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Epidemiological and Pathological Studies of Fascioliasis in Goats in Sylhet Region of Bangladesh and investigation on Effects of Different Liver Tonic on Pathology of Fascioliasis

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EPIDEMIOLOGICAL AND PATHOLOGICAL STUDIES OF FASCIOLIASIS IN GOATS IN SYLHET REGION OF BANGLADESH AND INVESTIGATION ON EFFECTS OF DIFFERENT LIVER TONIC ON PATHOLOGY OF FASCIOLIASIS

PhD THESIS

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JUNE, 2015

DEDICATED
TO MY
BELOVED PARENTS,
MY WIFE (Dr. RITA)
&
DAUGHTERS
ROSE AND SANVI

DECLARATION

I, hereby declare that the thesis entitled **"Epidemiological and pathological studies of fascioliasis in goats in Sylhet region of Bangladesh and investigation on effects of different liver tonic on pathology of fascioliasis"** is genuine record of research work carried out by me under the guidance and supervision of **Dr. Moizur Rahman,** Associate Professor, Department of Animal Husbandry and Veterinary Science, Faculty of Agriculture, University of Rajshahi, Rajshahi-6205. The thesis contains no materials which has been accepted for the award of any other degree or diploma elsewhere, and to the best of my knowledge, the thesis contains no material previously published or written by another person, except where due reference is made in the text.

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CERTIFICATE

This is to certify that the PhD dissertation entitled **"Epidemiological and pathological studies of fascioliasis in goats in Sylhet region of Bangladesh and investigation on effects of different liver tonic on pathology of fascioliasis"** submitted to the University of Rajshahi, Faculty of Agriculture, for the award of the degree of Doctor of Philosophy (PhD) in the Department of Animal Husbandry and Veterinary Science. This dissertation is written based on the original research work of **Kazi Mehetazul Islam**, a hard working PhD Research Fellow, carried out under our joint supervision. The dissertation has not been submitted to this University or to some other University before and is submitted now for the first time. It is further certified that this dissertation is fit to submit for the degree of Doctor of Philosophy (PhD) in the Department of Animal Husbandry and Veterinary Science and the candidate has fulfilled all the statutory requirements for the completion of the PhD Programme.

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The Author

LIST OF ABBREVIATIONS

ALP	=	Alkaline phosphatase
ALT/SGOT	=	Alanine transaferase/ Serum glutamate oxalate transaminase
AST/SGPT	=	Aspartate transferase/ Serum glutamate pyruvate transaminase
BBS	=	Bangladesh Bureau of Statistics
Bwt	=	Body weight
DLS	=	Directorate of Livestock Services
DMRT	=	Duncan's Multiple Range Test
DPX	=	Dextrin Plasticized Xylene
EPG	=	Eggs per gram
EDTA	=	Ethylene Diamine Tetraacetic Acid.
FAO	=	Food and Agriculture Organization
F. gigantica	=	*Fasciola gigantica*
GDP	=	Gross Domestic Product
Hb	=	Haemoglobin
g%	=	Gram Percentage
HH/SH	=	Household/Slaughterhouse
H & E	=	Hematoxylin and Eosin
L. auricularia	=	*Lymnaea auricularia*
L. snails	=	*Lymnaea snails*
MS	=	Meteorological Station
PCV	=	Packed Cell Volume
SPSS	=	Statistical Package for Social Services
%	=	Percentage
± SE	=	Plus minus Standard Error
TCBZ	=	Triaclabendazole
TEC/TLC	=	Total Erythrocyte Count/Total Leukocyte Count
U/L	=	Unit per Liter
WHO	=	World Health Organization
SAU	=	Sylhet Agricultural University

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ABSTRACT

An attempt was made to determine the prevalence, patho-surveillance and histopathology of caprine fascioliasis in five agro-ecologic zones in Sylhet region of Bangladesh. Goats were divided into young and adult groups. Rectal fecal samples from household live goats and livers from slaughtered goats were collected randomly and examined from July 2012 to June 2013. A survey was also conducted to determine the snail populations and to know the infection status of the developmental stages of *F. gigantica* in *Lymnaea* snails. In addition,, the effects of liver tonic, anthelmintic and their combination treatments on some indicator parameters were evaluated. Statistical comparisons, using SPSS analysis, Pearson's correlation and ANOVA statistics, were made to determine the differences in prevalence among seasons, age, sex and different upazillas.

A total of 1288 rectal fecal samples from household live goats and 2000 livers of slaughtered goats were examined. Four hundred five (31.75%) household live goats and 202 (10.10%) slaughtered goat livers were infected with *Fasciola gigantica*. The overall prevalence was 20.93%. The prevalence of fascioliasis in Biswanath (25.96%) was significantly ($P<0.001$) higher than Beanibazar (22.16%), Balaganj (20.23%), Jaintapur (19.45%) and Sylhet Sadar (16.84%). It was noticed that prevalence in household goats was higher than slaughtered goats. The infection rate in young household (39.05%) and slaughtered (15.58 %) goats was higher than adult household (23.22%) and slaughtered (9.59%) goats. The females showed high infection rate than male. The fascioliasis was more significantly recorded in females household (39.15%) and slaughtered (13.10%) goats than males (19.96% and 7.10%, respectively). Closer analysis of results indicated that there was statistically significant difference ($p<0.001$) in prevalence rates in different upazilla as well as between female and male goats. Seasonally, the highest prevalence (25.71%) was recorded during rainy season and the lowest (10.12%) was in summer season. The prevalence rate in rainy season was statistically significant ($p<0.001$) than winter and summer.

Intermediate hosts for *Fasciola gigantica*, the *L. auricularia var rufescens* was found in all agro-ecological sites at five different upazilla of Sylhet region. A total of 1865 *Lymnaea* snails were collected and examined. Of which 56 (3%) *Lymnaea* snails were infected with different developmental stages of *F. gigantica*. The infected snail

percentage was highest 4.08% in Biswanath followed by 3.16% in Beanibazar, 2.53% in Balaganj, 2.40% in Jaintapur and the lowest 1.83% in Sylhet Sadar. In month-wise data, the prevalence of snails infection was highest in May (5.06%) and August (5.61%) and the lowest in February (0.68%) and March (0.74%). No infection was observed in November to January. The larval stages of *F. gigantica* infection in *Lymnaea* snail's was highest in rainy (4.63%) season followed by summer (1.92%) and the lowest in winter (0.76%). The study revealed that the infection level of the developmental stages of *F. gigantica* in snails population decreases from November to January and increases from February to October and highest in August and September.

In gross examination, the infected livers were enlarged with rounded edges and thickened capsule. In acute cases, numerous hemorrhagic spots were observed on the parietal and visceral surfaces of the affected liver. In chronic form, liver was cirrhotic and reduced in size. The affected intra-hepatic bile ducts were protruded and engorged with flukes. Microscopically, migratory tracts were represented by the presence of hemorrhage, edema and infiltration of numerous eosinophil mixed with few lymphocytes. Fatty change, atrophy and necrosis of hepatocytes were recorded along with deposition of bile pigment in hepatic parenchyma and damage of portal tract area. The wall of bile ducts was thickened due to fibrosis and lining epithelia were hyperplastic. Cross sections of adult and immature flukes were found within the lumen of the thickened bile ducts and hepatic parenchyma respectively.

The efficacy of the anthelmintic, liver tonic and anthelmintic along with liver tonic against *F. gigantica* infection in goats and its effect on hematological and biochemical parameters were determined. Thirty five goats (2.0 to 2.5 years old) were randomly selected and divided into seven groups (namely T_0, T_i, and T_1 to T_5) based on fecal sample examination. The first group (T_0) kept as non infected control (negative control) and the second group (T_i) was infected control (positive control). Blood samples were collected to assess the hemto-biochemical parameters. The efficacy of anthelmintic, liver tonic and their combination treatment was evaluated by counting faecal egg per gram (EPG) and comparing hematological and biochemical parameters with pre-treatment values. On 7^{th}, 14^{th}, 21^{st} and 28^{th} days of treatment, a significantly ($P<0.001$) decreased EPG count was found in treated group of goats. The EPG count of positive control group (T_i) were significantly ($P<0.001$) increased. The packed cell volume (PCV), hemoglobin (Hb), total erythrocyte count (TEC) levels were gradually

decreased significantly (P< 0.001) in goats of positive control group. Conversely, hematological values were increased in treated group of goats, except the total leukocyte count (TLC) which was decreased significantly (p<0.001). This result was the indication of effective treatments. The level of serum glutamate pyruvate transaminase/aspartate transaminase (SGPT/AST), serum glutamate oxaloacetate transaminase/alanine transaminase (SGOT/ALT) and alkaline phosphatase (ALP) were declined significantly (P<0.001) in treated groups (T_1, T_3, and T_5) as compared to the infected control group (T_i).

The current research generated data on the epidemiology of *Fasciola gigantica,* pathology (gross and microscopic) caused by the immature and mature stages of the parasite in goats at Sylhet region of Bangladesh. This study was also provided information about the developmental stages of this parasite in its intermediate host (*Lymnaea auricularia* var *rufescens*) which is very important to design effective control program. A combined approach of administration of anthelmintic and liver tonics was applied to investigate how helpful the liver tonic is in regeneration of liver tissue which was damaged by fluke. The results showed that the liver tonic might have great impact on regeneration of hepatocytes and recommence of functional activities of the liver. Taken together, the findings of the present study have significant values and might helpful in establishing an effective treatment, control and eradication programs of fascioliasis in goats in Sylhet region of Bangladesh and also in similar temperate agro-ecological zones around the world.

CHAPTER-I

INTRODUCTION

The livestock population in Bangladesh is currently estimated to comprise 26.828 million cattle, 0.544 million buffalo, 16.242 million goat and 1.221 million sheep (BBS, 2010) which plays an important role in the rural economy (Kamaruddin, 2003). Livestock sub-sector contributed approximately 6.5% to the GDP and 13% to the foreign currency earning (Alam, 1993). More than 20% of the rural populations of our country were engaged in this sector (Samad, 1996). Approximately 10 million goats are reared in backyard systems by rural farmers in our country (Chowdhury *et al.,* 2003; Hossain, 2003) of which 2.46 million of goats are in Sylhet region of Bangladesh (personal contact with DLO, Sylhet, 2014).

Goat (*Capra*), a member of the Bovidae family and subfamily Caprinae is one of the oldest domesticated species. For thousands of years they have been used for their milk, meat, hair and skin over the world. Sylhet region is primarily an irrigated agro-ecological zones and animal treasure is one of the major sources of earning of farming community. Goat farming is an important source of livelihood for small, marginal and landless farmers as it plays an important role in providing food, fiber and manure etc. The rearing of goat had the added advantage of filling an important ecological niche being able to graze land on which sheep and cattle simply cannot thrive. Therefore, goats are the second most important livestock in Bangladesh which contributes in poverty alleviation and in supplying animal protein of high caloric value in the form of milk and meat.

The diseases are major setback to livestock industry. The disease producing organisms such as viruses, bacteria, protozoa, helminthes etc are implicated among the major causes of poor goat health and productivity (Nansen, 1991; Kusiluka *et al.,* 1998). Although they can cause diseases and deaths, the main economic losses result from chronic infections due to concurrent infections with several parasites that can cause unthriftness, poor feed utilization, poor growth, low resistance to some other infections, decreased meat and milk quality and infertility (Nansen, 1991; Kusiluka *et al.,* 1994). Faizal (1999) reported 1/3rd growth retardation in ruminants due to helminth infections. Herlich (1978) reported 5-10 % mortality and 10-20 % morbidity usually occur due to helminth parasites in small ruminants. Helminth infections are thought to be one of the

major factors hindering rural goat production in our country (FAO, 1992; Chowdhury *et al.,* 2003).

Fascioliasis is a serious helminthic disease caused by three trematodes *Fasciola hepatica* (the common liver fluke), *Fasciola gigantica* (the large liver fluke) and *Fasciola jacksoni* all over the world which is one of the most prevalent helminth infections of ruminants in different parts of the world (WHO, 1995; Okewole *et al*., 2000). These species overlaps in many areas of Asia and Africa (Mas-Coma *et al.,* 2005) but the economically important ones *F. gigantica* is regarded as one of the most important parasitic infections of ruminants (cattle, sheep and goat) in countries with tropical climates (Harrison *et al.,* 1996; Roberts and Suhardono, 1996; Tantrawatpan *et al.,* 2003). *F. hepatica* is more prevalent in temperate regions that causes economic losses of the livestock due to high mortality and morbidity (Ngategize *et al.,* 1993; Nonga *et al.,* 2009), reduction in growth rate, decrease in weight gain and work output in draught animals (Mandal, 1997), fertility, reduction in meat, milk production, liver condemnation and sudden death of heavily infected animals (Ngategize *et al.,* 1993; Mulcahy *et al.,* 1999). Prevalences of *F. gigantica* infection in some ruminants such as cattle, sheep and goat range up to 80-100% in many countries like West Africa (Schillhorn van Veen, 1980), Ethiopia (Yadeta, 1994; Mezgebu, 1995; Wassie, 1995), India (Sharma *et al.,* 1989), Pakistan (Kendall, 1954) and widespread in Bangladesh causing losses in lives and/or productivity (Rahman *et al.,* 1976; Qadir, 1981 and ADB Report, 1984). Liver cirrhosis (Marcos *et al.,* 2007) and significant losses of production is commonly occur in *Fasciola gigantica* infections (Vercruysse and Claerebout, 2001). The disease also has public health significance, causing human fascioliasis (Vassilev and Jooste, 1991; Lebbie *et al.,* 1994) in the world.

Fasciola has an indirect life cycle involving domestic and wild herbivorous and humans as definitive hosts and freshwater gastropods that is snails have been known to play an important role as intermediate hosts (Torgerson and Claxton, 1999; Walker *et al.,* 2008; Laxman *et al.,* 2011). A *Lymnaea* snail belonging to the family *Lymnaeidae* acts as an intermediate host of *F. gigantica* in all over the world including Bangladesh (Issia *et al.,* 2009). The common *Lymnaea* snails found in Bangladesh is *Lymnaea auricularia* var *rufescens* which appears to be the most suitable intermediate host for *F. gigantica* (Chowdhury *et al.,* 1994b) and in nearby countries Mathur, (1986); Morel and Mahato, (1987). The adequate amount of rainfall, relative humidity and favorable

temperature are required for the development of different developmental stages of *F. gigantica* within the snail and the reproduction of snails. The proper temperature ranges from 22-25º C where development within snail takes place in an efficient manner similarly humidity ranges from 55-70% is adequate for the development of the snail and parasite. In Bangladesh, incidence of *Fasciola* cercariae in *L. auricularia* var *rufescens* has been reported by Qadir, (1982) and studies on different fresh water snails have been carried out only in Savar Upazilla under the district of Dhaka, Bangladesh (Chowdhury *et al.,* 1994c). There is no information on cercarial infestations in *L. luteola* in our country, which is considered to be another intermediate host as *L. auricularia* var *rufescens* of *F. gigantica* in Bangladesh.

Egg count from fecal sample is a usual tool to investigate incidence and prevalence and to diagnose fascioliasis. Due to lack of sophisticated diagnostic tools in veterinary practice the intensity of liver damage is always ignored. Similarly information regarding epidemiology of fascioliasis based on liver pathology is almost nill though the liver damage is a key factor of health status, productive and reproductive performance and body immunity and mortality of the animal (Boray, 1982). Depending on the climatic conditions, the seasonal occurrence of fascioliasis varies from country to country. High incidence and clinical disease with high mortality are reported to occur in wet seasons. Domestic ruminants which are chronically infected are responsible for the spread of the disease by contaminating the pastures with liver fluke eggs. This is especially seen in areas that have favorable climatic conditions and suitable intermediate host (snails). Several factors are known to determine the epidemiological pattern of the associated disease condition including weather, husbandry practice and the physiological status of the animal (Tembely *et al*., 1997; Wall *et al*., 2004). There was several research work carried out on different aspects of fascioliasis in buffaloes (Alim *et al.,* 2000), cattle (Chowdhury *et al.,* 1994a), goats (Howlader *et al.,* 1991) and sheep (Alam *et al.,* 1994) in Bangladesh but limited study on goats. Considering above points, a thorough investigation is required to study the epidemiology and patho-surveillance of fascioliasis including gross and microscopic changes in liver of goats.

The gross pathological changes of the liver in chronic fascioliasis is characterized by increase in the size of the organ due to inflammatory changes in the parenchyma and fibrosis of the bile ducts containing the adult flukes and pin-point hemorrhages on the parietal surface of the liver (Okaiyeto *et al.,* 2012). Migration of immature flukes in the

hepatica parenchyma produces migratory tracts within which there is hemorrhage and necrosis (Soulsby, 1982). The histopathological changes was characterized by fibroblasts mixed with lymphocytes and few mononuclear cells in the area previously migrated by the young fluke. Huge proliferations of fibrous connective tissue associated with infiltration of lymphocyte and plasma cells in the portal area, atrophy, necrosis and fatty changes due to chronic fascioliasis (Howlader and Huq, 1997; Okaiyeto *et al.,* 2012). Thickening of the bile duct and fibrosis of the portal area were observed at post mortem due to the chronic nature of the infection. In chronic fascioliasis of dairy cattle, chronic hyperplastic chollangitis associated with chronic hepatic syndrome were noticed (Rahman *et al.,* 2007).

Fascioliasis also has effects on host's hematological and biochemical parameters (Rasool *et al.,* 1995; Iqbal *et al.,* 1998; Hayat *et al.,* 1996, 1999). A marked increase in plasma volume occurs during the first several weeks of infection which results with the rapid reduction of PCV (Berry and Dargie, 1978), mean haemoglobin concentration (Hb) and RBC (Kumar and Joshi, 1992; Khalid *et al.,* 2004; Eguale *et al.,* 2009; Egbe-Nwiyi *et al.,* 2000; Egbu *et al.,* 2013) and increased of ESR, Neutrophils, Eoesinphils and marked decreased lymphocytes count (Taimur *et al.,*1993; Egbe-Nwiyi *et al*., 2000; Molina *et al.,* 2006; Teleb *et al.,* 2007; Adama *et al.,* 2011).

Fascioliasis in goats also has an adverse effect on blood enzyme levels, which in turn suppresses goat production in different biological ways. The levels of alkaline phosphate (ALP), serum glutamate pyruvate transaminase/aspartate transaminase (SGPT/AST) and serum glutamate oxalate transaminase/alanine transaminase (SGOT/ALT) were reported to be increased significantly, whereas total serum protein (TSP) was reported to be significantly decreased due to leakage of protein through the bile duct epithelium in earlier report (Alam *et al.,* 1994; Chakraborty, 1994; Sharma *et al.,* 2001; Eguale *et al.,* 2009). To combat this problem efficiently, an understanding of the effect of liver flukes on hematological and biochemical parameters are essential.

Sylhet region of Bangladesh have wide water resources of large irrigated agro-ecological zones adjacent to river Surma, flowing water within the tea garden lakes, hilly area and fresh water but still there is no attention on snails study in this area. Sylhet region have favorable geological and climatic conditions for *Fasciola.* Average

temperature is 17°C and moisture is 80 % here. Highest rainfall occurred in this area of the country and has huge marshy area containing suitable snails.

The principal control of fascioliasis in developed countries is mainly based on good grazing land management practices that destroy the intermediate hosts, the snails, which is not always possible and strategic protective treatment helps to reduce grazing land contamination with fluke eggs and increase productivity (Bekele and Kasali, 1992; FAO, 1994; Hansen and Perry, 1994; Radostits *et al.*, 1994; Yilma and Malone, 1998). Effective control of this parasite includes strategic use of anthelmintic drugs and control of snail intermediate host. In our country there is no organized pasture and parasitic infection is controlled only using a routine prophylactic treatment (Radostits *et al*., 2000).

In fascioliasis the metabolic process of the liver is gradually reduced and causes the liver weak, damage liver cells and inhibits liver functions due to chronic fascioliasis in goats (Fikry *et al*., 1988). Liver tonic performs function of protecting the liver against toxins, poisons and pathogens, stimulates regeneration of liver cells, protects against inflammation. Liver tonic premix is hepato-protective; it stimulates sluggish liver parenchyma cells and restores liver function. It improves feed metabolism and its utilization, which results in better feed conversion, to enhance growth, weight gain, FCR and productivity.

In Bangladesh, effects of different types of liver tonic either single or combine used with anthelmintic in livestock are yet to investigate on pathology of fascioliasis in goats. Effective strategies for the control of fascioliasis are mainly based on the proper diagnosis and the use of very effective drugs. But, resistance of the liver fluke to flukicides is becoming a great problem to farmers (Estuningsih *et al.*, 1990; Sanyal, 1996; Mitchell *et al.*, 1998). A liver tonic helps to recover damaged liver and thereby improves liver functions. Therefore, combined use of anthelmintic and liver tonic might be an excellent therapeutic approach on fascioliasis of goat.

Information about epidemiology/patho-surveillance, pathology and treatment/control strategy against *F. gigantica* in the goat of Sylhet region of Bangladesh is still scanty. A plenty of work has been carried out on different aspects of ruminant parasitology, but no substantial work has been conducted on the epidemiology/patho-surveillance, climatic factors affecting different developmental stages of *F. gigantica* infection in

Lymnaea snails. So far no study was conducted about the effect of liver tonic against *F. gigantica* infection in the goat of Sylhet region. Hence the present work is aimed at to conduct different studies of fascioliasis in goats of Sylhet region and to correlate the results with the presence of *F. gigantica* in this host.

Thus, the present study was undertaken to fulfill the following objectives:

a) Generation of precise data on the prevalence of *Fasciola gigantica* infection in goats based on different age, sex and seasons at different upazilla of Sylhet.

b) Study on the pathology of *Fasciola gigantica* infection in liver of goat.

c) Determination of the effects of climatic factors on prevalence of developmental stages of *Fasciola gigantica* infection in *Lymnaea* snails (*Lymnaea auricularia* var *rufescens*).

d) Evaluation of the efficacy of anthelmintic, liver tonic and /or combination of liver tonic with anthelmintic on fascioliasis in goat.

e) Evaluation of the extent of liver damage by *Fasciola sp.* and healing by liver tonic in goat.

CHAPTER-II

REVIEW OF LITERATURE

Todate, a plenty of work has been conducted in this important area of research of Veterinary Parasitology. It is difficult to give a detailed account of the work done; therefore a brief account of literature available on related aspects of the present investigation has been critically reviewed and summarized below. The present study deals with the epidemiology of *Fasciola gigantica*, gross pathology, histopathology, clinico-pathology, developmental stages of *Fasciola gigantica* infection in *Lymnaea* snails and efficacy of different types of liver tonic in combination with anthelmintic on fascioliasis in goats, therefore the review of literature is presented separately with specific headlines.

2.1 *Fasciola gigantica*

Fasciola gigantica is a leaf shaped digenetic trematode that invades the liver before entering the bile ducts where maturity occurs (Soulsby, 1982). Its length may vary from 25 to 75 mm long by 15 mm wide (Soulsby, 1982) (Figure 2.1). It has an anterior shorter cephalic cone on which the oral and ventral suckers, which are approximately of equal size, are located. The intestine of the adult parasite is highly branched, with numerous diverticulae extending from the anterior to the posterior of the body. The pair of testes, also highly branched, is located in the posterior half of the body. The relative compact ovary is located just above the testes and is linked to a short convoluted uterus opening to a genital pore above the ventral sucker. The vitellaria are highly diffuse and branched in the lateral and posterior region of the body.

Members of the genus *Fasciola* are the digenean trematodes of the phylum Platyhelminthes. *Fasciola gigantica,* commonly known as the giant fluke, is a parasitic flatworm. The parasites cause disease known as fasciolosis (or fascioliasis) in domestic animals (cattle, sheep, goats, and donkeys), wild mammals (rabbits, beavers, deers, water buffalo and camels) and occasionally affects humans, thus considered as a zoonotic infection (WHO, 1995; Andrews, 1999; Okewole, *et. al.*, 2000; Aksoy *et al.*, 2006; Aldemir, 2006; Cucher, 2006; Ahmadi and Meshkehkar, 2010 and Alasaad, 2011).

Gross Microscopic

Figure 2.1 Adult stages of *Fasciola gigantica* (gross and microscopic)

It causes the disease tropical fascioliasis (Soulsby, 1982) and the most common trematode infection of domestic ruminants throughout the tropical and subtropical regions of Africa, in the United States, Far East South and South-east Asia including Bangladesh (Saleha, 1991; Cheesbrough, 2005). The flukes have overlapping distributions in some parts of the world especially in Asia and Africa. *Fasciola gigantica* usually occurs in tropical zones (McGarry *et al.,* 2007 and Alasaad, 2011). The two are more prevalent in areas with a high stock level of cattle and sheep, where they cause major economic losses through condemnation of infected organs at abattoirs as well as reduced production of meat and milk products (Aldemir, 2006; McGarry *et al.*, 2007; Ahmadi and Meshkehkar, 2010 and Alasaad, 2011). The eggs of *Fasciola gigantica* can reach sizes of 0.2 mm in length. (Kumar, 1998; Dangprasert *et al.*, 2001). Incidence in a particular region depends strongly on ecologic and climatic conditions (e.g. habitats for intermediate hosts and wild mammals, over wintering of the parasites in the environment, etc.) and on livestock management practices (stock density, grazing patterns, etc.).

According to Dunn (1978) and Soulsby (1982), the taxonomic classification of the organisms that cause fasciolosis is presented as follows:

Kingdom: Animalia

Phylum: Platyhelminthes,

Class: Trematoda,

Sub: class: -Digenea,

Order: Echinostomida

Family: Fasciolidae

Super Family: Fasciolidea,

Genus: *Fasciola*,

Species: *Fasciola gigantica* (Cobbold, 1855)

2.2 Life cycle of *Fasciola gigantica*

The life cycle begins when a final/definitive host ingests the parasite in the form of a metacercaria encysted on a water plant and these excyst in the small intestine, followed by migration through the intestinal wall, crossing of the peritoneum and penetration of the liver capsule. The immature fluke then migrates through the hepatic parenchyma for 6-8 weeks before entering the minor bile ducts and finally migrating to the larger bile ducts (and occasionally the gall bladder). This migratory phase gives rise to the acute form of the disease. Immature flukes then penetrate the liver and become mature in the biliary track. (Cheesbrough, 2005). The fluke then establishes and matures in the major bile ducts, where their continued presence causes the chronic form of fascioliasis. *Fasciola* spp are hermaphrodite and can either self or cross-fertilise. The pre-patent period is 13-16 weeks for *Fasciola gigantica* (Guralp *et al.* 1964). Eggs pass into the digestive tract via the bile before being excreted with faeces (Cheesbrough, 2005). Most adult flukes die within 9-12 months, however, some live 3-4 years in sheep, goat and cattle (Hammond and Sewell, 1974). Egg output varies, being highest within the first 12 weeks of egg production, before tailing off (Sewell, 1966). Time taken for the egg to embryonate in the external environment is temperature dependant, from 10 to 33 days. Hatching may take nine days and the optimal temperatures required are 22-26° C and this can take up to 14 weeks (Guralp *et al.,* 1964). Miracidia have only three hours to find a suitable intermediate host before they die. The miracidium enters the lymnaeid snails, where upon development (sporocyst) and asexual replication occurs (rediae), and giving rise to multiple cercariae (Figure 2.2) that escape the snail from 26 days post infection (Dinnik and Dinnik, 1956; 1964) and encyst on plants immersed in water, where they encyst and become the infective metacercariae. In the case of *Fasciola gigantica*, many cysts are free floating rather than attached to plants, and this has implications for the dispersal of the disease beyond areas inhabited by snails (Dreyfuss and Rondelaud 1997). Cyst survival is longer in moist, cool

conditions, and can be up to 6 months (Spithill *et al.*, 1999). The metacercaria are ingested by herbivorous animals that act as the final hosts (Figure 2.3). These metacercaria excyst in the small intestines as a result of low pH concentrations and soon find their way through the intestinal wall and peritoneum and finally into the liver capsule (Aksoy *et al.*, 2006). A minimum of 6-7 weeks is required for miracidia to form metacercaria. Under unfavorable circumstances this may take several months. Following infection in one snail with one miracidium, over 600 metacercariae may be produced. The immature flukes migrate through the parenchyma (a process that may take 6-8 weeks), make their way to the small bile ducts and eventually to the larger bile ducts (Aldemir, 2006). Here the parasite matures into an adult fluke and begins to produce eggs. Up to 20 000 eggs per fluke per day may be produced in the host resulting in infestations of epidemic proportions. In Bangladesh, the most common intermediate hosts are members of the genus *Lymnaea* (Aldemir, 2006), commonly known as fresh water snails or pond snails that includes the following *Lymnaea auricularis* var *rufescens* (Kendall, 1954, Qader, 1982 and Mathur, 1986).

The egg of *Fasciola gigantica* measures 150µm by 90µm in size (Soulsby, 1982). *Fasciola* eggs should be distinguished from the eggs of other flukes, especially from the large eggs of *Paramphistomum*. *Fasciola* eggs has a yellowish brown shell with an indistinct operculum and embryonic cells whereas *Paramphistomum* egg has transparent shell, distinct operculum with embryonic clear cells, and possess a small knob at their posterior end (Soulsby, 1982) (Figure 2.4).

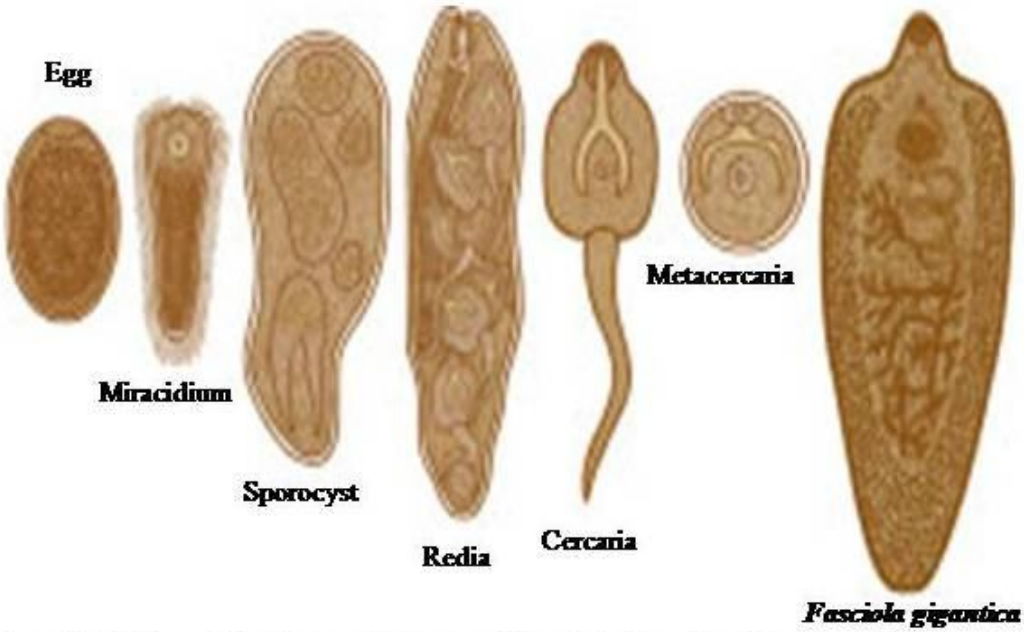


Figure 2.2 Different developmental stages of *Fasciola gigantica*, (http://parasite.org.au/para-site/contents/helminth-intoduction.html)

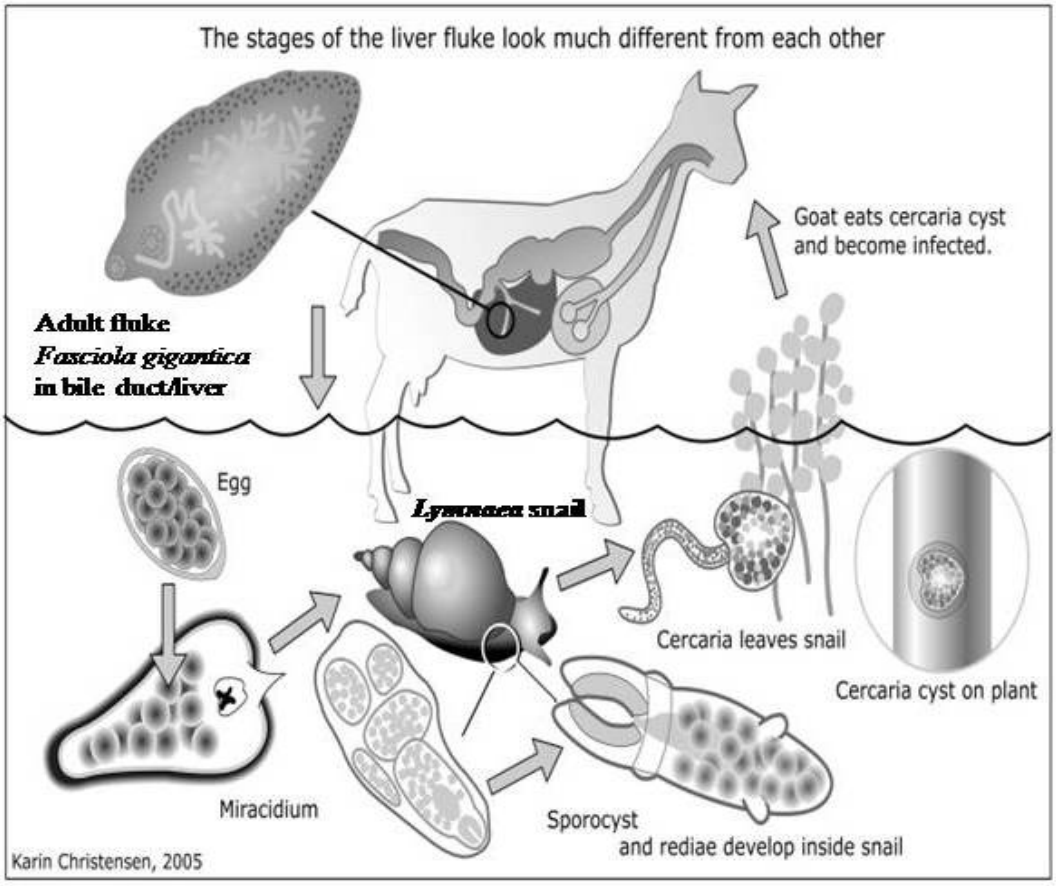


Figure 2.3 Life cycle of *Fasciola gigantica* in goat (http://www.goatbiology.com/parasites.html)

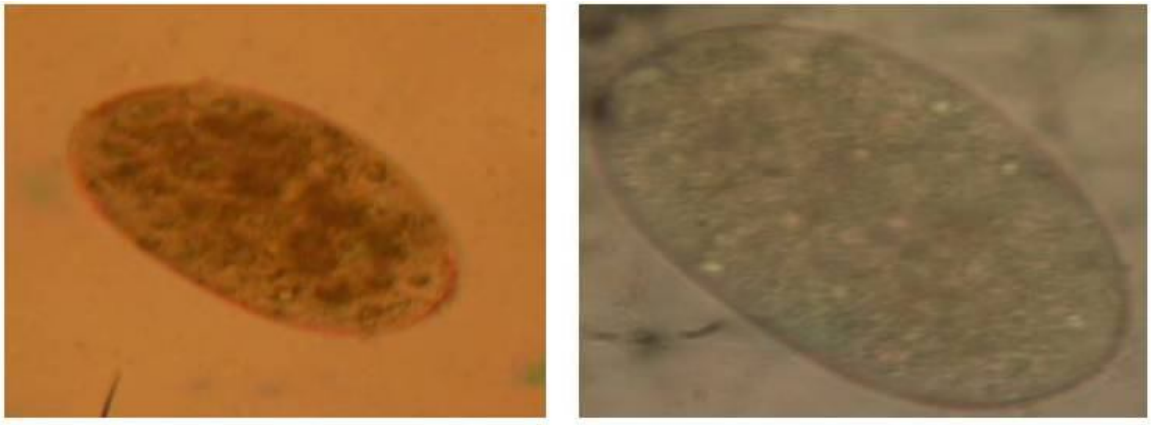


Figure 2.4 Eggs of *Fasciola gigantica* (10X & 40X)

2.3 The definitive host range and prepatent period

Fasciola spp have the potential to infect many different definitive hosts. However, they have evolved to survive best in certain preferred hosts. *Fasciola gigantica* is adapted to domestic cattle, with a longer adult survival time, higher levels of egg shedding and high infectivity compared to sheep and goats (Hammond and Sewell, 1974). However, wild ruminants (Hammond, 1972) and other wildlife (Walker *et al.,* 2011) can also be affected and could act as a reservoir in certain situation. Prevalence of around 50% have been found in Asia including Bangladesh and evidence suggests that the infection could survive in domestic ruminants such as cattle, sheep and goat and also deer (Masuduzzaman *et al.*, 1999; 2010). In ruminants, adult flukes die within 9-12 months and the duration of egg production is short, with resistance rapidly acquired (Ross 1968), although the mechanism for this may be partially dependent on the development of hepatic fibrosis rather than entirely immunological (Ross *et al.*, 1967).

The time for larval *Fasciola gigantica* to develop in the hepatic parenchyma and enter the bile ducts in ruminants (cattle, goat and sheep) about 89 days after infection (Guralp *et al.*, 1964). The prepatent period vary from 12 to 16 weeks (Guralp *et al.*, 1964; Sewell, 1966). Variations in the prepatent period may be due to the sensitivity of the egg detection method used, the size of the infecting dose, breed of host and strain of *Fasciola gigantica* (Spithill *et al.,* 1999). Egg output increases during the first 4 to 12 weeks after eggs appear in the feces (Sewell, 1966 and Prasitirat *et al.,* 1996). Survival of adults of *Fasciola gigantica* in

ruminants (cattle, goat and sheep) is usually less than a year but in some cases reaches 3 to 4 years (Hammond and Sewell, 1975).

2.4 Epidemiology

Abraham and Jude (2014) investigated fascioliasis among trade ruminants (cattle and goat) slaughtered at Calabar abattoirs using centrifugal method for fecal examinations. They found that 179 (44.8%) of 400 cattle and 126 (36.0%) of 350 goat had fascioliasis.

Ezatpour *et al.,* (2014) evaluated the contamination rate of slaughtered animals with fasciolosis at Lorestan abattoirs. A total of 356,605 livestock including 265,692 sheep and 90,913 goats were slaughtered in the 3-year period and overall 39,613 (11.1 %) livers were condemned. Fascioliasis was responsible for 6.3 % of total liver condemnations in this period. *Fasciola* spp infection in sheep (7.1%) was considerably higher than goats (3.9 %).

Jean-Richard *et al.,* (2014) observed a total of 880 animals of which 616 goats, 132 sheep and 130 cattle. The prevalence of adult *Fasciola gigantica* was 68% (CI 60-76%) in cattle, 12% (CI 10-16%) in goats and 23% (CI 16-30%) in sheep. All infected animals (n = 200), 53% (n = 106) were classified as lightly infected with 1-10 parasites, 18% (n =36) as moderately infected with 11-100 parasites and 29% (n = 58) as heavily infected with more than 100 parasites per animal.

Khanjari *et al.,* (2014) investigated a total of 2391 sheep and goats slaughtered at an abattoir in Amol region, northern Iran to determine the prevalence of the liver flukes infection based on season, sex and species of the animals. The prevalence of *Fasciola* spp was 6.6%. The seasonal prevalence of *Fasciola* spp. was highest during spring (8.3%) followed by autumn (8.1%), winter (5.9%) and summer (4.0%).

Usip *et al.,* (2014) was carried out the prevalence of fascioliasis and the economic loss of condemned liver due to *Fasciola* infection in cattle slaughtered at three abattoirs of Nigeria. A total of 185 males and 94 females cattle were examined by postmortem examination. The result showed that 38 (13.62%) of the cattle were infected with fascioliasis. The prevalence recorded for female cattle was 17.02% compared to the male 11.89%. There was no sex bias in infection among the animals examined (chi-square, $P>0.05$).

Abdi *et al.,* (2013) examined a total of 27242 indigenous animals including 17055 sheep, 5703 goats, and 4484 cattle, the prevalence of fascioliasis in IIam Province, Iran. The overall prevalence of *Fasciola hepatica* among 27242 slaughtered animals was 0.98%. Out of 267 domestic animals, 98 sheep, 28 goats and 141 cattle were infected with the parasite. The highest and lowest infection rate of 3.14% and 0.1% were cattle and goat respectively.

Bansal *et al.,* (2013) investigated a total of 960 goat and 445 sheep fecal samples from animals slaughtered at slaughter house, Mhow, Indore. Among the various helminths, the prevalence of *Fasciola* spp was 4.56 %. In monsoon season maximum prevalence (92.96 %) was recorded followed by winter (89.20 %) and summer (87.76 %). Age and sex wise prevalence was higher in less than one year of age group (91.05 %) and in females (90.96 %).

Mir *et al.,* (2013) find out the prevalence of fascioliasis in goats of different age, sex and seasons in Jammu and Kashmir State. A total of 284 livers were examined of which 15 were found to having *Fasciola hepatica* (5.28% prevalence). Seasonal prevalence of infections indicated that the infection was 1.56% in spring, 3.38% in summer 3.84% in autumn and10.84% in winter. Prevalence with respect to sex and age of the host were also recorded during the present study.

Nzalawahe and Komba, (2013) was estimated the prevalence of fascioliasis in cattle and goats slaughtered at Kasulu district abattoir. A total of 8410 cattle and 8424 goats were slaughtered and inspected; out of which 6376 (76%) and 2295 (26%) were found to be infected with fasciolosis respectively. The study observed significant seasonal pattern of fasciolosis in cattle with higher prevalence during the dry season.

Shitta, (2013) was examined a total of 350 fecal samples of cattle, of which 122 (34.90%) infected with one or more intestinal helminthes. The prevalence of *Fasciola gigantica* in cattle was 31 (8.90%). Of the 163 male cattle examined, a higher prevalence of 62 (38.04%) was observed when compared to the 60 (32.10%) of their female counterpart out of the 187 examined. The young cattle between the ages of 0-23 months were more susceptible to the intestinal parasites infection having a prevalence of 43 (45.30%) than the adult examined who had 79 (30.10%).

Squire *et al.,* (2013) determined the prevalence and intensity of gastrointestinal parasites of cattle from two institutional farms located in the Coastal Savanna and Transitional zones of Southern Ghana. A total of 309 (different ages, sex and breed) faecal samples were examined using the McMaster and sedimentation techniques. Overall, the cattle showed a very high prevalence (95.5%) of parasitic infections. Out of the prevalence of *Fasciola* was 51.1%.

Zeleke *et al*., (2013) had investigated the prevalence of ovine fascioliasis. A total of 1528 sheep were selected and sampled by using systematic simple random sampling and postmortem examinations were employed during the study period. Overall prevalence of ovine fascioliasis based on farm, abattoir and retrospective clinical study was 62.7% and 59.1% respectively. There was no statistically significant variations by sex and age of sheep in both farm and abattoir study.

