary
Small ruminant fasciolosis is a serious problem in animal production in different areas of the world especially in Ethiopia. It is a wide spread trematodal disease affecting small ruminants (sheep and goats) and also other species of animals. *Fasciola hepatica* and *Faciola gigantica* are the parasitic species belonging to Genus Fasciola under the phylum platyhelminths. *Fasciola hepatica* was shown to be the most important fluke species in Ethiopian livestock and it requires snail of the genus Lymnae for the completion of its life cycle and its biological features; mainly its external body structures such as the teguments and spines besides the enzymes it secrets for exsheathment are responsible for its pathogenicity. *Fasciola gigantica* which is tropical species can exist up to 2600m of elevation although an effective transmission cycle in a single year can only be maintained at elevation below 1700m. Availability of suitable ecology for snail; temperature, moisture and pH are factors influencing the agent and its epidemiology. The course of the disease runs from chronic long lasting to acute rapidly fatal. These give rise to application of different diagnostic methods including fecal egg examination, post-mortem examination, immunological assessment and serological liver enzyme analysis. Therefore, people have to be aware of the significance (both economic and public health significance) and have to do all their best so as to minimize the occurrence or control the disease by taking measures like forecasting the occurrence, reduction of snail population, prophylactic use of anthelmintic and immunologic approach.
Keywords: Fasciola, Biological characteristics, Small Ruminants, Epidemiology

1. INTRODUCTION

A number of factors limit efficient use of livestock production in different parts of the world including Ethiopia. Disease, malnutrition and other management problems are the major once. Among animal diseases, parasitic infections are considered to be important. In this regard Fasciolosis is one of the major prevalent Helminths diseases contributing to the losses in productivity worldwide (Scott and Goll, 1977; Smyth, 1994; WHO, 1995).
The agent responsible for the cause is Fasciola. As to the biology of the agent, members of this genus are commonly known as liver flukes, a leaf shaped approximately one inch long and somewhat less than one and half inch wide (David, 1990). The most important species concerned are *Fasciola hepatica* (*F. hepatica*) and *Fasciola gigantica (F. gigantica)*. The disease caused by Fasciola hepatica is one of the most sever Helminths infection for small ruminants causing wide spread morbidity, mortality, weight loss, anaemia and hypoproteinemia (Yadeta, 1994).

As to the epidemiology, the spread of hepatic fascioliasis to new area depends upon the spread of the host snail, the infected ruminants and the existence of suitable habitats for the snail distributing the disease (Heinonen and Kebede, 1995). Water logged and poorly drained areas with acid soils in the highlands are often endemic areas for Fasciolosis and since Ethiopia is one of the countries with recent expanding activities of small-scale traditional irrigation, this creates favourable conditions for fluke transmitting snail vectors and thereby favours the life cycle progression of the disease (Traore, 1989).

As the disease is of great economic significance, it causes great losses through mortality, ill thrift, liver condemnation at the abattoirs, predisposing to other disease etc. However, such great loss has to be minimized by implementing efficient diagnostic and control measures (Urquhart *et al.,* 1996).
Therefore the objective of this seminar paper is:

- To review the biology of Fasciola species and the epidemiology of fasciolosis among small ruminants
- To assess the epidemiology and ecology of the disease.

2. BIOLOGY OF THE AGENT

2.1. Taxonomy

Phylum platyhelminths contain the two classes of parasitic flat worms, the Trematoda and the Cestoda. The class Trematoda falls into two main subclasses, the Monogenia which have a direct life cycle, and the Digenia which require an intermediate host. There are many families in the class Trematoda and those which include parasites of the major veterinary importance are the Fasciolidae, Dicrocoeliidae, Paramphistomatidae and Schistosomatidae (Urquhart *et al.,* 1996). Of the lesser importance are the Troglotrematidae and Opisthorchiidae.

The most important by far are the Fasciolidae (Urquhart *et al.,* 1996).

According to Urquhart *et al.,* (1996), Fasciola belongs to the following taxonomic classification;

Kingdom:	Animalia
Phylum:	Platyhelminthes
Class:	Trematoda
Subclass:	Digenea
Order:	Echinostomida
Family:	Fasciolidae
Genus:	Fasciola
Species:	*F. hepatica; F. gigantica*

2.2. Morphology

Adult flukes are flattened and leaf like in its morphology measuring 30 by 13mm. These liver flukes are broader in the anterior region and possess an anterior cone shaped projection that is followed by a pair of prominent laterally directed shoulder (Hendrix and Robinson, 2006). The tegument is well armed with backwardly direct spines which together with the suckers serve as an effective mechanism to maintain the position of the parasite in the bile duct (Smyth, 1994).

