PREVALENCE OF FASCIOLIASIS IN SMALL RUMINANT AT SADAR UPAZILA OF DINAJPUR

A THESIS

BY

MD. HUMAYUN KABIR
REGISTRATION NO.: 1705446
SEMESTER: JULY - DECEMBER, 2018
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MASTER OF SCIENCE (M. S.)

IN

PARASITOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
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Submitted to the

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ABSTRACT

Fascioliasis plays an important role of major constraints to small ruminant production in Bangladesh. The study was conducted to investigate the prevalence of fascioliasis in small ruminant at sadar upazila of Dinajpur, Bangladesh during January to June 2019, using history, clinical signs, physical and coprological examinations. A total of 106 faecal sample were collected including 80 goat and 26 sheep sample from different area of sadar upazila of Dinajpur and the sample was considered as study population for the present study. Out of 106 sample 22 sample found positive and Overall prevalence of Fascioliasis was 20.75% whereas in case of goat it was 20% and 23.07% in sheep respectively. The age of the study population were divided into three groups i.e. young, adult, and old and their prevalence (%) of fascioliasis were found 15%, 20.58% and 23.07% in goat and 20%, 22,22% and 25% in sheep respectively. According to this study the highest number of positive fascioliasis was found in female goat, out of 45 female goat 10 were found positive and overall prevalence was equivalent to 22.22%%, while out of 35 male goat 6 were found positive and overall prevalence was equivalent to 17.14%. In case of sheep the highest number of positive fascioliasis was found in female, out of 14 female sheep 4 were found positive and overall prevalence was equivalent to 28.57%, while out of 12 male sheep 2 were found positive and overall prevalence was equivalent to 16.67%. In case of goat the higher prevalence of fascioliasis was recorded in poor animal 35.48% followed by healthy 10.20 %. In sheep the higher prevalence of fascioliasis was recorded in poor animal 33.33% followed by healthy 17.65%. The results indicate small ruminant of sadar upazila of Dinajpur area are very much susceptible to fascioliais. To control the disease in this area, appropriate preventive control strategies have to be designed to reduce the impact of the disease on small ruminant.

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LIST OF ABBREVIATIONS AND SYMBOLS

ALT	-	Alanine Amino Transferase
ASL	-	Aspartate Tgransaminase
ELISA	-	Enzyme Linked Immunosorbent Assay
HSTU	-	Hajee Mohammad Danesh Science and Technology University
iELISA	-	indirect Enzyme Linked Immunosorbent Assay
Ig	-	Immunoglobulin
Se	-	Sensitivity
Sp	-	Specificity
WHO	-	World Health Organization
PCR	-	Polymerase chain reaction

CHAPTER I

INTRODUCTION

Parasitism is one of the main causes limiting livestock production in most of the tropical and sub-tropical countries of the world. In Bangladesh, parasitism has been considered as one of the major constraints of livestock production (Jabbar and Hag, 1983). The incidence of parasitic diseases in the domestic ruminants of Bangladesh is also high and as a result hardly any livestock industry could develop here. There are about 60.23 million small ruminants (goat and sheep) in Bangladesh (FAO, 2018) which plays an important role in the rural economy and earn substantial amount of foreign currency by exporting skins and other by-products (Kamaruddin, 2003). A large number of helminth parasites are constantly deteriorating the health and productivity of the ruminants, *Fasciola gigantica* is by far the pre-dominant species of outstanding importance. The pathogenic effect of this parasite is extended over a large number of domestic ruminants; cattle, sheep, goats and buffaloes are mostly affected and drain a substantial economic loss to the country annually. Infection with *Fasciola gigantica* is regarded as one of the most common single helminth infection of ruminants in Asia and Africa (Hammond and Sewell 1990). This disease causes enormous economic losses all over the world and these losses are due to reduction in milk and meat production, condemnation of liver, loss of draught power, reproductive failure and mortality (Diaw *et al*., 1998). Worldwide losses in animal productivity due to fasciolosis were conservatively estimated at over US $3.2 billion per annum (Urquhart *et al*., 1996).