Abdulhakim and Addis (2012) investigated the prevalence and risk factors of fascioliasis in 1152 ruminants comprising of cattle, sheep and goats were subjected to routine post mortem examination for fasciolosis. The overall prevalence of fascioliasis was proved to be 21% (242/1152). The prevalence of fascioliasis in adult cattle, sheep and goats were confirmed to be 39.8%, 28.7% and 13.9% respectively and in young cattle, sheep and goats were proved to be 23.3%, 12.7% and 7.0% respectively. Significantly higher ($p<0.05$) prevalence of fascioliasis was seen in adult cattle, sheep and goats when compared to young ones.

Akhtar *et al.,* (2012) was observed the prevalence of *Fascioliasis* in sheep and goats of Dera Ismail Khan. A total of 380 sheep and 400 goats were screened to identify 100 infected animals (50 sheep, group-A and 50 goats, group-B) positive for fascioliasis. The faecal examination showed 13.39% and 12.50% fascioliasis in sheep and goats respectively.

Al-Nassir *et al.,* (2012) studied three slaughter- houses of Kerbala City and aimed to detect the most common parasitic infections of 20477 sheep carcasses, 4421 goat carcasses, 3993 cattle carcasses as well as 622 buffalo carcasses were examined. The prevalence of fascioliasis cattle, goats and sheep were 5, 4.07 and 4% respectively. Moreover, *Fasciola*

spp was also detected more frequently in buffalo liver (2.57%) than cattle (1.32%), goats (0.74%) and sheep (0.67%).

Asrafuzzaman (2012) had resolved the incidence of snail borne trematode infections of cattle in Rangunia of Chittagong district and Bandarban hill tract of Bangladesh and reported that cattle were infected with amphistomes (39.27%), *Fasciola gigantica* (6.91%) and *Schistosoma indicum* (10.18%).

Bogale *et al.,* (2012) was studied the prevalence and risk factors associated with ovine fascioliasis in 384 rectal faecal samples collected from sheep and examined using the sedimentation technique to detect *Fasciola* eggs. Out of them, 163 (42.44%) were positive for fascioliasis. Among the age groups higher prevalence was recorded in adults (51.40%) than young animals (17%) and the sex groups in which it was higher in females (53.06%) than in male animals (23.74%).

Gboeloh, (2012) conducted the seasonal prevalence of *Fasciola gigantica* in cattle slaughtered in major abattoirs in Port Harcourt City. The prevalence of *Fasciola gigantica* was significantly higher ($p<0.05$) than in the rainy season (May-September) and the dry season (November-March). The overall annual prevalence was higher (45.7%) and statistically significant ($p<0.05$).

Haq (2012) carried out the incidence of gastrointestinal helminth parasites of cattle at Mohangonj upazila, in Netrokona district, Bangladesh and recorded an overall 7.21%, 44.79% and 19.27% infection with *Fasciola gigantica*, amphistomes and *Schistosoma indicum*, respectively.

Kadir *et al.,* (2012) was determined the prevalence of fascioliasis in sheep 50518, cattle 16177, goats 7662, buffalos 48 and camels 24.in Kirkuk official slaughter house. In sheep, the rate of liver flukes 0.36% with the highest rate in summer 0.33%. In cattle, the rate of liver fluke was 1.27%, the highest was in winter 1.57%. In goats, the rate of liver fluke was 0.14%, with the highest rate was in winter 0.51%. In buffaloes and camels, only 1 case of fascioliasis was seen for each in spring.

Khan and Maqbool (2012) had investigated the epidemiology of fasciolosis at different managemental conditions. The prevalence was 22.6%, 17.5%, 10.82% and 8.76% respectively in slaughtered cattle, cattle at livestock farms, veterinary hospitals and household cattle. Overall highest seasonal prevalence in all types of cattle was recorded during autumn followed by spring and winter, while the lowest prevalence was recorded during summer. It was noticed that a higher infection rate was recorded in older and male cattle than in youngsters and females.

Mungube *et al.,* (2012) estimated the prevalence of fascioliasis in cattle, goats and sheep was 25.9%, 23.4% and 33.3%, respectively. Extensive grazing and age stratum 7-12 months were identified as potential fascioliasis risk factors with the latter being significantly associated with the disease.

Sangma *et al.,* (2012) revealed the prevalence of helminths of 190 sheep. Of which 154 (81.1%) were positive for one or more species of helminth parasites. Seven species of helminths were identified, one of them was trematodes, namely, *Fasciola gigantica* (8.4%), Prevalence of helminths was significantly ($p<0.01$) higher in young sheep aged >1-2 year (92.7%) than adult aged > 2 years (83.3%) and lamb aged ≤ 1 year (63.6%). Higher prevalence was recorded in female than in male sheep.

Yasin, (2012) had studied the incidence of gastro-intestinal helminth parasites of cattle at Shahjadpur upazila in Sirajgonj district, Bangladesh and reported 4.25% *Fasciola gigantica,* 31.32% amphistomes and 4.25% *Schistosoma* sp. infection.

Zgabeher *et al.,* (2012) investigated the prevalence of ovine fascioliasis in fecal samples randomly collected from 384 sheep revealed an overall prevalence of 39.5% (95% CI; 34.7-44.5%). The prevalence was higher in male than females and younger than adult ones; nevertheless, the differences were not statistically significant ($P>0.05$).

Ali *et al.,* (2011) investigated the prevalence of fascioliasis in a total of 267802 liver stock including 9066 cattle, 77912 sheep and 180824 goats. Overall 31954 (12%) livers were positive for fascioliasis. Fascioliasis was responsible for 4.8% of total liver condemnations. The prevalence of fascioliasis in females was more susceptible than males.

Kagira *et al.,* (2011) inspected a total of 989 sheep livers out of which 8 (0.8%) had liver flukes. Livers from a total of 954 goats were inspected, out of which 4 (0.4%) had liver flukes.

Mamun *et al.,* (2011) estimated the prevalence and economic losses caused by fascioliasis in ruminants. Out of the 6220 ruminants, 2319 were infected (37%) with *Fasciola sp.* The infection rate was highest in buffaloes (44.49%), cattle (37.90%), goat (32%) and lowest was recorded in sheep (30.91%). Maximum rate of infection was recorded ($p<0.05$) in autumn (47.34%) and lowest rate was recorded in summer (26.96%). *Fasciola sp.* was observed higher in older animals (39.63%) than younger (33.95%).

Mhoma *et al.,* (2011) studied the prevalence of *Fasciola gigantica* in goats. Randomly, 36 farmers from urban areas, with a total of 280 goats and 22 farmers from peri-urban areas with a total of 205 goats were identified for sampling. The prevalence of *Fasciola gigantica* was 4.2% in urban Mwanza City goats and in peri-urban Mwanza City the prevalence of *Fasciola gigantica* was 8.2%. The present study showed that the prevalence of *Fasciola gigantica* were significantly higher in urban compared to peri-urban areas.

Njoku-Tony, (2011) was determined the prevalence and distribution of fascioliasis in cattle. Out of 2,400 cattle examined, the prevalence was 23.3%. Infection level raise with increase in rainfall. Highest infection was observed at the peak of the rainy season (July-November) and dropping during the dry season (December-May). Results recorded higher infection in the males than the females.

Ozung *et al.,* (2011) observed the prevalence of fascioliasis of ruminants in a total of 2019 ruminant species of which 1020 (50.52%), 479 (23.72%) and 520 (25.76%) cattle, goats and sheep were infected by *Fasciola* respectively. The prevalence was 31.70%. On differential sex diagnosis, the rate of fascioliasis was higher in dams 77.75%, 55.95% and 80.77% than sires 22.25%, 44.05% and 19.23% for bovine, caprine and ovine species respectively.

Abouzeid *et al.,* (2010) investigated the prevalence of gastro-intestinal tract (GIT) parasites in 240 sheep in different area in the zoo garden and in Sinai district. The overall prevalence

of *Fasciola* sp. infection in sheep in Sinai and zoo garden was 10.0%. Lower age group animals were more prone to infection than adults.

Alam (2010) estimated the trematode infections in cattle and their vector snails at Patuakhali district and record that 18.10%, 32.86% and 8.57% cattle were infected with *Fasciola* sp., *Paramphistomum* sp. and *Schistosoma indicum* respectively.

Kabir *et al.,* (2010) stated the general clinical prevalence and comparative prevalence of diseases and disorders in cattle and goats in a total of 463, among them cattle were 348 and goats were 115. Clinical examinations detected 17 different types of diseases and disorders in 348 (75.16% of total animals) cattle, of which the prevalence of fascioliasis was 47 (13.51%). In goat the prevalence of fascioliasis was 12 (10.44%). Age wise prevalence in young and adult goat were 36 (31.30%) &79 (68.7%) respectively. Sex wise prevalence in male and female goat were 46 (40%) & 69 (60%) respectively.

Hossain *et al.,* (2011) studied the prevalence of fascioliasis in Black Bengal goats in a total of 318 livers of male and female goats collected randomly from slaughter house. Of which 66 were higher prevalence of *Fasciola gigantica.* The overall prevalence was 20.75%. Significantly fascioliasis was observed in older (58.33%), female goats (36.79%) and during the rainy season (26.16%).

Mellau *et al.,* (2010) examined a total of 115186 cattle, 61551 sheep and 37,850 slaughtered goats livers. Out of 18829 (16.3%), 10515 (17.1%) and 7011 (18.5%) livers of cattle, sheep and goats were found positive respectively.

Sutar *et al.,* (2010) investigated the helminth parasites of goats in a total of 400 fecal samples collected from different villages. Out of 400 samples, 251 were positive (62.75%). The prevalence of fascioliasis in goats was 9.25%. In rainy season, out of 150 fecal samples, 116 were positive (77.33%), while in winter out of 120 samples, 73 were positive (60.83%) and in summer out of 130 samples 67 were positive (51.53%).

Affroze (2009) had determined the epidemiology of fascioliasis in cattle at Netrakona district and recorded 31.14% cattle were infected with *Fasciola gigantica.*

Gadahi *et al.,* (2009) examined a total of 400 fecal samples comprising of 90 samples from sheep and 310 from goats. *Fasciola sp*. was found with prevalence of 4.44% in sheep. In case of goat the incidence of *Fasciola sp*. was 0.66 %.

Garg *et al.,* (2009) were recorded fascioliasis in cattle, buffaloes, sheep and goats respectively. The prevalence of infection in cattle and buffaloes was highest during the winter (11.84% cattle, 15.57% buffaloes) followed by summer and rainy respectively. However, the seasonal trends in sheep and goats were the reverse, with the peak prevalence during the rainy (4.60% sheep, 2.71% goats).

Anwar, (2008) reported 58.9% amphistomiasis, 15.6% fascioliasis and 13.7% intestinal *Schistosomiasis* in cattle in chars of the Jamuna river of Bangladesh.

Islam and Taimur, (2008) examined a total number of 136 Bengal sheep and 224 Bengal goats various parasitic infections. The prevalence of internal parasitic infections was higher in goats (74.55%) than in sheep (55.88%). Liver fluke (*Fasciola gigantica*) was more prevalent in goat (14.28 %) than in sheep (8.82%).

Islam *et al.,* (2008) reported that the overall incidence of 53% parasitic diseases. Among flukes infection was 22% and round worm infection was 17%. The animals were mostly affected in the age 2.5 years to above (14%) and 17% young animals were affected by round worms.

Kadir and Rasheed, (2008) evaluated the prevalence of hydatid cysts, liver flukes and lung worm among slaughtered sheep, goats, calves, cattle and buffaloes in Kirkuk slaughter house. The rate of liver flukes was observed in sheep (0.50%), goats (0.43%), cattle (2.63%) and 2(4%) in 50 buffaloes slaughtered. In addition to that there was seasonal fluctuation in the rate of helminthes infection. The rate of liver flukes in sheep and goats was highest in winter (0.88 & 0.68%), while in cattle was highest in autumn (5.0%) followed by winter (2.80%).

Mir *et al.,* (2008) studied the prevalence of trematodes by parasitological examination of 1,325 faecal samples collected from naturally grazing sheep in Kashmir Valley, India. The level of parasitism varied among 28.98% of the sheep that had at least one infection.

Fasciola gigantica (23.92%) and *Fasciola hepatica* (9.96%) were predominant found. Seasonal variations indicate that highest infections were recorded during the summer (13.94 %) followed by autumn (7.38%), spring (6.06%) and winter (1.41%). Highest (42.8 %) prevalence of trematodes parasites was observed in sheep that were more than 4 years old (42.8%) followed by 2-4 (37.7%) and 0-2 years (18.79%) of age groups respectively.

Mohsen and Ibrahem (2008) investigated some epidemiological features concerning parasitic infection of goats. Out of 350 fecal samples of goats examined, 321 animals proved to be infected with different internal parasites, representing an incidence rate of 91.7%. Trematodes infection represented as *Fasciola* eggs were detected in 3.4%. For studying the parasitic infection of goat's liver, 350 slaughtered goats were examined. Forty eight (13.7%) cases showed parasitic infection in their liver. Macroscopic examination of goats liver showed 6.28% *Fasciola gigantica* infection.

Saiful-Islam and Taimur, (2008) carried out a year-round study on 136 Bengal sheep and 224 Bengal goats with the aim to compare the species diversity and prevalence of infections with protozoa, flukes, tapeworms and nematodes parasitizing gastrointestinal tract and lungs of the small ruminants from various parts of Bangladesh. The prevalence of internal parasitic infections was higher in goats (74.55%) than in sheep (55.88%). Liver fluke (*Fasciola gigantica*) was more prevalent in goat (14.28%) than in sheep (8.82%).

Sheikh *et al.*, (2007) recorded the seasonal prevalence of bovine fascioliasis in different geographical areas of the Kashmir Velley. The overall prevalence of fascioliasis was 51%. More cases were observed in endemic areas (10) than in non-endemic area (59%). The prevalence of fascioliasis during spring, summer; autumn and winter seasons were 37%, 31%, 20% and 73% respectively.

Tasawar *et al.*, (2007) reported the overall prevalence of *Fasciola hepatica* in goats and relationship between body weight, age, breed of the host and also effect of parasite on the litter size of the host. The overall prevalence of *Fasciola hepatica* was 28.75%. Infection of parasite was more prevalent in Teddy than in Nachi goats (42.10 VS 16.67%; $P<0.05$). Results regarding the relationship between different age groups of goats and *Fasciola*

hepatica showed that highest prevalence (35.71%) of parasite was observed in age group of 13-24 months and it was lowest (18.18%) in age group of >36 months. The prevalence was significantly different (P<0.05) in different age groups.

Ansari-Lari and Moazzeni, (2006) estimated a total of 844,039 animals (cattle 131,716; sheep 577,090; goats 135,233) slaughtered in the 5-year period and overall 34,856 (4.1%) livers condemned. Fascioliasis was responsible for 54% of total liver condemnations. The prevalence of liver condemnations due to fascioliasis was decreased from 3.89, 3.20 and 2.63% in 1999-2000 to 1.07, 0.59 and 0.24% in 2003-2004 for cattle, sheep and goats respectively.

Jaiswal, (2006) studied on fascioliasis in ruminants at Dhanusa district based on examination for fecal sample brought to DLSO, Janakpur. A total of 2655 fecal samples were examined out of which 70.70% positive for overall parasitic infestation. The prevalence of *Fasciola* infection found in goat was 31.25%, in cattle was 49.36% and in buffalo was 56.02%.

Mungube *et al*., (2006) resolved the prevalence and economic losses caused by *Fasciola gigantica* and *Fasciola hepatica* in the ruminant production systems of Taveta division of Kenya. Liver condemnation rates differed significantly between bovines, caprines and ovines for *Fasciola gigantica* (26%, 6.6% and 5.2%, respectively) and for *Fasciola hepatica* (0.4%, 22% and 28%). The prevalence of amphistomes was very high all the year round and the rate of infection was 83.64%, 69.23% and 64.0% during monsoon, winter and summer season respectively.

Phiri *et al.,* (2006) examined the liver fluke burden and pathology in condemned and non-condemned livers and the correlation of fluke burden and fecal egg fluke counts. The authors showed that a significantly higher number of liver flukes (*Fasciola gigantica*) (P<0.001) were found in the condemned (mean SD= 100.616.7) than in the non-condemned livers (mean SD=0.70.5). Liver flukes found in 9.4% of the non-condemned livers suggest that abattoir records of liver inspection may underestimate *Fasciola gigantica* infections. Average fecal fluke egg counts from animals with condemned livers

(5 eggs per gram [EPG]) were significantly higher (P<0.001) than in animals with non-condemned livers (0.8 EPG).

Yadav *et al.,* (2006) resolved the fecal sample (n=520) from sheep (n=245) and goats (n=275) from R.S. Pura, Bishnah and Samba tehsils of Jammu district. A total of 83.07% gastro-intestinal parasite infection in which 83.24%, 80.00%, 84.72% and 80.55% infection were observed in sheep, lambs/hoggets, goats and kids respectively. The prevalence of *Fasciola sp.* was 3.08%. Seasonal variation was recorded throughout the year and was highest during rainy season (88.54%) followed by summer (83.15%) and winter (76.01%).

Ahmed *et al.,* (2005) investigated diversity and prevalence of helminth parasites of livers of sheep and goats in the district of Quetta. Overall trematodiasis was 23.75% in sheep and 27.90% in goats. Mixed infection of *Fasciola hepatica* and *Fasciola gigantica* was higher (12.26 and 20.93% in sheep and goats, respectively) compared to infection with parasites alone. An increase in prevalence and intensity of parasites was seen during and after the rainy season.

Masuduzzaman *et al.,* (2005) performed a total of 57 domesticated deer of various types examined to diagnose the fascioliasis in Chittagong zoo and some house deer of Chittagong city. By faecal examination out of 44 cases 34 (77.3%) were positive for fascioliasis. In post mortem examination, the overall incidence of fascioliasis in deer was 82.5%. In relation to sex the fascioliasis was significantly higher in female than male deer. The incidence of fascioliasis in various types of deer in relation to age was significantly higher in age group of above 2 years old than in age group below 2 years old.

Olupinyo and Ajanusi, (2005) evaluated a total of 200 gallbladders of sheep and goats at slaughter from Zaria abattoir and analyzed using sedimentation method for the presence of egg of *Fasciola* species. The prevalence of *Fasciola* species was 21% in sheep and 8% in goats. The prevalence of *Fasciola* species was 12% in ewes while in does, it was 10%. In the males, 18% of the rams had *Fasciola* species while bucks had 6%.

Waruiru *et al.,* (2005) estimated on the gastro-intestinal parasitic infection of sheep and goats in semi-arid area of Machakos district, Kenya. The overall prevalence of *Fasciola* spp infection in goats was 31.5%.

Alim *et al.,* (2004) reported on fascioliasis and biliary amphistomiasis in buffaloes in Bangladesh and recorded 50.5% buffaloes infected with Fasciola *gigantica*.

Njeruh *et al.,* (2004) examined a total of 1584 cattle, 989 sheep and 954 goats slaughtered originating from five out of the 8 provinces of Kenya. Among them 147 (9.3%) cattle, 8 (0.8%) sheep, 4 (0.4%) goats were shown to harbor liver fluke infection.

Profombuij, (2004) carried out the prevalence and economic importance of fasciolosis in a total number of 5,421,188 cattle, 1,700,281 sheep and 2,062,828 goats slaughtered, 427,931 cattle (8%), 61,955 sheep (3.6%) and 48,889 goats (2.4%) were infected with *Fasciola.*

Daoud and Sulaiman, (2003) were examined an 850 fecal samples of cattle from eight provinces in Ninevah governorate in Iraq. Of which 17.41% were infected with *Fasciola.* Season affected the prevalence of infection, with the highest incidence observed during spring (25%), and followed by winter (18.53%) and autumn (7.11%). Female cattle were more susceptible to *Fasciola gigantica* infection than male.

Koko *et al.,* (2003) investigated the prevalence of caprine fascioliasis and the epidemiological aspects, which might influence the prevalence of the disease in northern part of the Gezira State, Sudan. A total of 287 goats were collected fecal and serum samples of which 12.5% of goats positive for *Fasciola gigantica*.

Mazyzd and El-Nemr, (2002) evaluated the endoparasites of sheep, goats and Shephered in North Sinai Governorate, Egypt. They revealed an overall infection of 12.7% and the prevalence of *Fasciola* spp was 12.8%.

Molazadeh and Zohoor, (2002) inspected a total of 358 goats, 316 calves, and 269 sheep livers and carefully examined to observe *Fasciola*. The prevalence of *Fasciola hepatica*

was 0.9% in calves, 3.7% in sheep and 3 % in goats. Also, the prevalence of *Fasciola gigantica* was 0.9% in calves, 1.6% in sheep and 2.8% in goats.

Mutiiria, (2002) investigated a total of 2,062,828 slaughtered goats in Kenya of which, 48,889 were infected with *Fasciola* giving a national prevalence of 2.4%. The highest prevalence of 9.1% was recorded in Western province followed by Nyanza, Central, Eastern, Rift Valley, Coast and Nairobi province 4.7, 4.4, 2.7, 2.4, 0.5% and 0.5% infection.

Youssao and Assogba, (2002) determined the prevalence of cattle fascioliasis in the Niger River Valley of Benin varied from 7.5-52.4%**.** In Malanville slaughter houses, the prevalence of *Fasciola gigantica* infested livers was about 30% regardless of the season.

Jithendran and Bhat, (2001) observed the prevalence of gastrointestinal parasites in sheep and goats of Himachal Pradesh, India and found the prevalence *Fasciola sp.* infection in sheep and goats were 9.6% and 8.8% respectively.

Maqbool *et al.,* (2000) were examined a total of 600 fecal samples from live goats and 600 livers from slaughtered goats of which 173 (28.83%) fecal samples and 220 (36.66%) livers were found positive for *Fasciola gigantica.* Prevalence of fascioliasis was higher during autumn both in live 48% and slaughtered goats 63% while lowest during spring 21% and 25% respectively. The higher prevalence was recorded in adults (33.6%). Females were more affected than males.

Motaleb, (1996) investigated the status of parasitic diseases in buffaloes, cattle and goats in Anowara, Chittagong and reported that 38.5% buffaloes, 36.28% cattle and 13.3% goats were infected with *Fasciola gigantica*.

Saifuzzaman, (1996) reported the incidence of helminth parasites of cattle at Chandina, in Comilla district, Bangladesh and reported 21.1% *Fasciola gigantica* infection in cattle by fecal examination. The incidence of infection was the highest during the winter season after heavy rain fall.

Shrestha, (1996) determined the prevalence rate of fascioliasis both in large and small ruminants of 1994, 1001 and 1166 from cattle, buffalo and sheep and goats were examined for *Fasciola* ova of which 656, 460 and 411 were positive, respectively. The prevalence of the disease was 47, 46 and 35% in cattle, buffalo and sheep and goats, respectively.

Chowdhury *et al.,* (1993) recorded 3.7% fascioliasis and 19.8% paramphistomiasis in zebu cattle (*Bos indicus*) at savar, Dhaka, Bangladesh by coprological examination.

Mahato, (1993) **was** reported that the prevalence of *Fasciola* in 57.9% in buffalo, 44.8% in cattle, 22.4% in goats and 18.2% in sheep in the hills of Nepal. In the Terai area, the prevalence of *Fasciola* was 51.4% in cattle, 4.3% in buffalo and 13.3% in goats.

Nwosu and Srivastava, (1993) were investigated the prevalence of *Fasciola gigantica* eggs of cattle and small ruminants (sheep and goats) slaughtered in Maiduguri, Borno State, Nigeria. A prevalence rate of 42.7% was recorded for *Fasciola gigantica,* in 246 cattle and 718 sheep and goats examined. For sheep and goats, the prevalence of egg excretion was 28 (3.9%) for *Fasciola gigantica.*

2.5 Pathogenesis

The pathogenesis of *Fasciola gigantica* has been regarded as similar with that of *Fasciola hepatica* since both flukes have similar life cycle in the definite host (Soulsby, 1982). *Fasciola hepatica* and *F. gigantica* differ in some fundamental biological trait(s) which renders *F. gigantica* more susceptible to immune effector mechanisms (Spithill *et al.,* 1997; Piedrafita *et al.,* 2004). However, the relative rates of development of the *F. gigantica* and *F. hepatica* parasites, when compared to the development of host response factors, are poorly understood. A comparison of the humoral response during infection of Belle Islois sheep to *F. hepatica* and *F. gigantica* (Zhang *et al.,* 2004) as well as the modulation of lymphocyte and eosinophil responses (Zhang *et al.,* 2005a,b) suggested that sheep are less susceptible to *F. gigantica* compared with *F. hepatica*. Although studies have suggested that the host immune response to the parasite plays an important role in the susceptibility of sheep to *Fasciola spp*. (Wiedosari and Copeman, 1990; Roberts *et al.,* 1997a, b, c; Piedrafita *et al.,* 2004) it has been shown that parasite modulation of the

immune response may also play a role in immune evasion and subsequent host susceptibility (Zimmerman *et al.,* 1983; Chauvin *et al.,* 1995; Prowse *et al.,* 2002; Zhang *et al.,* 2005a, b). There are numerous studies of sheep breeds infected with *F. hepatica*, but few on the biological and immunological responses of sheep to *F. gigantic.* Pathogenesis depends primarily on the two different stages of development of the parasite in the liver of the host, the level of parasitemia, and if it is an acute, sub acute or chronic infection.

2.6 Snails (the intermediate host)

A unique feature of all fluke diseases is their obligatory association with a molluscan host and for *Fasciola* spp with freshwater pulmonate gastropods. Snails of the genus *Lymnaea* are the intermediate hosts for genus *Fasciola*. The epidemiology of fascioliasis is dependent on the ecology of the snail intermediate hosts. *Lymnaea* species is the most important in the transmission of *Fasciola gigantica.* The most important intermediate hosts of *Fasciola gigantica* are *Lymnaea natalensis and Lymnaea auricularia* (Urquhart *et al.,* 1996). *Lymnaea natalensis* is the recognized intermediate host for *Fasciola gigantica* (Yilma and Malone, 1998) Other species serving as secondarily hosts to this species are *Lymnaea rufescens and Lymnaea acuminate* (Indo-Pakistan) and *Lymnaea rubiginosa* (Malaysia).

Various lymnaeid snails are intermediate hosts for the liver flukes in different regions of the world. In Pakistan (including current Bangladesh) (Kendall, 1954) the prevalence of developmental stages of *Fasciola* cercariae in *Lymnaea auricularia* var *rufescens* (snails) were reported first time by Kendall (1954). Qadir (1982) reported *F. gigantica* in Bangladesh. In other nearby countries by *F. gigantica* were reported by Mathur (1986) and Morel and Mahato (1987). Another intermediate host, *Lymnaea luteola* are also infected by cercarial infestation of *Fasciola gigantica,* but is resistant to *Fasciola hepatica,* and *L. truncatula* is the host of *Fasciola hepatica,* but is resistant to *Fasciola gigantica* (Boray 1985).

2.7 Epidemiology of snail

Affroze *et al.,* (2013) identified a total of 278 aquatic snails collected from the study area. Among them 7 (2.52%) were infected with Gymnocephalous cercariae.

Cheruiyot and Wamae, (2013) observed the cercariae of *Fasciola gigantica* shed from experimentally as well as naturally infected *Lymnaca natalennsis* to encyst to varying degrees on different objects. They encysted on glass containers 47%, grass 35% and snail shells 14%. Some were found unattached at the bottom of the containers 2.4% or facing 1.6%.

Islam *et al.,* (2012) investigated different snail species and parthinate of trematodes carried by them, in some selected areas of Mymensingh Sadar, Mymensingh. Snails were collected by hand picking method and examined after crushing. Among 864 tested snails, it revealed the presence of six species of snails two of them like *Lymnaea auricularia* 145 (16.8*%),* *Lymnaea luteola* 205 (23.7%) in the study areas. Among them 5.8% *Lymnaea luteola* and 6.2% *Lymnaea auricularia* were infected with Gymnocephalous cercariae.

Reman *et al.,* (2012) carried out the snail intermediate hosts of *Fasciola gigantica* include *Radix (Lymnaea) auricularia* and *Radix (Lymnaea) luteola* distributed in various regions of Tamil Nadu. These snails were found to be susceptible to experimental infection with *Fasciola gigantica* miracidium. Various developmental stages of L*ymnaeid* snails from eggs to adult were studied under laboratory condition.

Arshad *et al.,* (2011) examined various species of snails which act as the intermediate host of the Schistosomes collected from the study area. A total of 10418 snails were collected of these 13.51% were found to be infected. Only 14.51% of 2350 snails collected from district Kasur were infected followed by 13.6% of 2882 from district Sheikhupura, then 13.4% of 2709 from district Sargodha and the lowest 12.51% of 2477 from district Lahore.

Hussein *et al.,* (2010) determined the egg, miracidium redia, cercaria and metacercariae of *Fasciola gigantica* from field infected *Lymnaea* (cailliaudi) *natalensis* snails, to analyze the differences between eggs and intra-mollusca stages of *Fasciola hepatica* and *Fasciola gigantica* in a zone of symparty. The egg of *Fasciola gigantica* has an umbilicus-like

invagination at the posterior end of the egg shell. The emerged Miracidium has an elongated conical body that has a broad anterior end and tapering posterior end. The surface was found to be covered with varied lengths of cilia except regions of lateral connection of epidermal plates. The radia of *Fasciola sp.* has a caudal papilliform process. Tail of cercaria was found to be provided with two fin folds. Steps of encystations of the cercaria were described as a variable morphological change in cercarial body and cyst wall.

Singh and Singh, (2009) collected a total of 1012 *Lymnaea auricularia*, 285 *Lymnaea luteola*, 1021 *Indoplanorbis exustus* and 115 *Gyraulus convexiusculus* from different areas in Faizabad, Uttar Pradesh, India. The snail samples were screened for the presence of trematode cercariae. Only 69 *Lymnaea auricularia* (6.8%) were involved in the transmission of fascioliasis. The number of snails shedding cercariae was also highest during the rainy season (95; 8.31%), followed by winter (53; 6.87%) and summer (12; 2.31%).

Hussein *et al.,* (2005) inspected a total number of 2237 specimens of lymnaeids, found in Qena Governorate, Upper Egypt. Fasciolid larval stages were detected in 35 individuals (1.56%), including very low rates of snail infection during July (0.48%) and September (0.63%), an appreciable rise in April (3.07%) followed by another increase in October (4.12%), and the highest percentage during November (12.96%) No fasciolid infection could be found in lymnaeids collected during the rest of the year

Salaby *et al.,* (2004) decided experimentally infections of pre-adult and adult *Lymnaea cailliaudi* snails using the same isolates of *Fasciola gigantica* miracidia performed under laboratory conditions. Pre-adult snails were divided into 3 groups kept at 18-20, 24-26 and 29-31^{o} C, respectively and the adult snails were represented by a single group kept at 24-26^{o} C to compare to its corresponding pre-adults ones. Prepatent period of *F. gigantica* inside its snail host was inversely related to temperature and markedly affected by age rather than the number of miracidia inoculated. In contrary, Temperature above 24 ^{o}C was suitable for high metacercarial production and a significant increasing in the percentage of the floating metacercariae was found at high temperature.

Mas-Coma *et al.,* (2001) examined the liver fluke and its lymnaeid snail host adapted to the extreme environmental conditions of the high altitude and succeeded in giving rise to high infection rates. When comparing the development characteristics of European *Fasciola hepatica* and *Lymnaea truncatula*, a longer cercarial shedding period and a higher cercarial production were observed, both aspects related to a greater survival capacity of the infected lymnaeid snails from the Altiplano. These differences would appear to favor transmission and may be interpreted as strategies associated with adaptation to high altitude conditions.

2.8 Infection of developmental stages of *Fasciola gigantica* in snail

Rondelaud *et al.,* (2014) investigated experimentally infected of *Lymnaea glabra* (two populations) with *Fasciola hepatica* and carried out during seven successive snail generations, to determine if prevalence and intensity of snail infection increased over time through descendants of snails already infected with *Fasciola hepatica*. The number of cercariae shed by infected snails compared to overall cercarial production noted in snails containing cercariae but dying without emission, the percentage was greater in *Galba truncatula* (69% instead of 52–54% in *Lymnaea glabra*). Even if most characteristics of *Fasciola hepatica* infection were lower in *Lymnaea glabra*, prevalence and intensity of parasite infection increased with snail generation when tested snails came from infected parents

Chontananarth and Wongsawad, (2013) studied a total of 2,479 snail individuals collected and classified into 7 families, 11 genera, and 14 species, Among them, 8 snails species were found to be infected with an overall prevalence of 17.27% (428/2 479), which infected with nine groups of cercariae; gymnocephalous cercaria, strigea cercaria, megalurous cercaria, monostome cercaria, parapleurolophocercous cercaria (Haplorchis cercaria), pleurolophocercous cercaria, furcocercous cercaria (Transversotrema cercaria), xiphidiocercaria, and virgulate cercaria. The parapleurolophocercous cercaria was found to be the dominant type among the cercarial infection in the snails (64.25%).

Imani-Baran *et al.,* (2013) carried out a total number of 6759 Lymnaeidae snails from 28 snail habitats; of these *Lymnaea gedrosiana* the prevalent snail (74.37%) which examined

for cercarial infection by shedding method. The overall infection rate was 8.03%. The most frequent trematode cercariae in the snail were xiphidiocercariae (81.98%), furcocercariae (32.26%), echinostome cercariae (5.19%), and monostome cercariae (1.24%). The highest infection rate in *Lymnaea gedrosiana* (100%) was with echinostome cercariae from Golestaneh in autumn.

Islam *et al.,* (2013) examined different snail species and parthinate of trematodes carried by them, in some selected areas of Mymensingh Sadar, Mymensingh. Snails were collected by hand picking method and examined after crushing. Among 864 tested snails, it revealed the presence of six species of snails among on of two like *Lymnaea auricularia* 145 (16.8%) and *Lymnaea luteola* 205 (23.7%). Among them 5.8% *Lymnaea luteola* and 6.2% *Lymnaea auricularia* were infected with Gymnocephalous cercariae. One or more species of snails were found to harbor single or mixed types of cercariae such as gymnacepahlus, echinostome and furcocercous. Population densities of different species of snails were determined in per unit time which were statistically significant ($p<0.01$).

Singh *et al.,* (2012) find out the infection of *Fasciola gigantica* larvae in *Indoplanorbis exustus* in Ramgarh Lake and GIDA pond. Higher infection of *I.ymnaea exustus* was observed in July to October. This study conclusively, shows that variant abiotic factors in different months of the year can significantly alter the infection rate and development process of larvae (sporocyst, redia and cercariae) in the snail *Indoplanorbis exustus.*

Vikram and Kakulte, (2012) reporte the digenetic larval trematodes infection with Molluscan snails in the water reservoir, ponds, ditches, lakes in and around Darna River, Nashik, Maharashtra, India. Molluscan snails prevailing to the ecological condition is studied in detail considering importance of environmental parameters such as physico-chemical examination i.e. temperature, humidity and rainfall, total hardness, total solids, total alkalinity, pH, chlorides.

Al-jibouri *et al.,* (2011) revealed the prevalence (%) significantly increased with temperature decreasing and the increase of miracidia dose, as well as the prevalence (%) significantly highest in largest snails. The prepatent period was significantly longest in small snails .Concerning the number of metacercariae production, the study reveals that the

production of metacercariae from snails increased at (25+1) while it decreased significantly at (19+1) and (30+1) and it also increased significantly with the increase of miracidia dose and the snails size. The floating metacercariae percentage significantly increased with the increase of temperature but there was no significant effect for miracidia dose and the size of snails.

Imani-Baran *et al.,* (2011) examined a total of 6759 *Lymnaeid* snails, 370(5.5%) *Lymnaea auricularia* snails identified. Cercariae infection was found in a number of 276 (74.56 %) snails. The results showed that removed cercariae from *Lymnaea auricularia* belonged to Echinostomcercariae (96.38 %) and Furcocercariae (3.62 %) which were found in two out of 28 sites during the course of study. In summer and fall, the highest prevalence of cercariae infection was recorded for both identified cercariae. It was concluded that *Lymnaea auricularia* could be an important intermediate host of large group digenian trematodes.

Sabeeh *et al.,* (2010) stated the infection of *Lymnaea auricularia* snails with *Fasciola gigantica* miracidia Cercarial shedding was irregular and considerable variation occurred in their output (169- 712 cercaria). Cercariae of *Fasciola gigantica* preferred the green parts of the grass *Echiochloa Crus-gall (Lymnaea)* than the brown, dead parts. Lower surface of submerged leaves had more metacercariae than the upper surface. There was a vertical zonation of metacercariae on grass in water. More metacercariae occurred on the upper part of submerged grass than on the lower parts. Vertical migration of cercariae after setting did not occur before encystment.

Garg *et al.,* (2009) revealed that 5.48% of the snails harboured larval stages of *Fasciola gigantica* from *Lymnaea auricularia* snails. Also, the snails in the tarai had a higher prevalence (7.28%) of infection compared to those in the plains (1.57%). The results of the present study may help to devise an effective control strategy against fascioliasis in north India.

Suhardono *et al.,* (2006) carried out the eggs of *Fasciola gigantica* in dung heaps that located in the shade or exposed to sun light, and examined at intervals for up to 14 weeks. The rate and extent of decline in viability of eggs were greater in dung exposed to sun light

than in shaded dung. This difference was attributed the higher temperature in dung in sun light, owing to the effect of direct sunlight and to a higher rate of fermentation in exposed than in shaded dung.

Pfukenyi *et al.,* (2005) reported the presence or absence of *Fasciola gigantica* metacercariae. Snails collected at the same time were individually checked for the emergence of larval stages of *Fasciola gigantica*. *Lymnaea natalensis*, the snail intermediate host of *Fasciola gigantica* was recorded from the study sites with the highveld having a significantly higher abundance of the snail species than the lowveld ($P < 0.01$). The snail population was low between December and March and started to increase in April reaching a peak in September/October.

Ashrafi *et al.,* (2004) examined *Lymnaea gedrosiana* and *Lymnaea palustris* the most prevalent lymnaeid snails. Of 4830 different snails studied, only seven *Lymnaea gedrosiana* were found to be infected with larval stages (rediae and cercariae) of *Fasciola* sp. Experimental infections of 15 common laboratory mice by metacercariae, obtained from those naturally infected snails, were carried out and all trematodes recovered at necropsy, 8 weeks post-exposure, appeared to be *Fasciola gigantica* based on morphology. The high temperature, moisture and rainfall during the year, especially in Bandar-Anzali, support the establishment and transmission of the disease in the zone.

Karimi *et al.,* (2004) determined two snail species belonging to genus *Lymnaea auricularia* and *Lymnaea gedrosiana*, were collected from 6 different districts of Shadegan area of Khoozestan. 8% of the total collections of the snails revealed the occurrence of larva in them. The prevalence of larva within the snails varied in different districts may be due to different geoclimatic conditions and availability of fresh water. Seasonal prevalence of snails and factors affecting snail populations including water temperature, light, water depth and pH under field conditions were also studied.

Rondelaud *et al.,* (2000) investigated a total number of 235 watercress beds in this study and found to contain 1 or 2 *Lymnaea* species as follows: *L. truncatula* only (84% of watering places), *L. glabra* only (6.3%), and both species (6.3%). In the populations of *L.*

truncatula, natural infections of snails with *Fasciola hepatica* were irregular and occurred up to six times over the 28-year period in the region of Limousin.

Charoenchai *et al.,* (1994) **s**tudied a total of 2408 *Lymnaea auricularia rubiginosa* collected and examined by shedding and crushing. Trematode infection occurred in 163 (6.77%) of 2,408 *Lymnaea auricularia rubiginosa* and some snails were infected with more than one cercarial species. 99 snails (4.11%) were infected with Echinostomes, while mixed infection of echinostomes with *Fasciola gigantica* and with schistosomes was found in 5 snails (0.21 %) and 2 snails (0.08%), respectively. Only I snail (0.04%), 19 snails (0.79%) and 37 snails (1.54%) were infected with *Fasciola gigantica*, respectively.

Chowdhury *et al.,* (1994b) examined a total of 4149 *Lymnaea auricularia* var *rufescens* and 401 *Lymnaea luteola* snails collected and examined in Savar, Dhaka, Bangladesh. *Fasciola* cercariae (Gymnocephalous cercariae) were found in 13 (0.31%) *Lymnaea auricularia var rufescens*, but was absent in ail *Lymnaea luteola* examined. Prevalence of *Fasciola* cercariae in the snails varied significantly (p lt 0.05) in different periods (months) of the year with higher prevalence in July-August (0.77%), followed by September-October (0.52%) and May-June (0.45%). Cercariae did not appear in the snails from November to April.

Woodruff and Uptham, (1992) reported the diseases include fascioliasis (liver fluke, *Fasciola gigantica*, is transmitted by *Lymnaeid* to cattle and sheep) in northeast Thailand.

2.9 Diagnosis

Diagnosis of fasciolosis may consist of tentative and confirmatory procedures. A tentative diagnosis of fasciolosis may be established based on prior knowledge of the epidemiology of the disease in a given environment; observations of clinical signs, information on grazing history and seasonal occurrence. Confirmatory diagnosis, however, is based on demonstration of *Fasciola* eggs through standard examination of feces in the laboratory; postmortem examination of infected animals and demonstration of immature and mature flukes in the liver. The latter is helpful in deciding the intensity of infection. There are other laboratory tests enzymatic and/or serological procedures used to qualify the infection

mainly for research purposes. Serological assays are often used to detect infections due to immature forms where fecal egg output is often nil. Such tests allow the detection of substance like cathepsin L proteases, excretory-secretory products, detection of Ag in milk, and ELISA detection of antibodies against the flukes plasma concentration of Gamma-glutamyltransferase (GGT), which are increased with the bile duct damage (Cornelissen *et. al.,* 2001) for example, Oxidative stress would be one of the consequences of the activity of inflammatory cells such as neutrophils, macrophages and eosinophils in producing oxygen-derived free radicals, nitric oxide and their products. A useful indicator of oxidative stress is the concentration of reduced glutathione (GSH) in cells. For chronic fasciolosis, confirmatory diagnosis could be easily carried out by coproscopic examination employing sedimentation technique. *Fasciola* eggs have high specific gravity and sedimentation is preferred to floatation. When the latter is employed, floating medium such as $ZnSo_4$ should be used. As *Fasciola* eggs may be confused with *Paramphistomum* eggs, addition of methyline blue in the feacal suspension will facilitate ease identification by providing a blue and contrasting microscopic field.

2.9.1 Clinical sings

The acute form of fascioliasis results from immature fluke migrating through the liver causing liver damage and hemorrhage. This can result in sudden death in younger animals following heavy infection, weakness, anemia and dyspnea. The chronic and sub acute form may however be more economically significant due to ongoing losses in productivity over a longer time period. The principal effects are bile duct obstruction, destruction of liver tissue, hepatic fibrosis, adult flukes ingesting blood and anemia. Clinical signs include oedema, anemia, progressive loss of condition, hypoalbuminaemia, emaciation, paler of the mucous membranes, edema (local swellings due to excess fluid) often as "bottle jaw"**,** digestive disturbances (diarrhea, constipation etc.), cachexia (wasting, i.e. weight loss, fatigue, weakness, loss of appetite, etc.) and ascities and decreased productivity, which often go undetected (Hossain *et al.,* 2005; Junqura 2013). Younger goats are usually more susceptible to fascioliasis caused by *Fasciola gigantica*, as older goats tend to develop some level of immunity or tolerance to infection (Keyyu *et al.,* 2003; Pfukenyi *et al.,* 2005 and Tasawar *et al.,* 2007).