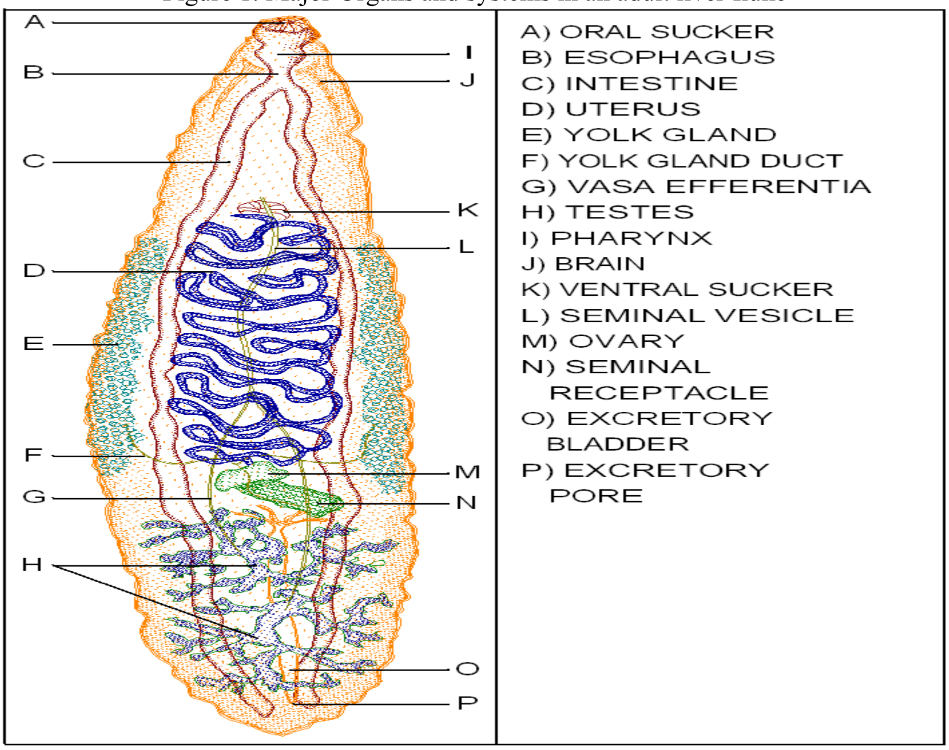
2.3. Structure and Function

The adult possesses two suckers for attachment. The oral sucker at the anterior end surrounds the mouth and the ventral suckers that is placed at the level of the shoulders of the fluke. The body surface is a tegument which is absorptive and is covered with spines (Urquhart *et al*., 1996).

The digestive system is simple, the oral opening leading to the pharynx, oesophagus and a pair of branched intestinal cecae which end blindly. Undigested material is presumably regurgitated. The excretory system consists of a large number of ciliated flame cells, which impel waste metabolic products along system of tubules which ultimately joins and opens to the exterior. The nervous system is simple consisting of a pair of longitudinal trunks connecting interiorly with two ganglia (Urquhart *et al*., 1996).

Trematodes in general are usually hermaphrodites and both cross- and self- fertilization may occur. The male reproductive system consisting of a pair of testes each leading to the vas-difference; these join to enter the cirrus sac containing a seminal vesicle and the cirrus, a primitive penis which terminates at the common genital opening. The female system has a single ovary leading into an oviduct which is expanded distally to form the ootype. There the ovum acquires a yolk from the secretion of the vitelline glands and ultimately a shell (Urquhart *et al*., 1996).