Fascioliasis can be affect and inhibits all ruminants. There are two species of liver fluke, which causes infection on ruminants. These species are: - *Fasciola hepatica* and *Fasciola gigantic*. *Fasciola hepatica* was occurred in worldwide distribution, but mostly it was common in desert or hottest zones. *Fasciola gigantic* is common in rainforest areas of African and Asian regions. May be, sometime the species of these two fasciolosis can be occurred in the same country as well as in the same areas of agro-ecological region of one country, this is may be due to availability of good air condition for their development of the intermediate hosts (Mas-Coma *et al*. 2005). Fascioliasis can causes different effects on animal that inhibit by this conditions, the common effects are: - decrease in milk production, decrease in meat and giving off springs in production systems and also it will not give a good income due to the body of animals that can be affected by these diseases was poor and it will cause economic loss for treatment of this condition (Hillyer and Apt, 1997). Fasciolosis has three clinical signs in general, the first sign is: - Acute fasciolosis: - this was occurring due to huge number of metecercariae infested on the hosts. In shoats, this condition of fasciolosis cannot sign clinical signs rather than death of the animals. in some cases it was shows abdominal pain , it will also causes rupture of liver walls and layer and finally it will causes blooding and death will occurs due to hemorrhages and jaundice (Urquhart., *et al*. 1989). The second sign is, Sub-acute fasciolosis this types of fasciolosis can be occurred due to infestations of medium numbers of metecercariae can affects the hosts and it shows, anemia, jaundice and species can be affected and damage livers and finally causes hemorrhage on affected animals and it was causes totally live damages (Urquhart *et al*. 1989). The third sign of fasciolosis is chronic fasciolosis and it is common in sheep's. This will happen when the flukes can reaches to reaches hepatic bile duct and it will show different signs. This sign are;- obstruction of bile duct, damage of liver tissue and fibrosis and anemia are the most common signs if this conditions and as well as the condition also causes edema and bottle jaw. Death eventually occurs when anemia becomes severe this type of fascioliasis can also cause as 'Black disease', which is clostridial species of bacterial infections (Radostits *et al.*, 2007).

The development of Fascioliasis involves the presence of an intermediate host, suitable habitats for mollusks and environmental factors such as high humidity, adequate temperature and rainfall. In livestock, fascioliasis is important for losses caused by either mortality in acute cases or weight loss, infertility and reduced production in chronic cases (Siddiki *et al*., 2010). The significance of helminth infestation has been increased many folds in developing countries. The disease is of paramount importance due to its broad distribution and definite hosts (Rondelaud *et al*., 2001). It is an emerging parasitic infection, having significant impacts on both veterinary and human health throughout the world (Lazara *et al*., 2010). Fascioliasis is an economically important parasitic disease of herbivorous mammals caused by trematodes of the genus Fasciola that migrate in the hepatic parenchyma and establish in the bile ducts (Troncy, 1989).

Therefore the present study was conduct for the fulfillment of the following objectives:

- To observe the overall prevalence of Fascioliasis in small ruminants at sadar upazila of Dinajpur district in Bangladesh.
- To determine the prevalence of Fascioliasis in relation to species age, sex and nutritional status.

CHAPTER II

REVIEW OF LITERATURE

2.1 Taxonomy of *Fasciola Sp.*

Taxonomic Classification

Kingdom *Animalia*

- **Phylum** *Platyhelminthes*
- **Class** *Trematoda*
- **Subclass** *Digenea*
- **Order** *Echinostomiformes*
- **Family** *Fasciolidae*
- **Genus** *Fasciola*
- **Species** *F. hepatica/ F. gigantica* (Soulsby, 1965)

Figure 1: Taxonomic Classification of *Fasciola Sp.*

2.2 Epidemiology of Fascioliasis

2.2.1 Etiology

Fasciolosis is caused by two digenetic trematodes *F. hepatica* and *F. gigantica*. Adult flukes of both species are localized in the bile ducts of the liver or gallbladder. The life cycle of these trematodes involves snail as an intermediate host (Walker *et al.,* 2008). Fascioliasis is a common disease of small ruminants caused by *F. hepatica* and *F. gigantica* (Rana *et al.*, 2014).