Complete organ failure may be occurred due to sudden migration of many immature flukes through the liver, which may lead to death of the healthy animals within a few days. Juvenile flukes adhering to the intestinal lining cause erosions those leads to the loss of electrolytes and proteins, resulting in foetid diarrhoaea. Anemia is not usually seen. Signs secondary to protein loss include edema and weight loss. Death and clinical disease are intermittent in endemic areas, but in acute outbreaks, mortality can be as high as 40% (Kimura 1961; Butler and Yeomans 1962). In milder infections clinical signs may or may not be readily observed, however, a decreased appetite and interference with post-absorptive metabolism of protein, carbohydrates and minerals, may have a significant effect on production. In addition to these, a condition known as *'black disease'* is a complication, which usually is fatal. Here, a secondary infection due to the bacterium *Clostridium novyi* Type B, proliferating in necrotic lesions produced by the young larvae migrating in the liver is responsible for the fatal outcome (Radostits *et al.,* 1994). Chronic fasciolosis provides the right environment in the liver for the germination of the spores of the bacterium.

2.9.2 Egg detection by fecal examination

Definitive diagnosis for *Fasciola* spp. is by detection of fluke eggs in feces. *Fasciola* spp. eggs from goats are a yellow/brown color and measure 129-204 µm (*Fasciola gigantica*) (Valero *et al.,* 2009). Differentiation of the eggs is aided by the use of contrast stains such as methylene blue. The most sensitive egg detection techniques involve various methods of direct smear, fecal filtration and sedimentation. These techniques have excellent specificity but sensitivity is variable depending on the intensity of eggs present in faeces. The 16 proportion of eggs recovered may vary between 18 and 75 % (Conceicao *et al.,* 2002, Suhardono *et al.,* 2006). Whilst sensitivity may reach 100% for fecal samples containing a high intensity of eggs per gram (EPG), it drops to 30% for less than 1.5 EPG (Conceicao *et al.,* 2002). In the case of *Fasciola* spp., the reduction in egg output after the first few weeks of patent infection makes fecal egg detection methods less sensitive (Spithill *et al.,* 1999). The qualitative examination of *Fasciola gigantica* eggs in feces and bile were compared with the detection of precipitating antibodies in sera by agar gel precipitation test (AGPT) in 1000 cattle slaughtered at the Bodija municipal abattoir in Ibadan, Nigeria. Faecal and

bile examination methods detected (196) 33.5% and (389) 38.9% of the animals as positive for fasciolosis, while (474) 47.4% were positive by AGPT. Both direct bile examination and faecal egg detection methods have high specificity and positive predictive value (100%) when compared with AGPT. However, lower values for sensitivity and negative predictive value were observed for both faecal egg examination (66.5% and 67.9% respectively) and bile examination (81.0% and 78.9% respectively). Fecal and bile examination failed to detect 33.5% and 19.0% of the cases detected by AGPT. The results of this study revealed that the AGPT could become a better test for the herd diagnosis of bovine fasciolosis (Adedokun *et al.,* 2008).

2.9.3 Gross and histopathological lesions

Abraham and Jude, (2014) investigated fascioliasis among trade ruminants (cattle and goat) slaughtered at Calabar abattoirs using histological preparations for liver tissue. They found that infected liver of the two ruminants were damaged. Damaged hepatic parenchyma resulting in severe hemorrhage, thickening and gross fibrosis of bile duct were observed. Dislodge hepatic cells became wandering cells amidst macrophages within the sinusoid. The central vein of cattle infected by *Fasciola hepatica* and *Fasciola gigantica* was enlarged and laden with debris resulting in obstruction of liver function such as protein synthesis.

Affroze *et al.,* (2013) determined the risk factors and gross pathology of liver fluke infection in cattle in Netrokona district of Bangladesh. At necropsy, *Fasciola* infected liver appeared larger with tensed capsule and bile ducts were dilated, thickened with fibrous tissue masses forming the characteristic pipe-stem liver.

Chiezey *et al.,* (2013) estimated the effect of the hepatic migratory phase of *Fasciola gigantica* infections in Yankasa ewes. Path-physiological signs included anorexia, progressive anemia, emaciation and significant hypoproteinemia. At post mortem hepatic fibrosis of the liver with intra-hepatic hemorrhage was observed.

Adama *et al.,* (2012) observed the pathological lesions in four *Fasciola gigantica* infected Yankasa sheep that died by an experimental infection at the RNAPRI, Shika-Zaria, Nigeria. The gross pathological lesions observed were hepatomegaly, appearance of migratory

tracts on the liver surface, marked distension of the gall bladder in which numerous flukes were present. The histopathological lesions were presented inform of intense hemorrhage both in the parenchyma and in the parasite tracts. There was fibrosis and distortion of the normal architecture of the hepatic cells. Observed clinical signs were inappetance, progressive anemia and emaciation. There was a marked reduction in albumin and total plasma protein levels in the blood of the infected sheep compared to their controls.

Badr and Nasr, (2009) decided bovine liver samples from El-Warak abattoir which appeared grossly infested with mature *Fasciola* worms. The histopathological examination of acute hepatitis revealed hepatic necrosis and degeneration with presence of multiple variable sized abscesses in the hepatic parenchyma consisted of homogenous structurless mass of necrotic cells surrounded by heavy aggregations of inflammatory cells mainly neutrophils, histiocytes and lymphocytes and the abscesses bounded by fibrous connective tissue capsule. While the chronic hepatitis revealed increased fibrous connective tissue proliferation in the portal triads, around small and large bile ducts and in the Glisson's capsule. The biliary epithelium was hyperplastic with formation of large numbers of newly formed bile ductless and presence of mature *Fasciola* worm within the lumen of the main bile ducts.

Siddiquq and Ved, (2009) revealed the pathology of fasciolosis due to *Fasciola gigantica* in goats of Kanpur region. On post-mortem examination of 240 livers samples from slaughtered goats were collected randomly, of which 12(5%) livers were found pathologically changed and many lesions were observed on the surface. Grossly, the livers were hard, firm and revealed grayish-white discoloration on the surface.

Masuduzzaman *et al.,* (2005) studied a total of 57 domesticated deer of various types examined to diagnose the fascioliasis in Chittagong zoo, Chittagong city, Bangladesh. The gross examination revealed enlarged livers with round edges and thickened capsule with numerous haemorrhagic spots on the parietal surface. In chronic form, the livers were cirrhotic and reduced in size. The affected intra-hepatic bile ducts were protruded and were engorged with flukes. Microscopically the migratory tracts were represented by the presence of hemorrhage, edema and infiltration with numerous eosinophil mixed with few

lymphocytes. The wall of the bile ducts was thickened with fibrous tissue proliferation and the lining epithelium showed hyperplastic changes.

Pérez *et al.,* (1999) observed the lesions produced by *Fasciola hepatica* in the liver, gall-bladder and hepatic lymphnodes (HLNs) of four groups of five, one group of goats received a single oral dose of metacercariae, but the other four groups received four or five doses at different intervals over a period of 11 weeks. Goats with numerous hepatic calcareous granulomas showed the most severe hepatic damage, including marked cirrhosis, with a striking infiltrate of CD3+ T lymphocytes and lambda IgG- plasma cells, replacing extensive areas of hepatic parenchyma, in which hypertrophy of the smooth endoplasmic reticulum of hepatocytes was evident.

Chakraborty and Chaudhury, (1992) discovered elephants infected with *Fasciola jacksoni* autopsied at Assam State Zoo, India. The parasites were attached to biliary epithelium. Microscopy demonstrated that the biliary epithelium was distorted by necrotic tissue which contained erythrocytes and ova of *Fasciola jacksoni*. The epithelium was analyzed by X-ray microanalysis, which showed that the infected epithelium contained aluminum, silicon, calcium and iron, while non-infected, normal biliary epithelium contained only phosphorus and sulfur. Scanning electron microscopy demonstrated that both the dorsal and ventral surfaces of the parasite possessed spines.

Wiedosari *et al.,* (1991) examined the pathological changes in the liver induced by *Fasciola gigantica* in sheep. Lesions associated with the migration of immature flukes through the parenchyma were a prominent feature of infection with *Fasciola gigantica*. The size of the hepatic lesions increased during the course of infection and the formation of progressively larger areas of scar tissue in the liver. Microscopically, cellular infiltration, proliferation of bile ductless and fibrosis were occurred in adjacent portal triads and interlobular septa. In bile ducts infected with *Fasciola gigantica* there was more extensive desquamation of the epithelium, more intense mucosal infiltration with lymphoid cells and fewer eosinophil, less severe glandular hyperplasia, more free blood in the lumen and a thicker duct wall.

Wiedosari and Copeman, (1990) find out the experimentally induce to *Fasciola* in sheep. This animal developed pathologically altered values from 12 weeks post infection, coincident with the period of greatest hepatic hemorrhage and destruction of hepatic tissue by migrating flukes and their entry into bile ducts.

2.9.4 Clinico-pathology

2.9.4.1 Hematological parameters

Abraham and Jude, (2014) investigated fascioliasis among trade ruminants (cattle and goat) slaughtered at Calabar abattoirs using haemocytometer for blood. They found that excessive leucocytosis with marked eosinophilia was observed in infected animal blood. It is important to examine carcasses of ruminants slaughtered in abattoirs before presentation for public consumption.

Egbu *et al.,* (2013) determined the hematological changes due to the infection of fascioliasis in cattle. Statistical analysis revealed high significant differences between the packed cell volume (PCV), hemoglobin (Hb) and red blood cells (RBC) of infected and non-infected cattle ($p<0.05$). Significant differences existed between the white blood cells (WBC) ($p<0.05$). There was notable reduction in PCV, Hb and RBC with increase in worm load and a multiple regression analysis revealed significant negative correlation between worm load and RBC, Hb and PCV with correlation coefficient values, r = -0.616, -0.592 and -0.615, respectively. Levels of neutrophils, eosinophils, monocytes and lymphocytes increased progressively as worm load increased. Only basophils showed no change.

Zeleke *et al*., (2013) revealed a total of 1528 sheep selected and sampled by using systematic simple random sampling, Haematocrite centrifugation were employed during the study period. The mean PCV value of *Fasciola* species in infected and uninfected sheep were 24.04 ± .381 and 25.87 ± .516 respectively.

Adama *et al.,* (2011) examined the experimentally infected with *Fasciola gigantica* in Yankasa sheep and hematological parameters evaluated using twelve Yankasa ewes aged 10-12 months. Mean packed cell volume (PCV), hemoglobin concentration and total plasma protein of the infected group were significantly lower than the controls ($p<0.01$).

Eosinophilia featured in the infected sheep. Mean White Blood Cell (WBC), neutrophils and lymphocyte levels of the infected sheep were significantly ($p<0.05$) different from those of the controls at the advanced periods of the infection (12-14weeks pI). The findings of this study revealed the potentials of hematological parameters as a veritable diagnostic tool in the management of ovine fascioliasis.

Rahman *et al.,* (2007) estimated the hematological changes of natural concurrent infection of *Fasciola gigantica* in cattle at Soba west Irrigated Agricultural Scheme, Khartoum State. They were observed anemia represented by a declined hemoglobin concentration (Hb %) and a fall in PCV were encountered.

Teleb *et al*., (2007) studied the hematological changes in twenty five months old Farafra sheep. The hematological study showed a significant decrease ($P<0.05$) in red blood cell (RBC) counts, hemoglobin (Hb) concentration, percentage of packed cell volume (PCV %) and monocytes counts in sheep infected with *Fasciola gigantica* compared to the control. Moreover, white blood cell (WBC) counts, eosinophils and neutrophils counts were significantly higher ($P< 0.05$) in infected groups than the control.

Vengust *et al.,* (2003) decided the hematological constituents as well as the liver can be affected by *Fasciola hepatica* in deer. Blood samples were taken from 62 male and female fallow deer that were shot, aged 6 months in Slovenia. Significant differences in blood were found in the mean cell volume. Mean cell hemoglobin concentration, eosinophils, basophils, lymphocytes, monocytes, alanine aminotransferase, urea, glucose and copper in blood were significantly higher in animals with fascioliasis. It has been concluded that some of the values in fallow deer like in other domestic ruminants are the sensitive indicators of liver cell damage in fascioliasis.

Wiedosari and Copeman, (1990) find out the experimentally induce to *Fasciola* in sheep. Infected and non-infected sheep had similar values for packed cell volume, mean corpuscular volume, and mean corpuscular hemoglobin concentration, serum glutamate dehydrogenase, serum gamma glutamyl transferase and serum aspartate transferase throughout the trial, except for one animal infected with 500 metacercariae from which the highest recovery of flukes (55) was made.

2.9.4.2 Biochemical parameters

Chiezey *et al.,* (2013) determined the effect of the hepatic migratory phase of *Fasciola gigantica* infections in Yankasa ewes. Hepatic dysfunction indicated by the significant increases in liver enzymes; aspartate transaminase, alanine transaminase and alkaline phosphatase levels in infected ewes and at post mortem hepatic fibrosis of the liver with intra-hepatic hemorrhage was observed.

Adama *et al.,* (2011) carried out the biochemical responses of Yankasa ewes to experimental infection of *Fasciola* gigantica at the RNAPRI, Shika-Zaria, Nigeria. The pathogenic effect was also evident based on fluke biomass, mean fluke length and width, degree of liver damage, serum AST, ALT, ALP and electrolytes responses. There was an increased level of AST from the 4th week post-infection indicating greater damage to the liver parenchyma while the levels of ALT began to rise at the 9th week post-infection among the infected group indicating epithelial damage in the bile ducts. The finding of this study reveals the diagnostic potentials of liver enzymes in the early detection of ovine fasciolosis.

Ghanem *et al.,* (2010) studied on fifteen male baladi goats from Giza Governorate (about 12 months age) free from previous infection with *Fasciola gigantica*. It was divided into 5 groups, the first group (G1) was vaccinated with unbound fraction proteins, the second group (G2) was vaccinated with natural glutathione-S-transfers (GST), the third group (G3) control group was vaccinated with Freund's adjuvant, The fourth group (G4) kept as control positive and the fifth group (G5) kept as control negative. Blood samples were collected to assess the AST, ALT, and ALP, Total protein, Serum albumin and Serum globulin. During vaccination period, no significant changes were detected for all groups regarding production of AST, ALT and ALP. After challenge, enzymes levels were higher in infected groups (G3 and G4) than vaccinated groups (G1 and G2).

El-Shazly, (2008) examined in chronic *Fasciola* (cholestatic and non-cholestatic) patients of serum lipids, liver enzymes and total bile acids. Variations in the biochemical parameters between infected and control groups were detected and typified by considerably higher serum triglycerides (TG, $P < 0.001$), alkaline phosphatase (ALP, P<

0.001) and total bile acids (TBA, P < 0.001) in the infected group. For cholestatic patients, TG, very low density lipoprotein (VLDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyl transferase (GGT) and ALP were statistically higher (P < 0.001 for each comparison) and total cholesterol (TC); low density lipoprotein (LDL) and high density lipoprotein (HDL), were statistically lower than non-cholestatic patients (P = 0.02, < 0.001, < 0.001 respectively).

Phiri *et al.,* (2007) evaluated the influence of *Fasciola hepatica* and *Fasciola gigantica* infection on serum glucose and beta-hydroxybutyrate (beta-HOB) in sheep. This was done by setting up two groups of sheep. The results of weight gain, parasitological and serum liver enzymes activity (glutamate dehydrogenase [GLDH] and gamma glutamyl transferase [gamma-GT]) used in monitoring the infection showed that all infected animals developed fasciolosis.

Raadsma, (2007) investigated the early biochemical changes in plasma, comparative host-immune responses and parasite recovery data in Merino sheep during infection with *Fasciola gigantica* and *Fasciola hepatica.* One group of sheep was uninfected, four groups of sheep received incremental challenge doses of metacercariae of *Fasciola gigantica* (50, 125, 225 and 400, respectively) and the sixth group was challenged with 250 *Fasciola hepatica* metacercariae. At 6 wpi, elevated levels of plasma GLDH were observed in the *Fasciola gigantica* infected groups compared to the uninfected sheep ($p<0.005$) whereas the *Fasciola hepatica* challenged group had four-fold higher levels of GLDH compared to the *Fasciola gigantica* infected group ($p<0.001$). Elevated levels of GGT as an indicator of epithelial damage in the bile duct was only seen in the group challenged with *Fasciola hepatica* at 10 wpi when it rose from below 100 IU/l to approximately 250 IU/l ($p<0.0001$) whereas no detectable increase in GGT was observed in any of the groups challenged with *Fasciola gigantica.*

Teleb *et al.,* (2007) investigated serum biochemical parameters in twenty five months old Farafra sheep. Moreover, significant elevations in serum total bilirubin, activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT) were also observed in infected sheep comparing with control one.

Khalil *et al.,* (2005) examined a total of 200 local Iraqi male goats naturally infected with fascioliasis. The results of experiment showed that: hemoglobin (Hb), eosinophils counts, SGOT, SGPT, SLDH, fibrinogen, bilirubin and E.P.G.S were convenient parameters to assess anthelmintic activity in chronic cases of infection. There were gradual significant increase in Hb concentration and significant decrease in SGOT, SLDH and eosinophils count on the 3rd week post treatment with triclabendazole, while the result didn't show any significant changes in serum proteins.

Mbuh and Mbwaye, (2005) decided the biochemical parameters of blood in healthy and infected goats. The serum minerals potassium, chlorine and bicarbonate did not show any significant differences ($P>0.05$) between the infected and control groups of goats, but there was a significant difference ($P<0.0001$) in sodium, calcium and urea levels. For serum lipids cholesterol and triglyceride, there were significant ($P<0.0001$) differences between the infected and control group. This goes same for serum proteins, albumin and total proteins. However, serum enzymes, serum glutamic oxaloacetic transaminase (SGOT) and gamma glutamyl transferase (GGT) were not significantly different ($P>0.05$) between the infected and control groups. There was a significant difference ($P<0.0001$) in alkaline phosphatase (ALP) and serum glutamic pyruvic transaminase (SGPT) between the infected and control groups.

Waweru *et al.,* (1999) examined 32 sheep aged between 6 and 9 months, acquired from a fluke-free area and sheep of each breed divided into two equal groups of six. Each animal in one group of each breed was experimentally infected with 400 viable metacercariae of *Fasciola gigantica*. The other groups acted as uninfected controls. Blood samples were taken at weekly intervals for the determination of serum bilirubin, albumin, and gamma glutamyl transferase levels. However, serum bilirubin and gamma glutamyl transferase (GGT) in the infected animals were elevated significantly more in the Dorper than in the Red Masai sheep.

Chakarborty and Lodh, (1994) investigated the blood biochemical profiles in fascioliasis in goats and they recorded a decrease in the total serum protein and serum albumin and marked increase in serum globulin concentration in all infected goats.

Ferre *et al.,* (1994) determined the effects of experimental fascioliasis at various stages of development on the daily food intake of sheep. Significantly increase in serum glutamate dehydrogenase (GLDH) activity from 40 days post-infection and in aspartate aminotransferase (AST) activity from 60 days post-infection. Both enzyme activities reached maximum levels in the serum of infected animals at 80 days and then progressively decreased. Serum gamma-glutamyl transferase (GGT) activity was significantly increased from 80 to 120 days post-infection. The coincidence of decreased food intake with the period of significant increase, both in AST and GLDH activities, indicated that damage caused around the time of migration of immature flukes through the liver parenchyma involved in appetite depression.

Goraish *et al.,* (1991) observed experimentally infected with 100 viable metacercariae of *Fasciola gigantica* in goats and treated with rafoxanide (Ranide, 7.5 mg/kg) at week 4. The plasma enzyme activity of aspartate amino-transferase (AST), glutamate dehydrogenase (GD) and sorbitol dehydrogenase (SD) increased to a similar extent with primary and challenge infections. However, the plasma antibody response to *F. gigantica* was less pronounced in reinfected goats.

2.10 Fluke control

Like other diseases of livestock, there are various methods of disease management at individual and herd levels. Possible options for control of *Fasciola* spp. are reviewed by (Roberts and Suhardono, 1996). Currently, anthelmintics are the only method used in most tropical and subtropical regions (Fabiyi and Adeleye, 1987). Oxyclozanide combined with levamisole (Ratnaparkhi *et al.,* 1993; Mahbub, 1996; Stevenson *et al.,* 2002; Hassan *et al.,* 2012), triclabendazole (Abdul Hadi *et al.,* 1996; Apt *et al.,* 2005) and nitroxylin (Keyyu *et al.,* 2008) are three anthelmintics that are available in Bangladesh and have reasonable (although not complete) efficacy against adult flukes and thus in controlling the chronic form of disease (Keyyu *et al.,* 2008). However, their efficacy against acute fascioliasis is poor as they are ineffective against immature fluke. Careful consideration of local economic and animal management conditions is required when designing a control programme, and for small scale farming, grazing management may provide the best control option if good information is available (Keyyu *et al.,* 2005; 2006).

2.11 Efficacy of anthelmintic

Ayaz, (2014) determined the efficacy of albendazole, oxyclozanide and triclabendazole in sheep. The efficacies were measured by the fecal egg count reduction test (FECRT).The prevalence of fascioliasis was 38% in sheep. The *Fasciola hepatica* was the major specie with prevalence (26%) and *Fasciola gigantic*a (9%). It is concluded that *Fasciola hepatica* is the major specie and the flukicides like Albendazole, Oxyclozanide and Triclabendazole, are indicative against fasciolosis in sheep. The present study is aimed to assess the comparative efficacy of commonly available flukicides viz Albendazole, Oxyclozanide and Triclabendazole in sheep under field conditions is also evaluated.

Shareef *et al.*, (2014) concluded the triclabendazole (TCBZ), the anthelmintic drug active against both mature and immature liver flukes, used to the effect of *in vivo* treatment on the tegumental surface of juvenile *Fasciola gigantica*. Five goats were infected with 150 *Fasciola gigantica* metacercariae each by oral gavage. Four of them were treated with single dose of TCBZ at 10 mg/kg at four weeks post-infection. They were euthanized at 0 (untreated), 24, 48, 72 and 96 h post treatment. Juvenile flukes were manually retrieved from the goat livers and processed for scanning electron microscopy. The present study further establishes the time-course of TCBZ action *in vivo* with 100% efficacy against the juvenile tropical liver fluke.

Jamila and Nabila, (2013) studied the in vivo effects of ethanolic extract of *B. egyptiaca* on goats naturally infected with fascioliasis, through investigation of egg/gm (EPG) feces and hematological analysis. The effect of ethanolic extract of *B. egyptiaca* was compared with the effects of triclabendazole (TCBZ) which is the ideal anthelmentics against fascioliasis. The results indicated that using of ethanolic extract of *B. egyptiaca* in treatment of infected animals improved their health condition, through decreasing the number of egg of *Fasciola*/gm feces and improving the hemogram levels in the infected goats.

Shokier *et al.*, (2013) evaluated the efficacy of five different anthelmintics against *Fasciola* species in naturally infected cattle in Beni-Suef, Egypt. On zero day each group was administered one of the five anthelmintics; albendazole, rafoxanid, oxyclozanide, oxyclozanide and combination of levamisole and triclabendazole. The

efficacy of the used anthelmintics was decided by fecal egg count reduction percent in which the fecal egg was investigated by sedimentation technique. The results in groups of oxyclozanide, oxyclozanide and levamisole combination and triclabendazole indicated that, these anthelmintics were found of high efficacy against fascioliasis through, 100% reduction in fecal egg count in the day 14 post-treatment. However, the results for albendazole and rafoxanid groups yielded lower efficacy levels, with faecal egg count reductions between 75% and 80.58%, over the period 7–84 days post-treatment.

Carneiro *et al.*, (2012) estimated the efficacy of triclabendazole in the treatment of clinical fasciolosis in goats on a property in Jerônimo Monteiro, ES after parasitological diagnosis. Twenty-eight goats with signs of fasciolosis, created a system of semi-confinement property in the flooded area and the presence of the intermediate host of *Fasciola hepatica* were treated with medications based on triclabendazole at a dose of 10mg/kg after confirmation of infection by stool examinations. The triclabendazole at a dose of 10mg/kg body weight was effective in the clinical treatment of fascioliasis in goats.

Geurden *et al.,* (2012) decided the efficacy of a pour-on solution containing moxidectin plus triclabendazole (MOX plus TCBZ) against immature and adult stages of the liver fluke in cattle and compare the efficacy with other commercially available preparations. Efficacy against 8-week old and adult flukes was >99.5%. For 6-week old immature fluke, the efficacy was 98.0% and for 4-week old immature fluke the efficacy was 90.9%. The efficacy was 29.7, 43.4, 53.2 and 99.2% against 4-week, 6-week and 8-week old immature flukes and adult flukes, respectively.

Hassan *et al.,* (2012) stated the efficacy of selective anthelmintics against ecto- and endoparasites of Black Bengal goats and their treatment effects on body-weight gains and hemato-biochemical indices, a field trial was conducted at Pahartali Thana in Chittagong district. A reduction of eggs per gram count was very significant from day 7 (91.3% reduction) through day 28 (100%) with the treatment of ivermectin. The reduction rate of eggs per gram was also significant with the treatment of triclabendazole along with levamisole (75.8%-94.7%).

Roy, (2012) resolved the efficacy of Fasinex® and Levanid® against mixed infection of Fascioliasis and Paramphistomiasis were 40% and 20% respectively. Lezol® was 10% effective against single type of infection with *Paramphistomu sp., Fasciola sp.* and Nematodes. The efficacy of Fasinex®, Levanid® and Nitronex® against fascioliasis only was 87.5%, 85.7% and 10% respectively. These diferences were not statisticaly significant.

Villegas *et al.,* (2012) reported a single administration of triclabendazole (10 mg/kg). Interviews to assess the occurrence of adverse events conducted on treatment day, one week later, and one month after treatment. Further parasitological screenings were performed three months after treatment and again two months later (following a further treatment) in order to evaluate the efficacy of the intervention. Ninety infected children were administered triclabendazole. Adverse events were infrequent and mild. Observed cure rates were 77.8% after one treatment and 97.8% after two treatments, while egg reduction rates ranged between 74% and 90.3% after one treatment and between 84.2% and 99.9% after two treatments. The proportion of high-intensity infections (≥400 EPG) decreased from 7.8% to 1.1% after one treatment and to 0% after two treatments.

Goral *et al.,* (2011) regulated a case of *Fasciola gigantica*-induced biliary obstruction and cholestasis diagnosed and treated via endoscopy and triclabendazole treatment. This is the first case of *Fasciola gigantica* treated via endoscopic biliary extraction during ERCP and drug treatment reported from Turkey.

Martínez-Valladares *et al.,* (2010) investigated the efficacy of triclabendazole (TCBZ) and nitroxynil against a TCBZ-resistant *Fasciola hepatica* strain in a naturally infected sheep flock. The efficacies were measured by the faecal egg count reduction test. The level of *Fasciola hepatica* antigens was tested in faeces; and hematological indices such as total proteins (TP), albumin, hepatic enzymes and total IgG were also studied. The results confirmed the resistance of *F. hepatica* against TCBZ in the flock with an efficacy during the first month post-treatment between 59.4% and 73.8%. In the nitroxynil group, the efficacy during the same period ranged between 81.3% and 86%, likely because the efficacy of this drug against 7- to 9-week-old immature stages is only 50-90%. Anemia was showed in all groups and white blood cells were always higher than the reference

range. The results conclude that nitroxynil could be an alternative in case of TCBZ resistance.

Halferty *et al.,* (2009) evaluated the efficacy of triclabendazole (TCBZ), sulphone (TCBZ . SO_2) and its sulphoxide (TCBZ . SO) against *Fasciola hepatica*. All three compounds induced changes to the surface morphology of the fluke, the changes comprising swelling and blebbing to a greater or lesser extent in different regions of the fluke. TCBZ.SO_2 was more disruptive anteriorly and TCBZ.SO posteriorly. The results demonstrate that both TCBZ and TCBZ . SO_2 are capable of disrupting the fluke *in vitro* and are not the inactive compounds they were assumed to be previously. They may well contribute to drug action *in vivo* as well, indicating that drug action is due to the additive effects of several metabolites, rather than being due to a single active metabolite, namely, TCBZ.SO.

McConville *et al.,* (2009) revealed the efficacy of the experimental fasciolicide, compound alpha (15 mg/kg) against triclabendazole (TCBZ)-resistant and TCBZ-susceptible *Fasciola hepatica* infections of sheep. The results showed that, whilst compound alpha was very active against adult TCBZ-susceptible flukes, producing a 100% reduction in faecal egg counts, it only caused a 62.5% reduction in fluke burden against juvenile flukes. Moreover, compound alpha was not effective against any stage of infection with TCBZ-resistant *Fasciola hepatica* in sheep. However, they reached the bile ducts more quickly and their eggs appeared in the feces >2 weeks earlier.

Mooney *et al.,* (2009) inspected the efficacy of four different anthelmintics against *Fasciola* in a naturally infected hill sheep flock in the west of Ireland. The number of *F. hepatica* eggs per gram of feces was determined using the sedimentation technique and the efficacy of each anthelmintic was calculated in terms of the percentage reduction in egg count at each time point. The results for closantel, oxyclozanide and nitroxynil indicate that these drugs are effective with fecal egg count being reduced by 100% by day 14 post-treatment. These results are highly indicative of triclabendazole resistant *Fasciola hepatica* in sheep on this farm.

Keiser *et al.,* (2005) reviewed of triclabendazole includes an overview of the pharmacodynamics and pharmacokinetics, toxicology and efficacy against the major food-borne trematodes in laboratory animals. Efforts to facilitate broader registration of this drug should go hand-in-hand with research and development on novel drugs against food-borne trematodiasis, better access to improve sanitation, sound health education and the development of new technologies for assuring food safety.

Maqbool *et al.,* (2000) evaluated the comparative efficacy of triclabendazole and albendazole in fascioliasis of goats. The result revealed that triclabendazole was more effective than albendazole in the treatment of fascioliasis in goats.

Moll *et al.,* (2000) find out on a sheep farm died from sub acute and chronic liver fluke disease despite four previous treatments with triclabendazole (TCBZ) in the province of North Holland, Netherlands. The results showed a significant reduction of 99.7, 98.1 and 99.2%, respectively, in fluke egg output at 21 days in all non-TCBZ treated animals. TCBZ treatment produced percentage decreases of 15.3, 4.3 and 36.6%, respectively. These results are highly indicative of the presence of TCBZ-resistant *Fasciola hepatica* in sheep and cattle on this farm.

Okewole *et al.,* (2000) investigated the efficacy of three anthelmintic (Albendazole, triclabendazole and a combination of diamphenethide and rafoxanide) prophylactic treatment against ovine heminthosis were compared in a *Fasciola*-endemic old Dairy Project farm. Hematological, coprological, growth rate, conception rate and other economic production parameter assessments rated rafoxanide plus diamphenethide combination formula albendazole triclabendazole suspension in their chemoprophylactic efficacies. The wider (nematocidal and fasciolicidal) anthelmintic spectra and enhancing pharmacokinetic disposition of both the rafoxanide-diamphenethide combination formula and albendazole gave them an edge over triclabendazole, while the ineffectiveness of the daily prophylactic dose regime of albendazole on the immature flukes lowered its overall efficacy when matched with the combination formula.

Martínez-Moreno *et al.,* (1997) estimated the efficacy of triclabendazole against all stages (early immature, late immature and mature) of *Fasciola hepatica* infected goats. The

influence of triclabendazole treatment on the path-physiology of the disease, in terms of hematological parameters and serum enzyme levels, and in the dynamics of production of specific antibodies to excretory/secretory products (ESP) of *F. hepatica* was also examined. Treatment with triclabendazole, by eliminating most of these flukes, largely reduced hematological alterations. Serum levels of the enzymes aspartate aminotransferase, lactate dehydrogenase and gamma-glutathione transferase reflected hepatic damage during goat fasciolosis. Early treatment prevents the development of both parenchyma and bile ducts lesions.

Ghimir and Karki, (1996) examined fecal samples of buffalo and revealed a high prevalence of fasciolosis in the adult buffalo population of rice belt area (82□94%) of Nanglebhare and Lapsiphedi VDCs of Kathmandu district. An evaluation showed that the efficacy of available anthelmintics was in the order of Rafoxamide (67%), Oxyclozanide (65%), Triclabendazole (52%) and Abendazole (48%) against Fasciolosis in buffaloes

Jemli *et al*., (1993) studied the experimental infestation of twenty-six lambs performed using 200 *Fasciola hepatica* metacercaria. Biochemical and hematological study of the lambs showed an important increase in the number of eosinophils and an increase in the enzymatic activities of GLDH and GGT in the blood. Treatment with a fasciolicide, triclabendazole, is effective in normalizing blood parameters within a few weeks.

Mahato and Rai, (1992) decided the efficacy of triclabendazole against *Fasciola gigantica* in naturally infected buffaloes. The results indicated that the drug was not effective against *Fasciola gigantica* infection in buffaloes at the dose rate of 12 mg/kg body weight gain orally. When the dose rate was increased to 24 mg/kg body weight, the drug was found to 100 percent effective.

Maqbool *et al.,* (1992) evaluated the efficacy of rafoxanide, albendazole and triclabendazole in sheep naturally infected with *Fasciola gigantica* and *Fasciola hepatica*. The results indicated that triclabendazole is virtually 100% effective against fascioliasis whereas the efficacy of rafoxanide and albendazole was 97.1 and 95.7%, respectively.

Richards *et al.,* (1990) investigated a total number of 274 cattle and used to assess the efficacies of triclabendazole, albendazole, clorsulon, nitroxynil, oxyclozanide and rafoxanide against *Fasciola hepatica*. Clorsulon, nitroxynil and rafoxanide administered at recommended dose rates showed negligible activity against these stages of the parasite. The mean efficacies of triclabendazole at 12 mg/kg were 87.5 and 95.7%, respectively. The efficacies of triclabendazole, clorsulon, nitroxynil and rafoxanide against *Fasciola* spp were 100, 99.0, 99.1 and 90.1% respectively. Albendazole and oxyclozanide showed poor efficacy against *Fasciola*.

2.12 Efficacy of liver tonics

Debnath *et al.,* (2014) estimated the effect of polyherbal liver tonic Xlivpro premix (M/S Ayurvet Limited, India) on growth and performance in broilers. Statistical analysis of results showed a significant (P>0.05) increase in live weight (g) and live weight gain (g) in herbal liver tonic Xlivpro premix supplemented in comparison to control group. Plasma concentration of total protein (g/dl) and enzymes (AST/ALT) (U/L) was found to be non-significantly (P>0.05) different between the both groups, though numerically high in Xlivpro premix supplemented group. The trial investigation revealed better results in herbal liver tonic Xlivpro premix supplemented group in comparison to the untreated group.

Harmon *et al.,* (2014) evaluated the efficacy of ferrous carbonate and ferrous sulfate in purified and unsupplemented purified diets in maintaining normal hemoglobin values of swine. Ferrous carbonate was ineffective as oral hematinics supporting no higher hemoglobin than found in pigs receiving an unsupplemented purified diet. None of these levels supported hemoglobin values greater than the unsupplemented diets. These pigs had significantly greater hemoglobin values after 8 to 11 days on the new regime than the pigs remaining on ferrous carbonate. The current study confirms that ferrous carbonate is ineffective as oral hematinics even at levels well in excess of the suggested requirement value.

Khorramshahi *et al.,* (2014) determined the effect of *Cynara scolymus L.* (artichoke) on protection against carbon tetrachloride (CCl4) induced hepatotoxicity in Japanese quail.

Results showed that artichoke powder significantly decreased concentration of ALT in blood serum, while CCl4 significantly increased concentration of AST ($P<0.05$) in serum. Moreover, the interaction effects of artichoke and CCl4 showed that artichoke significantly decreased serum concentration of ALP ($P<0.05$). Results of liver histopathology showed that CCl4 damaged liver cells by causing formation of bubble-like structures in the liver tissue, shrinking of the sinusoid space and inflammation in parts of the liver parenchyma as well as abnormality of the hepatic artery and bile duct in liver tissue. Therefore, results of this study demonstrated that application of *Cynara scolymus L.* powder had a significant protective effect from serious hepatocellular injury in birds from hepatotoxicity.

Sahoo *et al.,* (2012) regulated the efficacy of herbal formulation Yakrifit (Ayurvet Limited, India) on growth rate of post-weaned twenty one growing Ghungroo piglets, National Research Centre. The trend of body weight indicated that initially there was no difference between body weight of various groups, however after 2 weeks of treatment with Yakrifit liquid and bolus was significantly ($P<0.05$) better than the control group. Overall, the growth rate was significantly higher in all the treatment groups.

Buraimoh *et al.,* (2011) evaluated the hepatoprotective effect of ethanolic leave extract of *Moringo oleifera* on the histology of the liver of wistar rats. Twelve (12) h after administration, the rats were sacrificed and the liver was fixed immediately in Formalin. The liver tissues was processed and stained in Hematoxylin and Eosin (H&E). The histological observations showed that the leave extract of *Moringa oleifera* was hepatoprotective.

Chaudhary *et al.,* (2011) observed the methanol extracts of *M. indica* studied for hepatoprotective activity against albino rats with liver damage induced by carbon tetrachloride (CCl4). It was found that the methanol extract of *M. indica* at a dose of 300 mg/kg body weight exhibited moderate protective effect by lowering the serum levels of Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum bilirubin and Serum alkaline phosphate (SALP) to a significant extent.

Das *et al.,* (2011) inspected a total of 1200 chicks of day old age (Cobb 400) randomly divided into three groups with one control and two treatments having 400 birds in each

group with 4 replicates of 100 broilers each. The control (T0) was offered feed without liver tonic whereas, other two groups were supplemented with chemical liver tonic (Toxol, Vesper) 0.500 g/kg diet (Tl), and herbal liver tonic (Hepaceaf, Concept) 0.500 g/kg diet (T2) from day old age till the end of trial (42 days). It was evident that there was significant decrease in liver marker enzymes aspartate aminotransferase and alanine aminotransaminase (SGOT and SGPT), total triglycerides and cholesterol in treated groups than control ($P<0.05$) indicating better health of the birds.

Sadashiv and Krishna, (2011) estimated the efficacy of the powder of *Achyranthes Aspera L* leaves on CCl4 induced liver dysfunction in Wistar rats. The examination of regeneration of liver cells is well supported by liver and tissue biochemistry. OECD guidelines were strictly followed during acute toxicity and efficacy study. The leaves powder of this plant is compared with Silymarin by standard protocol and is found to have better hepatoprotective properties, thus it may act even in humans as a potent liver tonic.

Sonkusale *et al.,* (2011) determined a total of 75 Cobb day old broilers divided into three equal groups (n =25) whether the supplementation of herbal products Superliv liq. and Repchol (supplied by M/S Ayurvet Limited, Baddi, India) efficacious in treatment of liver and kidney due to CCl4. A significant ($p<0.01$) decrease in feed conversion ratio was observed in group C as compared to group B. A significant ($p<0.01$) decrease in level of cholesterol, triglycerides, HDL, VLDL, creatinine, liver enzymes (SGOT and SGPT) and increase in serum protein level was recorded in group C as compared to group B on 28th and 42nd day of experiment, which was in confirmation with the values of negative control group A, falling into normal range. Gross and histopathology of liver and kidney revealed that there was mild congestion and negligible degenerative changes in group C in contrast to severe changes of FLKS syndrome in group B.

Hadiya *et al.,* (2010) stated the efficacy of herbal liver tonic formulations on growth and body weight gain in 48 Jaffrabadi buffalo calves. A significant ($P<0.05$) increase in body weight gain in groups supplemented with herbal formulations Yakrifit (M/s Ayurvet Ltd. Baddi, India) @ 1 bolus/calf/day, was observed in comparision to untreated control. Supplementation of herbal liver tonic products improves liver function, feed assimilation and digestibility of ration ultimately leading to gain in body weight.

Chaudhari *et al.,* (2009) revealed the hepatoprotective activity of hydroalcoholic extract of *Momordica charantia* Linn.leaves against carbon tetrachloride induced hepatotoxicity in albino wistar rats. Hydroalcoholic extract of *Momordica charantia* leaves (100mg/kg and 200mg/kg p.o.). The hepatoprotective activity of hydroalcoholic extract of *Momordica charantia* leaves were evaluated by estimation of SGOT, SGPT, ALP and total bilirubin. In the hydroalcoholic extract of *Momordica charantia* leaves treated animals, the toxic effect of carbon tetrachloride was controlled significantly by restoration of the increased levels of SGOT, SGPT, ALP and total bilirubin as compare to the toxicant control. The hydroalcoholic extract of leaves of *Momordica Charantia* showed significant hepatoprotective activity.

Sharma *et al.,* (2008) examined the efficacy of herbal liver tonic and growth promoter products on overall growth, performance and carcass quality parameters in 180 day-old broiler chicks. A significant ($P<0.05$) improvement in growth performance traits was observed in treated groups. The results also indicated significant ($P<0.05$) and notable improvement in livability, overall carcass yield & carcass quality parameters.

Singh and Singh, (2009) evaluated the efficacy of polyherbal growth promoter and liver tonic product *Superliv* concentrate premix simultaneously on performance of commercial broiler chicks. It was observed that the mean total body weight gain and feed conversion ratio (FCR) were significantly ($P=0.05$) different in treated group compared to control. Percent protein, energy and ether extract retention were significantly higher in group supplemented with herbal liver tonic product than control. It may be concluded that supplementation of Superliv concentrate of feed is beneficial for commercial broiler rearing and optimum feed utilization

Sharmin *et al.,* (2004) studied the effects of hematinics on body weight and certain hematological values in 9 female Black Bengal goats. The result showed that body weight gain of hematinics treated groups significantly increased than untreated control and hematological examination showed increased hemoglobin, packed cell volume, total erythrocytes count, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in the treated groups.

Ravikumar and Bhagawat (2002) determined the present field trial indicate that the use of Liv.52 Protec liquid in broiler as a growth promoter improves profitability through better-feed efficiency, faster growth rate and reduced mortality.

CHAPTER-III

MATERIALS AND METHODS

3.1 Study area

This study was conducted in Sylhet region of Bangladesh. Sylhet is located in North-East part of Bangladesh and between $24^0$30' North latitude and $91^0$40’ East longitudes. The division has an area of 3490.40 square kilometers. More than three quarter consists of mostly tea garden, flowing water within the tea garden, lakes, hilly, water logged low lying areas and haor (Figure 3.1). Different areas of Sylhet region was selected based on irrigated agro-ecological zones such as Sylhet Sadar, Balaganj, Beanibazar, Biswanath and Jaintapur upazilla (Figure 3.2).