Figure 1. Major Organs and systems in an adult liver fluke



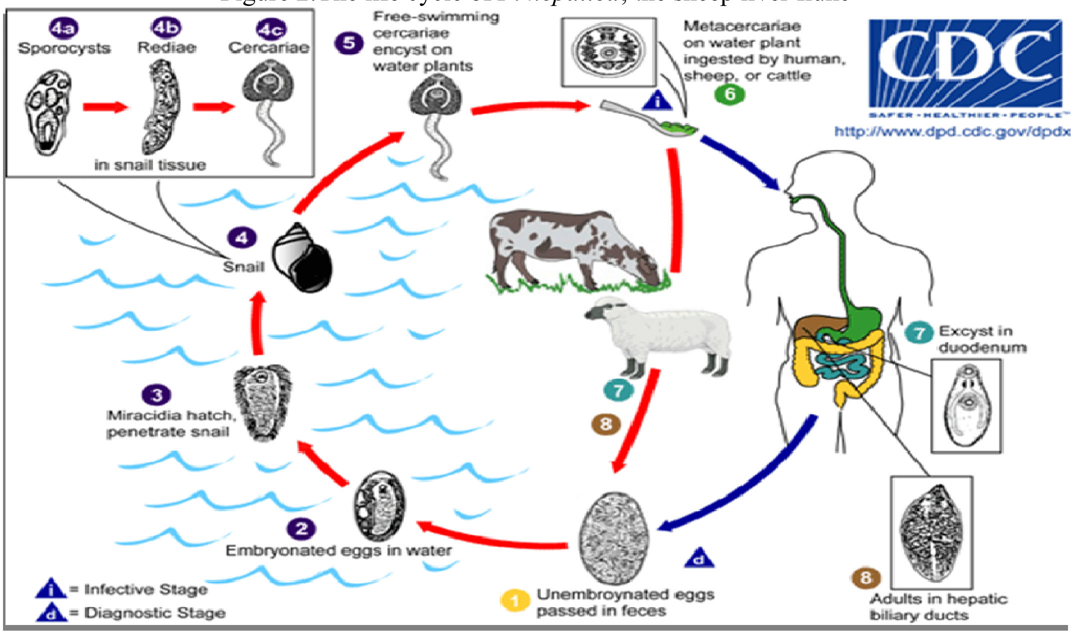
(Source: Urquhart *et al.,* 1996).

2.4. Life Cycle and Host Range

2.4.1. Life cycle of Fasciola

Immature eggs are discharged in the biliary ducts and in the stool. Eggs become embryonated in water; eggs release miracidia, which invade a suitable snail intermediate host, including the genera Galba, Fossaria and Pseudosuccinea. In the snail the parasites undergo several developmental stages (sporocysts, rediae and cercariae). The cercariae are released from the snail and encyst as metacercariae on aquatic vegetation or other surfaces. Mammals acquire the infection by eating vegetation containing metacercariae. Humans can become infected by ingesting metacercariae-containing fresh water plants, especially watercress. After ingestion, the metacercariae excyst in the duodenum and migrate through the intestinal wall, the peritoneal cavity and the liver parenchyma into the biliary ducts, where they develop into adults (http://www.dpd.cdc, 2010 and Urquhart *et al.,* 1996).

Figure 2.The life cycle of *F. hepatica*; the sheep liver fluke



(Source: http://www.dpd.cdc, 2010)

2.4.2. Host range

Fasciolosis is a disease of sheep, goats, and cattle and occasionally affects humans. It is coursed by genus Fasciola commonly called liver fluke. The two most important species are *F. hepatica* and *F. gigantic* (Dunn, 1978). *Fasciola gigantica* is exclusively tropical and measures (27 to 75mm) x (3 to 12mm) whereas, *F. hepatica* is found in temperate areas (high altitude regions in east Africa) and measures approximately (20 to 30mm) x (10mm) (Brown, 1980).

Intermediate host: *Galba truncatula*, the most common intermediate host of *F. hepatica* in Europe and South America Intermediate hosts of *F. hepatica* are freshwater snails from family Lymnaeidae (Torgerson and Claxton, 1999; Graczyk *et al*., 1999). Snails from family Planorbidae act as an intermediate host of *F. hepatica* very occasionally (Mas-Coma *et al*., 2005). The following are important species involved in the transmission of *F. hepatica* and are responsible for the development of miracidium to cercaria stages of Fasciola larvae (Smyth, 1994).