2.2.2 Geographical Distribution

F. hepatica measures 2 to 3 cm and has a cosmopolitan distribution. *F. gigantica* measures 4 to 10 cm in length and the distribution of the species is limited to the tropics and has been recorded in Africa, the Middle East, Eastern Europe and south and eastern

Asia (Torgerson, and Claxton, 1999). Special conditions are needed for fascioliasis to be present in an area, and its geographic distribution is very patchy (focal). The eggs passed in the stool of infected mammals have to develop (mature) in a suitable aquatic snail host to be able to infect another mammalian host. Requirements include sufficient moisture and favorable temperatures (above 50°F) that allow the development of miracidia, reproduction of snails, and larval development within the snails. These factors also contribute to both the prevalence and level (intensity) of infection. Infective Fasciola larvae (metacercariae) are found in contaminated water, either stuck to (encysted on) water plants or floating in the water, often in marshy areas, ponds, or flooded pastures. People (and animals) typically become infected by eating raw watercress or other contaminated water plants. The plants may be eaten as a snack or in salads or sandwiches. People also can get infected by ingesting contaminated water, such as by drinking it or by eating vegetables that were washed or irrigated with contaminated water. Infection also can result from eating undercooked sheep or goat livers that contain immature forms of the parasite. In domestic livestock in Japan, diploid (2n = 20), triploid (3n = 30) and chimeric flukes (2n/3n) have been described, many of which reproduce parthenogenetically. As a result of this unclear classification, flukes in Japan are normally referred to as Fasciola spp (Sakaguchi, 1980). Recent reports based on mitochondrial genes analysis has shown that Japanese Fasciola spp. is more closely related to *F. gigantica* than to *F. hepatica* (Itagaki and Tsutsumi, 1998). In India, a species called F. jacksoni was described in elephants (Singh *et al.*, 1994).

2.2.3 Host Range of Fascioliasis

Fascioliasis occurs in many areas of the world and usually is caused by *F. hepatica*, which is a common liver fluke of sheep and cattle. In general, fascioliasis is more common and widespread in animals than in people. Even so, the number of infected people in the world is thought to exceed 2 million. *Fasciola hepatica* is found in more than 50 countries, in all continents except Antarctica. It is found in parts of Latin America, the Caribbean, Europe, the Middle East, Africa, Asia, and Oceania. *Fasciola gigantica* is less widespread. Human cases have been reported in the tropics, in parts of Africa and Asia, and also in Hawaii. In some areas where fascioliasis is found, human cases are uncommon (sporadic). In other areas, human fascioliasis is very common (hyperendemic). For example, the areas with the highest known rates of human infection are in the Andean highlands of Bolivia and Peru (Valero and Mas-Coma, 2000).

2.2.4 Source and Mode of Transmission

Favourable ecological conditions consisting of ideal temperature and presence of natural water sources such as ponds, lakes, streams, canals, rivers, etc., necessary for the survival of the snail intermediate hosts and presence of definitive host allows the parasite complete its life cycle. The infected definitive hosts contaminate the environment by spreading parasite eggs with their faeces. By an estimate, a sheep with mild clinical infection can contaminate a pasture with more than 500,000 eggs a day, and one with moderate infection can shed nearly 3 million eggs a day. Similarly, a moderately infected buffalo passes 7 to 10 million eggs per day. Temperatures of above 10^0 C and up to 28^0 C promote the development and hatching of miracidium in 2 weeks, which infect the appropriate snail intermediate host. The infected snail starts shedding cercariae in 4 to 8 weeks depending on temperature range. The cercariae encyst on the pasture or the aquatic vegetation or even water surface. Animals acquire infection by ingesting the metacercariae while grazing on the infested pasture or with contaminated water and aquatic vegetation (Gupta and Singh, 2002).

2.2.5 Risk Factors

Many factors enhanced the persistence of fascioliasis: The suitability of the climate and canals for the intermediate host; the resistance of metacercariae for dissociation, especially with the presence of shallow water, enough vegetation, and/or humidity; and continued exposure of the animals to encysted metacercariae, grazing habits, and movement between the infected and treated localities (El-Bahy, 1998). Climatic factors are of supreme importance influencing epidemiology of Fascioliasis. (Claxton *et al*., 1997; Rangel-Ruiz *et al*., 1999; Phiri *et al*., 2005; Ansari-Lari and Moazzeni, 2006).