Figure 3.1 Map of Bangladesh indicating the location of study area (star mark)

Figure 3.2 Map of Sylhet indicating sampling sites at different upazilla (star mark)

3.2 Study period

The research work was conducted for the two years six months from July 2012 to December 2014. Although three seasons of Bangladesh are prominent such as summer, rainy and winter. The experiment was done during these three seasons.

3.3 Research laboratories

The study was carried out in the Laboratory under the Department of Parasitology, Anatomy and Histology, Pharmacology and Toxicology, Faculty of Veterinary and Animal Science, Sylhet Agricultural University, Sylhet-3100, Bangladesh.

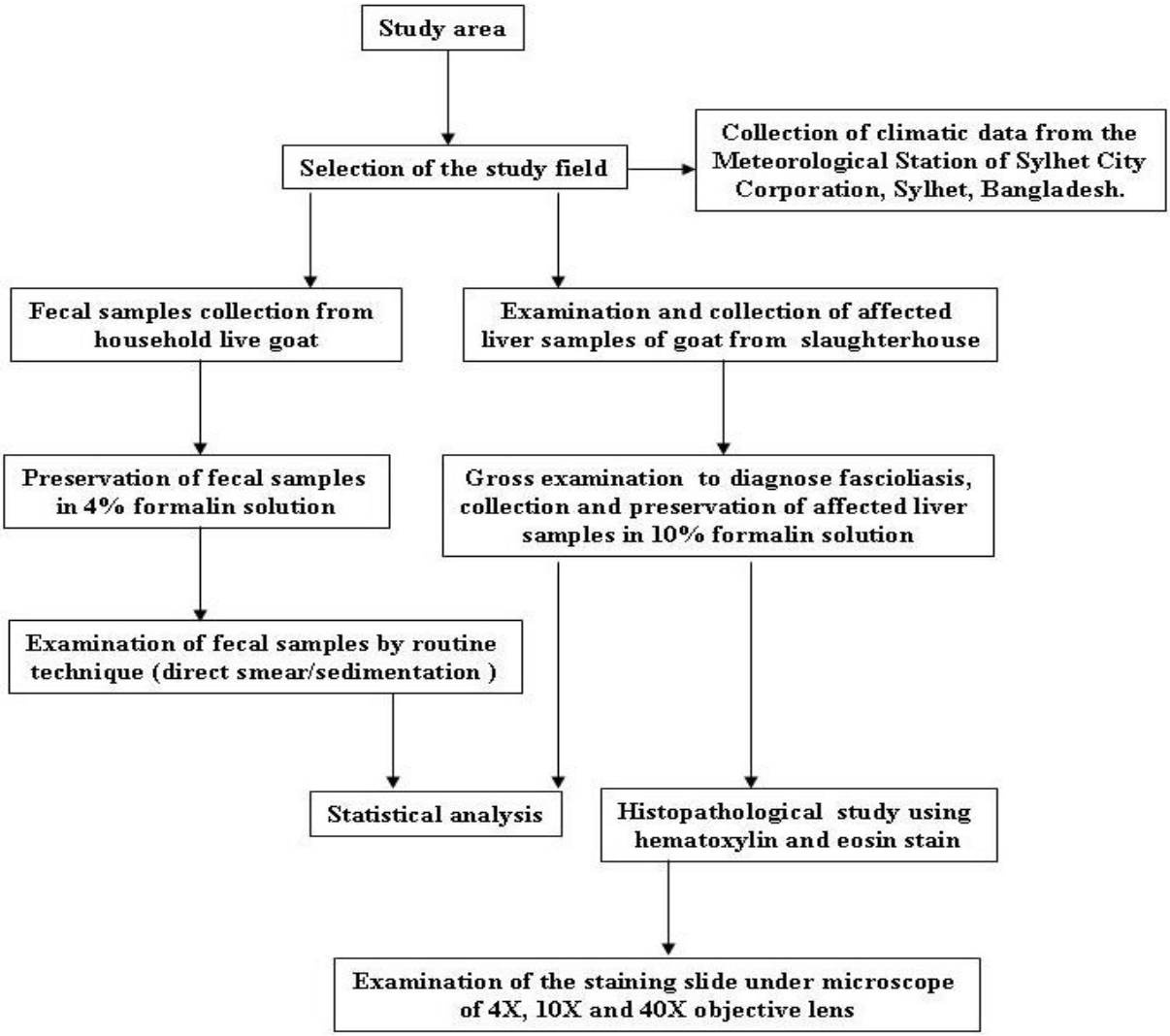
3.4 Experimental samples and animals

Fecal, blood and liver samples were collected directly household live and slaughtered goats from farmers and slaughterhouse respectively. The goats of various local breeds, sexes and age groups were selected randomly from small holder farmers. The age of the

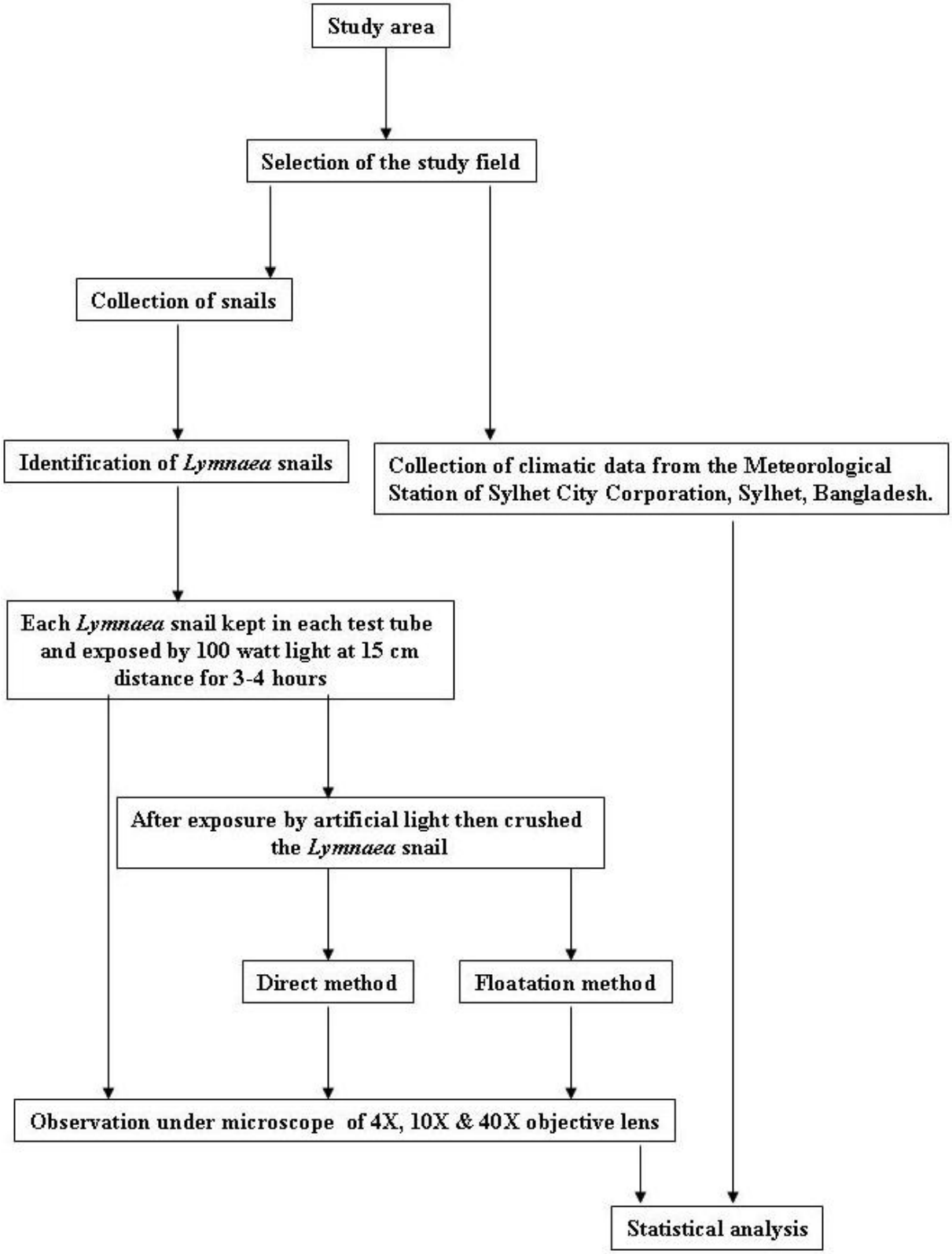
goats were determined by dentition. Goats were divided into two age groups such as young (<1.5 years) and adult (≥1.5 years) age. The sexes of the goats were recorded by examining presence of penis or uterus.

3.5 Experimental layout

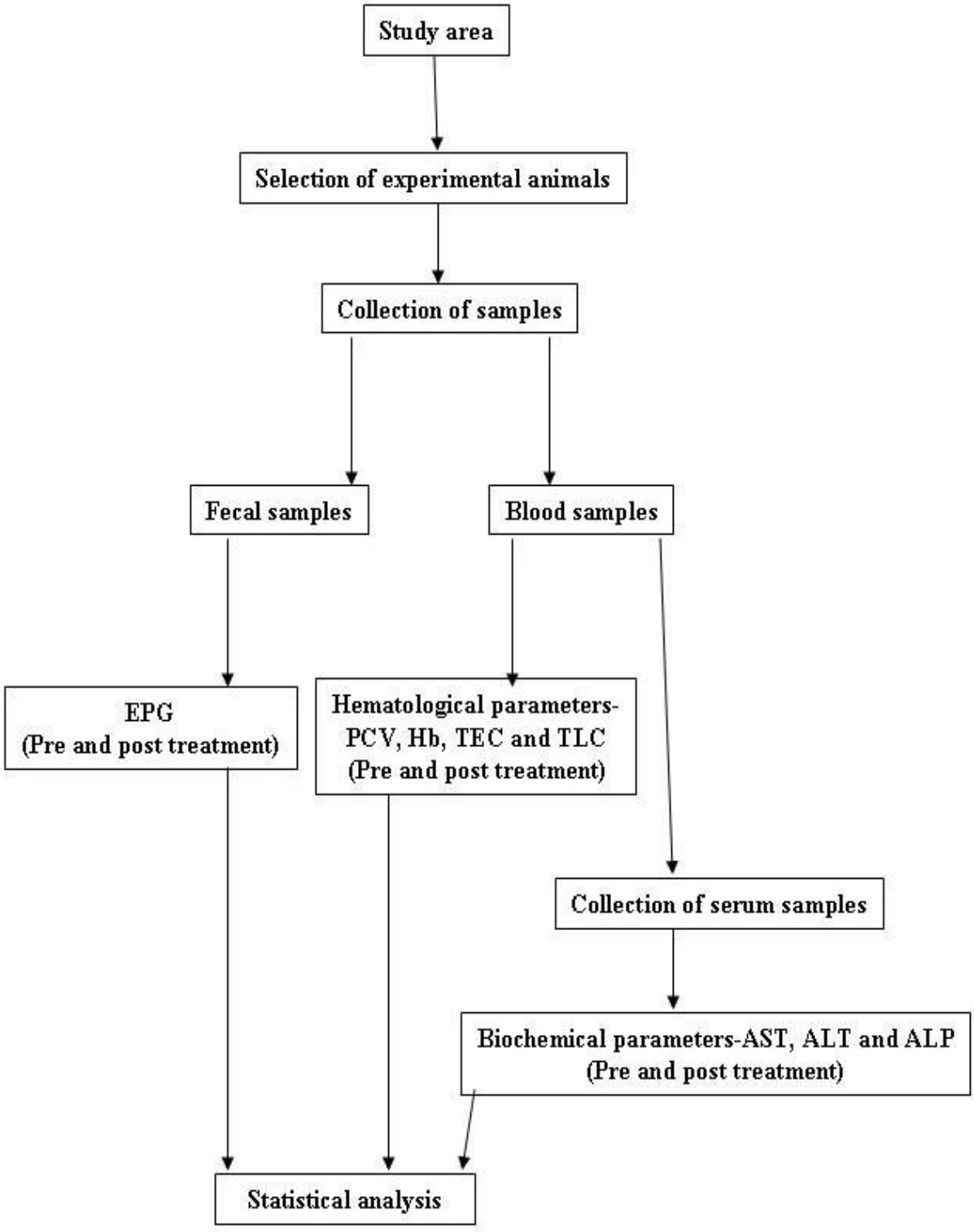
3.5.1 Epidemiological and pathological studies of fascioliasis in goats



3.5.2 Effect of climatic factors on prevalence of infection status of the developmental stages of *Fasciola gigantica* in *snails*



3.5.3 Efficacy of different types of liver tonic and anthelmintic on pathology of fascioliasis in goats



3.6 Epidemiological studies of fascioliasis in goats

3.6.1 Study period and climatic condition

The experiment was conducted from July 2012 to June 2013. The average maximum and minimum temperatures were 23^{o}C and 7^{o}C respectively. The annual average rainfall was 3334 mm and humidity was 80% (MS, 2013).

3.6.2 Collection of fecal samples

During the study period, a total of 1288 household live goat fecal samples were collected from five different upazillas of Sylhet region. The fresh fecal samples were collected from the rectum of goats for feces examinations. Suitable containers like screw-capped wide mouthed glass bottles were used for collection of fecal samples and transported to laboratory for further examination. Sometimes plastic bottles and polythene bags were also used. These were made air tight as much as possible in order to prevent the rate of development and hatching of eggs. The samples collected from the remote areas were preserved in 4% formalin and then transported to the laboratory. 20 gm of fecal samples were collected from each goat and kept at 4 ^{0}C before further examination (Figure 3.3). Identification was performed based on morphology of *Fasciola gigantica* egg (Soulsby, 1986) (Figure 3.3).

3.6.3 Examination of fecal samples

3.6.3.1 Physical examination

The color, consistency, presence of blood, mucous, dead worms and others were looked before proper examination of fecal sample. The physical examination was done by naked eyes.

3.6.3.2 Microscopic examination

Examination of feces for *Fasciola gigantica* eggs may vary from a simple direct smear to more complex methods involving centrifugation and McMaster techniques (Figure 3.3).

3.6.3.2.1 Examination by direct smear method

This method was found useful only in the cases of heavy infections, in this method a small portion of fecal matter was directly placed on a grease free slide by a glass rod and 1-2 drop of saline was added to make a uniform suspension and then a cover slip was placed onto the smear. The slide was examined under low power microscope (10X) (Figure 3.3).

3.6.3.2.2 Examination by indirect method

3.6.3.2.2.1 Concentration methods

The concentration method was done in order to separate the parasitic objects from the bulk of the material in the specimen. For this purpose only the sedimentation method was employed.

3.6.3.2.2.2 Concentration by centrifugation

This method was found reliable for all types of parasitic eggs specially trematodes. 5 to 6 gm of faecal samples were taken and thoroughly mixed with 15 to 20 ml of water, the emulsion was strained through a sieve to remove all coarse particles. This filtrate was poured into a centrifuge tube. The centrifugation was done at the speed of 1500 rpm for 5 minutes. After centrifugation the supernatant was discarded and the sediment was examined microscopically (10X) by placing a drop of sediment on slide and covered with a cover slip.

3.6.4 Examination of slaughtered goats in slaughterhouse

During the study year, 2000 goats at slaughterhouses were examined to record the prevalence of the disease in a systematic survey in five different upazilla of Sylhet region. Post-mortem examinations of slaughtered goats were carried out and livers were checked for the presence of liver flukes. *Fasciola gigantica* was identified based on morphology (Soulsby, 1986) (Figure 3.4).

Figure A. Collected goat fecal samples

Figure B. Slide preparation for direct smear method.

Figure C. Slide prepared for microscopic examination.

Figure D. Examination of slide under microscope

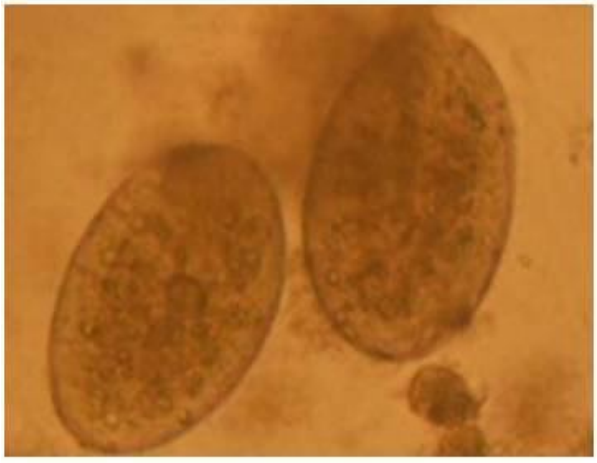


Figure E. Egg of *Fasciola gigantica* 10X

Figure F. Egg of *Fasciola gigantica* 40X

Figure 3.3(A-F) Examination of goat fecal samples by direct smear method

Figure A. Carcass of goats in slaughterhouse

Figure B. Normal goat livers

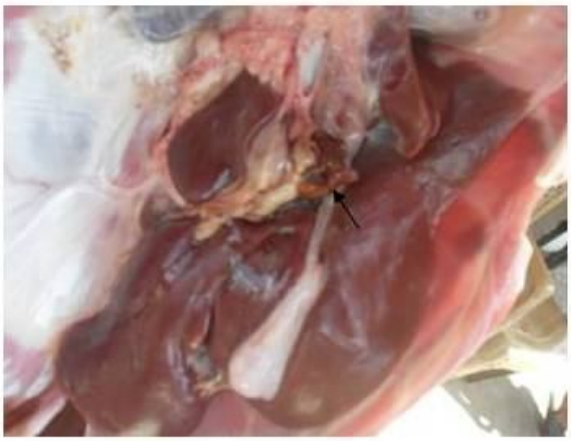


Figure C. *Fasciola* gigantica infected goat liver (indicated by arrow)

Figure 3.4(A-C) Examination of slaughtered goat s in slaughterhouse.

3.7. Effects of climatic factors on prevalence of infection status of the developmental stages of *Fasciola gigantica* in *Lymnaea snails* (*Lymnaea auricularia* var *rufescens*)

3.7.1 Duration of study

The research was conducted from the period of March 2013 to February 2014.

3.7.2 Temperature, rainfall and humidity

The average maximum and minimum temperatures were 34^{o}C and 13^{o}C, respectively. The annual average rainfall was 3854.2 mm and humidity was 68.92% (SM, 2014).

3.7.3 Collection of snails

A total of 1865 *Lymnaea* snails were collected randomly from five different upazilla in Sylhet region of Bangladesh (Figure 3.5).

3.7.4 Identification and examination of *Lymnaea* snails

The snails were collected from five different upazilla in Sylhet region with hand-picked, brought to the laboratory and washed thoroughly, cleaned from mud, debris and ciliates. *Lymnaea* snails were identified according to the shell morphology (Figure 3.5) as described by El-Gindy, (1960) and Malek, (1984). After collection, *L.* snails were kept individually in test tubes containing distilled water and exposed to a 100-W light bulb at a distance of 15cm for 4-6 hours for cercarial shedding (Faltynkova *et al.*, 2008). The snails which did not shed cercariae on the first exposure were re-exposed on the second day. The water of these tubes was examined against the light, with naked eye or with a hand lens, every hour interval during the day and on the following. Cercariae were characterized by morphological and biometrical examinations as described by Frandsen and Christensen, (1984) and Dawes, (1968). The infected snails were segregated from the rest as described by El-Gindy, (1960) and Malek, (1984). The snails those could not shed cercaria under the artificial light; were crushed and examined their internal contents for the presence of other developmental stages of *Fasciola gigantica* (Shaikh and Rahman, 1968). The types of cercariae were identified by Cable, (1950).

Figure -A

Figure- B

Figure- C

Figure- D

Figure- E

Figure- F

Figure 3.5 *Lymnaea auricularia* snail, the intermediate host of *Fasciola gigantica*

3.8 Collection of parasite and liver samples for pathological study

3.8.1 Collection of liver samples

During the study period, a number of 2000 slaughtered goats at slaughterhouses in five different upazilla of Sylhet regions were examined to record the prevalence of the disease in a systematic survey. Post-mortem examinations of slaughtered goats were carried out and livers with gall bladder were closely examined at the laboratory of parasitology, SAU, Sylhet for gross pathology and collection of liver samples (Figure 3.6B).

3.8.2 Collection of parasite from infected liver

During collection of parasite, the infected goats liver with gall bladders were carried out in the laboratory of parasitology, SAU, Sylhet. The liver and gallbladder were subjected to thorough investigation and collection of parasites following the procedure of Ross (1967). *F. gigantica* was identified based on the morphology (Soulsby, 1986) (Figure 3.6A).

Figure A: Collection of *Fasciola gigantica* from infected goat liver

Figure B: Collection and preservation of infected liver samples.

Figure 3.6(A-B): Collection of parasites and preservation of infected liver

3.8.3 Pathological examination

3.8.3.1 Gross examination

The gross examination was carried out in the laboratory of parasitology, SAU, Sylhet. The gross changes were recorded carefully and preserved at 10% formalin solution in the laboratory for histopathological study.

3.8.3.2 Histological examination

The histopathological examination was carried out in the laboratory of Anatomy and Histology, SAU. The well fixed liver tissues of goats having *Fasciola gigantica* infection was processed, embedded in paraffin wax, cut in appropriate thickness and stained with hematoxylin and eosin as per standard methods described by Luna, (1968) (Figure 3.7).

3.8.3.2.1 Staining of liver sample (hematoxylin and eosin)

Materials required

1. Paraffin
2. Hand gloves
3. Microtome machine
4. Timer
5. Fume hood
6. Absorbent liner
7. Water bath with drain
8. Pasteur pipette
9. Slides with mounted tissues
10. Cover slips
11. Mounting medium-DPX/CBS
12. Staining jars
13. Alcohol (ethanol)
14. Clearing agent
15. Eosin stain
16. Hematoxylin stain
17. Xylene
18. Cotton and tissue papers

I) Fixation: The infected liver tissues were fixed in 10% formalin solution for 7-10 days.

II) Processing of the liver tissue: After fixation, the liver tissues were cut into cubic sizes and tagged with thread then rinsed by running tap water over night (Figure 3.7).

III) Dehydration and clearing: After rinsing, each tissue sample was dehydrated by submerging in a series of concentrated ethanol (70%, 80%, 90%, 95%, 98%, and 100%). The tissues were kept for 3 hours in each grade of ethyl alcohol and then transferred to a hydrophobic clearing agent, xylene for two changes to remove the ethyl alcohol. In both cases the tissues were kept for 40 minutes.

IV) Embedding: The tissues were infiltrated through two changes of liquid paraffin in the oven taking 2 hours in each step to remove the xylene from tissues. When the tissues were dehydrated, cleared and infiltrated, they were placed in liquid paraffin for embedding and allowed for hardening.

V) Sectioning: The paraffin blocks containing the tissue were sectioned at 6 micro meter thickness using a microtome (Microm GmbH, type HM 325, Germany) and the sections were floated on a water bath at 45º C for flatten out. Then floated sections were picked up carefully on glass slides, smeared with egg albumin and dried on the slide warmer. The good sections were chosen to ensure the different position of tissue about every 21st section. Four tissue sections on each slide were taken from liver for histological analyses (Figure 3.7).

VI) Staining procedure

a) Deparaffinization: The tissue sections were deparaffinized by two changes in xylene; 15 minutes in each case.

b) Rehydration: Then tissue sections were transferred through descending grades of ethanol (100%, 95%, 90%, 80%, and 70%) for 3 minutes in each.

c) Rinsing: Rinsed in distilled water (5 minutes)

d) Staining: Stained in hematoxylin (6 minutes)

e) Rinsing: Rinsed in running tap water (20 minutes)

f) Staining: Counter stained in eosin (15 seconds)

g) Dehydration: Dehydration is usually carried out by passing material through increasing strengths of alcohol. Dehydration is the process of replacing water specimen

with an anhydrous solution. The stained specimens were passed through ascending grades of ethanol (100%, 95%, 90%, 80%, and 70%) for 3 minutes in each. However the specimens were given two washes in 100% alcohol. The time required in the different grades of ethyl alcohols for dehydration depends upon the specimens and the permeability of its integument.

h) Dealcoholization and Clearing: All traces of water have been removed from the specimens; it is then transferred to a clearing reagent (xylene) which renders it transparent and miscible with a resinous mountant. After complete dehydration, the slides were transferred to xylene for clearing. Two washes in xylene were given to ensure complete dealcoholization.

VII) Mounting: The specimens cleared in xylene were mounted in Canada balsam or DPX (Dextrin Plasticized Xylene). The specimens were properly placed on the slide and a sufficient amount of mountant was added. Then a proper sized cover-glass was lowered on the specimen to avoid displacement of specimen or formation of air bubble. After mounting, cover-glass on the slides was allowed to dry thoroughly before final cleaning. The excess of the mountant was removed from the slide with a razor blade. The remaining resin film was wiped away with a cloth moistened in xylol.

VIII) Labelling: Slides were labelled on the left end with all the information of the material.

IX) Storing Slides: The prepared slides were stored in wooden boxes, protected from dust, dirt and sunlight. If stored in open boxes or trays, the stains may fade very rapidly. The slides were kept flat during storage by standing the boxes on end to avoid the gradual drifting of the material through the mountant.

X) Photomicrography The slides were carefully studied and various pathological lesions were examined. Photographs of whole mounts or important parts were taken with the help of Digital Olympus Camera (4X, 10X and 40X).

Figure A. Processing of the *Fasciola gigantica* infected goat liver

Figure B. Blocking of liver tissue

Figure C. Sectioning of the blocking liver tissue by microtome

Figure D. Withdrawal of tissue from hot water bath

Figure E. Drying of the tissue contain slides

Figure F. Examination of slide under microscope

Figure 3.7 (A-F) Processing of liver tissue for histopathology study

3.9 Efficacy of different types of liver tonic and anthelmintic on pathology of fascioliasis in goats

3.9.1 Experimental place

Among 5 upazilla, Biswanath was selected as experimental place. The highest number of goat population, high prevalence of fascioliasis (*Fasciola gigantica* infection) of household live and slaughterhouse goats (previously described), good co-operation of the Veterinary Surgeon and easy communication were the criteria behind the selection. More than three quarter of the upazilla consists of mostly high plain, water logged and low lying areas. In addition, the semi-scavenging systems rearing of goats were the most popular with the aim of having kids and meat as a source of subsistent family income. The semi-scavenging goats were usually feed on the grasses of low lying surface, near the flowing water lake, roadside land and the leaves of jackfruits, neem leaves; mango leaves with a minimum irregular supplementation of concentrated cereal feed (wheat bran, rice polish, boiled rice, soya bean meal and pea bran etc).

3.9.2 Duration of study

The experiment was conducted from the period of May 2014 to August 2014.

3.9.3 Selection and management of the experimental goats

A total of 7 household farmers, having 35 goats, were selected randomly from Biswanath upazilla of Sylhet region. Each farm consisted of 5 goats, with an individual age of 2.0-2.5 years old were included in the sampling frame. All goats belonging to the 7 household farms were brought under parasitic screening. Five to ten grams of feces per goat was taken aseptically from the rectum. The direct smear methods described previously (Soulsby, 1986), followed by the modified McMaster counting technique described earlier (Kassai, 1999), were used to screen *Fasciola gigantica* eggs in this study.The goats infected with at least 450 *Fasciola gigantica* eggs per gram (EPG) were considered for the selective drug trial.

3.9.4 Grouping of experimental goats

The goats were randomly divided into 7 groups using a random number table; each consisted of 5 goats. The sample size for each group was statistically sufficient.

Specific treatment groups were selected by tossing a coin. The groups were designated as T_0, T_i, and T_1 to T_5.

3.9.5 Experimental trials

The group T_0 (negative control-non infected) and T_i (positive control-infected) were treated only with PBS. T_1 was treated with anthelmintic, T_2 and T_3 group treated only with liver tonic for 3 successive days, T_4 and T_5 group treated with anthelmintic along with liver tonic for 3 successive days. All experimental animals were infected with *Fasciola gigantica* except group T_0. The details of either liver tonic or anthelmintic or anthelmintic along with liver tonic treatments schedules are presented in Table 3.2.

Table 3.1 Composition of liver tonics

Name of component	**Liver tonics**	
	Liva-vit	**Hepamin forte**
Vitamin B_1	500mg	2.5 mg
Vitamin B_2	200 mg	-
Vitamin B_6	200 mg	-
Vitamin B_{12}	1000 mcg	0.75 mcg
Vitamin K_3	65 mcg	-
Folic acid	30 mg	-
Nicotinamide	6000 mg	-
Liver extract	20 mg	125 mg
Pantothenic acid	1100 mg	-
Niocinone	-	28 mg
dl-Panthenol	-	2.5 mg
Inositol	100 mg	35 mg
Choline chloride	5000 mg	2000 mg
Yeast extract	-	80 mg
dl-Methionine	5000 mg	-

Table 3.2 Infection status and treatment schedule of naturally infected experimental goats

Group of goats	Status of infection	Treatment status	Anthelmintic /Liver tonic	Company name	Doses	R/A
T_0	Non infected	PBS (Non infected control)	-	-	PBS	Oral
T_i	Infected-control	PBS (Infected control)	-	-	PBS	Oral
T_1	Infected	Triclabendazole	Fasinex	Novartis Bangladesh Ltd.	12 mg/kg	Oral
T_2	Infected	Liver tonic (for successive 3days)	Hepamin forte	Novartis Bangladesh Ltd.	10 ml/goat	Oral
T_3	Infected	Liver tonic (for successive 3days)	Liva-vit	Square Pharmaceuticals Ltd.	5 ml/goat	Oral
T_4	Infected	Triclabendazole + Liver tonic-T_2 (for successive 3days)	Fasinex + Hepamin forte	Novartis Bangladesh Ltd.	12 mg/kg + 10 ml/goat	Oral
T_5	Infected	Triclabendazole + Liver tonic T_3 (for successive 3days)	Fasinex + Liva-vit	Novartis Bangladesh Ltd. Square Pharmaceuticals Ltd.	12mg/kg + 5ml/goat	Oral

3.9.6 Collection of samples

Fecal samples of all goats were examined as per the protocol in order to assess the parasitic load on day 0 (before treatment) and days 7, 14, 21 and 28 (during the trial). Fecal and blood samples were taken from each goat following the same schedule (Table 3.3 and Figure 3.8 A-B). Five ml of blood sample was collected aseptically from the live goats from jugular vein with the help of syringe, of which 3 ml of blood was transferred to a sterile vial containing EDTA (1mg/ml of blood) to estimate routine blood parameters procedure followed by Sharma and Singh (2000). Remaining 2 ml of blood was transferred to vacutainer tube for serum separation which was carried to laboratory in an ice-cabinet for further analysis (Figure 3.9 A-B).

Table 3.3 Schedule for the collection of feces and blood from experimental goats

Sampling occasion	Fecal samples	Blood samples
Day 0	20 gm	5 ml
Day 7	20 gm	5 ml
Day 14	20 gm	5 ml
Day 21	20 gm	5 ml
Day 28	20 gm	5 ml

3.9.7 Fecal egg count

The fecal egg counts were of great importance in experimental and diagnostic work in which the comparison of counts in various goats provides great information on worm burden. Animals having higher number of eggs per gram (EPG) of faeces were selected for hematological and biochemical parameters. McMaster's egg counting technique was employed for counting of eggs (Figure 3.8).

McMaster's egg counting: This was done by modified McMaster Counting Chamber as described by Urquhart *et al*. (1996). This technique is useful in the determination of number of eggs per gram (EPG) of faeces. In this technique 3 gm of faecal samples were taken and 42 ml of floatation solution was added to the sample and thoroughly mixed so that it forms a homogenous mixture. The solution was then transferred through a sieve in order to remove the coarse particles and filled in test tube and centrifuged at 2000 rpm for 2 minutes. The supernatant was poured off and the

sediment was agitated and again the tube was filled to the previous level with floatation solution. The upper fluid portion was poured in both chambers of McMaster slide with the help of pipette and no fluid was left in the pipette, as the eggs would rise quickly in the floatation fluid. Both chamber of slide was examined and numbers of eggs were multiplied by 100 to determine eggs per gram (EPG).

Figure A. Experimental goats

Figure B. Fecal samples collected from goats

Figure C. Preparation of McMaster slide for examination

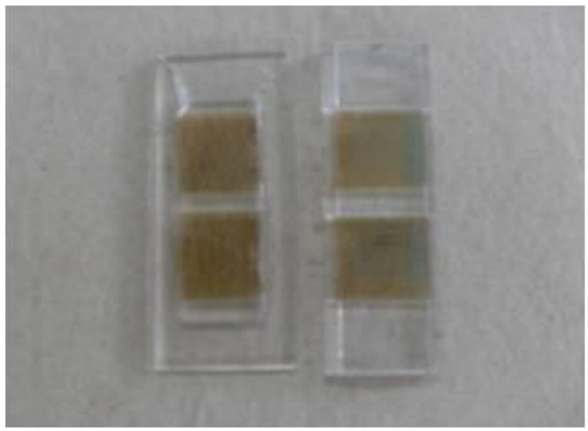


Figure D. Fecal sample in McMaster slides

Figure E. Examination of sample under microscope

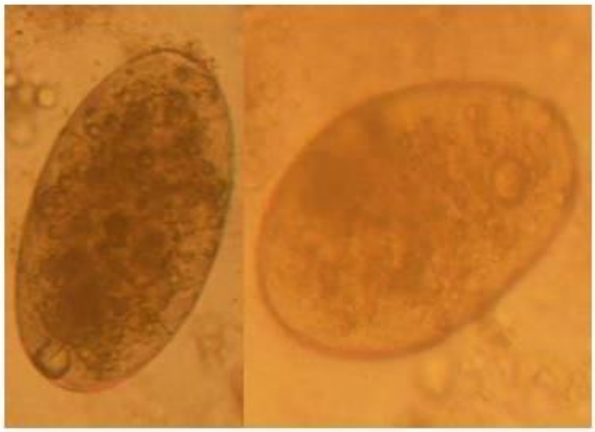


Figure F. Eggs of *Fasciola gigantica* (10X)

Figure 3.8(A-F) Collection and examination of fecal samples for detection of eggs of *Fasciola gigantica*

3.9.8 Determination of hematological parameters

A portion of the blood from each sample was used to evaluate routine hematological parameters. The packed cell volume (PCV) was determined using microhaematocrit centrifuge technique. Hemoglobin concentration (Hb) was determined using cyanomethemoglobin methods (Coles, 1980). Total leukocyte count was conducted on weekly basis using haemocytometric method and the total erythrocytes were counted similarly monitored (Figure 3.9 and Figure 3.10).

3.9.8.1 Hematocrit or Packed cell volume (PCV):

This was obtained by centrifuging blood (containing 5mg/ml EDTA) in a graduated tube until corpuscles were packed down to a constant volume. The volume of packed cell was then expressed as a percentage of the original volume of blood. With the aid of capillary pipette a Wintrobe's haematocrit tube was filled to the 100 mark with the anticoagulated blood and centrifuged for 5-10 min at 7,000 rpm. As the original column of blood in the tube is 100 mm long, the volume of packed cell is read directly as percentage. The analysis was done according to England and Walford (1972) (Figure 3.9).

3.9.8.2 Estimation of hemoglobin concentration (Hb): The hemoglobin was estimated by Cyanomethemoglobin method (ICSH, 1973). In this method, Ferricyanide present in the Drabkins solution converts ferrous (Fe^{2+}) iron of haemoglobin to the ferric (Fe^{3+}) state to form methemoglobin. Methemoglobin reacts with potassium cyanide to form Cyanomethemoglobin. The color developed was measured spectrophotometrically at 540 nm (Wharton and McCarty, 1972; Van Assendelf, 1974) (Figure 3.10).

Drabkin's solution used was prepared by mixing the following reagents in the given proportion:

Sodium bicarbonate: 1.0 gm.

Potassium cyanide: 0.05 gm.

Potassium ferricyanide: 0.2 gm.

Distilled water: 1000 cc.

Calculation: Hb (g/100ml) = A540 test sample X 15.06 (Std. conc. as stamped on the vial X 0.261)/ A540 Standard

3.9.8.3 Total erythrocyte count (TEC): Total erythrocyte count estimates the total number of red blood cells in a cubic millimeter of blood. An improved Neubaurs chamber was used for counting RBC (Baker and Silverson, 1982) (Figure 3.10).

The Hayem's dilution fluid which was used had following composition:

Mercuric chloride ($HgCl_2$): 0.5gm

Sodium Chloride (Nacl): 1.0gm

Sodium sulphate (Na_2SO_4): 5.0gm

Distilled water (H_2O): 200ml

Blood was drawn upto the 0.5 mark in the RBC diluting pipette. The tip of the pipette was cleaned and RBC dilution fluid was drawn up to 101mark.The resulting solution was shaken for 3 minutes. The first few drops of the solution were discarded and then chamber was loaded by one or two drops of blood solution. RBC was counted in four corner and central squares having smallest 80 squares (40X) and the calculations were made:

RBC count = Number of cells counted X dilution factors X depth of chamber)/Area counted

Where dilution factor is one in 200, depth is 1/10mm and area counted = 80/400 =1/5 sq. mm

RBC count = (Number of cells counted X 200 X 10) ÷ 1/5, RBC /cu.mm = Number of cells counted x 10,000

3.9.8.4 Total leukocyte count (TLC)

Total leukocyte count (TLC) estimates the total number of white blood cells in a cubic millimeter of blood. WBC diluting fluid or Turk' fluid contains a weak acid to lyses the red blood cells and gentian violet stain for staining the nucleus of white blood cells. This was done in the same manner as the RBC count was done. For leukocyte classification the nomenclature of England and Bain (1976) was followed (Figure 3.10).

Turk's WBC dilution fluid was used which had the following composition:

Glacial acetic acid (CH3COOH): 1.5 ml

1% aqueous solution of Gentian violet: 1.0ml

Distilled water: 100ml

This fluid contains two things, weak acid which lyse the RBC cells and stain which gives color to the nucleus of WBC.

Neubaur's haemocytometer (Baker and Silverson, 1982) was used for counting leucocytes. The blood was sucked up in the WBC diluting pipettes upto the 0.5mark and then WBC dilution fluid was drawn upto the 11 mark of pipette. Solution was mixed gently and bubbling was avoided. The Neubaur's chamber was charged by the resulting mixture. The cells were counted under microscope of 40x objective lens.

TLC = (Cells counted X Blood dilution X Depth of Chamber)/Areas of chamber

TLC = (Cells counted X 20 X 10)/4, TLC/cu.mm.= Cells counted x 50

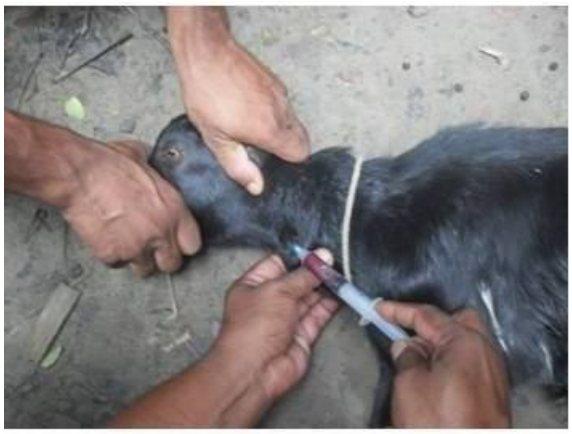


Figure A. Collection of blood from experimental goat

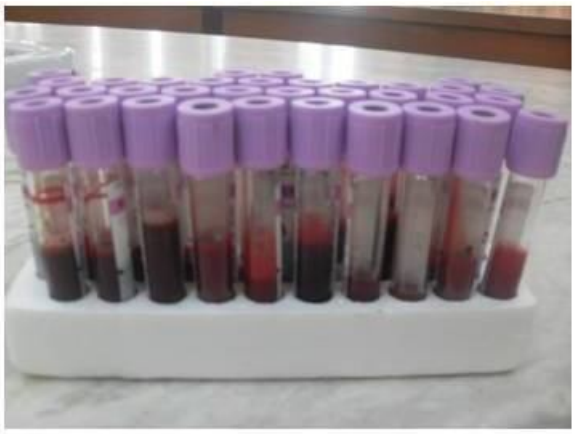


Figure B. Collection of blood into evacuated tube

Figure C. Blood filling into the haematocrite tube

Figure D: Determination of PCV (%) after centrifugation

Figure 3.9(A-D) Collection and processing of blood to determine hematological parameters

Figure A: Determination of Hb (gm%) using cyanomethemoglobin method

Figure B: Preparation of haemocytometer chamber for TEC and TLC

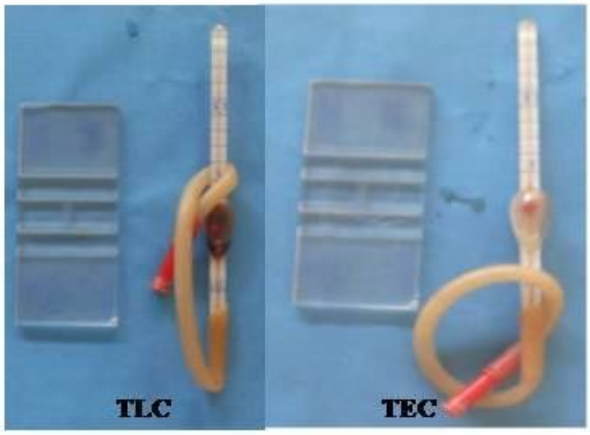


Figure C: Hematocytometers chamber for examination

Figure D: Determination of TEC and TLC

Figure 3.10(A-D): Determination of Hb (%), TEC and TLC

3.9.9 Determination of biochemical parameters

A portion of the blood serum from each sample was used to evaluate routine biochemical parameters. Serum samples were then separated by centrifugation at 1500 rpm for 10 min. These samples were evaluated for biochemical parameters such as aspertate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) using Rx Monza analyzer (Humalyzer-3000, USA) (Figure 3.11). A brief description of these is as follows.

3.9.9.1 Aspartate transaminase (AST)

The aspartate transaminase (AST) has been found in the cytoplasm and the mitochondria of cells. Severe tissue damage will result in more mitochondrial enzyme being released. AST is usually tested alongside ALT to diagnose liver damage. AST levels are sometimes compared directly to ALT and an AST/ALT ratio is calculated. This ratio may be used to distinguish between different causes of liver damage.

3.9.9.2 Alanine transaminase (ALT)

The enzyme alanine transaminase (ALT) has been found to be in highest concentrations in the liver, with decreasing concentrations found in the kidneys, heart, skeletal muscle, pancreas, spleen and lung tissue respectively. ALT measurements are used in the diagnosis and treatment of certain liver diseases. ALT is often tested in combination with AST as part of a liver panel with ALT levels being higher in most types of liver disease.