Fig 3: Snail intermediate host

Table 1: Snail intermediate host and their distribution

Snail intermediate host	Country
Lymnae truncatula	Europe
Lymnae stagnalis	Europe
Lymnae viator	Peru
Lymnae columella	New Zealand
Lymnae tomentosa	Australia
Lymnae natalensis	West and Eastern Africa
Lymnae rufescens	West Africa
Lymnae glarba	Europe

(Source: Smyth, 1994)

Figure 4. Shell layers of *Galba truncatula*; intermediate host of *Fasciola hepatica*

(Source: Torgerson and Claxton, 1999)

Final host: The final hosts are sheep, goats, cattle, horse, deer, man and other mammals in which its usual site is the liver (Taylor*,* 2007). The juvenile stage migrates and became mature within the biliary system of the liver. When the agent becomes mature, it plugs the duct system. The adult one gives egg that is released with bile secretion in to gut where it was excreted with faeces (Urquhart *et al.,* 1996).

3. EPIDEMIOLOGY

3.1. Geographic Distribution

Human and animal fasciolosis occur worldwide (Torgerson and Claxton, 1999). While animal fasciolosis is distributed in countries with high cattle and sheep production, human fasciolosis occurs, excepting Western Europe, in developing countries. Fasciolosis occurs only in areas where suitable conditions for intermediate hosts exist (Torgerson and Claxton, 1999)

3.2. Ecology of Intermediate Host

The *Lymnae truncatula* is the widest spread and important species evolved in the transmission of *F. hepatica*. The snails are amphibious and although they spend hours in shallow water, they periodically emerge on to the surrounding mud. They are capable of withstanding summer drought or winter freezing for several months by respectively aestivating and hibernating deep in the mud. Optimal conditions include a slightly acid pH environment and a slowly moving water medium to carry away waste products. They feed mostly on algae and the optimum temperature range for development is 15 to 22^{0}C; below 5^{0}C development ceases (Urquhart *et al*., 1996). This snail is commonly seen in poorly drained land, drainage ditches and areas of seepages of spring or broken drains, muddy gateways, vehicle wheel ruts, wet and muddy places near drinking troughs and hoof prints of animals on clay soil (Soulsby, 1968).

3.3. Factors Influencing the Agent.

The main factors determining the timing and severity of metacercariae accumulating on herbage are listed below. In particular temperature and moisture (rainfall) affect both spatial and temporal abundance of snail host and the rate of development of fluke egg and larvae. Four main factors necessary for the outbreak of fasciolosis influencing the production of metacercariae are: the availability of suitable snail and its habit, temperature, moisture and pH (Radostits *et al*., 2007; Urquhart *et al*., 1996).

3.3.1. Availability of suitable snail and its habit

Lymnae truncatula prefers wet mud to free water and permanent habitats includes the banks of ditches or streams and the edges of small ponds, following heavy rainfall or flooding, temporary habitats may be provided by hoof marks, wheel ruts, or rain ponds. Though slightly acid pH environment is optimal for *Lymnae truncatula*, excessively acid pH levels are detrimental (Radostits *et al*., 2007; Urquhart *et al*., 1996; Rowcliff and Ollerenshow, 1960).

3.3.2. Temperature

Temperature is an important factor affecting the development rate of snails and the stages of parasites outside the host. The mean day and night temperature of 10^0C or above is necessary for the snail host to breed and for the F. hepatica to develop within the snail (Radostits *et al*., 2007). All activities cease at temperature below 5^0C. This is also minimum range for the development and hatching of F. hepatica eggs. However, it is only when temperature rises to 15^0c and is maintained above this level that a significant multiplication of snails and flukes larvae stages ensures (Urquhart *et al*., 1996)

3.3.3. Moisture

The ideal moisture condition 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, for miracidia searching for snails and for the dispersal of cercariae being shed from the snail (Urquhart *et al*., 1996)

3.3.4. PH

Fields with clumps of rushes are common sites having a slight pH. Eggs incubated at 27^0C will develop and hatch within a pH range of 4.2 to 9.0, but development is prolonged when pH exceeds 8.0 (Rowcliff and Ollerenshow, 1960)

3.4. Significance of Small Ruminant Fasciolosis

3.4.1. Economic significance

The economic losses due to fasciolosis throughout the world are enormous and these losses are associated with mortality, morbidity, reduced growth rate, condemnation of fluky, liver, increased susceptibility to secondary infections and expense due to control measures (Urquhart *et al.,* 1996).

Economical effect of fasciolosis in sheep consists in sudden deaths of animals as well as in reduction of weight gain and wool production (Sinclair, 1962; Roseby, 1970). Importance of cattle fasciolosis consist in economic losses caused by condemnation of livers at slaughter and production losses especially due to reduced weight gain (Phiri *et al*., 2006).