2.2.6 Prevalence of Fascioliasis

Hossain *et al*. (2011) conducted a study in Sylhet district of Bangladesh to determine the prevalence of fascioliasis in Black Bengal goats of different age groups, sex and in seasons. In this study, livers of male and female goats were collected randomly from slaughter house during a period of 1 (one) year (October, 2007 to September, 2008). A total of 318 livers examined of which 66 were found to contain *Fasciola gigantica*. The overall prevalence rate was 20.75%. Fascioliasis was observed significantly higher in older (58.33%), female goats (36.79%) and during the rainy season (26.16%). The estimated economic losses due to condemnation of liver were 5.59% which amounted to US$ 115.44 per thousand liver of slaughtered goat. The prevalence was significantly different (P≤0.05) in different age groups and sex of the animals. The present study indicates that Fasciola infection in Black Bengal goats associated with age and sex of the animals; and seasons of the year.

Khan *et al*. (2015) was conducted a study to determine the prevalence of fascioliasis in sheep in Quetta district from January to June 2007. Two hundred and fifty liver samples along with gall bladder and bile ducts were collected from sheep. Over all prevalence of liver fluke infestation in sheep was recorded as 30% in young and adult and 32% in aged sheep. Out of these positive samples Fasciola hepatica was found 50% and *Fasciola gigantica* 10% in case of young whereas in adults 20% with *Fasciola hepatica* and 14%with *Fasciola gigantica* and in aged sheep the prevalence was recorded as 33.33% and 13.33% with fasciola hepatica and *Fasciola gigantica* respectively. Variable degree of gross lesions were noted in affected livers included cholangitis (63.15%), biliary obstruction (57.89%), fibrosis (68.42%), hyperplasia (60%), haemorrhages (47.36%), enlargement (52.63%), fibrinous exudates (63.15%) and oedema (57.0%) in sheep during the study period.

Swarnakar and Sanger (2014) conducted a study of epidemiological aspects of domestic ruminants; cow, buffaloes and goat with liver fluke infection in Udaipur district. Infection of liver fluke cause mortality in domestic ruminants and lead to great economic losses. An epidemiological study was conducted in Udaipur district from April 2013 to march 2014. Total 379 samples, 255 stool samples and 124 liver samples of freshly slaughtered domestic ruminants were collected from different villages of Udaipur district. The stool examinations determine eggs counts per gram of feces to identify liver fluke spp., *Fasciola hepatica* and *Fasciola gigantica* and focusing on control procedures of infection in domestic ruminants. Out of 379 samples of domestic ruminants, 42 buffaloes were found infected with liver fluke, resulting rate of 11.08%. And this study showed infection of *F. gigantica* was more harmful as compared to *F. hepatica*. The appearance of infection of liver fluke also fluctuates seasonally. The infection also found throughout the year in different seasons, in summer (6.67%), winter (11.70%) and autumn (6.38%) and maximum in monsoon (14.11%). Sex wise occurrence revealed that the female of domestic ruminants (12.04%) were more susceptible to the infection as male of domestic ruminants (10.11%). Appearance with breed showed that buffalo had highest *Fasciola* infections (12.68%), followed by cow (9.70%) and goat (7.5%). The study showed that the infection in domestic ruminants with liver fluke depends on sex, season and breed also.

Getabalew *et al*. (2019) was conducted a study in Debre Berhan Agricultural Research Center (DBARC) to estimate the prevalence of ovine fasciolosis, and to assess the associated risk factors for the disease. Sedimentation technique was employed to detect fasciola eggs during the study period. Atotalof121faecal samples were examined, in which 85(70.2%) were found to be positive for fasciolosis. Breed wise study was conducted and 57.6%, 35.3%, and 7.1% prevalence's were recorded for Local, Cross and Exotic sheep breeds, respectively. The difference in the prevalence of the disease among breeds was statistically significant (X2=32.277; df=2; p=0.000). The prevalence of ovine fasciolosis within each breed were 83.33%, 87.5%, 23.92%, 24%, and 66.67% in Menze, Local X Awasi, Local X Dorper, pure Dorper and Washera, respectively. From local sheep, the prevalence was high in Menz (83.33%) compared to Washera (66.67%). Out of cross breed sheep, the prevalence was higher in Local X Awasi (87.5%) when compared to Local X Dorper (23.92%). The lowest prevalence (24%) was recorded in Exotic breed (Dorper).