3.9.9.3 Alkaline phosphatase (ALP)

Alkaline phosphatase is diagnostic use in the investigation and treatment of hepatobiliary disease such as hepatitis, liver disease, damaged liver cells release high levels of ALP into the blood. ALP levels are usually much lower than both AST and ALT however when bile ducts become blocked due to gallstone or others etc

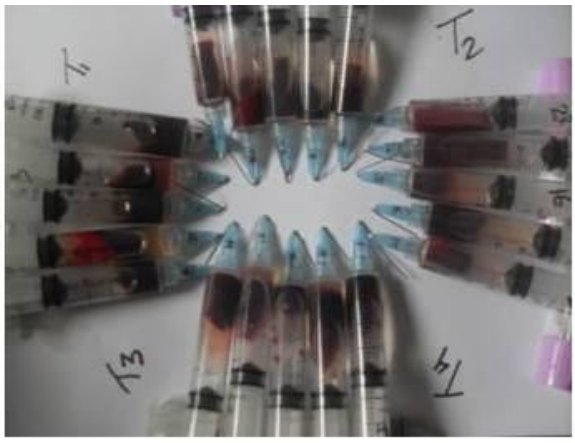


Figure A: Collected blood samples.

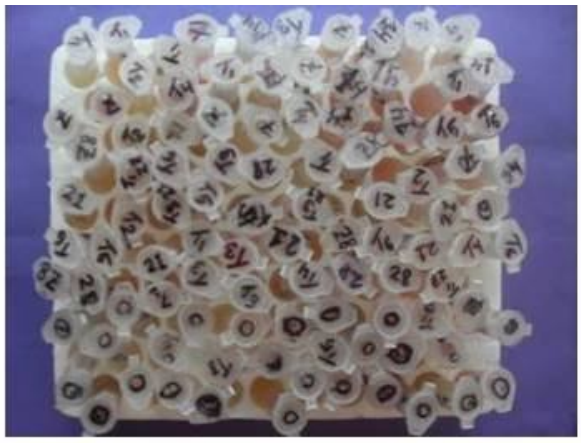


Figure B: Collected serum samples

Figure C: AST, ALT and ALP Randox kits

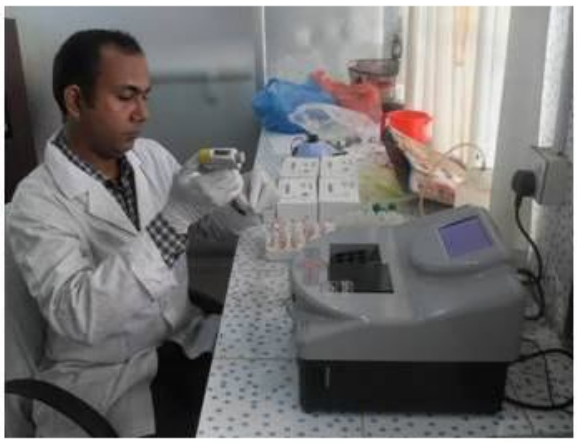


Figure D: Preparation of serum samples for analysis

Figure E: Sample setting into the Rx Monza analyzer machine

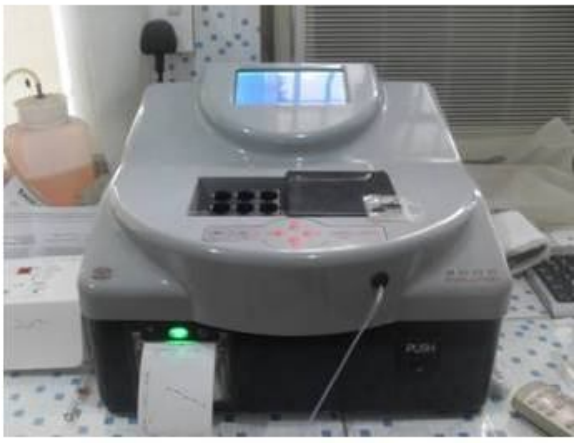


Figure F: Sample analysis by Rx Monza analyzer machine

Figure 3.11(A-F): Determination of biochemical parameters using Rx Monza analyzer

3.10 Collection of climatic data

Climatic data regarding temperature (^{o} C), rainfall (mm) and relative humidity (%) of the selected areas were collected from the meteorological station which located within the Sylhet City Corporation, Sylhet, Bangladesh during the period from July 2012 to February 2014 to determine the effect of climatic change on the prevalence of fascioliasis, infection of developmental stages of *Fasciola gigantica* in *Lymnaea* snails and prevalence of *Lymnaea auricularia* var *rufescens*.

3.11 Statistical analysis

Variations in the prevalence of fascioliasis in different age groups, season, sex and location were determined by statistical analysis. The prevalence of infection of developmental stages of *Fasciola gigantica* in *Lymnaea* snail's in different month, season, and efficacy of either liver tonic or anthelmintics or combined uses of anthelmintic and liver tonic were also subjected to statistical analysis. The analysis was performed by logistic regression using statistical software SPSS (Version 15.2, SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2007. Values of $p<0.001$ and $p<0.05$ were considered as significant at 99.99% and 95% confidence interval, respectively. Meteorological data including average temperature (°C), relative humidity (%) and rainfall (mm) was obtained from meteorological station. Their correlation with the prevalence of infection was worked out by using Pearson's correlation.

CHAPTER-IV

RESULTS

The present study was aimed to determine the epidemiology, pathology, infection status of different developmental stages of *Fasciola gigantica* in *Lymnaea* snails and evaluation of the efficacy of different types of liver tonic in combination with anthelmintic on fascioliasis in goats in Sylhet region of Bangladesh. For this purpose goats were examined from July 2012 to December 2014 for the period of thirty months. For the proper understanding, the observations were divided into four main parts. The agro-climatic and geographical conditions of Sylhet region are conducive for goat rearing. However, losses due to infectious disease are not uncommon. Mostly one of them trematodes, *Fasciola gigantica* was encountered during present study. For a clear understanding, the observations have been divided into following headings dealing with various aspects of the study.

4.1 Epidemiological study of fascioliasis (*Fasciola gigantica*) in goats

The epidemiology of the *Fasciola gigantica* infected household live and slaughterhouse goats in Sylhet region has been studied taking into consideration the overall prevalence, prevalence of different upazilla, age wise prevalence, sex wise prevalence and seasonal prevalence with the associated risk factors.

4.1.1 The overall prevalence

During the study year, a total of 1288 household live and 2000 slaughterhouse goats were examined of which 405 (31.75%) household live and 202 (10.10%) of slaughterhouse goats were found to be positive for *Fasciola gigantica*. The overall prevalence was 20.93%. It was noticed that prevalence of household live (31.75%) was higher than slaughterhouse goats (10.10%). Household live goats were 3.14 times more susceptible to *Fasciola gigantica* infection than slaughterhouse. Moreover, the prevalence of fascioliasis was higher in household live goats than slaughterhouse in each upazilla of Sylhet region, Bangladesh (Figure 4.1). The overall prevalence of Sylhet Sadar, Balaganj, Beanibazar, Biswanath and Jaintapur upazilla were 16.84%, 20.23%, 22.16%, 25.96% and 19.45% in household live and slaughterhouse goats respectively (Table 4.1 and Figure 4.1).

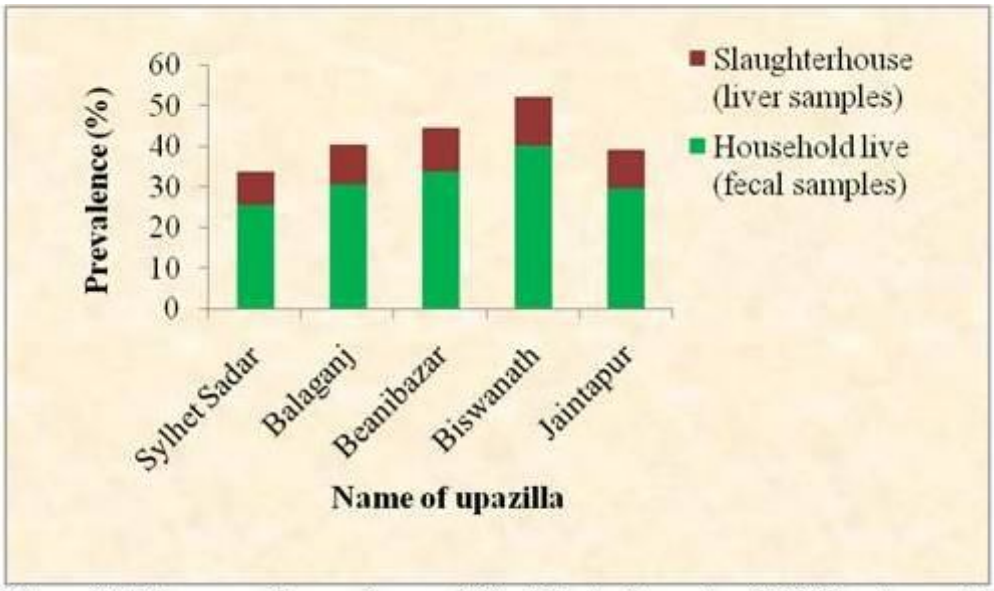


Figure 4.1 The overall prevalence of fascioliasis in goats of different upazilla

4.1.2 Prevalence of fascioliasis in different study areas

The present study revealed a correlation of disease prevalence with five different upazilla in Sylhet region of Bangladesh. The prevalence of fascioliasis in Sylhet Sadar, Balaganj, Beanibazar, Biswanath and Jaintapur upazilla was 25.43%, 30.45%, 33.57%, 39.91% and 29.39% in household live goats and 8.25%, 10.00%, 10.75%, 12.00% and 9.50% in slaughtered goats (Table 4.1 and Figure 4.2). There were significant differences between the prevalence of the infection in the different region, Biswanath and Beanibazar upazilla having a higher prevalence than Balaganj, Jaintapur and Sylhet Sadar in both household live and slaughterhouse goats. Sylhet Sadar showed a significantly lower prevalence than the other regions.

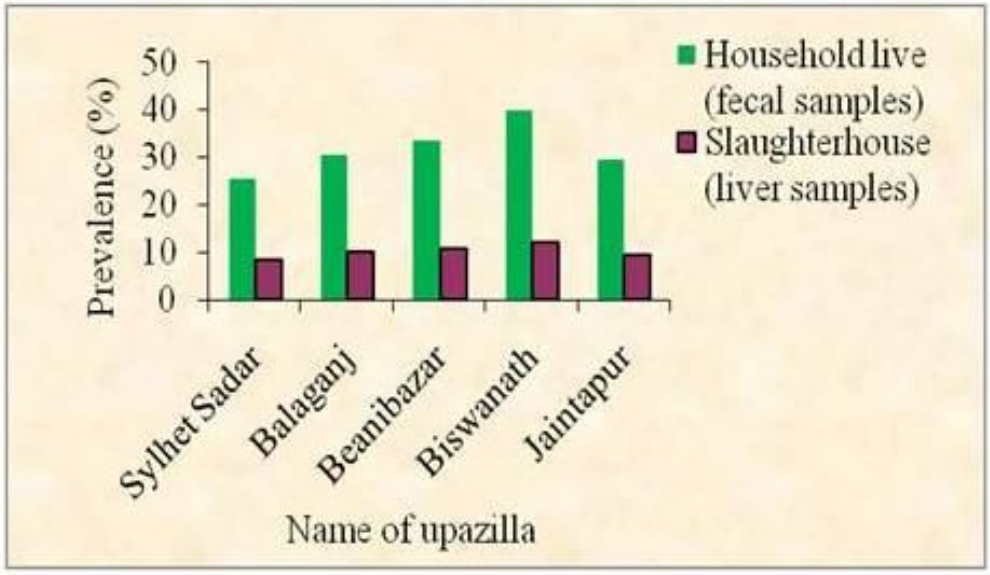


Figure 4.2 Prevalence of fascioliasis in household live (fecal samples) and slaughterhouse (liver samples) goats of different upazilla.

Table 4.1 Prevalence of fascioliasis in goats in different study areas

Upazilla	Household live goat			Slaughterhouse goat			Overall (%)
	No. examined	No. Positive	Prevalence (%)	No. examined	No. positive	Prevalence (%)	
Sylhet Sadar	291	74	25.43	400	33	8.25	16.84
Balaganj	266	81	30.45	400	40	10.00	20.23
Beanibazar	280	94	33.57	400	43	10.75	22.16
Biswanath	223	89	39.91	400	48	12.00	25.96
Jaintapur	228	67	29.39	400	38	9.50	19.45
Average	1288	405	§31.75±2.42***	2000	202	§10.10±0.63***	20.93

***Significant differences between groups $p<0.001$, § ± standard deviation

4.1.3 Age-wise prevalence of fascioliasis (*Fasciola gigantica*) in goats

To observe the age specificity, the samples were divided into two age groups ranges from birth to 3 years. The overall prevalence of fascioliasis was more frequently recorded in young of household live (39.05%) and slaughterhouse (15.58%) than adult household live (23.22%) and slaughterhouse (9.59%) goats. The highest prevalence of

fascioliasis was in young household live goats (47.66%) and slaughterhouse goats (18.37%) of Biswanath upazilla and the lowest prevalence was in household live goats (31.79%) and slaughterhouse goats (11.77%) of Sylhet Sadar Upazilla (Table 4.2 and Figure 4.3). The highest prevalence of fascioliasis in adult goats was 29.47% (household live) and 11.11% (slaughterhouse) in Biswanath upazilla and the lowest prevalence was 18.57% (household live) and 9.72% (slaughterhouse) in Sylhet Sadar (Table 4.3 and Figure 4.3). Young's were 2.58 times more susceptible to *Fasciola gigantica* infection than adult. Significant differences (between age groups $p<0.001$, household $p<0.002$ and slaughterhouse $p<0.004$) were also observed in prevalence with regard to different upazilla.

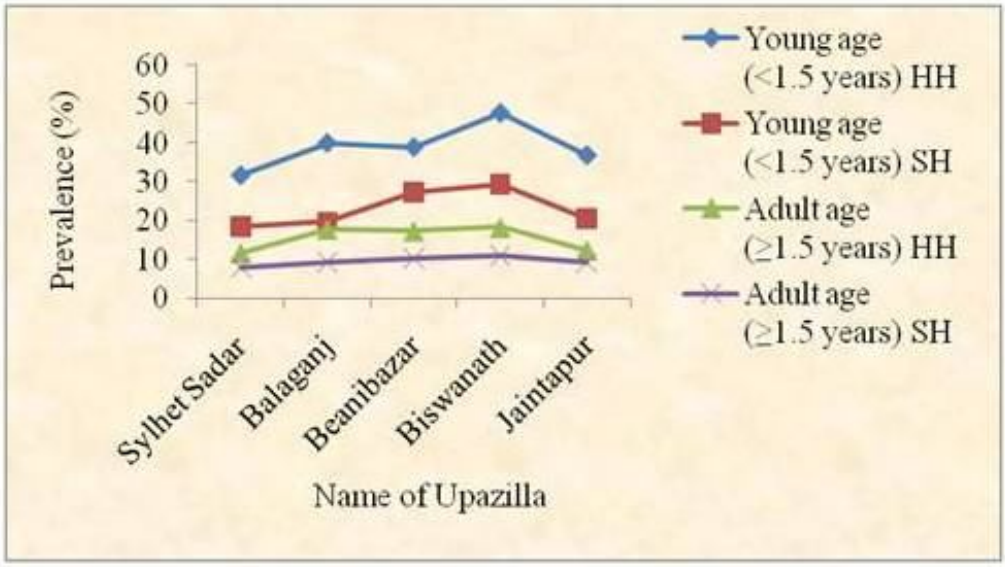


Figure 4.3 Age wise prevalence of fascioliasis in goats at different upazilla (HH-Household live and SH-Slaughterhouse)

4.1.4 Sex-wise prevalence of fascioliasis (*Fasciola gigantica)* in goats

In this study, it was found that the female goats were infected higher than males. The overall prevalence of fascioliasis was 39.15% in females of household live and 13.10% in slaughterhouse (Table 4.4 and Figure 4.4). The prevalence in male household live and slaughterhouse goats was 19.96% and 7.10%, respectively (Table 4.5 and Figure 4.4). The highest prevalence in female goats was 46.71% in household live and 15.00% in slaughterhouse of Biswanath. The lowest prevalence was 33.33% in household live and 10.50% in slaughterhouse goats of Sylhet Sadar (Table 4.4). Females were 2.16

times more susceptible to *Fasciola gigantica* infection than males. The highest prevalence of fascioliasis of male goats was 25.35% and 9.00% in Biswanath and the lowest prevalence was 14.17% in household live and 6.00% in slaughterhouse goats of Sylhet Sadar (Table 4.5). Significant differences ($p<0.001$) were also observed in prevalence with regard to different upazilla.

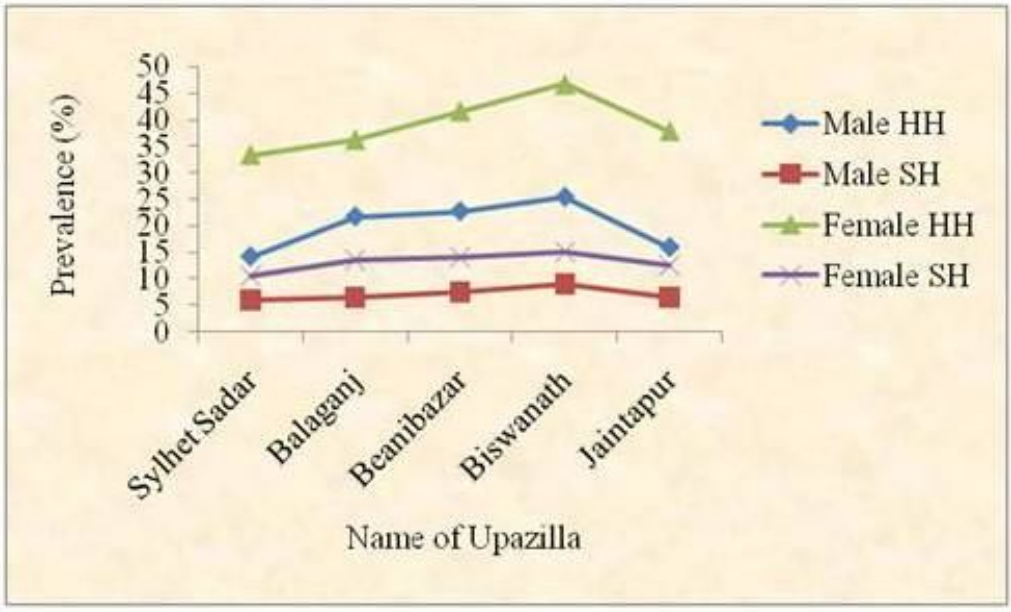


Figure 4.4 Sex wise prevalence of fascioliasis in household live (HH) and slaughterhouse (SH) goats at different five upazilla.

Table 4.2 Prevalence of fascioliasis in household live and slaughterhouse goats in relation to young age

Upazilla	Young age (<1.5 years)						Overall prevalence (%)
	Household live goat			Slaughterhouse goat			
	No. examined	No. positive	Prevalence (%)	No. examined	No. positive	Prevalence (%)	
Sylhet Sadar	151	48	31.79	34	04	11.77	33.61±1.74***
Balaganj	140	56	40.00	28	05	17.86	
Beanibazar	149	58	38.93	23	04	17.39	
Biswanath	128	61	47.66	49	09	18.37	
Jaintapur	122	45	36.89	40	05	12.50	
Average	690	268	39.05±2.57**	174	27	15.58±1.42**	

***Significant differences between age groups p<0.001, **household groups p<0.002 and **slaughtered groups p<0.004

Table 4.3 Prevalence of fascioliasis in household live and slaughterhouse goats in relation to adult age

Upazilla	Adult age (≥1.5 years)						Overall prevalence (%)
	Household live goat			Slaughterhouse goat			
	No. examined	No. positive	Prevalence (%)	No. examined	No. positive	Prevalence (%)	
Sylhet Sadar	140	26	18.57	366	29	7.92	13.04±0.93***
Balaganj	126	25	19.84	372	35	9.41	
Beanibazar	131	36	27.48	377	39	10.35	
Biswanath	95	28	29.47	351	39	11.11	
Jaintapur	106	22	20.76	360	33	9.17	
Average	598	137	23.22±2.19**	1826	175	9.59±0.54**	

***Significant differences between age groups p<0.001, **household groups p<0.002 and **slaughtered groups p<0.004

Table 4.4 Prevalence of fascioliasis in female household live and slaughtered goats

Upazilla	Female						Overall prevalence (%)
	Household live goat			Slaughterhouse goat			
	No. examined	No. positive	Prevalence (%)	No. examined	No. positive	Prevalence (%)	
Sylhet Sadar	171	57	33.33	200	21	10.50	24.52±1.35***
Balaganj	160	58	36.25	200	27	13.50	
Beanibazar	161	67	41.62	200	28	14.00	
Biswanath	152	71	46.71	200	30	15.00	
Jaintapur	140	53	37.86	200	25	12.50	
Average	784	306	39.15±2.32***	1000	131	13.10±.77***	

***Significant differences between sex's groups p<0.001

Table 4.5 Prevalence of fascioliasis in male household live and slaughtered goats

Upazilla	Male						Overall prevalence (%)
	Household live goat			Slaughterhouse goat			
	No. examined	No. positive	Prevalence (%)	No. examined	No. positive	Prevalence (%)	
Sylhet Sadar	120	17	14.17	200	12	6.00	11.33±0.91***
Balaganj	106	23	21.70	200	13	6.50	
Beanibazar	119	27	22.69	200	15	7.50	
Biswanath	71	18	25.35	200	18	9.00	
Jaintapur	88	14	15.91	200	13	6.50	
Average	504	99	19.96±2.12***	1000	71	7.10±.53***	

***Significant differences between sex's groups p<0.001

4.1.5 Seasonal influence on fascioliasis (*Fasciola gigantica*) in goats

The seasonal influence of *Fasciola gigantica* infection is shown in the Figure 4.5. The overall prevalence of *Fasciola gigantica* was highest in rainy season (25.71%) followed by winter (16.84%) and summer (10.12%). In rainy season, the highest prevalence of fascioliasis was 47.87% and 21.01% in Biswanath; the lowest prevalence was 33.33% and 13.57% in Sylhet Sadar in household live and slaughterhouse goats, respectively (Table 4.6). In winter season, the highest prevalence of fascioliasis was 38.75% and 8.33% in Biswanath; the lowest prevalence was 25.74% and 6.80% in household live and slaughterhouse goats in Sylhet Sadar, respectively (Table 4.7). In summer season, the highest prevalence of fascioliasis was observed in Biswanath and lowest in Sylhet Sadar in household live and slaughterhouse goats, respectively (Table 4.8). Significant differences ($p<0.001$) were also observed in prevalence with regard to different upazilla. Prevalence and meteorological factors such as temperature, humidity and rainfall were significantly correlated.

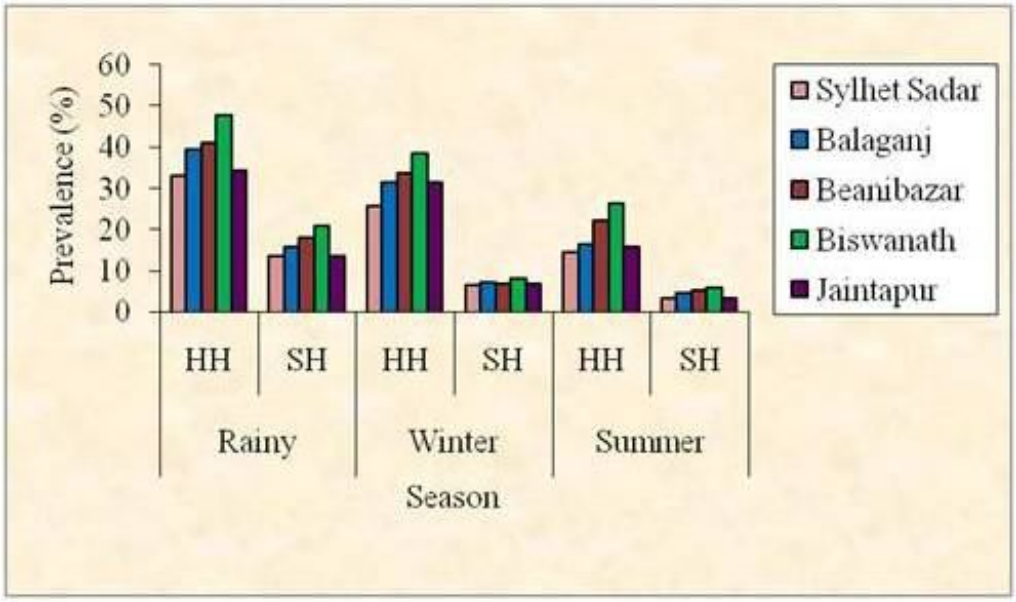


Figure 4.5 Season wise prevalence of fascioliasis in household live (HH) and slaughterhouse (SH) goats at different upazilla.

Table 4.6 Prevalence of fascioliasis in household and slaughtered goats in rainy

Upazilla	Rainy season						Overall prevalence (%)
	Household live goat			Slaughterhouse goat			
	No. examined	No. positive	Prevalence (%)	No. examined	No. positive	Prevalence (%)	
Sylhet Sadar	108	36	33.33	140	19	13.57	25.71 [a] ±1.98***
Balaganj	101	40	39.60	149	24	16.11	
Beanibazar	109	45	41.28	149	27	18.12	
Biswanath	94	45	47.87	138	29	21.01	
Jaintapur	102	35	34.31	189	26	13.76	
Average	514	201	39.28[a]±2.63***	765	125	16.51[a]±1.40***	

[a, b, c:] In a column among seasons and groups with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99.99% level of significance ($p<0.001$). ***Significant differences in different season's and groups $p<0.001$

Table 4.7 Prevalence of fascioliasis in household and slaughtered goats in winter

Upazilla	Winter season						Overall prevalence (%)
	Household live goat			Slaughterhouse goat			
	No. examined	No. positive	Prevalence (%)	No. examined	No. positive	Prevalence (%)	
Sylhet Sadar	101	26	25.74	147	10	6.80	16.84 [c] ±0.79***
Balaganj	92	29	31.52	148	11	7.43	
Beanibazar	95	32	33.68	142	10	7.04	
Biswanath	80	31	38.75	144	12	8.33	
Jaintapur	76	24	31.58	129	09	6.98	
Average	444	142	32.25±2.09***[a]	710	52	7.32±0.27***[b]	

[a, b, c:] In a column among seasons and groups with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99.99% level of significance ($p<0.001$). ***Significant differences in different season's and groups $p<0.001$

Table 4.8 Prevalence of fascioliasis in household and slaughtered goats in summer

Upazilla	Summer season						Overall prevalence (%)
	Household live goat			Slaughterhouse goat			
	No. examined	No. positive	Prevalence (%)	No. examined	No. positive	Prevalence (%)	
Sylhet Sadar	82	12	14.63	113	04	3.54	$10.12^{b} \pm 0.89^{***}$
Balaganj	73	12	16.44	103	05	4.85	
Beanibazar	76	17	22.37	109	06	5.50	
Biswanath	49	13	26.53	118	07	5.93	
Jaintapur	50	08	16.00	82	03	3.66	
Average	330	62	$19.19^{b} \pm 2.26^{***}$	525	25	$4.70^{b} \pm 0.48^{***}$	

[a, b, c:] In a column among seasons and groups with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99.99% level of significance ($p<0.001$). ***Significant differences in different season's and groups $p<0.001$

4.2 Effects of climatic factors on prevalence or infection status of developmental stages of *Fasciola gigantica* in *Lymnaea* snails (*Lymnaea auricularia* var *rufescens*)

4.2.1. The prevalence of developmental stages in snail

The prevalence of both *Fasciola gigantica cercariae* and sporocyst infection in *Lymnaea auricularia* var *rufescens* in different upazilla in Sylhet region of Bangladesh is shown in the Table 4.9 and Figure 4.6. In the present study, a total of 1865 *Lymnaea* snails were collected randomly and examined during the period of one year, of which 56 (3%) *Lymnaea* snails were found positive for *Fasciola cercariae* and sporocyst. The overall prevalence was 3.00%. On the other hand, the highest prevalence was found in Biswanath upazilla (4.08%) followed by Beanibazar (3.16%), Balagonj (2.53%), Jaintapur (2.40%) and Sylhet Sadar (1.83%). The prevalence of different developmental stages of *Fasciola gigantica* varied from 1.83 to 4.08 during March 2013 to February 2014 in different upazilla of Sylhet region in Bangladesh. The highest prevalence was observed in Biswanath (4.08%) and lowest in Sylhet Sadar (1.83%) upazilla.

Table 4.9 Infection status of developmental stages of *F. gigantica* in *Lymnaea* snails

Upazilla	No. examined	Different developmental stages (positive)			Total positive	Prevalence (%)
		Sporocyst	Redia	Cercaria		
Sylhet Sadar	219	0	0	4	4	1.83
Balagonj	396	1	0	9	10	2.53
Bishwanath	515	3	0	18	21	4.08
Beanibazar	443	2	0	12	14	3.16
Zoyantapur	292	1	0	6	7	2.40
Overall	1865	7	0	49	56	3.00

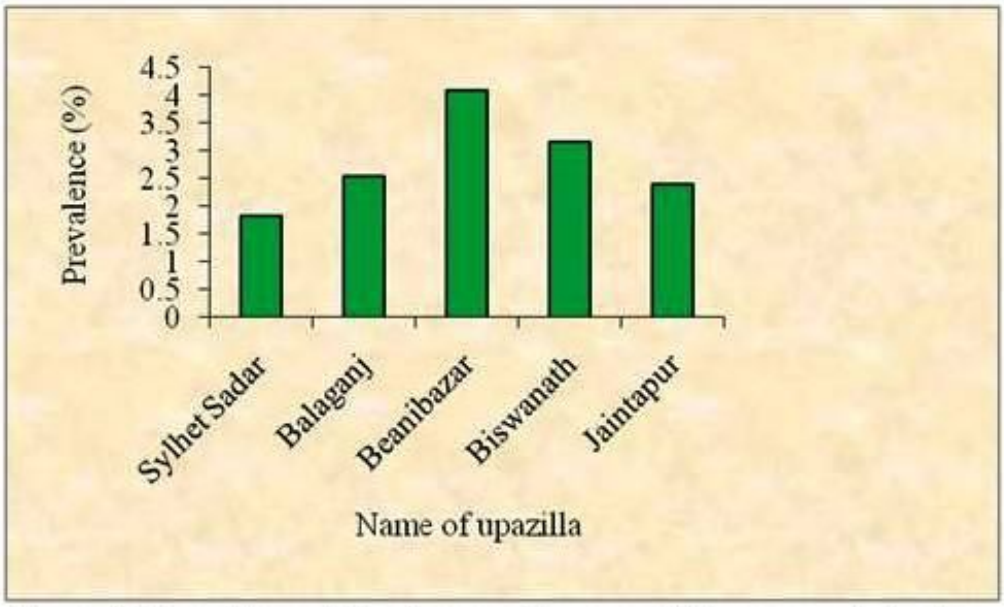


Figure 4.6 Prevalence of developmental stages of *F. gigantica* infection in *Lymnaea* snails of different upazilla.

4.2.2 Microscopic features of sporocyst and cercaria

In the present study, both the sporocyst and *cercariae* of *Fasciola gigantica* were found in *Lymnaea* snails during the period of study. Microscopically, *Fasciola* sporocyst was found as a sac like structure (Figure 4.7) and *Fasciola cercariae* were large heart shaped body with a simple long tail. The body of cercaria had a characteristically thick wall and was surrounded by minute spines all over its surface. The ventral sucker was

well developed and larger than the oral sucker. The alimentary canal was rudimentary and consists of a mouth followed by pharynx surrounding the esophagus that leads to intestine. The later bifurcates into two simple branches that extend around the ventral sucker to a level below the posterior border of the ventral sucker (Figure 4.8).

Figure A. Sac like structure of a sporocyst (10X)

Figure B. Sac like structure of a sporocyst (10X.)

Figure C. Sac like structure of a sporocyst (40X).

Figure D. Sac like structure of a sporocyst (40X)

Figure E. Sac like structure of a sporocyst (40X)

Figure F. Sac like structure of a sporocyst (40X)

Figure 4.7 Microscopic feature of a sporocyst. (A+B)

Figure A. Heart shaped body, well develop ventral and oral sucker, a simple long tail (4X)

Figure B. Heart shaped body, well develop ventral and oral sucker, a simple long tail (4X)

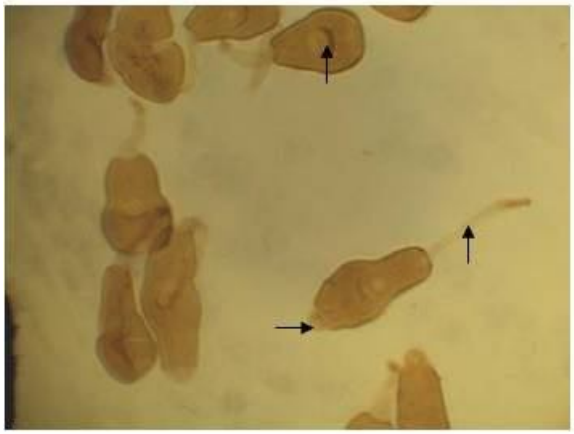


Figure C. Heart shaped body, well develop ventral and oral sucker, a simple long tail (0X.)

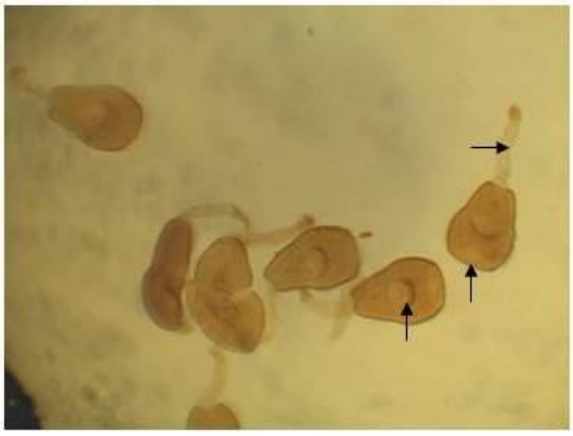


Figure D. Heart shaped body, well develop ventral and oral sucker, a simple long tail (10X)

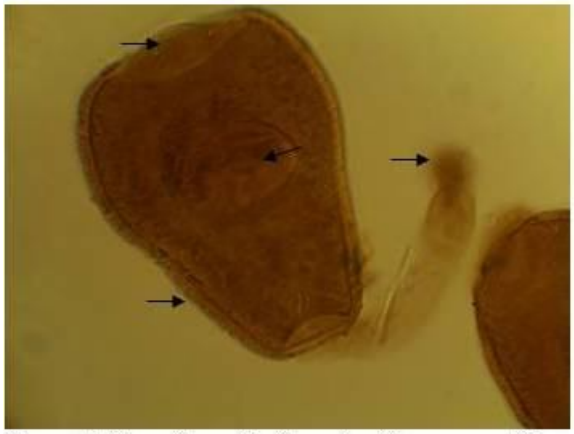


Figure E. Heart shaped body and surface cover with spines, well develop ventral and oral sucker, a simple long tail (40X)

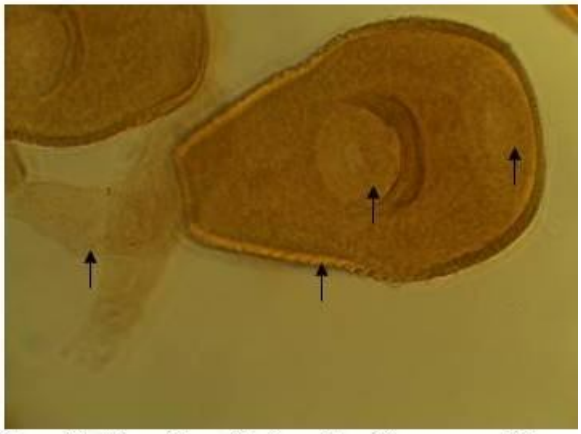


Figure E: Heart shaped body and surface cover with spines, well develop ventral and oral sucker, a simple long tail (40X)

Figure 4.8 Microscopic feature of a cercaria (A+F) indicated by arrow

4.2.3. Month wise prevalence of developmental stages of *F. gigantica infection* in *Lymnaea* snails

During the present study, month-wise data showed highest infection in *Lymnaea* snails were in May (5.06%) and August (5.61%) when the average temperature, average relative humidity and rainfall dropped to 29.55 and 29.70°C, 78 and 80%, 958.2 and 727.2 mm respectively. The prevalence of both sporocyst and cercarial stages increasing from April to September, decreasing from February to March and no infection was observed from November to January (Table 4.10 and Figure 4.9).

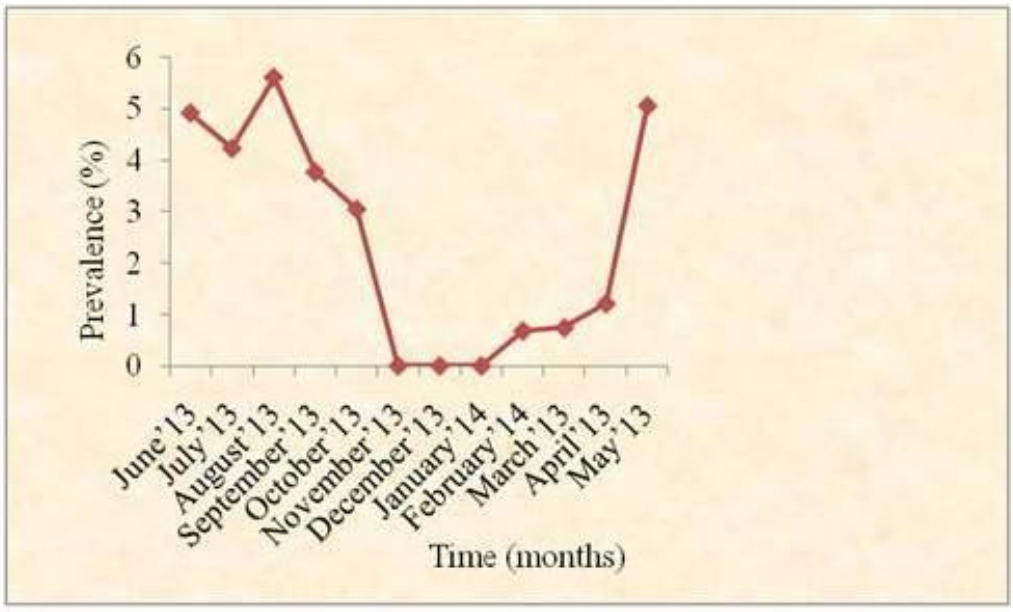


Figure 4.9 Prevalence of infection status of developmental stages of *F. gigantica* in *Lymnaea* snails

4.2.4 Season wise prevalence of developmental stages of *F. gigantica infection* in *Lymnaea* snails

The seasonal variations in the infection status of snails were studied from March 2013 to February 2014. The highest prevalence of developmental stages infection of *F. gigantica* in snails population was found in rainy season (4.63%) followed by summer (1.92%) and then winter (0.76%). This result is shown in the Table 4.10 and Figure 4.10.

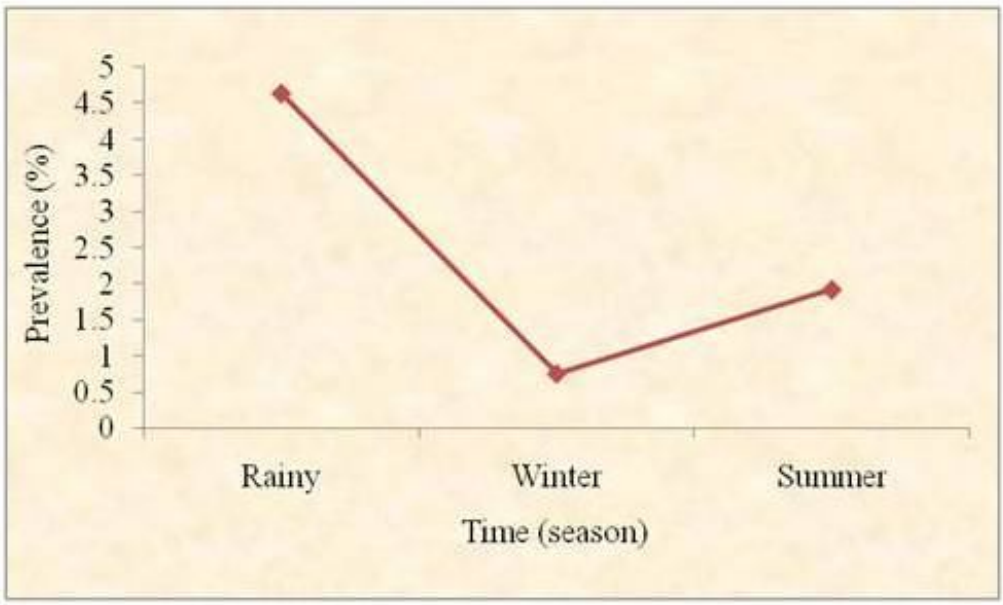


Figure 4.10 Season wise prevalence of developmental stages of *F. gigantica* infection in *Lymnaea* snails

4.2.5 Correlation between *Lymnaea* snail's infection, meteorological factors and snail population

The present study revealed a Pearson's correlation between snail's infection and meteorological factors (temperature, relative humidity, and rainfall) which was found highly significant (Table 4.10 and Figure 4.11). The different developmental stages of *Fasciola gigantica* infection in snail populations decreases from November 2013 to January 2014 and increases from February 2014 to October 2013 and was highest in August 2013 and September 2013 (Table 4.10 and Figure 4.12).

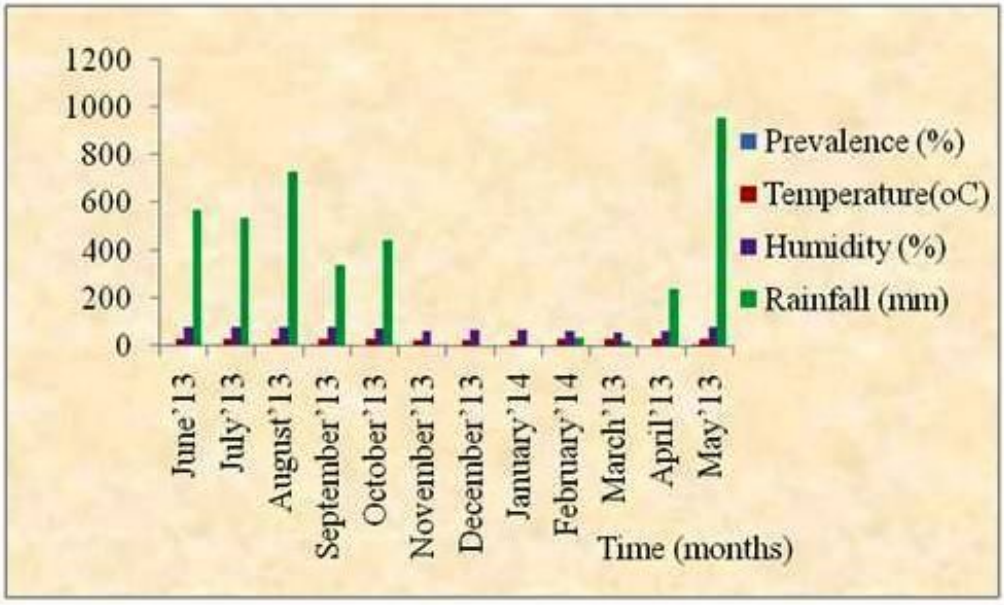


Figure 4.11 Month-wise prevalence of temperature (oC), relative humidity (%) and rainfall (mm) in Sylhet region of Bangladesh.