3.4.2. Public health significance

Studies carried out in recent years have shown human fasciolosis to be an important public health problem (Chen and Mott, 1990). Human *F. hepatica* infection is determined by the presence of the intermediate snail hosts, domestic herbivorous animals, climatic conditions and the dietary habits of man (Radostits *et al*., 2007; Chen and Mott, 1990). Because *F. hepatica* cercariae also encyst on water surface, humans can be infected by drinking of fresh untreated water containing metacercariae (Chen and Mott, 1990). In addition, an experimental study suggested that humans consuming raw liver dishes from fresh liver infected with juvenile flukes could become infected (Taira *et al.*, 1997).

4. PATHOGENESIS AND CLINICAL SIGNS

4.1. Pathogenesis

The development of infection in definitive host is divided into two phases which are the parenchymal (migratory) phase and the biliary phase (Dubinský, 1993). The parenchymal phase begins when excysted juvenile flukes penetrate the intestinal wall. After the penetration of the intestine, flukes migrate within the abdominal cavity and penetrate the liver or other organs. F. hepatica has a strong predilection for the tissues of the liver (Behm*,* 1999). Occasionally, ectopic locations of flukes such as the lungs, diaphragm, intestinal wall, kidneys, and subcutaneous tissue can occur (Chen and Mott, 1990; Boray, 1969). During the migration of flukes, tissues are mechanically destroyed and inflammation appears around migratory tracks of flukes. The second phase (the biliary phase) begins when parasites enter the biliary ducts of the liver. In biliary ducts, flukes mature, feed on blood, and produce eggs. Hypertrophy of biliary ducts associated with obstruction of the lumen occurs as a result of tissue damage (Taylor*,* 2007; Urquhart *et al.,* 1996).

Fig 5: Adult Fasciola on the bile duct liver from sheep examined at post mortem

4.2 Clinical Signs

Clinical signs of fasciolosis are always closely associated with infectious dose (amount of ingested metacercariae). In sheep, as the most common definitive host, clinical presentation is divided into 4 types (Radostits *et al.*, 2007; Behm*,* 1999; Urquhart *et al.,* 1996 and Dubinský, 1993). The four clinical types of fasciolosis in small ruminants are described below.

A) Acute Type I Fasciolosis**:** infectious dose is more than 5000 ingested metacercariae. Sheep suddenly die without any previous clinical signs. Ascites, abdominal haemorrhage, icterus, pallor of membranes, weakness may be observed in sheep (Radostits *et al.*, 2007; Behm*,* 1999).

B) Acute Type II Fasciolosis: infectious dose is 1000-5000 ingested metacercariae. As above, sheep die but briefly show pallor, loss of condition and ascites (Urquhart *et al* 1996).

C) Sub acute Fasciolosis**:** infectious dose is 800-1000 ingested metacercariae. Sheep are lethargic, anaemic and may die. Weight loss is a dominant feature (Radostits *et al.*, 2007 and Urquhart *et al.,* 1996).

D) Chronic Fasciolosis: infectious dose is 200-800 ingested metacercariae. Asymptomatic or gradual development of bottle jaw and ascites (ventral edema), emaciation, weight loss, anemia, hypo-albuminemia and eosinophilia may be observed in all types of fasciolosis (Behm, 1999; Dubinský, 1993).

5. DIAGNOSIS

Diagnosis is based predominantly on feces examinations and immunological methods. However, clinical signs, biochemical and hematological profile, season, climate conditions, epidemiology situation, and examinations of snails must be considered (Torgerson and Claxton, 1999; Dubinský, 1993). Based on this information, a number of techniques are used to diagnose the disease like fluke egg count, liver enzyme detection, post mortem examination (Urquhart *et al*., 1996).

5.1. Post Mortem Examination

Examination of fresh carcasses is the best method of diagnosis if liver fluke is suspected. As untreated animals provide the most accurate indication of the liver fluke and periods of challenge, post mortem examination will also demonstrate any lesions resulting from concurrent disease such as black disease or parasitic gastro enteritis (Urquhart *et al.,* 1996). There is no cure and death follows quickly. As Clostridium novyi is common in the environment, black disease is found wherever populations of liver flukes and sheep overlap. Even though, it is impossible to detect Fasciola in live animals, liver examination at slaughter or necropsy was found to be the most direct, reliable and cost effective technique for the diagnosis of Fasciolosis (Urquhart *et al*., 1996).