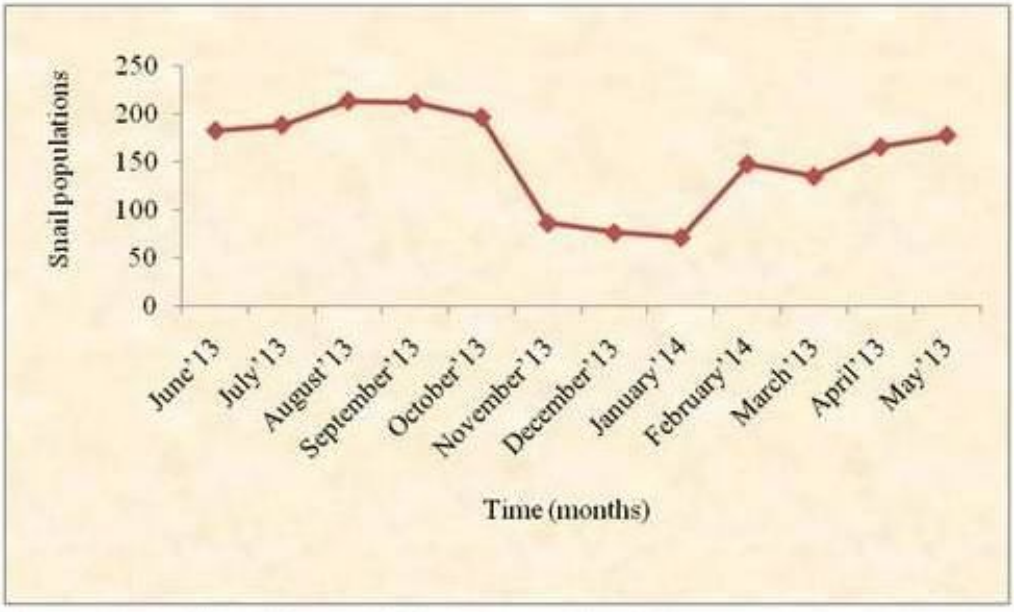


Figure 4.12 Population of *Lymnaea* snails (*Lymnaea auricularia* var *rufescens*) in Sylhet region of Bangladesh.

Table 4.10 Seasonal influence on snail population and infection status of developmental stages of *F. gigantica* in snail

Season	Month	No collected	Positive	Prevalence (%)	Seasonally	Temperature (^{o}C)		Humidity (%)	Rainfall (mm)
						Minimum	Maximum		
Rainy	June'13	183	09	4.92	4.63 [a]±0.40**	25.8	33.9	75	567.8
	July'13	189	08	4.23		25.8	33.3	78	534.1
	August'13	214	12	5.61		25.6	33.6	80	727.2
	September'13	212	08	3.77		25.3	32.8	78	337.1
Winter	October'13	197	06	3.05	0.76 [b]±0.76**	24.6	31.5	74	444.1
	November'13	86	00	0.00		17.4	30.1	63	nill
	December'13	76	00	0.00		14.6	26.2	68	nill
	January'14	71	00	0.00		13.1	27.1	66	nill
Summer	February'14	148	01	0.68	1.92 [b]±1.05**	18.4	31.6	62	34.2
	March'13	135	01	0.74		19.7	34.2	58	15.4
	April'13	166	02	1.21		21.9	33.0	59	236.1
	May'13	178	09	5.06		24.7	34.4	78	958.2

[a, b] In a season column with same or without superscripts do not differ significantly as per DMRT, data were calculated at 5% level of significance ($p<0.02$). **Significant differences in different seasons $p<0.02$, A Pearson's correlation of prevalence (%), temperature (^{o}C), humidity (%) and rainfall (mm) were significant at the 0.01 level.

4.3 Pathological changes in liver of goat due to fascioliasis

Clinical examination of the *Fasciola gigantica* infected goats showed pale visible mucous membrane to a large extent where a very few infected goats suffer from 'bottle jaw' syndrome. Acute *Fasciola* infection causes extensive hemorrhage in liver parenchyma and ingestion of blood by adult flukes leading to pale mucous membrane. On the other hand, the massive *Fasciola gigantica* infection causes sufficient liver destruction leading to cessation of protein synthesis causes 'bottle jaw' syndrome occasionally.

4.3.1 Gross pathology

The gross pathological changes of the liver in acute form of fascioliasis was characterized by slightly swollen, soft in consistency, enlarged livers with round edges and thickened capsule with numerous hemorrhagic spots on the parietal and visceral surface of the liver (Figure 4.13). In chronic form, affected livers were cirrhotic and reduced in size with irregular and granular surfaces (Figures 4.14, 4.15 and 4.16), hemorrhagic, ulcerative and congestive (Figure 4.13, 4.14 and 4.15). The affected bile duct was moderately distended and contained both pre-adult and few adult flukes mixed with dirty bile and tissue debris (Figure 4.14 and 4.15). The color of the livers became pale, the capsule was thick, opaque, rough and was closely adhering with parenchyma. The parietal surface of the liver covered with whitish color fibrous connective tissue and the parenchyma was somewhat tough to cut due to the presence of fibrous tissue and healing of migratory tracts caused by the immature flukes (Figures 4.13, 4.14, 4.15 and 4.16). The affected intra-hepatic bile duct was protruded and engorged with pre-adults and adult flukes. In majority cases, the gall bladder was highly distended with bile. The bile duct was cut to tough due to proliferation of fibrous tissue and the lumen was of dirty appearance (Figure 4.7, 4.8 and 4.9). Hepatic lymphnodes were hemorrhagic, congested and swollen (Figures 4.14, 4.15 and 4.16). Adult liver flukes were attached with the hepatic parenchyma (Figure 4.14 and 4.15).

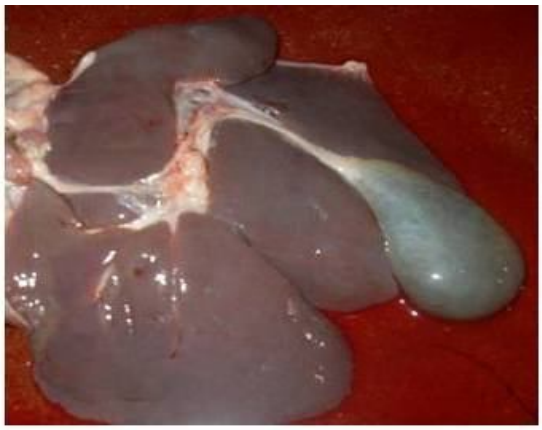


Figure A. Normal goat liver (visceral surface)

Figure B. Normal goat liver (parietal surface)

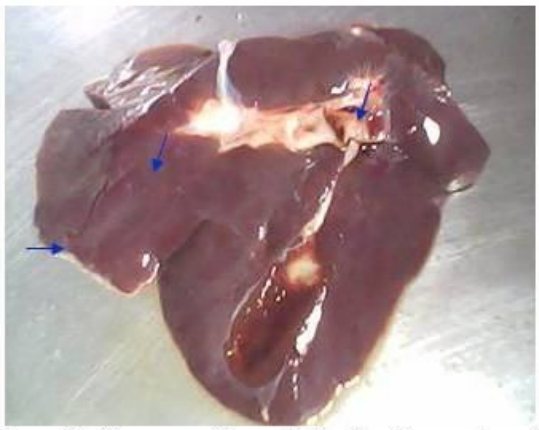


Figure C. Presence of rounded edge, hypernia and adult flukes on the visceral surface of liver

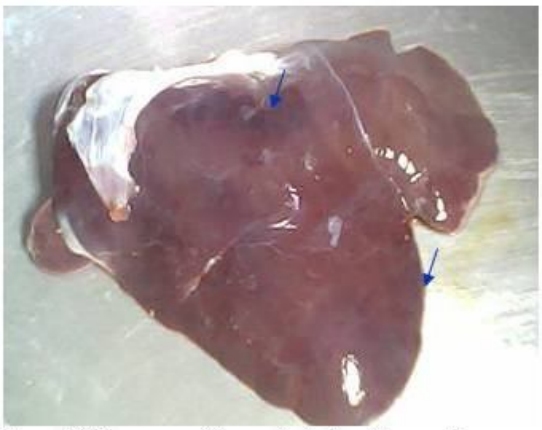


Figure D. Presence of rounded edge, hypernia congestion and hemorrhage on the parietal surface of liver

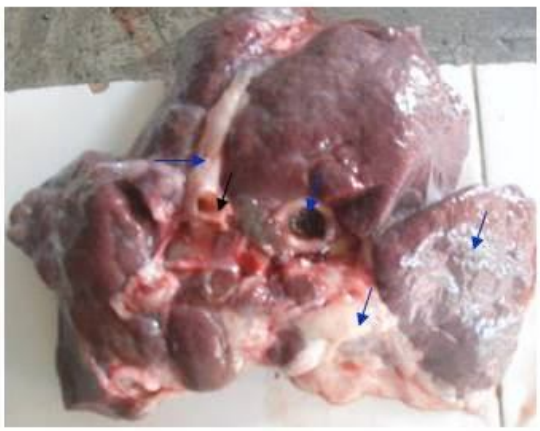


Figure E. Presence of rounded edge, hypernia hemorrhage, adult flukes, bile duct engorged with *F. gigantica* on the visceral surface of liver

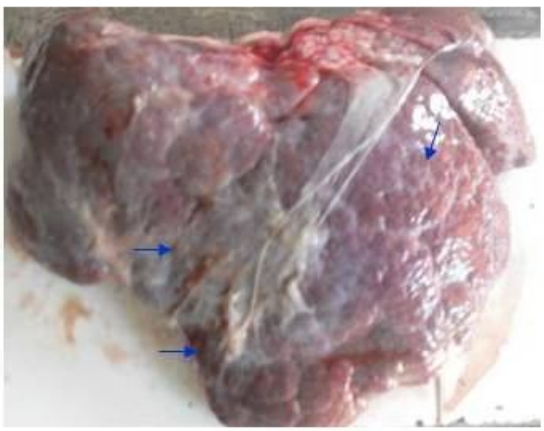


Figure F. Presence of rounded edge, hypernia hemorrhage irregular surface and fibrin formation on the parietal surface of liver

Figure 4.13 Gross examination of normal (A+B) and affected goat liver (C+F) in acute case (indicated by arrow)

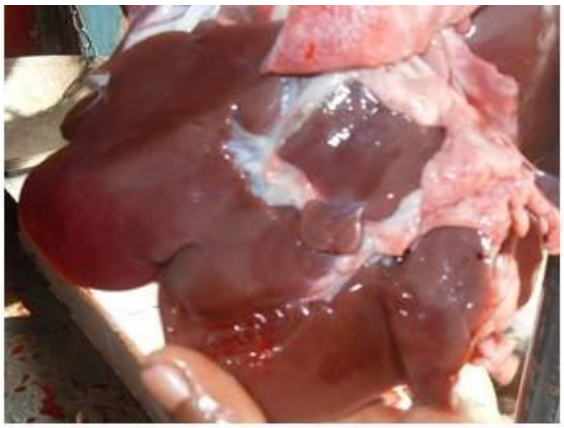


Figure A. Normal goat liver (visceral surface)

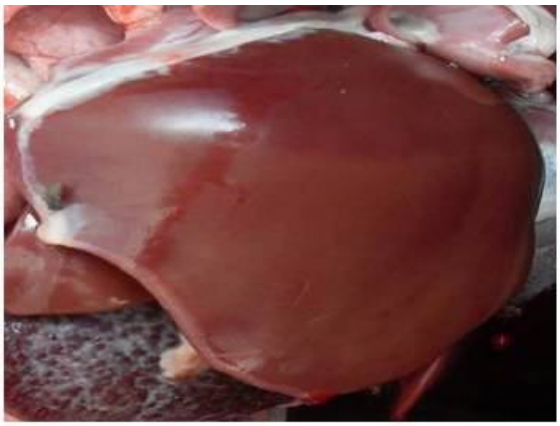


Figure B. Normal goat liver (parietal surface)

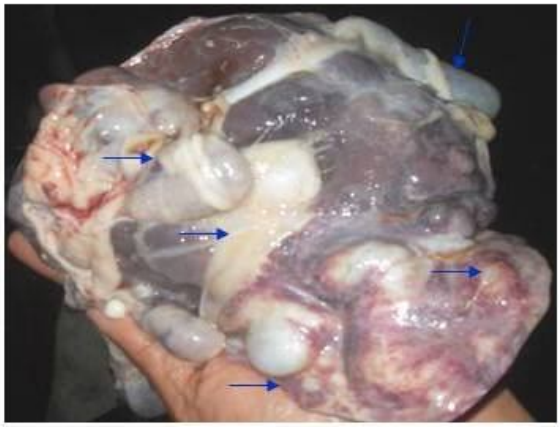


Figure C. Presence of hemorrhagic hepatic lymphnode, fibrosis, hemorrhage, bscess, distended gallbladder and rounded edge (visceral surface)

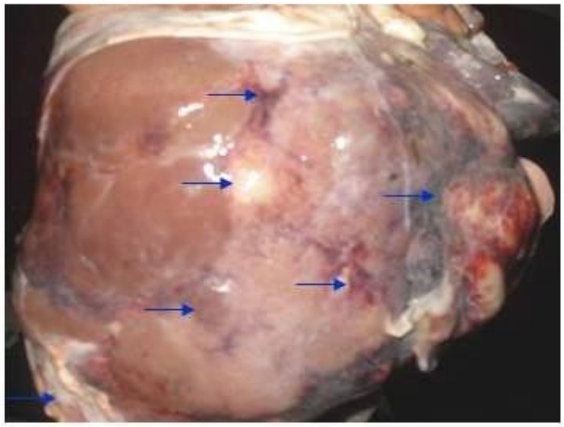


Figure D. Presence of hemorrhage, rounded edge, congestion, abscess and fibrin (parietal surface)

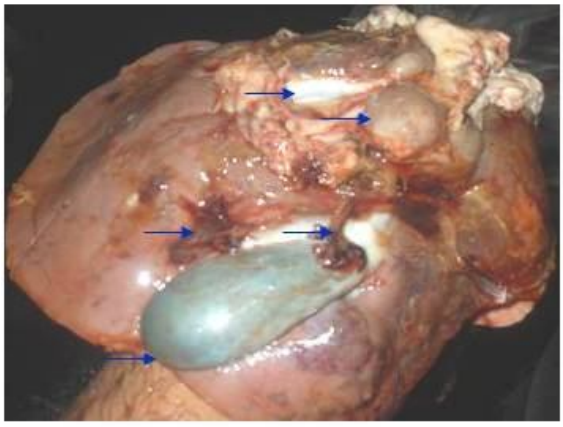


Figure E. Presence of hemorrhagic hepatic lymphnode, fibrosis, hemorrhage, abscess, distended gallbladder and rounded edge (visceral surface)

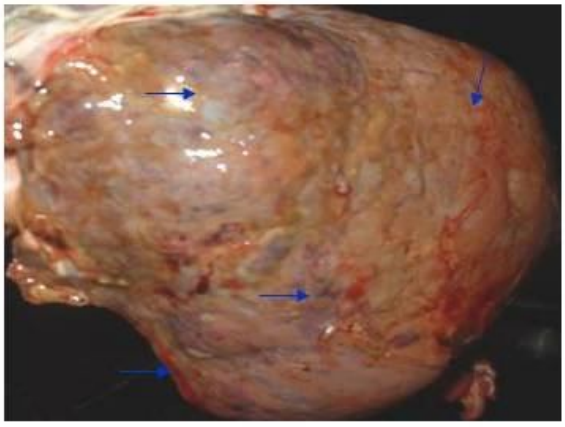


Figure F. Presence of hemorrhage, congestion, abscess, rounded edge and fibrin formation (parietal surface)

Figure 4.14 Gross examination of normal (A+B) and affected goat liver (C+F) in chronic case (indicated by arrow)

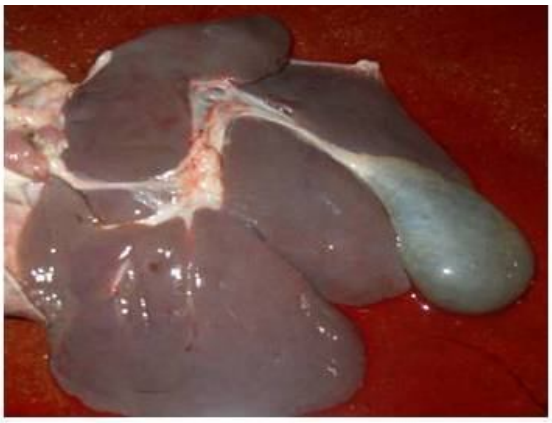


Figure A. Normal goat liver (visceral surface)

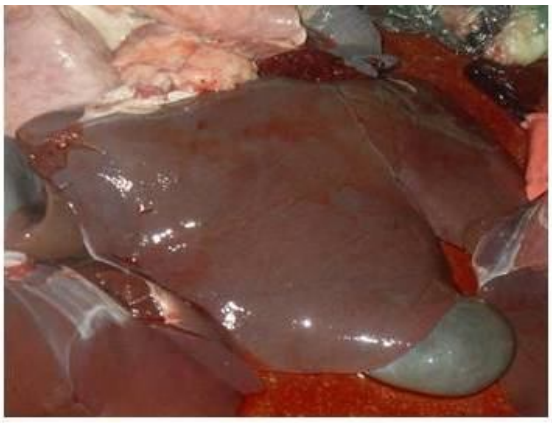


Figure B. Normal goat liver (parietal surface)

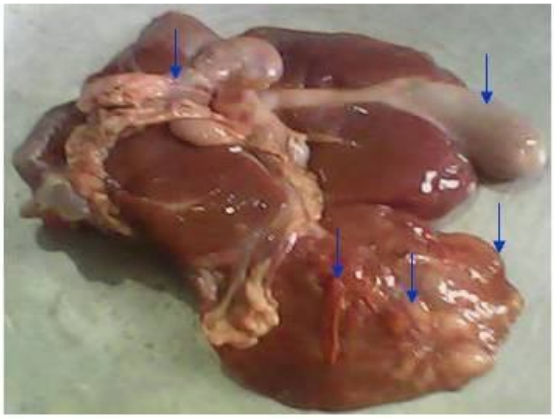


Figure C. Presence of adult *F. gigantica* hemorrhagic hepatic lymphnode, fibrosis, hemorrhage, abscess, distended gallbladder and rounded edge (visceral)

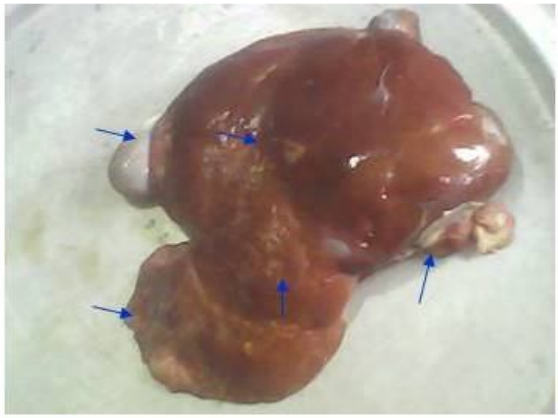


Figure D. Presence of hemorrhage, abscess, distended gallbladder and rounded edge (aparieta surface)

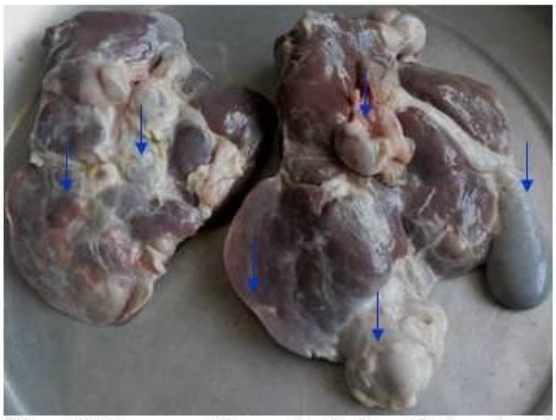


Figure E. Presence of hemorrhagic hepatic lymphnode, fibrosis, hemorrhage, abscess rounded edge, ulceration And distended gallbladder, (visceral surface)

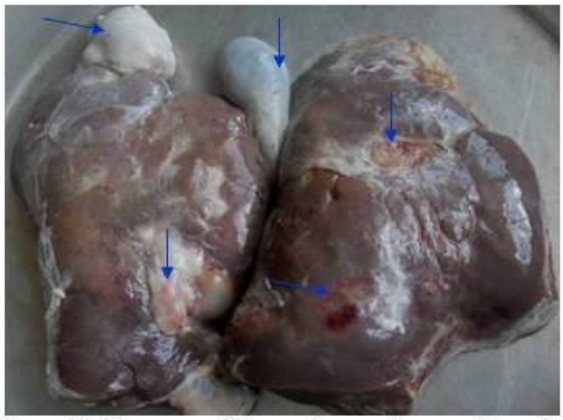


Figure F. Presence of hemorrhage, abscess, distended gallbladder, ulceration and rounded edge (parietal surface)

Figure 4.15 Gross examination of normal (A+B) and affected goat liver (C+F) in chronic case (indicated by arrow)

Figure A. Normal goat liver (visceral surface)

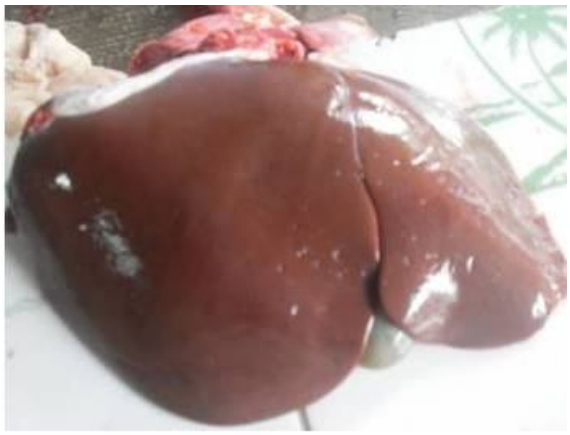


Figure B. Normal goat liver (parietal surface)

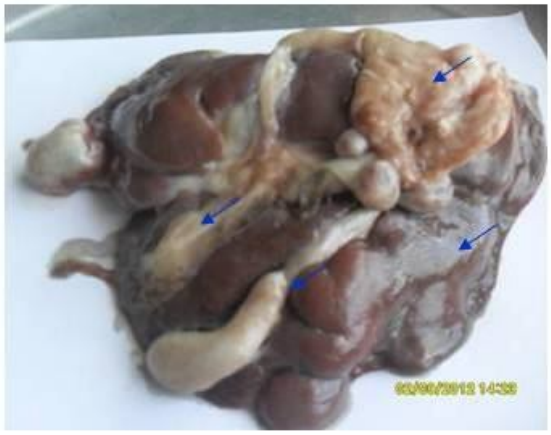


Figure C. Presence of hemorrhagic hepatic lymphnode, fibrosis, hemorrhage, distended gallbladder and rounded edge (visceral surface)

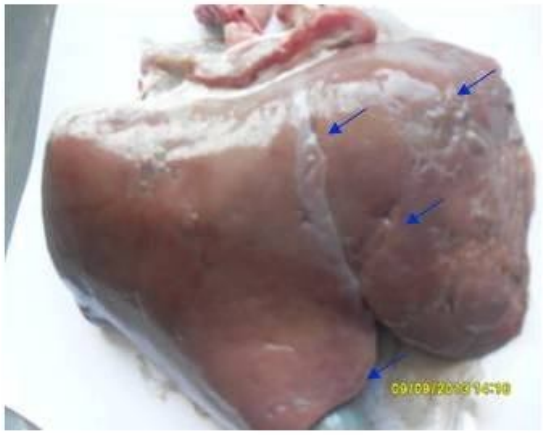


Figure D. Presence of hemorrhage, fibrin formation, irregular surface and rounded edge (parietal surface)

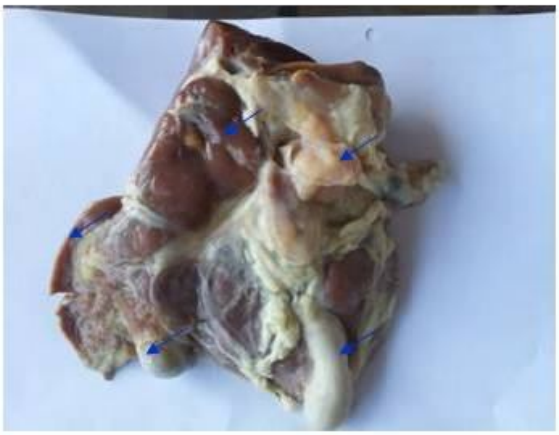


Figure E. Presence of fibrosis, hemorrhage, abscess, distended gallbladder and rounded edge (visceral)

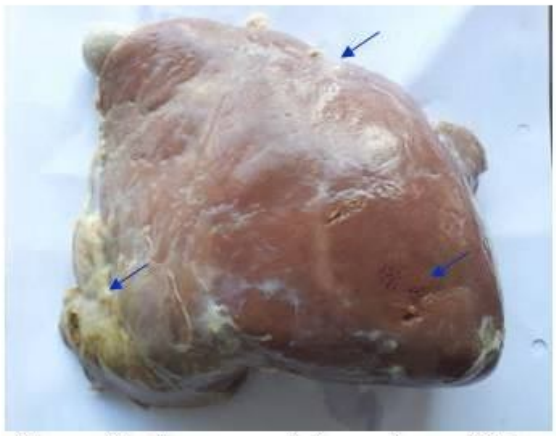


Figure F: Presence of hemorrhage, fibrin formation and rounded edge (parietal surface)

Figure 4.16 Gross examination of normal (A+B) and affected goat liver (C+F) in chronic case (indicated by arrow)

Figure A. Sectioned of goat liver affected by *Fasciola gigantica*

Figure B. Collected adult *Fasciola gigantica* from goat liver

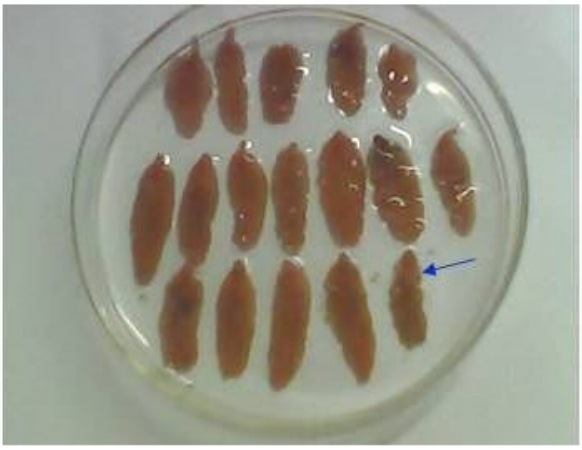


Figure C. Collected adult *Fasciola gigantica*

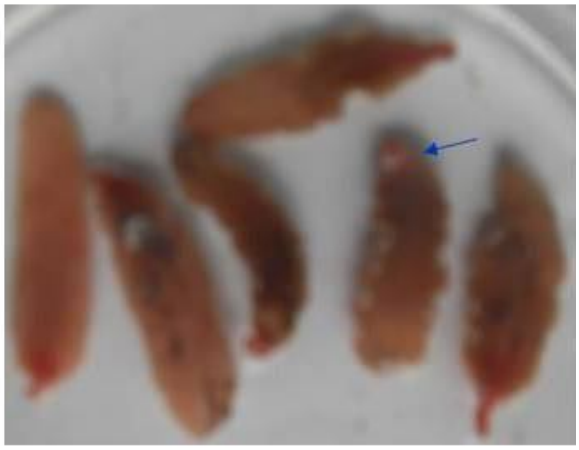


Figure D. Collected adult *Fasciola gigantica*

Figure 4.17 *Fasciola gigantica* collected from dissected goat liver

4.3.2 Histopathology

In acute form, the migrating tract produced little reaction in the host tissue and in the later stages they became surrounded by macrophages, eosinophils and granular tissue. In early stage, the flukes into the bile ducts resulted in proliferation of the epithelium producing a granular mucosa which was found in the present study. On the other hand, microscopically the wall of the bile ducts was thickened with fibrous tissue proliferation and the lining epithelium showed hyperplastic changes (Figure 4.18). Formation of new bile ducts, fatty changes, deposition of bile pigment and hemorrhage in the hepatic parenchyma and hepatic cord were seen (Figure 4.19). Infiltrations with numerous eosinophils mixed with few lymphocytes in the hepatic parenchyma were observed. Pyknotic nuclei and more acidophilic cytoplasm were observed in the hepatic

parenchyma known as coagulation necrosis due to chronic fascioliasis (Figure 4.20). The migratory tracts were represented by the presence of hemorrhagic, edema and infiltration with numerous eosinophils mixed with few lymphocytes, atrophy, necrosis, haemorrhage, fatty changes, blood vessel abnormalities, damage to portal tract area. Eosinophilic infiltration with few lymphocytes was more common in the migratory tract (Figure 4.21). The cross section of adult and immature *Fasciola gigantica* was found in the lumen of the thickened bile ducts and hepatic parenchyma respectively (Figure 4.21 and 4.22). In most advanced stages, these hyperplastic changes in some of the larger bile ducts or wall of bile ducts or gallbladder appeared as gland like structure and the dilated ducts produced pressure atrophy, necrosis and fatty changes of surrounding hepatocytes (Figure 4.18). Considerable proliferation of fibrous connective tissue was observed due to continuous irritation, infiltration of inflammatory cells and coagulation necrosis in the portal areas. There was little lymphocytic infiltration of fibrous strands. Portal veins were dilated and irregular in shape. Sub-capsular and hepatic parenchyma hemorrhage was also observed in the peripheral surface of the livers due to trauma. Hepatitis of goat liver characterized by presence of plasma cells, lymphocytes and there was also presence of hemosiderin pigment. Parasitic cholecystitis of a goat liver was characterized by parasite within gall bladder surrounded by fibrous connective tissue and infiltration of inflammatory cells. In this study, no calcification was observed in the wall of the bile ducts in chronic fascioliasis of goat.

The liver is one of the vital organ of the body. Gross and microscopic pathology caused by *Fasciola gigantica* might affect the health status of the animal. Hepatocytes are damaged to cause impairment in protein synthesis of the liver which may results edema (bottle jaw) and anemia. Hyperplasia may results obstruction of bile duct which is responsible for jaundice as well as impairment in the digestion and absorption of fatty acids. The liver damage may also cause poor growth rate, emaciation and cachectia as the proteins are inadequate in the animal body.

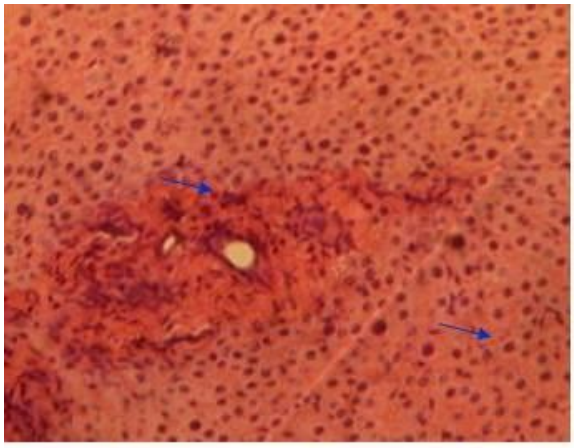


Figure A. Normal bile duct and hepatocytes of the hepatic parenchyma (10X)

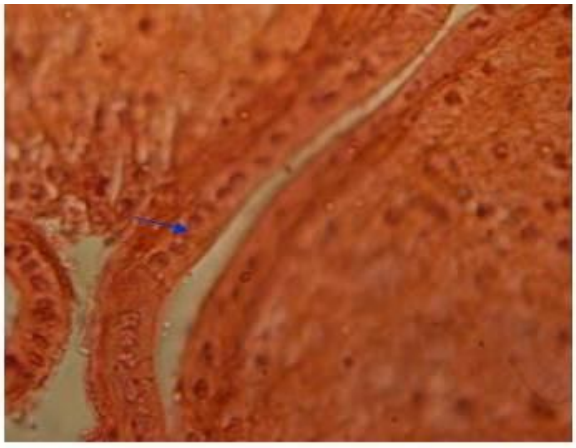


Figure B. Normal bile duct of the hepatic parenchyma (40X)

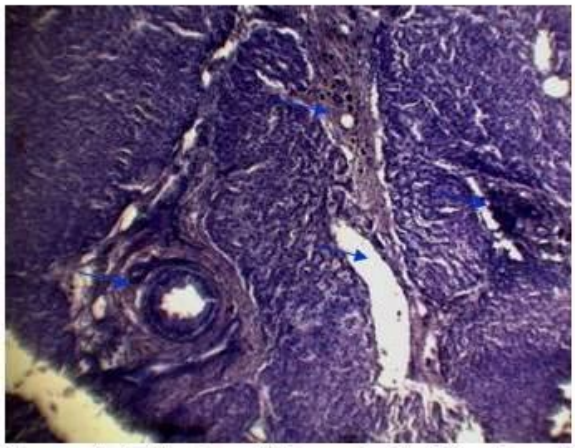


Figure C. Dilated and hyperplastic bile duct, bile pigment, fibrosis, fatty changes, loss and atrophy of hepatocytes of the hepatic parenchyma (10X)

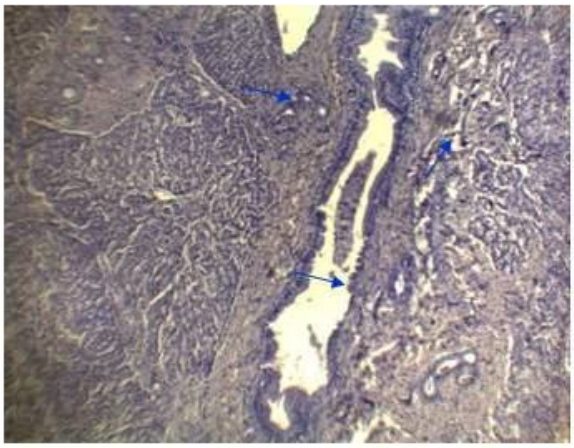


Figure D. Dilated and hyperplastic bile duct, fatty changes, fibrosis, loss and atrophy of hepatocytes of the hepatic parenchyma (10X)

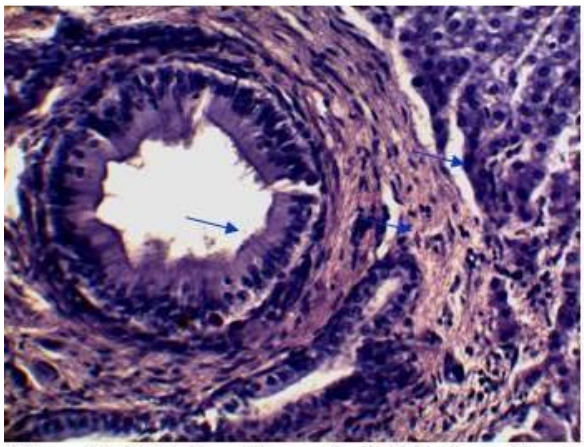


Figure E. Dilated and hyperplastic bile duct, fibrosis, loss and atrophy of hepatocytes of the hepatic parenchyma(40X)

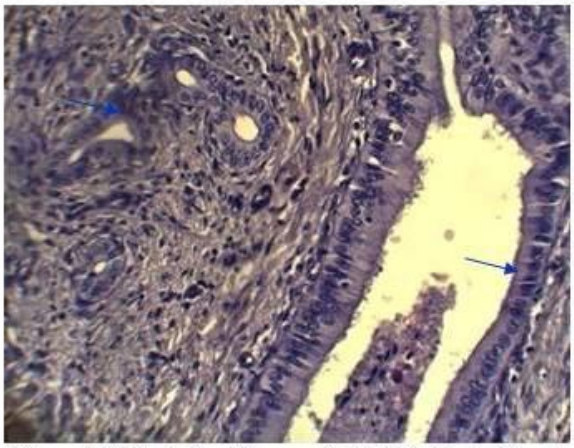


Figure F. Dilated and hyperplastic bile duct, fibrosis, loss and atrophy of hepatocytes of the hepatic parenchyma(40X)

Figure 4.18 Histological examination of normal (A+B) and affected goat liver (C+F) due to *Fasciola gigantica* (indicated by arrow)

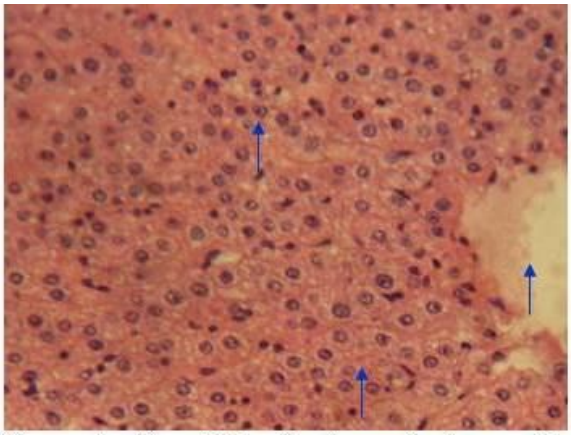


Figure A. Normal hepatocytes, cytoplasm and central vein of the hepatic parenchyma (40X)

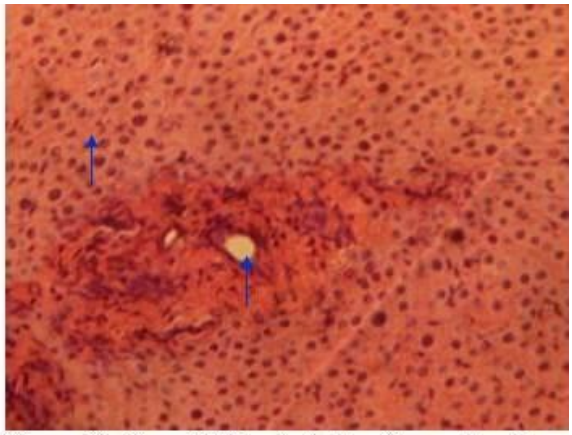


Figure B. Normal bile duct, hepatic cord and hepatocytes of the hepatic parenchyma (40X)

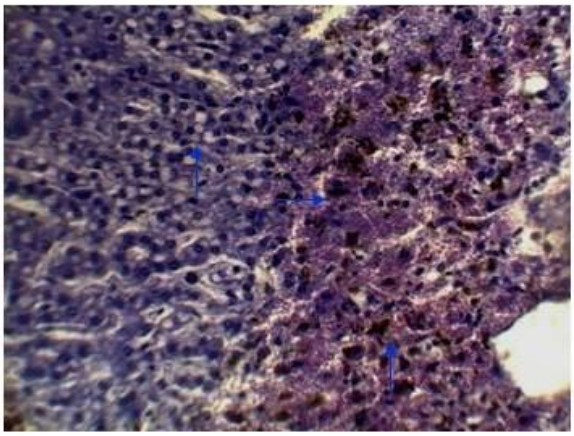


Figure C. Fatty change, hemorrhage and bile pigment of the hepatic parenchyma (40X)

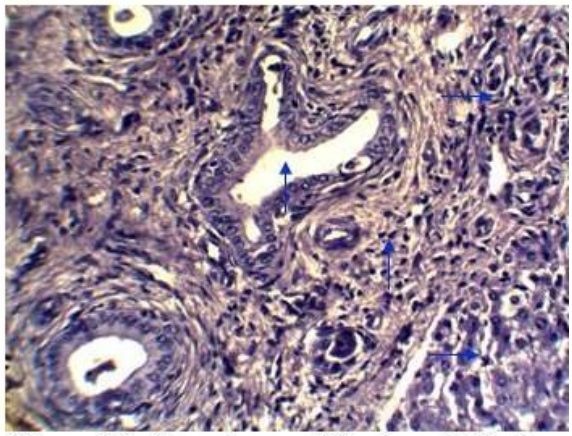


Figure D. Excessive proliferation, thickening ,dilated and hyperplasia of bile duct, atrophy of hepatocytes and infiltration of inflammatory cells of the hepatic parenchyma (40X)

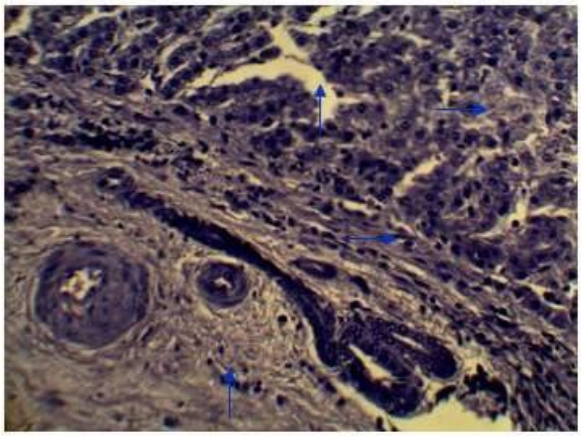


Figure E. Fibrosis, loss of hepatocytes, atrophy of hepatocytes, and infiltration of inflammatory cells of the hepatic parenchyma (40X)

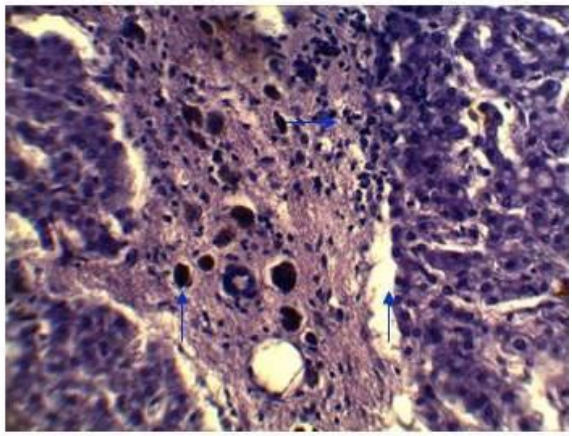


Figure F. Bile pigment within Fibrosis, loss and atrophy of hepatocytes, and infiltration of inflammatory cells of the hepatic parenchyma (40X)

Figure 4.19 Histological examination of normal (A+B) and affected goat liver (C+F) due to *Fasciola gigantica* (indicated by arrow)

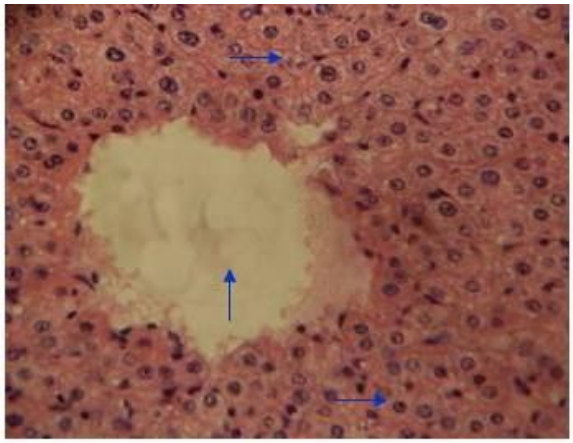


Figure A. Normal hepatocytes, cytoplasm and central vein of the hepatic parenchyma (40X)

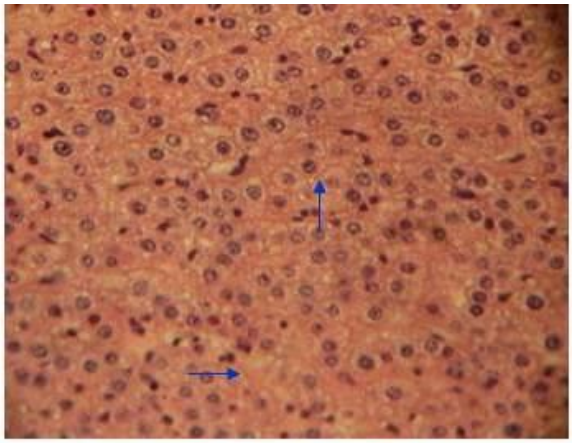


Figure B. Normal hepatocytes and cytoplasm of the hepatic parenchyma (40X)

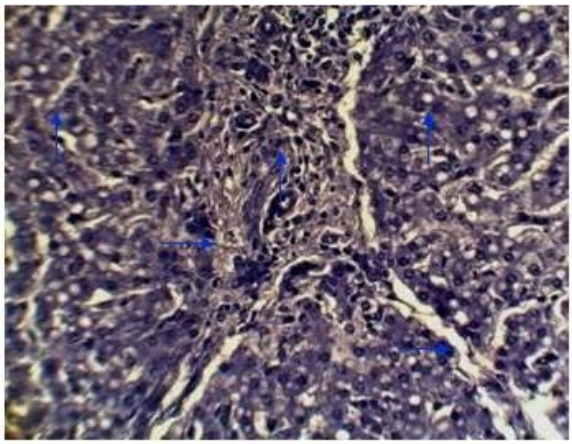


Figure C. Fatty changes, fibrosis of the hepatic cord, loss and atrophy of hepatocytes and infiltration of inflammatory cells of the hepatic parenchyma (40X).

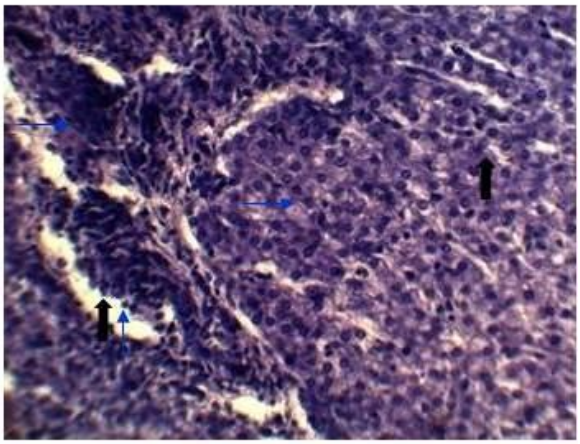


Figure D. Fatty changes, loss and atrophy of hepatocytes and infiltration of inflammatory cells of the hepatic parenchyma (40X).

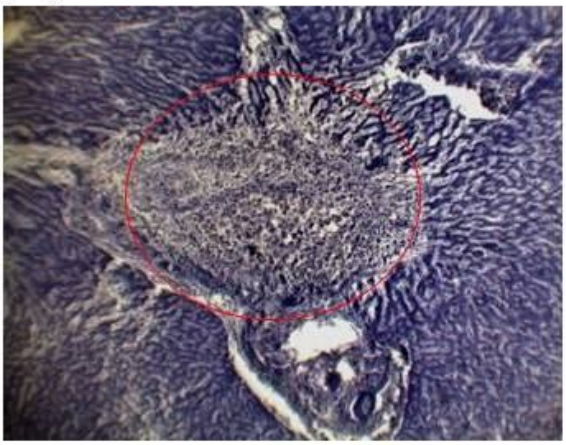


Figure E. Coagulation necrosis of the hepatic parenchyma (10X)

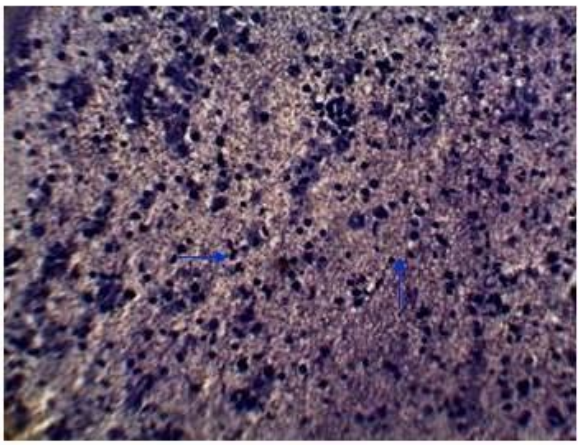


Figure F. Pyknotic nuclei and more acidophilic cytoplasm of hepatocytes of the lparenchyma (40X)

Figure 4.20 Histological examination of normal (A+B) and affected goat liver (C+F) due to *Fasciola gigantica* (indicated by arrow)

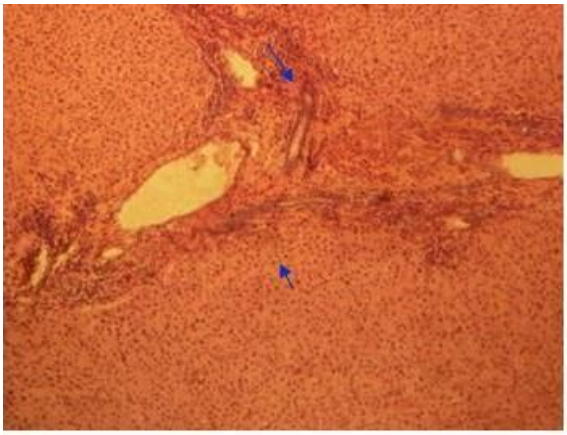


Figure A. Normal hepatocytes and hepatic cord within the hepatic parenchyma (4X)

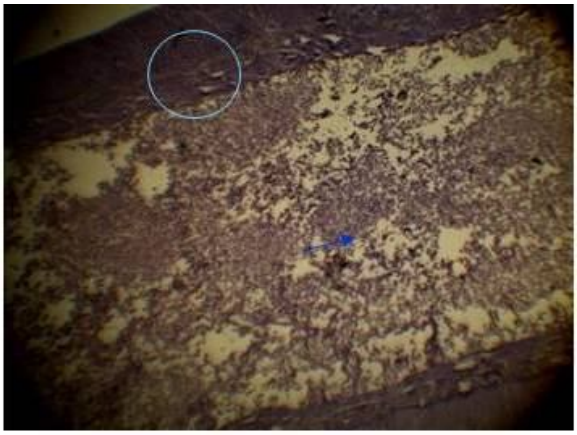


Figure B. Migratory tract, hemorrhage, tissue debris and loss of hepatocytes within the hepatic parenchyma (4X)

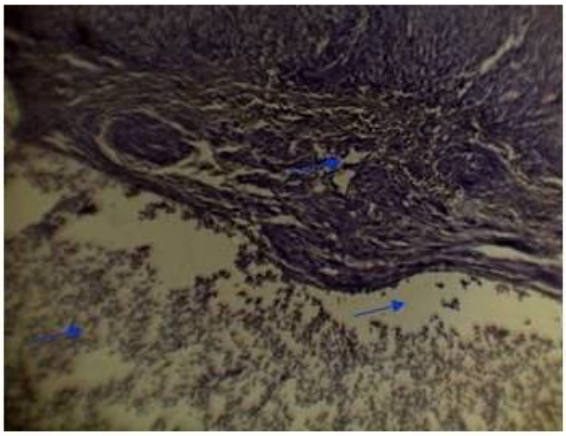


Figure C. Migratory tract contain hemorrhage, tissue debris and loss of hepatocytes from the hepatic parenchyma (10X)

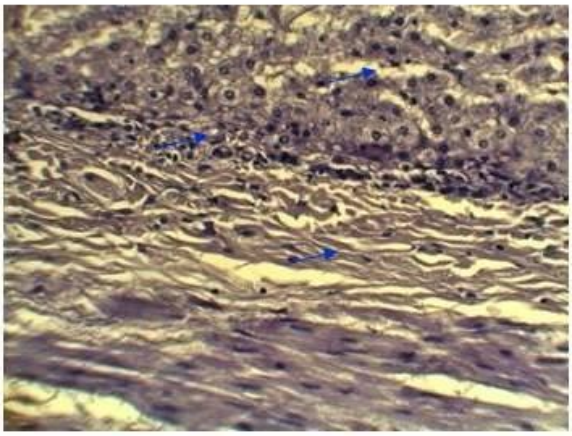


Figure D. Infiltration of inflammatory cells, atrophy of hepatocytes and fibrous tissue proliferation within the hepatic parenchyma (40X).

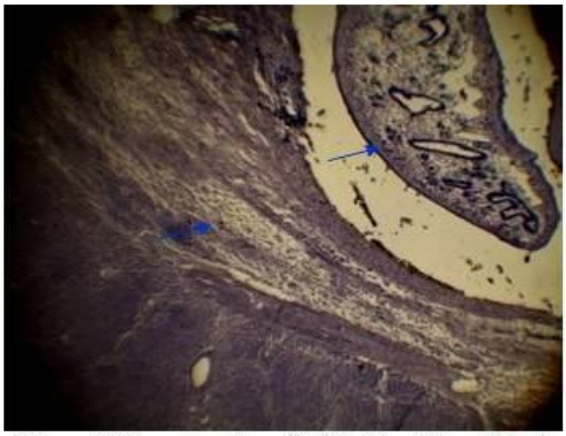


Figure E. Cross section of adult *Fasciola* within the hepatic parenchyma and loss of Hepatocytes of the hepatic parenchyma (10X)

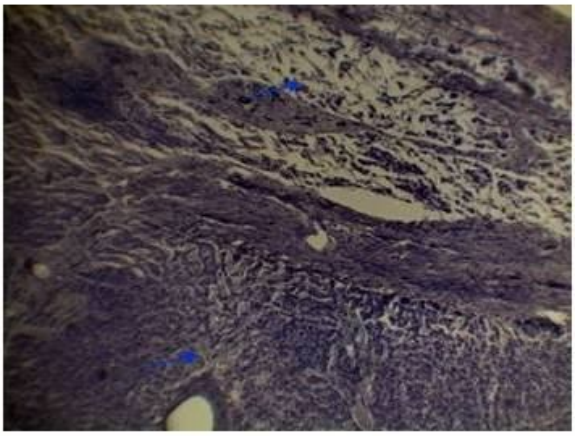


Figure F. Loss of hepatocytes from the hepatic parenchyma (10X)

Figure 4.21 Histological examination of normal (A) and affected goat liver (B-F) due to *Fasciola gigantica* (indicated by arrow)

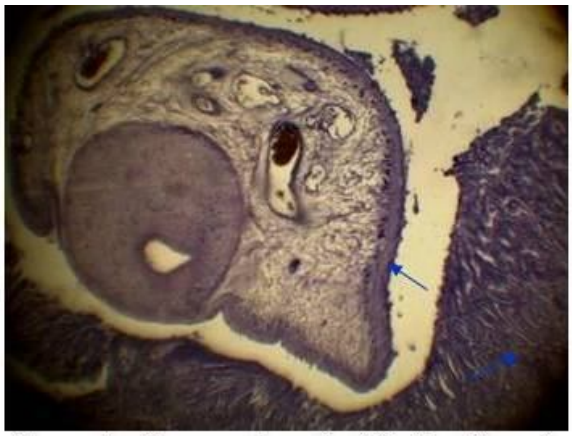


Figure A. Cross section of adult *Fasciola* and proliferation of glands within the bile duct/gall bladder (10X)

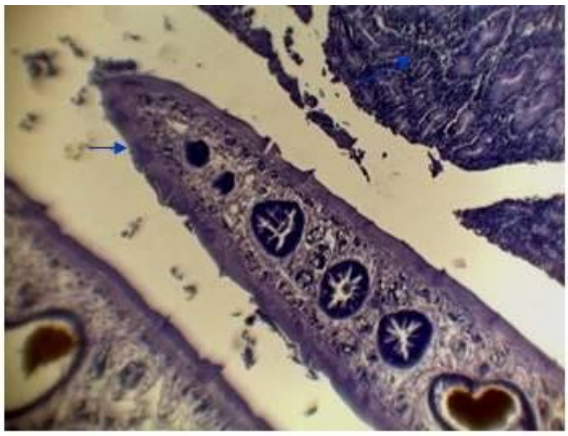


Figure B. Cross section of adult *Fasciola* and proliferation of glands within the bile duct/gall bladder (10X)

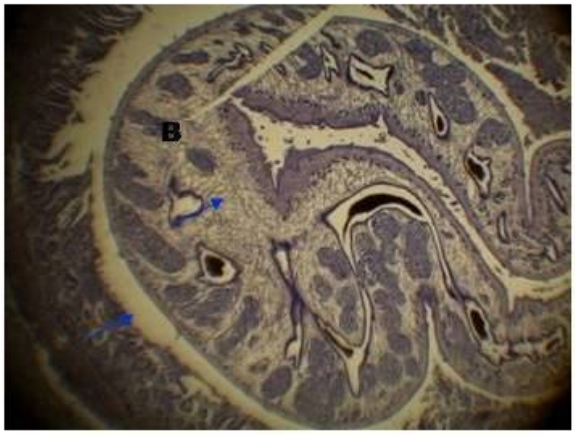


Figure C. Cross section of adult *Fasciola* within the bile duct/gall bladder and thickening of wall (10X)

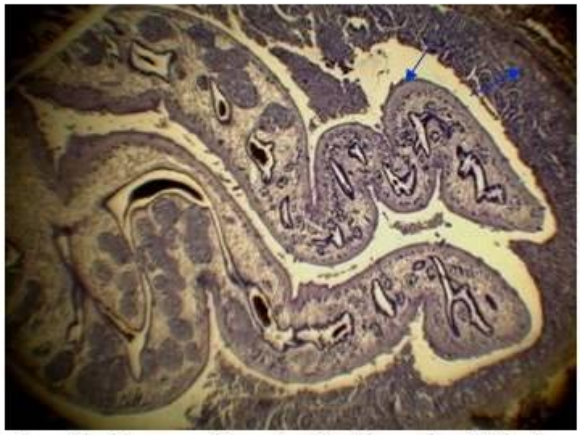


Figure D. Cross section of adult *Fasciola* within the bile duct/gall bladder and thickening of wall (10X)

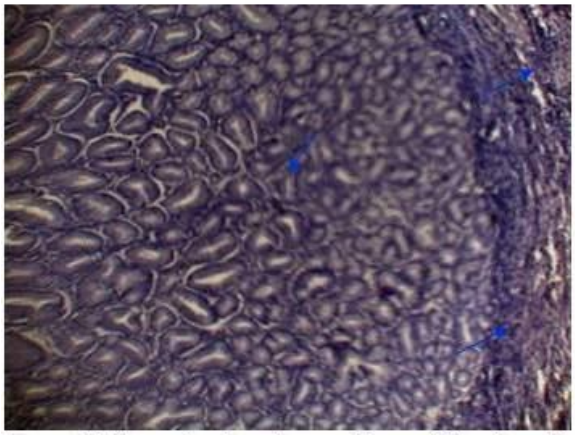


Figure E. Hyperplastic and excessive proliferation of glands, thickening of the wall of gall bladder (10X),

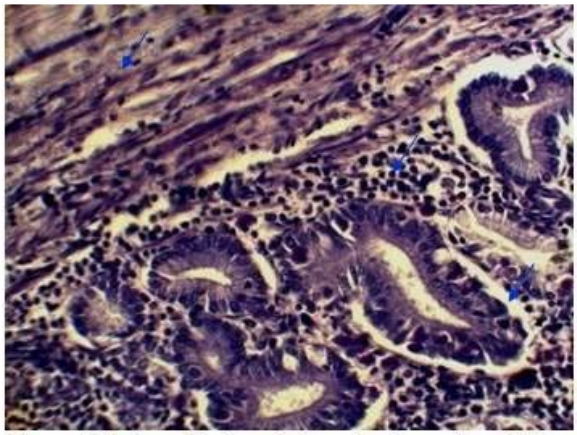


Figure F. Hyperplastic of the glands, eosinophilic infiltration and thickening of the wall of gall bladder due to proliferation of fibrous tissue (40X)

Figure 4.22 (A+F) Histopathological study of goat liver infected with *Fasciola gigantica* (indicated by arrow)

4.4 Determination of the efficacy of different types of liver tonic combined with anthelmintic and their effect on pathology of fascioliasis in goats

4.4.1 Counting of egg per gram (EPG) of feces

The average EPG load (*Fasciola* eggs-Figure 4.26) of goats was 0 for T_0, 740 for T_i, 860 for T_1, 830 for T_2, 570 for T_3, 690 for T_4 and 1000 for T_5 before the trial. The results showed that the level of EPG in feces was highly decrease in the groups treated with anthelmintic (Triclabendazole-TCBZ) combined with liver tonic (Hepamine forte). EPG in feces was decreasing from 7th day and reached to zero on 21st days of the treatment). The groups treated TCBZ combined liver tonic (Liva vit) also showed decrease EPG in feces at 7th day and reached to zero EPG in feces on 28th day of infection. A significant average reduction percentage in EPG in feces was observed from day 7th through day 28th in the treatment groups following treatments. The reduction rate was statistically identical between the treatment groups at different observational periods (P<0.001). Conversely, in the control group of infection, the EPG in feces load was sharply increased in only liver tonic treatment groups (Table 4.11 and Figure 4.23).

Table 4.11 Egg per gram (EPG) of feces in experimental goats

Group	EPG-(Mean±SE)				
	Day 0	Day 7	Day 14	Day 21	DAY 28
T_0	00^{c}	00^{c}	00^{c}	00^{d}	00^{d}
T_i	740^{ab}±193.91	970^{a}±172.19	1140^{a}±142.65	1280^{a}±127.80	1380^{a}±88.88
T_1	860^{ab}±108.86	610^{ab}±112.25	330^{c}±93.00	180^{d}±68.19	80^{d}±51.48
T_2	830^{ab}±78.42	910^{a}±76.49	960^{a}±48.48	1030^{b}±51.48	1040^{b}±43.01
T_3	570^{b}±80.00	580^{ab}±60.42	660^{b}±67.82	680^{c}±66.33	750^{c}±74.16
T_4	690^{ab}±181.93	440^{b}±166.88	280^{c}±118.95	170^{d}±80.00	60^{d}±40.00
T_5	1000^{a}±50.00	620^{ab}±48.99	320^{c}±66.33	00^{d}	00^{d}

a, b, c, d: In a column among control and treatment groups with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99.99% level of significance (p<0.001),***Significant differences in different days and groups p<0.001.

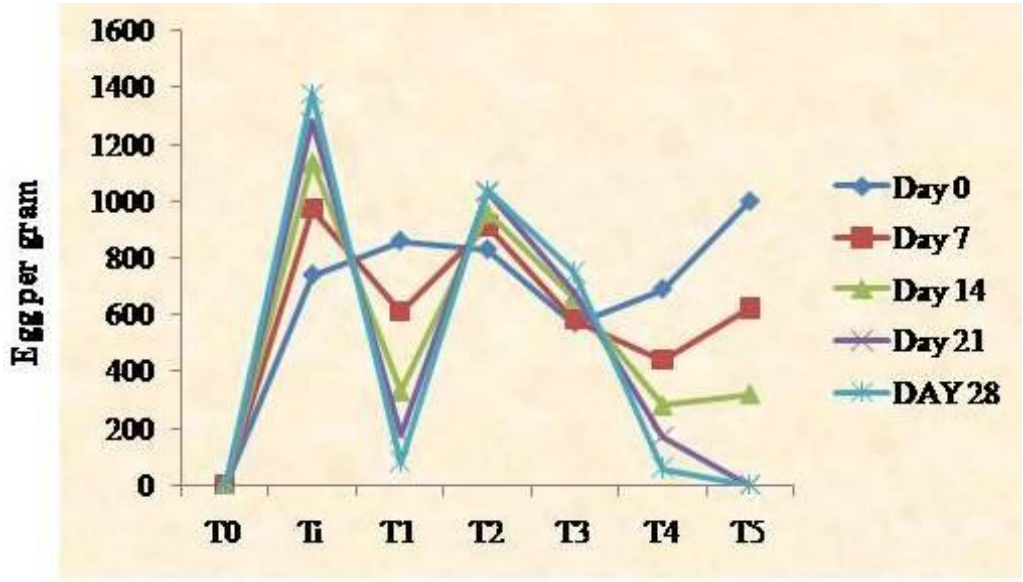


Figure 4.23 Egg per gram (EPG) of feces from the experimental goats

4.4.2 Determination of hematological parameters

Hematological parameters were improved significantly with the anthelmintic along with liver tonics and then only anthelmintic treatment groups. The PCV, Hb, TEC and TLC levels were significantly varied between anthelmintic (triclabendazole-TCBZ) or anthelmintic along with liver tonics and infected control groups. Another things, only liver tonics treatment and infected control groups were equal in these levels. In the infected control group, the TLC sharply increased and PCV, Hb and TEC decreased during the experimental period. Analysis among the results of the treatment groups at day 28th showed a significant difference in mean PCV, Hb, TEC and TLC values ($P < 0.001$). But detected significantly higher values in the treated groups compared to the positive control (T_i).

4.4.2.1 Hematocrit or Packed cell volume (PCV)

The results of PCV at different days, showed that, treatments TCZB with Hepamine forte® improved the PCV increased from 18.60 on day zero and reached to 29.40 on the 28th day. The TCBZ with Liva-vit® also PCV increased from 19.00 on day zero and reached to 25.40 on the day 28th. Only TCBZ treated group was increased PCV from 18.40 to 23.80 on the day of 28th of treatment. The infected control groups showed detectable decline in PCV at the end of the experiment and were significantly decreased

level of PCV compared to the non infected control and other groups (Table 4.12 and Figure 4.24).

Table 4.12 Packed cell volume (PCV) level in blood of the experimental goats

Group	PCV (%)-(Mean±SE)				
	Day 0	Day 7	Day 14	Day 21	DAY 28
T_0	28.60^{a}±0.75	29.20^{a}±0.58	29.20^{a}±0.58	29.80^{a}±0.58	29.80^{a}±0.37
T_i	18.20^{b}±0.66	17.60^{e}±0.51	17.00^{e}±0.32	16.00^{f}±0.32	14.80^{f}±0.37
T_1	18.40^{b}±0.51	20.60^{cd}±0.60	21.60^{d}±0.51	23.40^{c}±0.51	23.80^{c}±0.66
T_2	18.80^{b}±0.58	18.00^{e}±0.63	18.00^{e}±0.00	18.60^{e}±0.87	18.20^{e}±0.66
T_3	20.00^{b}±0.45	20.20^{d}±0.37	21.60^{d}±0.40	21.20^{d}±0.49	21.20^{d}±0.49
T_4	19.00^{b}±0.45	22.00^{bc}±0.45	23.20^{c}±0.58	25.20^{b}±0.58	25.40^{b}±0.40
T_5	18.60^{b}±0.75	23.40^{b}±0.68	25.60^{b}±0.75	29.00^{a}±0.71	29.40^{a}±0.68

a, b, c, d, e. f: In a column among control and treatment groups with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99.99% level of significance ($p<0.001$),***Significant differences in different days and groups $p<0.001$.

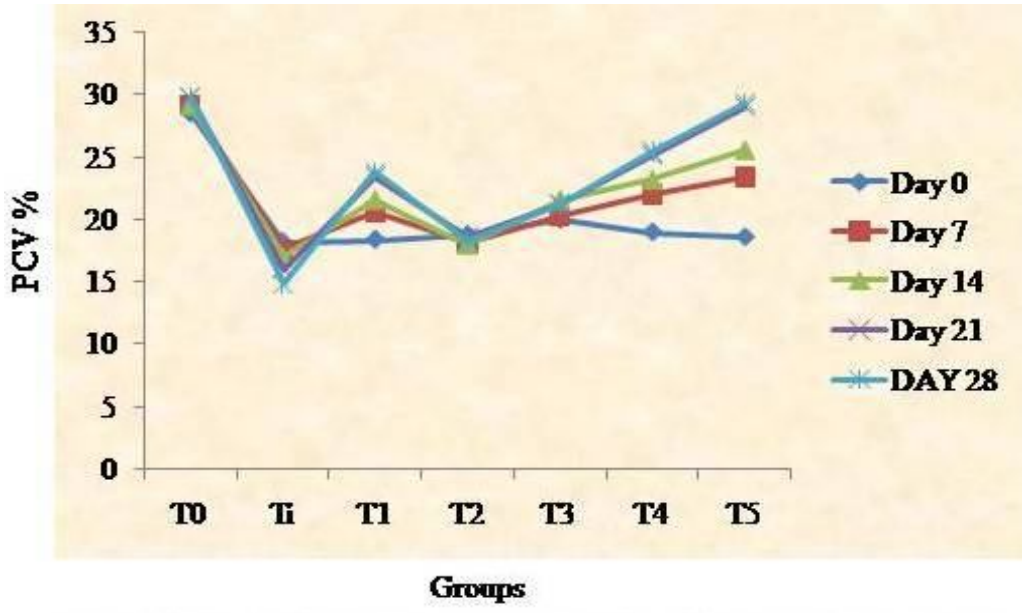


Figure 4.24 Packed cell volume (PCV) level in blood of the experimental goats

4.4.2.2 Estimation of hemoglobin concentration (Hb)

Our results indicated that, the treatment including TCBZ with Hepamine forte® improved the Hb concentration from 6.12 on day zero and reached to 8.52 on the 28th day. The TCBZ and Liva-vit® treatment increased Hb concentration from 6.60 on day zero and reached to 8.16 at the end of the experiment, while only TCBZ treated group increased Hb concentration from 6.44 on day zero and reached to 7.88 on the 28th day (Table 4.13 and Figure 4.25).

Table 4.13 Hemoglobin (Hb) concentration in blood of the experimental goats

Group	Hb (g%)-(Mean±SE)				
	Day 0	Day 7	Day 14	Day 21	DAY 28
T_0	9.32^{a}±0.19	9.56^{a}±0.17	9.72^{a}±0.15	9.92^{a}±0.10	10.04^{a}±0.12
T_i	6.44^{bc}±0.26	6.08^{d}±0.22	5.76^{d}±0.19	5.52^{e}±0.16	5.08^{f}±0.14
T_1	6.44^{bc}±0.21	6.88^{bc}±0.24	7.44^{b}0.23	7.68^{b}±0.19	7.88^{c}±0.19
T_2	6.36^{bc}±0.07	6.28^{cd}±0.16	6.20^{cd}±0.06	6.16^{d}±0.13	6.12^{e}±0.10
T_3	6.72^{c}±0.14	6.76^{bc}±0.18	6.64^{c}±0.15	6.80^{c}±0.17	6.68^{d}±0.16
T_4	6.60^{bc}±0.21	7.16^{b}±0.23	7.60^{b}±0.21	7.80^{b}±0.23	8.16^{b}±0.21
T_5	6.12^{b}±0.10	6.88^{bc}±0.10	7.48^{b}±0.14	7.92^{b}±0.10	8.52^{b}±0.10

a, b, c, d, e. f: In a column among control and treatment groups with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99.99% level of significance ($p<0.001$),***Significant differences in different days and groups $p<0.001$.

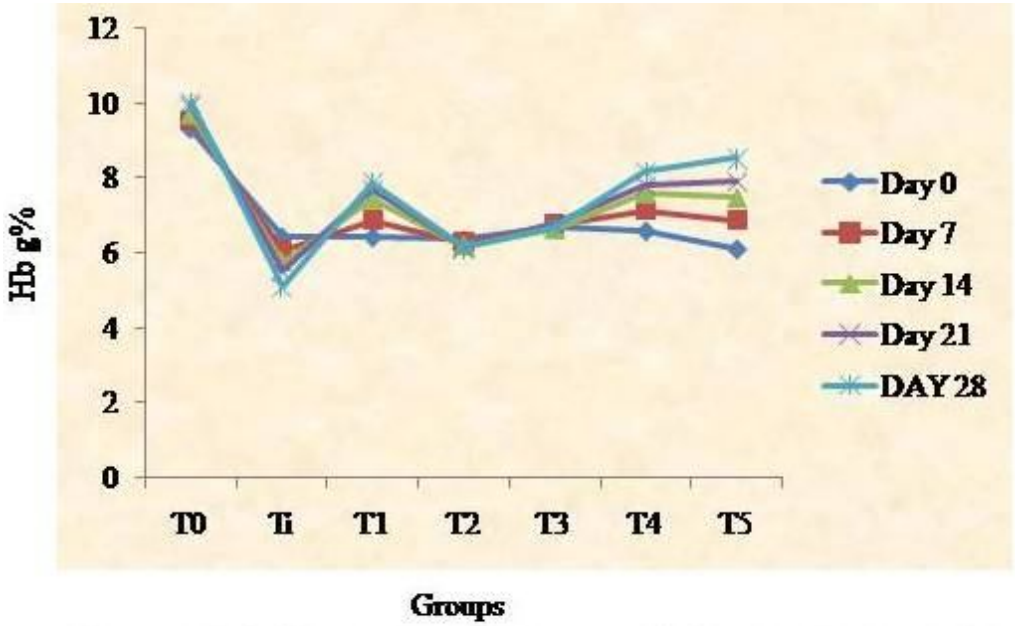


Figure 4.25 Hemoglobin (Hb) concentration in blood of the experimental goats

4.4.2.3. Total erythrocyte count (TEC)

The results were showed that the most efficient treatments of fascioliasis in goats that improved the TEC level TCBZ along with Hepamine forte® increased TEC from 6.67 on day zero and reached to 12.40 on the 28th day. The TCBZ with Liva-vit® treatment increased TEC level from 7.34 on day zero and reached to 10.08 on the 28th day. Another thing only TCBZ treated group increased the level of TEC from 7.19 on day zero and reached to 9.94 on the 28th day. The results also indicated that the control infected non-treated of lower TEC count than the control non-infected and other treated groups (Table 4.14 and Figure 4.26).

Table 4.14 Total erythrocyte count (TEC) level in blood of the experimental goats

Group	TEC(million/cu mm)-(Mean±SE)				
	Day 0	Day 7	Day 14	Day 21	DAY 28
T_0	11.48^{a}±0.28	11.82^{a}±0.26	12.05^{a}±0.22	12.36^{a}±0.17	12.47^{a}±0.18
T_i	7.16^{b}±0.38	6.64^{e}±0.34	6.17^{e}±0.30	5.88^{e}±0.26	5.54^{e}±0.24
T_1	7.19^{b}±0.32	7.88^{cd}±0.39	8.61^{c} ±0.19	9.51^{b}±0.23	9.94^{b}±0.14
T_2	6.86^{b}±0.32	7.12^{de}±0.38	7.21^{d} ±0.34	7.11^{d}±0.23	7.26^{d} ±0.14
T_3	7.33^{b}±0.37	8.46^{c}±0.24	8.49^{c} ±0.25	8.81^{c}±0.19	8.99^{c}±0.23
T_4	7.34^{b}±0.30	8.19^{c}±0.29	8.73^{c}±0.28	9.56^{b}±0.24	10.08^{b}±0.33
T_5	6.67^{b}±0.16	10.15^{b}±0.24	10.69^{b}±0.28	11.82^{a}±0.22	12.40^{a}±0.28

a, b, c, d, e: In a column among control and treatment groups with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99.99% level of significance ($p<0.001$),***Significant differences in different days and groups $p<0.001$.

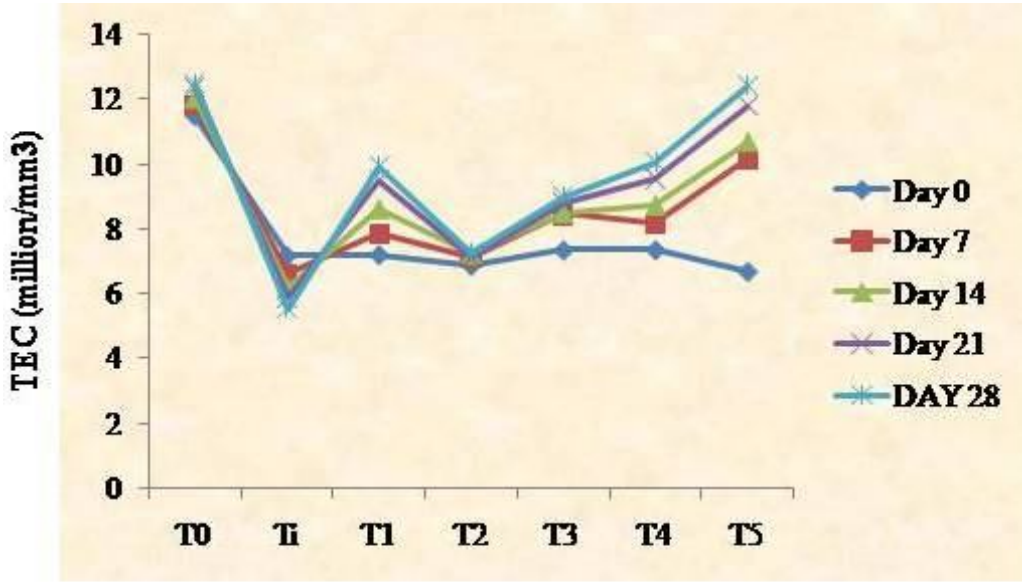


Figure 4.26 Total erythrocyte count (TEC) level in blood of the experimental goat

4.4.2.4 Total leukocyte count (TLC)

The results indicated that, the most efficient treatment of fascioliasis in goats that improve the TLC level was TCBZ along with Hepamine forte®. TCBZ with Liva vit® and only TCBZ groups were improved the TLC level compared to others treatment. There was a significant ($p<0.001$) rise in the levels of TLC level among the infected control group increased TLC from 7.76 on day zero and reached to 10.40 on the 28th day of the experiment than non infected control group increased TLC from 5.81 on day zero and reached to 6.50 on the 28th day of the experiment (Table 4.15 and Figure 4.27).

Table 4.15 Total leukocyte count (TLC) level in blood of the experimental goats

Group	TLC (thousand/cu mm)-(Mean±SE)				
	Day 0	Day 7	Day 14	Day 21	DAY 28
T_0	5.81^{e}±0.32	6.10^{c}±0.29	6.21^{e}±0.26	6.38^{d}±0.23	6.50^{d}±0.21
T_i	7.76^{d}±0.16	8.30^{ab}±0.17	9.04^{a}±0.21	10.11^{a}±0.25	10.40^{a}±0.27
T_1	8.74^{bc}±0.27	7.63^{b}±0.29	7.34^{d}±0.20	6.19^{d}±0.17	6.00^{de}±0.20
T_2	8.43^{cd}±0.46	8.82^{a}±0.42	8.90^{ab}±0.42	9.06^{b}±0.24	8.58^{b}±0.39
T_3	8.84^{d}±0.11	8.34^{ab}±0.25	8.42^{abc}±0.17	8.30^{c}±0.21	7.98^{bc}±0.08
T_4	9.57^{ab}±0.03	8.52^{a}±0.08	8.23^{bc}±0.09	7.82^{c}±0.18	7.60^{c}±0.16
T_5	10.05^{a}±0.36	8.03^{ab}±0.28	7.67^{cd}±0.26	5.86^{d}±0.20	5.60^{e}±0.17

a, b, c, d, e: In a column among control and treatment groups with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99.99% level of significance ($p<0.001$),***Significant differences in different days and groups $p<0.001$.

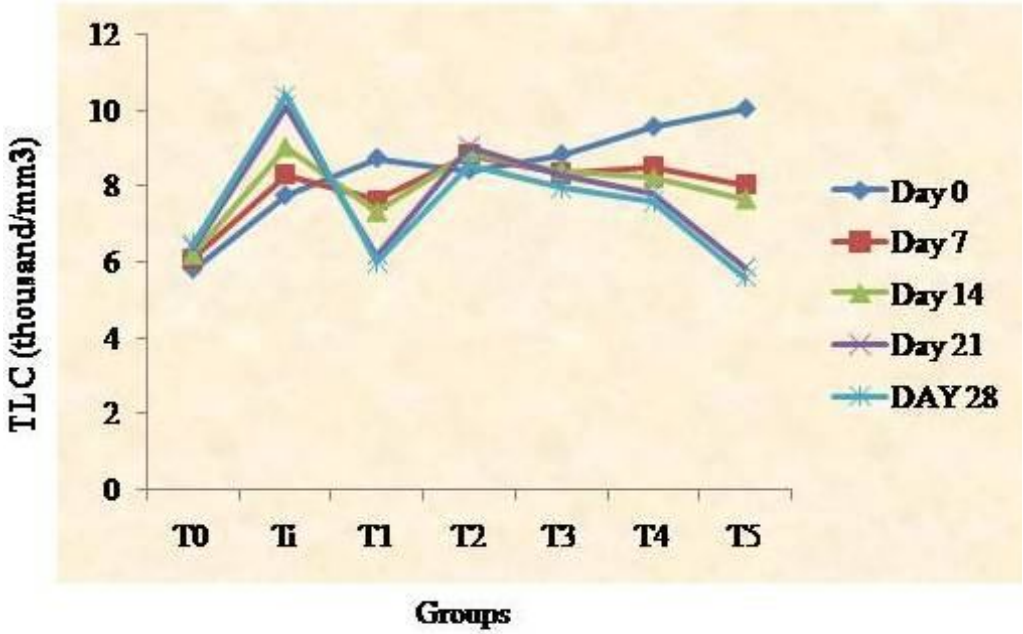


Figure 4.27 Total leukocyte count (TLC) level in blood of the experimental goats

4.4.3. Biochemical parameters

The levels of biochemical parameters (AST, ALT and ALP) varied significantly at different observational periods within the individual control and treatment groups ($P<0.001$). The post hoc test suggested that these values decreased significantly on days 7^{th}, 14^{th}, 21^{st} and 28^{th} compared to day 0 ($P<0.001$). The values of biochemical parameters were significantly lower in the treatment groups than in the positive control group which suggests the removal of *Fasciola gigantica* from the affected liver and regeneration of liver parenchyma. T_1, T_4 and T_5 were also showed the same values of biochemical parameters. This might be suggested that liver tonic alone has no significant effect on improvement of biochemical parameters in *Fasciola* affected liver.

4.4.3.1 Aspartate transaminase (AST)

Our results indicated that, the most efficient treatments of infection of *Fasciola* in goats that improve the AST levels was TCBZ along with Hepamine forte®. TCBZ with Liva vit® and only TCBZ groups were also improved the AST levels compared to others treatment. There was a significant ($p<0.001$) rise in the levels of AST among the infected control group AST increased from 41.20 on day zero and reached to 104.40 on the 28th day of the experiment than non infected control group AST increased from 3.60 on day zero and reached to 6.00 on the 28th day of the experiment. In T_4 and T_5 groups the AST decreased sharply (Table 4.16 and Figure 4.28).

Table 4.16 Aspartate transaminase (AST) concentration in serum of the experimental goats

Group	AST(U/L)-(Mean±SE)				
	Day 0	Day 7	Day 14	Day 21	DAY 28
T_0	3.60^{d}±0.40	3.80^{d} ±0.37	5.00^{e} ±0.32	6.00^{d}±0.32	6.00^{d} ±0.45
T_i	41.20^{c} ±2.52	51.80^{ac} ±3.81	69.40^{a} ±2.46	87.00^{a} ±4.44	104.40^{a}±4.97
T_1	55.20^{b} ±4.00	47.60^{b} ±3.76	14.00^{d}±1.52	8.60^{d} ±1.08	7.40^{d}±0.51
T_2	65.00^{ab} ±3.86	63.60^{a} ±3.78	63.40^{a}±3.59	63.40^{b} ±3.59	58.40^{b}±3.37
T_3	65.60^{ab} ±3.50	62.60^{a} ±3.06	46.40^{b}±3.30	44.00^{c} ±3.27	43.20^{c}±2.99
T_4	65.20^{ab} ±7.64	56.80^{ab} ±7.56	29.00^{c} ±3.78	13.80^{d}±1.83	8.80^{d}±0.75
T_5	74.60^{a} ±2.54	63.40^{a} ±2.29	14.20^{d} ±1.56	6.20^{d}±0.58	6.00^{d}±0.32

a, b, c, d, e: In a column among control and treatment groups with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99.99% level of significance ($p<0.001$),***Significant differences in different days and groups $p<0.001$.

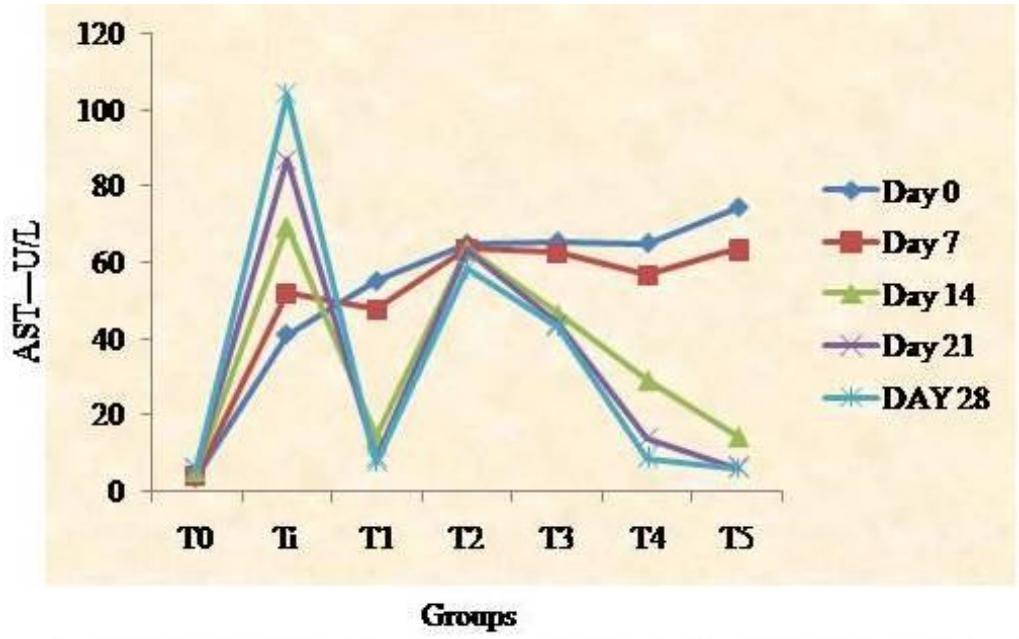


Figure 4.28 Aspartate transaminase (AST) concentration in serum of the experimental goats

4.4.3.2 Alanine transaminase (ALT)

The results of ALT at different days showed that, the most efficient treatments on ALT was TCBZ along with Hepamine forte®. TCBZ with Liva vit® and only TCBZ groups were also improved the ALT compare to others. Elevated level of ALT, as an indicator of epithelial damage in the bile ducts, began to rise significantly ($p<0.001$) in the levels of ALT among the infected control group ALT increased from 27.20 on day zero and reached to 35.0 on the end of the experiment. On the other hand in the non infected controls group ALT increased from 9.40 on day zero and reached to 16.40 on the end of the experiment. In T_4 and T_5 groups the ALT decreased sharply (Table 4.17 and Figure 4.29).