5.2. Fluke Egg Count

Diagnosis of fasciola is confirmed by finding the eggs in the feces. These eggs must be distinguished from the eggs of other flukes especially the large eggs of paramphistomes (Soulsby, 1968) The Fasciola eggs are oval, yellow brown and measures (130 to 150µm by 60 to 90 µm). Each egg will possess a distinct operculum (Hendrix and Robinson, 2007)

5.3. Serological Detection

Various serological techniques on blood samples including ELISA can be used to detect antibodies of *Fasciola hepatica* with high level of specificity (Urquhart *et al.,* 1996). Antibodies against components of flukes are detected in serum or milk samples, the ELISA and passive hem agglutination test, being the most reliable (Taylor, 2007).

5.4. Detecting Liver Enzymes
Two enzymes are usually measured, glutamate dehydrogenase (GLDH), is released when parenchymal cells are damaged and levels become elevated within the first few weeks of infection. The other gamma-glutamyl transferase (GGT) indicates damage of epithelial cells lining the bile ducts and raised levels are maintained for longer periods (Taylor, 2007). Elevation of liver enzyme activities, such a glutamate dehydrogenase (GLDH), gamma-glutamyl transferase (GGT), and lactate dehydrogenase (LDH), is detected in subacute or chronic fasciolosis from 12-15 week after ingestion of metacercariae (Anderson *et al*., 1981; Sykes *et al*., 1980).

6. TREATMENT
A number of drugs have been used in control fasciolosis in animals. Drugs differ in their efficacy, mode of action, price, and chemical name, trade name and availability on the market. According to Fairweather and Boray (1999), the Fasciolicides (drugs against Fasciola species) fall into five main chemical groups as described below.

A. Halogenated phenols: bithionol (Bitin), hexachlorophene (Bilevon), nitroxynil (Trodax).
B. Salicylanilides: closantel (Flukiver, Supaverm), rafoxanide (Flukanide, Ranizole).
C. Benzimidazoles group: triclabendazole (Fasinex), albendazole (Vermitan, Valbazen), mebendazol (Telmin), luxabendazole (Fluxacur)
D. Sulphonamides: clorsulon (Ivomec).
E. Phenoxyalkanes: diamphenetide (Coriban) Triclabendazole (Fasinex) is considered as the most common drug due to its high efficacy against adult as well as juvenile flukes.

In general, Triclabendazole is used in control of fasciolosis of livestock in many countries. Nevertheless its long-term veterinary, it caused appearance of resistance to F. hepatica. In animals, its resistance was first described in Australia (Overend and Bowen, 1995), later in Ireland (O'Brien, 1998) and Scotland (Mitchell et al., 1998) and more recently in the Netherlands (Moll et al., 2000). Considering this fact, scientists have started to work on the development of new drug. Recently, a new Fasciolicide was successfully tested in naturally and experimentally infected cattle in Mexico. This new drug is called 'Compound Alpha' and is chemically very similar to triclabendazole (Ibarra et al., 2004).

7. CONTROL AND PREVENTION
Control of fasciolosis may be approached in different ways (Urquhart *et al.,* 1996) where some of them are listed below.

7.1. Reduction of Snail Population
A survey of the area for snail habitats should be made to determine whether these localised or wide spread. The best long term method of reducing mud snail population such as *Lymnae truncatula* is drainage since it ensures permanent destruction of snail habitats. When snail habitats are limited, a simple method of control is to fence off this area or treat annually with a molluscicide ($CuSO_4$) (Taylor *et al*., 2007 and Urquhart *et al*., 1996). When intermediate snail host is aquatic, such as *Lymnae tomentosa*, good control is possible by adding a molluscicide to the water habitats of the snail (Urquhart *et al.,* 1996)

7.2. Anthelmintic Therapy
The prophylactic use of fluke anthelminthics is aimed at reducing pasture contamination by fluke eggs at a time most suitable for their development which ranges from April to August in tropics. The removing fluke population at a time of heavy burden or at a period of nutritional and pregnancy stress to the animal are another treatment strategies. To achieve these goals, the proper controlling program is recommended for years with normal or below average rainfall. Since the time of treatment is based on the fact that most metacercareae appear in autumn and early winter, it may require modification for use in other areas (Thomas, 1983). The precise timing of the spring and autumn treatment will depend on lambing and service dates (Urquhart *et al*., 1996)

7.3. Forecasting the Occurrence
The life cycle of liver flukes and the prevalence of fasciolosis is dependent on climate. This has led to the development of forecasting systems based on meteorological data which estimates the likely timing and severity of the disease. The forecast is used to issue an early warning of disease by calculating data from May to August, so that control measures can be introduced prior to shedding of cercareae (Taylor, 2007). Although this technique is mainly applied to summer infection of snail, it is also used for forecasting the winter infection of snail by summating the value for August, September and October. The other technique used is 'a wet day'

forecast. This compares the prevalence of Fasciolosis over a number of years with the number of rain days during the summer of these years. In essence, wide spread fasciolosis is associated with 12 wet days (over 1.0mm of rainfall) per month from June to September where temperature do not fall below the seasonal normal (Urquhart et al., 1996).

7.4. Immunological Approach

Antibody is produced by the host against the antigen in the exsheathing fluid and against enzymes created by the worm. Immunity could be produced by vaccinating with antigen material derived from helminthes. Helminthes immunity is usually less efficient and more transient than the immunity to microorganisms because, they don't reproduce in the host as do bacteria, virus and protozoa. Even though cattle develop a strong immune reaction to Fasciola hepatica than sheep, the severe reaction in cattle results in hepatic fibrosis, hyperplasia and calcification of the bile ducts (Radostits *et al*., 1994).

8. CONCLUSION AND RECOMMENDATIONS

The suitable agro ecologic area is responsible for the growth and multiplication of the parasites and their snail intermediate hosts. Ethiopia is one of the countries having the suitable habitats for the intermediate hosts, is severely affected by Fasciolosis. The presence of water in combination with favourable temperature and soil types in Ethiopia is the main factor influencing the snail distribution. Physical changes of the landscape have also created a favourable biotype for the establishment of the snail. This suggests that the extension of irrigation networks in arid zones has enlarged the geographic area for the snail and snail transmitted diseases such as Fasciolosis. Direct action of reducing or eliminating snail population as well as irrigation schemes has to be instituted for the effective prevention and control of Fasciolosis and other water born animal and human diseases. In light of the above conclusion, the following recommendations are forwarded:

- ✓ Epidemiological studies on the prevalence of Fasciolosis are recommended by taking agro ecological situation into consideration in a given area so as to take effective control measure.
- ✓ Study on the biology of the agent and its snail intermediate host as well as identifying their appropriate habitats are recommended for treatments of animals and ecology.
- ✓ Locally available control strategies like planting and use of trees and shrubs having molluscicidal activity like “Endode” along streams shall be encouraged from economic point of view.
- ✓ Strict adherence in proper dosage of fluckcidal and molluscicidal drugs in treatment and control strategies are recommended to avoid drug resistance.

9. REFERENCES

Anderson, P.H., Matthews, J.G., Barrett, S., Brush, P.J., Patterson, D.S. (1981). Changes in plasma enzyme activities and other blood components in response to acute and chronic liver damage in cattle. *Res Vet Sci*. 31: 1-4.

Behm, C.A. and Sangster, N.C. (1999): Pathology, pathophysiology and clinical aspects. In Dalton, J.P. (Ed.), Fasciolosis. CAB International Publishing, Wallingford, Pp. 185–224.

Boray, J. C. (1969). "Experimental fascioliasis in Australia". *Adv. Parasitolo*. 7: 95–210

Brown, D.S. (1980): Fresh water snails of Africa and their medical importance, Taylor and France Ltd., London. P. 487.

Chen, M.G., Mott, K.E. (1990): Progress in assessment of morbidity due to *Fasciola hepatica* infection: a review of recent literature. *Trop. Dis. Bull*. 87:1–38.

David, C. (1990): The veterinary book for sheep farms. 2nd ed. Butler and Tanner, Ltd. London, UK. Pp. 521-533.

Dubinský, P. (1993). Trematódy a trematodózy. PP. 158–187

Fairweather, I., Boray, J.C. (1999): "Fasciolicides efficacy, actions, resistance and its management". *Vet. J.* 158: 81–112.

Graczyk, TK. and Fried B. (1999). "Development of Fasciola hepatica in the intermediate host". In: Dalton, J.P. Fasciolosis. Wallingford, Oxon, UK: CABI Pub. Pp. 31–46.