Table 4.17 Alanine transaminase (ALT) concentration in serum of the experimental goat

Group	ALT(U/L)-(Mean±SE)				
	Day 0	Day 7	Day 14	Day 21	DAY 28
T_0	9.40^{c}±0.51	12.00^{c}±0.95	15.80^{b}±0.73	12.20^{d}±0.66	16.40^{ab}±0.93
T_i	27.20^{b}±2.0	30.80^{a}±1.74	32.40^{a}±2.80	36.20^{a}±1.85	35.00^{c}±3.23
T_1	26.60^{b}±1.81	14.80^{c}±1.32	13.80^{b}±1.24	13.20^{cd}±0.20	15.60^{b}±0.75
T_2	26.80^{b}±0.86	22.80^{b}±1.28	22.40^{c}±1.29	21.60^{b}±1.22	20.80^{a}±0.80
T_3	26.20^{b}±0.970	20.00^{b}±0.69	18.20^{b}±0.97	17.80^{b}±0.74	17.40^{ab}±0.75
T_4	32.00^{a}±1.34	13.00^{c}±0.32	16.40^{b}±1.08	16.20^{bc}±0.58	15.40^{b}±0.60
T_5	31.40^{a}±1.44	15.60^{c}±2.62	15.00^{b}±0.55	14.00^{cd}±1.41	14.80^{b}±2.27

a, b, c, d: In a column among control and treatment groups with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99.99% level of significance ($p<0.001$),***Significant differences in different days and groups $p<0.001$.

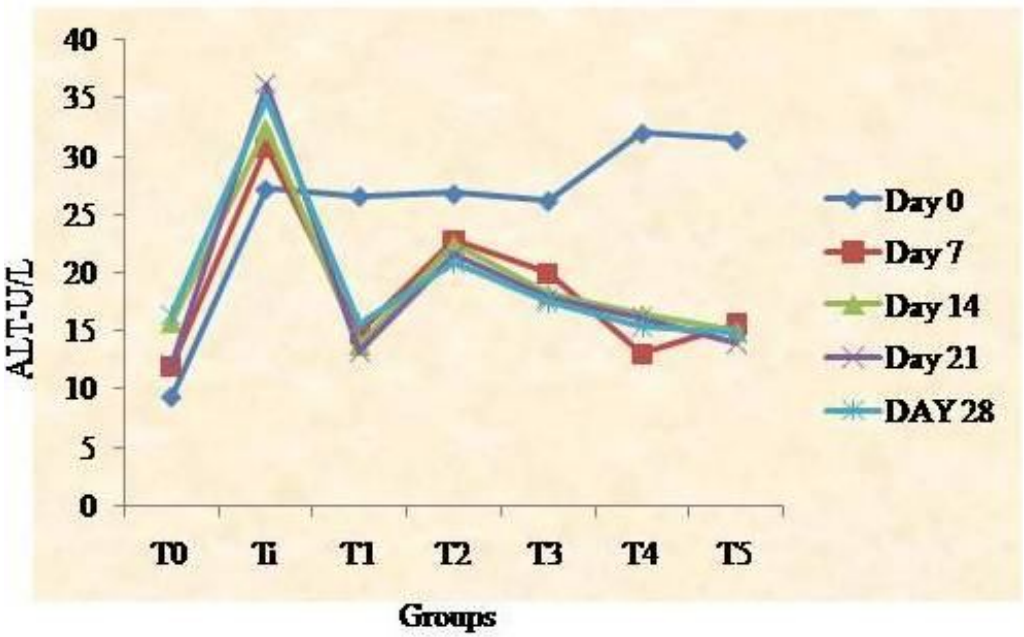


Figure 4.29 Alanine transaminase (ALT) concentration in serum of the experimental goat

4.4.3.3 Alkaline phosphatase (ALP)

The results were showed that the most efficient treatments of fascioliasis in goats that improve the ALP level was TCBZ along with Hepamine forte®. TCBZ with Liva vit® and only TCBZ groups only were also improved the ALP level compare to others treatment. There was a significant ($p<0.001$) rise in the levels of ALP in the infected control group, ALP increased from 243.40 on day zero day and reached to 535.80 on 28^{th} day of the experiment. Whereas in non infected control group ALP increased from 82.0 on day zero day and reached to 86.80 on 28^{th} day of the experiment. On the other hand T_4 and T_5 groups the level of ALP reduced a great extent (Table 4.18 and Figure 4.30).

Table 4.18 Alkaline phosphatase (ALP) concentration in serum of the experimental goat

Group	ALP(U/L)-(Mean±SE)				
	Day 0	Day 7	Day 14	Day 21	DAY 28
T_0	82.00^{c}±5.89	88.60^{d}±4.41	95.40^{e}±3.20	86.20^{d}±5.81	86.80^{c} 4.54
T_i	243.40^{b}±22.99	280.40^{ab}±24.37	352.80^{a}±23.91	379.80^{a}±14.54	535.80^{a}±23.76
T_1	252.40^{b}±14.56	162.40^{c}±12.32	143.40^{de}±11.04	129.00^{cd}±10.01	86.80^{c}±10.88
T_2	288.40^{ab}±38.29	287.60^{ab}±38.19	248.40^{bc} ±32.59	244.40^{b}±32.30	235.00^{b}±31.14
T_3	300.20^{ab}±38.74	260.60^{ab}±37.65	273.00^{b}±29.24	264.60^{b}±28.72	256.80^{b}±29.85
T_4	343.00^{a}±21.15	317.20^{a}±19.11	267.40^{b}±10.88	249.00^{b}±14.07	196.40^{b}±28.15
T_5	343.00^{a}±21.15	227.00^{c} 17.63	197.00^{cd}±17.11	173.00^{c}±17.48	76.40^{c}±7.49

a, b, c, d, e: In a column among control and treatment groups with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99.99% level of significance ($p<0.001$),***Significant differences in different days and groups $p<0.001$.

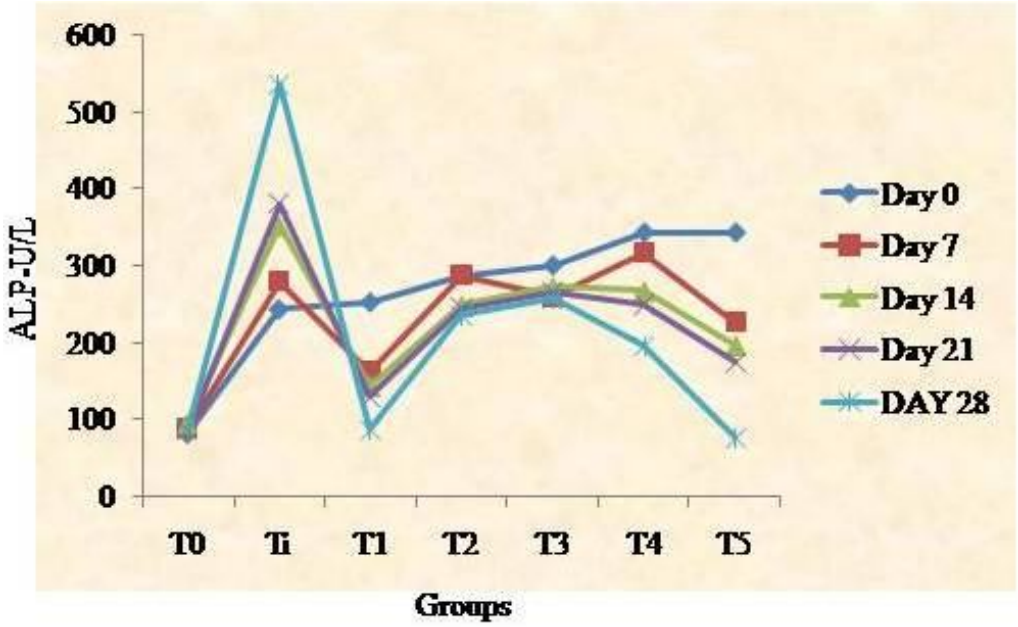


Figure 4.30 Alkaline phosphatase (ALP) concentration in serum of the experimental goat

CHAPTER-V

DISCUSSION

Fascioliasis is widespread ruminant health problems and causes significant economic losses to the livestock industry in Bangladesh. In the present study, irrespective of the seasons and irrigation status of the grazing lands, the high infection prevalence of fascioliasis was recorded in the Sylhet region of Bangladesh. The findings, therefore, strongly suggest that the climatic factors in the Sylhet region is more favorable for the propagation and activity of the snail intermediate hosts and progression of the parasitic life cycle in most part of the year. The overall prevalence was significantly high ($p<0.001$). This indicated the irrigation may help to maintain optimal wetness required for the development of both the snail intermediate host and intra-mollsucian parasite phases. The availability of water, which is the most important enhancing factor, was responsible for the increase seasonal prevalence of fascioliasis. Similar observations and assertions were previously made by Richter *et al., (*1999). The presence of adequate moisture from irrigated channels in these areas created favorable condition needed for the progression of the lifecycle, survival and multiplication of the snail intermediate hosts and consequently attributed to the increased fascioliasis prevalence.

In domestic ruminants, the adverse effect of acute or chronic fascioliasis includes decreased weigh gain and milk production, decreased female fertility, work power and mortality. Hepatic pathology, even when only limited areas of the liver are damaged, results in significant disturbances in mitochondrial bioenergetic metabolism of carbohydrates, proteins, lipids and steroids, as well as bile flow and bile composition (Calléja *et al.,* 2000). Sheep and goat are very susceptible to acute fascioliasis and the damage results from the immature flukes tunneling through the liver parenchyma with extensive tissue damage and hemorrhage that culminate in severe clinical disease and high mortality in the grazing sheep in Africa (Okewleo, 2000). During the movement of the immature stages of *Fasciola sp,* which may continue for months, symptoms may include abdominal pain, enlarged liver, fever, and diarrhea. Mitchell (2001) indicated that the pathology associated with diseases are caused by the inflammation of the bile ducts which causes thickening of the lining and eventually leads to fibrosis that results in reduced flow of the bile and back pressure builds leading to atrophy of the liver

parenchyma and cirrhosis. Occasionally the flukes penetrate the wall of bile duct into the liver parenchyma causing liver abscesses.

Control of fascioliasis is based on good grazing land management practices that destroy the intermediate hosts, the snails, which is not always possible. Strategic anthelmintic treatment helps to reduce grazing land contamination with fluke eggs and increase productivity (FAO, 1994; Yilma and Malone, 1998). In addition to avoiding pasture contamination and improving productivity of animals, anthelmintic combined with liver tonic was used as effective treatment of fascioliasis for the first time in Bangladesh that successfully limited the parasitic infection in goats of small holder farmers in the Sylhet region of Bangladesh. Among different anthelmintic triaclabendazole is one of the most widely used anthelmintic which is administrated orally at the dose rate of 10mg/kg (Ramisz, *et. al.,* 1990; Hansen and Perry, 1994). It has been shown to be highly effective at removing all stages of liver fluke in goat (Fawcett, 1990; Hansen and Perry, 1994) and liver tonic helps to recover damaged liver and thereby improves liver functions. Therefore, use of anthelmintic along with liver tonic might be an excellent therapeutic approach to fascioliasis of goat.

5.1 Epidemiological study of fascioliasis (*Fasciola gigantica*) in goats

5.1.1 The prevalence of fascioliasis in goats

The prevalence of fascioliasis in goats in study area was 31.75% based on fecal samples examination and 10.10% based on liver samples examination. The highest prevalence of fascioliasis found in Biswanath upazilla which was 39.91% in fecal and 12.00% in liver samples. The lowest prevalence of fascioliasis found in Sylhet Sadar which was 25.43% in fecal and 8.25% in liver samples, respectively. The prevalence of five different upazilla was correlated with the findings of previous study.

The results of our study showed similarities with the results obtained by Maqbool *et al.,* 2000; Mohsen *et al.,* 2008; Mamun *et al.,* 2011 and Mungube *et al.,* 2012 in which the prevalence of *Fasciola gigantica* in goats was range from 23.4 to 91.74% determined by fecal samples examination. Selim *et al.* 1997; Mungube *et al.* 2006; Kadir and Rasheed, 2008; Mohsen *et al.,* 2008; Saiful Islam *et al.,* 2008; Abdulhakim and Addis, 2012; Kadir *et al.,* 2012; Jean-Richard *et al.,* 2014 and Khanjari *et al.,* 2014 also

reported that the prevalence of *Fasciola gigantica* in goat was range from 6.6 to 14.28% by liver samples examination.

The prevalence determined by fecal samples examination is mostly similar to previous findings but disparity among the prevalence of the liver samples examination might be due to the geo-climatic conditions together with the water logged and low lying pasture of study area which are highly favorable for the development and multiplication of *Fasciola s*pecies and their intermediate hosts (snails).

5.1.2 Age wise prevalence of fascioliasis (*Fasciola gigantica*) in goats

In this study, the prevalence of fascioliasis in young (<1.5 years) age group of household live (39.05%) and slaughterhouse (15.58%) goats were significantly higher than adult (≥1.5 years). Kuchai *et al.,* (2010) and Lone *et al.,* (2011) were stated that young animals showed greater susceptibility (50 and 58%) to helminths than adults (33.33 and 34%). Tasawar *et al.,* (2007) also found that the prevalence of *Fasciola gigantica* was significantly higher in young goats (35.71%) than adult (18.18%). Likewise, a number of studies had reported the susceptibility of young goats than adults (Patel *et al.,* 2001; Magona and Musini, 2002; Keyyu *et al*., 2003; Lateef *et al.,* 2005; Tariq *et al.,* 2008; Abouzeid *et al.,* 2010; Chakiso *et al.,* 2014; Kuchai, *et al.,* 2011a; Kuchai, *et al.,* 2011b and Sangma *et al.,* 2012).

The highest prevalence of fascioliasis in young age group may be due to the factor of primary infection in which there is a chance of delay in onset of immunity which results successful establishment of parasites in the host body. Undernourishments and generally poor husbandry may also be another factor. Developments of significant immunity in adults make them relatively resistant to reinfection which is thought to be the factor with regard to low prevalence. However, constant exposure of a degree of infection may be required to maintain their resistant status.

Winkler (1982) also reported that host may recover from parasitic infection with increasing age and hence become resistant. On the other hand, young age groups of goats were more infected than adults may be due to low level of immunity.

5.1.3 Sex wise prevalence of fascioliasis (*Fasciola gigantica*) in goats

The prevalence of fascioliasis was more in females of household live (39.15%) and slaughterhouse (13.10%) goats in compare to their male counter partners (19.96 and 7.10%, respectively). The highest prevalence of fascioliasis of female goats was 46.71% in household live and 15.00% in slaughterhouse goats of Biswanath upazilla and the lowest prevalence was 33.33% in household live and 10.50% in slaughterhouse goats of Sylhet Sadar.

Recently, similar findings obtained in a study conducted by Kuchai, *et al.,* (2011a), Affroze *et al.,* (2013) and Ardo *et al.,(*2013). They stated that the prevalence of fascioliasis was significantly higher in females (38.07, 41.36 and 23.6%) than males (29.09, 13.85 and 18.2%). Oyeduntan *et al.,* (2008) also reported that the annual prevalence of *Fasciola gigantica* was higher in females (63.7%) than in males (36.3%). The similar findings were also observed by many anthers females more infected than males (Chowdhury *et al*., 1994c; Selim *et al.,* 1997; Maqbool *et al.,* 2000; Patel *et al.,* 2001; Shahiduzzamam *et al*., 2003; *Masuduzzaman et al., 2005;* Fatima *et al.,* 2008; Hossain *et al.,* 2011; Kuchai, *et al.,* 2011b; Bhutto *et al*., 2012 and Bogale *et al.,* 2012).

Physiological peculiarities of female animals which usually constitute stress factors like calving and lactation reduced their immunity to infections. Females are usually weak and malnourished and consequently are more susceptible to infections besides some other reasons (Blood and Radostits, 2000). Based on the present study and the work of other researchers, it seems that sex plays a significant role in the preponderance of helminth infection. The influence of sex on the susceptibility of animals to infections could be attributed to genetic predisposition and differential susceptibility owing to hormonal control.

5.1.4 Seasonal influence on fascioliasis (*Fasciola gigantica*) in goats

The seasonal influence of fascioliasis was highly observed in rainy season (39.28 and 16.51%) followed by winter (32.25 and 7.32%) and summer (19.19 and 4.70%) of household live and slaughterhouse goats, respectively. A study conducted by Waruiru *et al.,* (2005) revealed the seasonal variation throughout the year found that the highest prevalence during rainy season (88.54%) followed by summer (83.15%) and winter (76.01%). The high prevalence of fascioliasis (74.38 and 92.96%) in rainy season

followed by winter (57.2 and 89.20%) and summer (24.4 and 87.76%) was also reported by Singh *et al.,* 2009 and Bansal *et al.,* 2013. Khanjari *et al.,* 2014 reported that the seasonal prevalence of *Fasciola* spp. was highest during rainy season (16.4%) followed by winter (5.9%) and summer (4.0%) in both sheep and goats. Besides these many studies have also been reported about high prevalence of fascioliasis in rainy season followed by winter and then summer (Jithendran and Bhat, 1999; *Maqbool et al., 2002;* Tamloorkar *et al.,* 2002; Mungube *et al.* 2006; Oyeduntan *et al.,* 2008; Hossain *et al.,* 2011; Kuchai *et al.,* 2010; Mellau *et al.,* 2010; Sutar *et al.,* 2010; Kuchai, *et al.,* 2011a; Kuchai, *et al.,* 2011b; Mamun *et al.,* 2011; Khan and Maqbool, 2012).

Climatic factors, particularly rainfalls are frequently associated with differences in the prevalence of fascioliasis because this is suitable for intermediate hosts like snails to reproduce and to survive longer under moist conditions (Ahmed *et al.,* 2007).

Moreover, Bangladesh has a rainy season for four months, which facilitates parasitic survival in such an environment. The prevalence of *Fasciola gigantica* was found to be significantly higher during the wet season than that of dry season. The proportion of animals passing fluke eggs increased gradually from the early dry season and peaked at the end of the dry season and the early part of the rainy season (Keyyu *et al.,* 2005).

5.2 Prevalence of infection of developmental stages of *Fasciola gigantica* in *Lymnaea* snails

5.2.1 The prevalence

Lymnaea auricularia var. *rufescens* belonging to genus *Lymnaea* act as intermediate host of *Fasciola gigantica* which infect livestock population mainly ruminants. Both human being and livestock population are infected by various species of trematodes (Platyhelminthes: Trematoda) in which snails are the intermediate host. In the present study, the prevalence of infected *Lymnaea* snails was determined as 3%. The findings of both sporocyst and cercaria infection in *Lymnaea* snails is higher (3%) than the findings of Chowdhury *et al.,* (1994c) and lower than the Qadir, (1982). Chowdhury *et al.,* (1994c) examined 4149 *Lymnaea* snails in which 0.31% was infected. Qadir, (1982) examined only 730 snails and incidence was 3.70%. This finding showed similarities with the studies of Garg *et al.,* (2009); Imani-Baran *et al.,* (2013) and Islam *et al.,* (2012) those reported the prevalence of developmental stages of *Fasciola*

gigantica (cercariae) in *Lymnaea* snails was 2.35%, 5.5% and 16.8%, respectively. The variation of infection rates in *Lymnaea* snails among the findings of different investigators was thought to be due to the total number of *Lymnaea* snails examined, level of *Fasciola gigantica* infection in the animals, duration of the study period, meteorological factors that govern the breeding, life span of infected snails and development of different developmental stages within the snails.

In Biswanath upazilla, there were enough marshy areas with flooded pasture which favor the development of *Lymnaea* snails. The highest prevalence at Biswanath upazilla indicated that the environmental conditions are more suitable for the snail breeding in this study area. Thus, the environmental and managemental conditions might have great impact on infection status of developmental stages of *Fasciola gigantica* in snails. Similar observation was also reported by Tanveer, (1990b) and Rondelaud *et al.*, (2007).

5.2.2 Microscopic features of a sporocyst and cercaria

The microscopic features of both sporocyst and cercaria of *Fasciola gigantica* were observed. The characteristic sac like structure of sporocyst and large heart shaped body with a simple long tail of cercaria was showed high similarities with the observations of Belding, (1965) and Koie *et al.*, (1977).

5.2.3 Month wise prevalence of infection status of developmental stages of *Fasciola gigantica* in *Lymnaea* snails

Prevalence of different developmental stages of *Fasciola gigantica* infection in the *Lymnaea* snails varied significantly in different months of the year. Developmental stages of *Fasciola gigantica* in the *Lymnaea* snails were not available around the year. Sporocyst and cercariae were started to appear in the snails from April, reached a peak during May to October then low in February to March and disappeared during November to January. In the collected *Lymnaea* snails, only sporocysts were found during the period of February to April. Chowdhury *et al.*, (1994c) and Qadir, (1982) were observed too much higher incidence of gymnocephalous cercariae (*Fasciola* cercariae) in *Lymnaea* snails in different months except November to April. *Lymnaea* snails become infected during the period when conditions are favorable for infestation. The cercariae might have died by November and there was no more infection in snails.

During winter (November to January), development and hatching of eggs ceased or took a long time due to low temperature. Therefore, snails were not infested during this period and cercariae were not found in snails by April.

There were favorable temperature, humidity and rainfall from May to October onward for the development and hatching of *Fasciola gigantica* eggs and other developmental stages of *Fasciola* within the snails. The development and hatching of *Fasciola gigantica* eggs takes 16-30 days at 20 ^{0}C, 11-25 days at 25 ^{0}C and 12-24 days at 30 ^{0}C (Gosh, 1985) and development of cercariae stages of *Fasciola gigantica* within the snails takes 75 days (Gosh, 1985). Thus, cercarial infestation in the snails began to increase from April and continued until October due to warm, wet and humid conditions.

5.2.4 Season wise prevalence of developmental stages of *Fasciola gigantica infection* in *Lymnaea* snails

The peak infection of *Lymnaea* snails were recorded in rainy season followed by summer and winter season. *Lymnaea auricularia* var *rufescens* was the predominant snail species encountered in this study. Water, muddy, marshy area, clear water, presence of small number of aquatic weeds and an abundance of algae are the optimum habitats for development and infection in *Lymnaea* snails. The high density of snail intermediate hosts and the irrigated areas was additional evidence for a higher infection rate in these localities making animal feeds obtained from the irrigation canals a potent source of infections larva to the snails. The findings shown that the abundance of the development stages of *F. gigantica* infection in *Lymnaea* snail was higher in the irrigation channels because the water temperature, rainfall and humidity. Therefore, these results were in agreement with Tanveer, (1990a); Mansourian, (1992) and Karimi, (2003).

5.2.5 Correlation between *Lymnaea* snail's infection, meteorological factors and snail population

Effect of climatic factors on the prevalence was found to be highly significant. In relation to the effect of meteorological factors, it was observed that late summer providing the optimum temperature required for the breeding and reproduction of snails. It indicated that temperature had a great effect on prevalence of snail population. The

similar finding was also described by Imani-Baran *et al.*, (2013). During the late summer a large number of mature snails oviposit and die consequently. As the summer merges into rainy season an increment was observed in snail population. These observation was critically justified the findings of Qadir, (1982) and Chowdhury *et al.*, (1994a). They reported that ova production of snails is not affected by increasing the period of day light and darkness has no harmful effect on it which was also stated by Mansourian, (1992) and Karimi, (2003).

5.3 Pathological study of fascioliasis (*Fasciola gigantica*) in goats

5.3.1 Gross pathology

The gross pathological changes of liver revealed that swollen, enlarged livers with rounded edges and thickened capsule with numerous hemorrhagic spots on the parietal surface of the liver which is partly due to the inflammatory changes and later fibrosis of the liver parenchyma, Most of the gross lesions of fascioliasis in goats were observed and similar to the findings obtained in the study conducted by Adama *et al.*, 2012 in sheep, Masuduzzaman *et al.*, 1999; Ahmedullah *et al.*, 2007; Rahman *et al.*, 2007; Okaiyeto *et al.*, 2012; Affrose *et al.*, 2013 in cattle, Alim *et al.*, 2000 in buffalo, Masuduzzaman *et al.*, 2005 in deer.

Liver trauma is the abrasion caused by cuticular spines and the prehensile action of the suckers of fluke and appears to account for the majority of the damage caused in the liver. Death of the host is a consequence of the hemorrhage induced by this damage. The oral sucker of fluke is the route by which they obtain most of their nutrition. It appears to cause considerable damage to liver tissue and macerated hepatic cells have been observed inside the sucker and pharynx. The oral sucker extends during migration and feeding from the earliest stages is capable of disrupting cells. The muscular pharynx assists in this process and oral sucker is a major organ involved in tissue disrupting (Behm and Sangster, 1999). Therefore, the lesions of fascioliasis were produced.

5.3.2 Histopathology

The histopathological changes observed in this study were characterized by hemorrhagic, edema and infiltration with numerous eosinophils mixed with few

lymphocytes. Atrophy, necrosis, hemorrhage, fatty changes, blood vessel abnormalities, damage to portal tract area are also common. Similar changes were observed by Masuduzzaman *et al.,* 2005 and Okaiyeto *et al.,* 2012. In chronic fascioliasis, fibrosis of portal area and thickening of the bile duct were observed at histopathological examination. When a section of the bile duct was cut through, there was no evidence of calcification in goats. These findings showed similarities with the previous results obtained in chronic fascioliasis of sheep, goat, pig and deer in which there was no calcification in the wall of bile ducts (Masuduzzaman *et al.,* 2005 and Chiezey *et al.,* 2013). But the calcification was seen in cattle liver (Badr and Nasr, 2009 and Okaiyeto *et al.,* 2012). Calcification is not common might be due to variation of species.

5.4 Efficacy of different types of liver tonic along with anthelmintic on pathology of fascioliasis in goats

Fasciola gigantica infection in goats has important implications for animal health and welfare, farming economics and meat production all over the world. It is considered to be among the main causes of reduced livestock productivity. One unique and perhaps the most important prophylactic attribute is the anthelmintic along with liver tonics for the prompt removal and recovery of the *Fasciola gigantica* affected goats. The findings of this study indicate that administration of liver tonics combined with anthelmintic (Triaclabendazole-TCBZ) had significant effect on affected liver. Hematological parameters and LFT (Liver function test) were done to determine the effect of liver tonics along with anthelmintic on the functional status of liver. The results of this study are in the line of findings reported by Helfgot, *et al.,* 1990; Aydin *et al.,* 2003 and Schwaiger *et al.,* 2004**.** Significant changes were observed in hematological levels and serum level of AST, ALT and ALP in goats after liver tonics and/or anthelmintic administration.

5.4.1 Counting of egg per gram (EPG) of feces

The *Fasciola* eggs count is a clear factor that gives the estimate of the presence adult flukes to explain the level of success in the experiment. The results for anthelmintic (TCBZ) along with liver tonics and only anthelmintic (TCBZ) treated goats indicated that these drugs were highly effective on *Fasciola gigantica* infection. The egg per gram of feces (EPG) was reduced to 100%. The anthelmintic (TCBZ) along with liver tonics and only anthelmintic treated goats had nearly negative EPG at the end of the

experiment (28 days after treatment). This clinical field test for egg reduction affords valuable information, even though the number of flukes removed is unknown (Wood *et al.,* 1995). Triclabendazole (TCBZ) along with Liva vit® was effective as EPG of feces being reduced to 100% at day 21 post-treatment. Triclabendazole (TCBZ) along with Hepamin forte® and only triclabendazole (TCBZ) reduced the EPG of feces to 100% on day 28^{th} of treatment. The present findings were coincided with the earlier researcher Ratnaparki *et al.,* (1992) who reported the 80% efficacy of triclabendazole in cattle.

5.4.2 Determination of hematological parameters (PCV, Hb, TEC and TLC)

After treatment with anthelmintic (TCBZ) along with liver tonics (Hepamin forte® and Liva vit®) the blood parameters (PCV, Hb and TEC) were significantly increased and TLC values decreased than others groups, which could be due to the elimination of *Fasciola gigantica* from the affected goats. The increase of PCV values after treatment might be associated with the increase of Hb%, as these parameters are closely associated and correlated with each other. Anthelmintic along with liver tonics showed better effect than anthelmintic alone. Anthelmintic combined Liva vit® may be used as the most efficient drug for removal of *Fasciola gigantica* from infected goats as well as to regenerate the liver in its normal condition. Hassan *et al.,* (2012) stated the improvement of PCV (from 19.90 to 20.90), Hb (from 6.7 to 7.0), TEC (from 7.4 to 10.5) and decreased TLC mainly eosinophil (from 10.0 to 6.7) on the 28^{th} day of anthelmintic treatment which is similar to the findings of this study. Some other reports (Yousif, *et al.,* 1988; Kumar, *et al.,* 1992; Khalid, *et al.,* 2004 and Islam *et al.,* 2003) also stated the similar findings.

Triclabendazole might cause death of *Fasciola gigantica* into the hepatic parenchyma resulting protection of severe hemorrhage caused by extensive migration of the young flukes and blood sucking of the adult. Liver tonics may be improved the hematopoietic activity resulting in increased production of erythrocytes due to neutralization of unknown toxic substances, storages of iron to increased hemoglobin level, maintenance of plasma and blood volume to increased packed cell volume as well as high level of erythrocytes and prevent secondary bacterial infection as well as decreased level of TLC.

5.4.3 Determination of biochemical parameters (AST, ALT and ALP)

Aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are the enzymes that mostly produced by the liver (Smith and Sherman, 1994), and predictive of the health status of goats (Otto *et al.,* 2000; Ikhimioya and Imasuen, 2007; Pierce *et al.,* 2007; Mahgoub *et al.,* 2008). Several studies have been conducted on blood parameter levels in goats (Pambu-Gollah *et al.,* 2000; Kannan *et al.,* 2003; Stella *et al.,* 2007) and several others on the use of EPG in faeces (Hoste *et al.,* 2005; Stella *et al.,* 2007; Rumosa Gwaze *et al.,* 2009) in monitoring the health status of goats, but none have been focused on the relationship between these enzymatic parameters. Fascioliasis considered the main cause of liver damage which hampers the functional activities of liver. All these enzymes are intracellular and are being located in mitochondria or cytoplasm or both and when cell's function altered, damaged or destroyed, the enzyme escapes into the blood (Doxey, 1971; Connor *et al.,* 2003 and Grünwaldt *et al.,* 2005). The *Fasciola gigantica* infection in goats had elicited enough hepatic insults and then other enzymes would have been elevated as well, which was found in this study. After treatment, the levels of AST, ALT and ALP were decreased than control groups, which suggest the removal of *Fasciola gigantica* and regeneration of liver tissue of the affected goats. Hassan *et al.,* (2012) stated the improvement of AST (from 36.3 to 8.3) and ALT (from 11.20 to 5.50) on the 28^{th} day of anthelmintic treatment which is similar to the findings of this study. Some other reports (Ragab *et al.,* 1981; Chaudhri, *et al.,* 1988 and Alam *et al.,* 1994) also stated the similar findings. The anthelmintic along with liver tonics or anthelmintic alone were showed some positive effect against *Fasciola gigantica* in the experimental goats which improved the level of biochemical parameters. But there was no significant effect observed in biochemical parameters when goats were only treated with liver tonic as the parasite were not destroyed and continue their liver damage.

The present findings suggest that the treatments have shown satisfactory performances in terms of the removal of *Fasciola gigantica*. This indicate that the liver recovered his normal functional activities as the parasite removed from the liver and hepatocytes might be enhanced the power of regeneration. Therefore, the efficacy of triclabendazole has been variously acknowledged against adult flukes (Dorchies *et al.,* 1983; Gadzhiev and Aliev, 1983; Sanchez Alvarez *et al.,* 1988; Beriajaya and Knox, 1988; Gupta *et al.,* 1989). Triclabendazole is a proven drug against *Fasciola sp.* and liver tonic has no

effect on destruction of parasite. The objective of the study is to investigate how helpful the liver tonic is in regeneration of liver tissue which was damaged by fluke. T4 and T5 groups showed considerable positive effect on liver tissue regeneration. This may be suggested that liver tonic should be used shortly after anthelmintic treatment. Thus, use of liver tonic after few days of anthelmintic treatment might have great impact on regeneration of hepatocytes and recommence of functional activities of the liver. Probably it is a new finding and needs further detail study.

CHAPTER-VI

SUMMARY

The present study which deals with the patho-surveillance (epidemiology and pathology), infection status of developmental stages of *Fasciola gigantica* in *Lymnaea* snail (*Lymnaea auricularia* var *rufexcens*) and efficacy of different liver tonics combined with anthelmintic against natural *Fasciola gigantica* infection in goat in Sylhet region of Bangladesh.

A total of 1288 household live goat feces and 2000 slaughtered goats liver were examined, of which 405 feces (egg) (31.75%) and 202 (10.10%) liver (fluke) were positive for *Fasciola gigantica*.

The present study revealed that the infection level of *Fasciola gigantica* in goat is high. That means the geographical and climatic conditions of the Sylhet region are suitable for growth, development and multiplication of *Fasciola gigantica*.

The overall prevalence of fascioliasis was found to be 20.93%. The overall prevalence of household live and slaughterhouse goats was 16.84% (107/691) from Sylhet Sadar followed by 20.23% of 666 from Balaganj, 22.16% of 680 from Beanibazar, 25.96% of 623 from Biswanath and 19.45% of 628 from Jaintapur upazilla, respectively. This variation might be due to the differences in husbandry or management practices in different upazillas in which regular anthelmintic therapy and hygienic measures may play critical role in establishment of infection.

The infection level in household live goats was 3.14 times higher than the slaughterhouse goat. This might be justified by the healthy condition, slaughtering of more male and adult goats in slaughter house.

The young goats were 2.58 times higher than adults regarding susceptibility to *Fasciola gigantica* infection. The young ages are an important factor in the onset of infection because immunity plays a great role in the establishment of parasites in the host body. Undernourishments and generally poor husbandry also has impact on parasitic infection. In adult animals, the prevalence of helminth is low due to the development of significant immunity, which is initially low but increases with the intensity and duration of exposure of infection. Therefore, young animals need special attention

because of their high susceptibility to infection. They should be included in deworming programming supplemented with liver tonics.

The study also revealed that maximum *Fasciola gigantica* infection was observed in females as compared to males. Physiological stress, influence of sex on the susceptibility of animals to infections could be attributed to genetic predisposition and differential susceptibility owing to hormonal control.

In seasonal pattern, the highest prevalence was found during rainy season (25.71%) followed by winter (16.84%) and summer (10.12%). The optimum temperature, humidity and rainfall in rainy season may favor the growth development and multiplication of *Fasciola gigantica*.

Lymnaea auricularia var. rufescens belonging to genus *Lymnaea* act as intermediate hosts of *Fasciola gigantica* infecting both human being and livestock populations by various species of trematodes (Platyhelminthes: Trematoda), which are required as intermediate hosts of snails (*Gastropoda*).

A total of 1865 *Lymnaea* snails were collected and examined, of which 56 snails were found to having infected with different stages of *Fasciola gigantica* from different areas in Sylhet region of Bangladesh during the period of one year.

The overall infection rate was 3.00%. Only 4.08% infected of 515 snails were collected from Biswanath upazilla followed by 3.16% of 443 from Beanibazar, 2.53% of 396 from Balagonj then 2.40% of 292 Jaintapur upazilla and the lowest 1.83% of 219 from Sylhet Sadar due to different geoclimatic conditions and availability of meteorological factors.

In month-wise data, the prevalence of snails infection was observed to be the highest in May (5.06%) and August (5.61%) and the lowest in February (0.68%) and March (0.74%), but there was no infection observed in November to January.

The infection status of developmental stages of *Fasciola gigantica* in snail was highest inb rainy (4.63%) season followed by summer (1.92%) and winter (0.76%). Significant correlation was observed between snails infection and meteorological factors like temperature (^{o}C), relative humidity (%) and rainfall (mm). The infection status of developmental stages of *Fasciola gigantica* was observed in snails population

(*Lymnaea* snails) decreases from November to January and increases from February to October and highest in August and September.

Grossly, in acute form, there is enlarged liver with rounded edges and thickened capsule with numerous hemorrhagic spots on the parietal surface. In chronic form, liver was cirrhotic and reduced in size. The affected intra-hepatic bile ducts were protruded and engorged with flukes.

In histopathology, the migratory tracts were represented by the presence of hemorrhage, edema and infiltration with numerous eosinophils etc. The wall of bile ducts was thickened. The lining epithelium becomes hyperplastic. The section of adult and immature *Fasciola gigantica* was found in the bile ducts and within hepatic parenchyma, respectively.

The effects of either anthelmintic (Triaclabendazole-TCBZ) along with liver tonic or only anthelmintic trial, revealed a significant decrease ($p< 0.001$) in egg per gram of feces (EPG) output was seen in anthelmintic combined liver tonic (T_4 and T_5) and only anthelmintic (T_1) treated groups as compared to untreated infected control group (T_i).

The results of hematological parameters (PCV, Hb and TEC) at different days showed that, the most efficient treatments on PCV, Hb and TEC was TCBZ along with liver tonics (T_4 and T_5) as it improve the hematological parameters. The infected control group (T_i) showed significantly decreased level of PCV, Hb, TEC and increased TLC compared to the non infected control (T_0) and other groups. The groups that treated with TCBZ along with liver tonics (T_4 and T_5) showed gradual decrease in TLC count, TCBZ (T_1) also showed a lower level of TLC at the end of the experiment. The results indicated that the infected control groups (T_i) showed detectable elevated in TLC at the end of the experiment. Infected control group (T_i) showed significantly increased TLC compared to the non infected control (T_0) and other treated groups.

In addition, similar significant trends were depicted in improvements of biochemical parameters (AST, ALT and ALP) between TCBZ and liver tonics (T_4 and T_5) and only TCBZ group (T_1). During the experimental period, goats received only liver tonics (T_2 and T_3) showed slightly better biochemical parameters as compared to the infected control group (T_i). The infected control groups showed significantly increased level of

AST, ALT and ALP compared to the non infected control and other groups at the end of the experiment.

The current study generated data on the epidemiology of *Fasciola gigantica,* pathology (gross and microscopic) caused by the immature and mature stages of the parasite in goats at Sylhet region of Bangladesh. The study also provided information on the developmental stages of this parasite in its intermediate host (*Lymnaea auricularia* var *rufescens*) which is very important to design effective control program. A combined approach of using anthelmintic and liver tonics was tested to treat the infected goats. Therefore, the results of the present study have significant effects in establishing an effective treatment, control and eradication programs of fascioliasis in goats in Sylhet region of Bangladesh and also in similar temperate agro-ecological zones around the world.

CHAPTER-VII

CONCLUSIONS

It is evident that comprehensive data on the impact of fascioliasis over several years is difficult and expensive to obtain. This is due mostly to the long term and chronic nature of the disease, its multiple effects on productivity and the difficulty of making an accurate diagnosis. Fascioliasis devastates the small ruminants and continually drains the economic gains from the animals in Sylhet region of Bangladesh. The geo-climatic conditions along with the water logged and low lying areas in Sylhet region of Bangladesh are highly favorable for the development and multiplication of *Fasciola giagntica* and their intermediate hosts *Lymnaea auricularia* var *rufescens*. The effective methods are required to control fascioliasis in goats.

This study showed the patho-surveillance, gross and histopathological changes, infection of developmental stages of *Fasciola gigantica* in *Lymnaea* snails and efficacy of anthelmintic with liver tonics in goats. This give an insight for better understanding of the effect of the disease and help to adopt treatment/control strategies against fascioliasis in Sylhet region as well as in Bangladesh.

Study on snail populations and infection status might be useful to control snail population which is important in controlling fascioliasis. Further study should be needed to develop an effective control strategy based on the findings of this study to protect the domesticated animal from the harmful effect of *Fasciola gigantica* in Bangladesh.

The study on liver tonic is first time in Bangladesh. There was a positive effect of liver tonic on hematological and biochemical values. The liver tonic might have some functions which could help to recover liver in normal state. Use of liver tonic after a short period of administration of anthelmintic might have significant effect. Further study should be needed to know the details about effect of liver tonic.

Taken together, this study might be valuable regarding development of an effective control/treatment strategy against fascioliasis in domesticated animals which will be helpful to overcome economic losses due to fascioliasis in Bangladesh.

CHAPTER-VIII

RECOMMENDATIONS

Diseases are major constraint of livestock development. Due to illness there are losses in productivity, interference in agricultural development and human well-being as well as poverty alleviation in many regions of the developing world. The most important and readily measurable direct effects of disease are often losses in productivity. These include the effects due to death, illness leading to condemnation, poor weight gain and poor feed conversion. Disease of livestock have many additional direct and indirect impacts on human nutrition, community development and socio-cultural and also in reduction in farm income, contributing to food insecurity and poor nutrition. On the basis of outcome of the present study the recommendations are as bellows:

Regular screening of animals for the presence of fascioliasis should be carried out to understand the disease potential. Awareness building is vital regarding regular use of anthelmintic followed by liver tonic by livestock farmers to eliminate the *Fasciola gigantica* infection from the host. Water, being the source of disease causing parasites, should be supplied in pure form. Supply of contaminated water to goats and other domestic animals may lead to infection with *Fasciola gigantica*. Overcrowding at farms should be avoided and good sanitary conditions should be provided.

Based upon epidemiological principles, *Three Check Points* to manage and control fascioliasis in goats in Sylhet region of Bangladesh are suggested and recommended. (1) Pre grazing deworming early in spring before the animals are sent to pastures for grazing. (2) Deworming during the middle of grazing months (July-August). (3) Post grazing deworming before the onset of winter. For prevention of spread of *Fasciola gigantica*, the contamination of pastureland should be prevented by treating the hosts with anthelmintics.

To produce healthy goats balanced feed should be provided containing more than 3% protein. To maintain the healthy condition of liver, liver tonic might be useful regarding functional activities of hepatocytes. Use of liver tonics with active ingredients is also recommended for regular interval or at least for few days after anthelmintic therapy.

Reducing the number of snail population in pasture is essential. It can be done by breaking the life cycle of *Lyamnaea aruricularia var. rufescens.*

New herd of goats should be accepted after fulfilling the quarantine terms and conditions. This will be helpful to prevent the incoming of fascioliasis.

The animal shed should not be kept wet, moist or humid. These all provide better opportunity to *Fasciola gigantica* for their growth and development. Therefore, shifting of the goats from wet sheds to dry sheds may reduce chances of re-infection or even mass infection among goats.

Awareness building among the livestock farmers/entrepreneurs and public butchers regarding the ill effect of infection by *Fasciola gigantica* can also lead to minimization of the spread of these fascioliasis.

Students and mass media like radio, television, news papers, etc can play a key role in creating awareness about the concerned population.

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