Hendrix, C. M. and Robinson, E. (2006). Diagnostic parasitology for veterinary technicians. 3rd ed. Pp. 107 – 109.

http://www.dpd.cdc.gov/DPDX/HTML/Fascioliasis.htm. Life cycle of fasciola. Date of access, March 6, 2010.

http://www.msu.edu/course/zol/316/f.hep tax.htm. Taxonomic classification of fasciola. (date of access March 6, 2010).

Ibarra, F., Vera, Y., Quiroz, H., (2004): "Determination of the effective dose of an experimental fasciolicide in naturally and experimentally infected cattle". *Vet. Parasitolo*.120: 65–74.

Mas-Coma, S., Bargues, M. D., Valero, M. A. (2005): "Fascioliasis and other plant-borne trematode zoonoses". *Int. J. Parasitol*. 35 : 1255–1278.

Mitchell, G. B., Maris, L., Bonniwell, M.A. (1998). "Triclabendazole-resistant liver fluke in Scottish sheep". .

*Vet. Rec*143: 399.
Moll, L., Gaasenbeek, C.P., Vellema, P., Borgsteede, F.H. (2000): "Resistance of Fasciola hepatica against triclabendazole in cattle and sheep in The Netherlands". *Vet. Parasitol*. 91: 153–158.
O'Brien, D.J. (1998): Fasciolosis: a threat to livestock. *Iris Vet. J.* 51: 539–541.
Overend, D.J., Bowen, F.L. (1995): "Resistance of Fasciola hepatica to triclabendazole". *Aust. Vet. J.* 72: 275–276.
Phiri, I. K., Phiri, A. M., Harrison, L. J. (2006): "Serum antibody isotype responses of Fasciola-infected sheep and cattle to excretory and secretory products of Fasciola species". *Vet. Parasitolo*. 141 : 234–342.
Radostits , O. M., Gay, C.C., Hinchcliff, K.W., Constable, P.D., (2007): A text book of the disease of cattle, horse, sheep, pigs and goats. 10th ed. Pp. 1576-1579
Roseby, F.B., (1970). The effect of fasciolosis on the wool production of merino sheep. *Aust. Vet. J.* 46: 361–365.
Rowcliff, S.A. and Ollerenshow, C. B. (1960): Observation on the bionomics of the eggs of *Fasciola hepatica*. *Ann. Trop. Med. Parasite*. 54: 172 -181.
Scott, J.M. and Goll, P.H. (1977): The epidemiology and anthematic control of ovine Fasciolosis in Ethiopian central highlands. *Bri. Vet. J.* 133: 273-280
Sinclair, K.B. (1962). Observations on the clinical pathology of ovine fascioliasis. *Bri. Vet. J.* 118: 37–53.
Smyth, J.D. (1994): Introduction to animal parasitology. 3rd ed. Pp.206.
Soulsby, E. J. L., (1968): Helminthes, arthropods and protozoa of domestic animals. 6th ed. Pp. 22 – 34.
Sykes, A.R., Coop, A.R., Robinson, M.G. (1980). Chronic subclinical ovine fascioliasis: plasma glutamate dehydrogenase, gamma glutamyl transpeptidase and aspartate aminotransferase activities and their significance as diagnostic aids. *Res. Vet. Sci*. 28: 71–78.
Taira, N., Yoshifuji, H., Boray, J. C. (1997): Zoonotic potential of infection with Fasciola spp. by consumption of freshly prepared raw liver containing immature flukes. *Int. J. Parasitolo.* 27, 775–779.
Taylor, M. A. and Coop, R. L. (2007): Veterinary Parasitology. 3rd ed. Pp. 85-89.
Thomas, A. P. (1983). The life history of the liver fluke (*Fasciola hepatica). Quarterly J. Microscopic. Sci*. 23: 99-133.
Torgerson, P. And Claxton, J. (1999). "Epidemiology and control." In: Dalton, J. P. Fasciolosis. Wallingford, Oxon, UK: CABI Pub. Pp. 113–49
Traore, A. (1989): Incidence and control of Fasciolosis around Niono, ILCA. *Bulletin Anim. Prod*. 33: 18-20.
Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A.M., Jennings, F. W. (1996): Veterinary parasitology 2nd ed. Blackwell, Science, UK. Pp.103-113.
WHO. (1995). Control of Food born Trematode Infections. WHO Anonymous Technical Series No. 849. WHO, Geneva, P.157