Chekol and Girma (2018) conducted a study on the prevalence of ovine fasciolosis was conducted in Wadla district from October 2016 to June 2017. A total of 384 fecal samples were randomly collected directly from the rectum of individual animals. Parasitological investigation was performed using sedimentation technique. From a total of 384 copro-logically examined samples 130 sheep were found positive for fasciolosis with an overall prevalence of 33.85%. From the study peasant association Kone, Dorera, Betehar, Beteyohannis and Gashena were accounted for 34.61%, 32.47%, 32.89%, 35.06% and 34.21 % respectively. The difference in the prevalence in the five peasant associations was not statistically significant (p> 0.05). The prevalence of ovine fasciolosis was computed for the different age, sex and body condition categories. The prevalence rate of fasciolosis in young sheep 32.43% was less than 34.75% and the difference were not statistically significant (p>0.05). The prevalence of fasciolosis in sex groups in the present study was 34.69%, 32.98% in female and male respectively. On the other hand in the present study area the prevalence of fasciolosis was found to be higher in sheep with poor body condition than those with medium and good body conditions with prevalence of 36.5%, 33.88% and 27.03% respectively. There is no statistically difference ($p>0.05$) between three types of body condition scores.

Abduleziz Jema (2017) was conducted to determine the prevalence and risk factors associated with small ruminant fasciolosis in Haramaya Districts from November 2013 to 2014. Fecalsamples were examined by using sedimentation technique from 431 small ruminants (291 sheep and 140 goats). The study revealed that the overall prevalence of fasciolosis was 34.8%. The prevalence offasciolosis based on species were 40.5% and 22.9% in Ovine and Caprine, respectively and a significant difference was observedbetween sheep and goats. Based on ages and body conditionsof the animals there were no significance differences.

The prevalence of fasciolosis based on address varied with highest prevalence recorded in Haramaya (39.3%) followed by Adele (35.34%). Low prevalence was observed in Ifa Bate (26.2%). there was no significance difference ($p < 0.05$) between both address during study. Prevalence of infection based on sex indicated that the prevalence of fasciolosis was higher in female (39.5%) than males (24.6%) and statistically significant difference was found between males and females.

Dawit Kassye et al. (2017) was conducted a study to assess the prevalence of fasciolosis, associated risk factors, identification of *Fasciola* species and sensitivity estimation of direct smear and sedimentation technique for detection of *Fasciola* eggs. An overall prevalence of 13.88 % (23.26% in sheep and 4.12% in goats), 16.91% (27.22% in sheep and 6.1% in goats) and 22.22% (16.91% in sheep and 6.7% in goats) was obtained up on direct smear, sedimentation and postmortem examination respectively. Statistically significant difference ($P<0.05$) was observed in occurrences of *Fasciola* between animals species, body condition and among the origin of animals. The prevalence of fasciolosis was higher in sheep than goats and poor conditioned compared to those with good body condition score. There was statistically significant difference ($P<0.05$) between different place of origin, highest prevalence was observed in animals brought from Harar (57.14%) than Jigjiga (7.24%). Both species of *Fasciola* were recovered from sheep and goats of all study areas*; F. hepatica* being predominant (69.31%) compared to *F. gigantica* (19.31%) and mixed infection (11.36%).

2.3 Pathogenesis

Pathogenesis in fascioliasis commences with juvenile fluke entering the hepatic tissues. The course of pathogenesis in different hosts is similar but may vary in severity with the number of metacercariae ingested, the species involved and the stage of the parasitic development. The juvenile flukes migrate in the parenchyma extensively and cause traumatic lesions with haemorrhage and inflammation. Lesions produced by *F. gigantica* are more severe with fewer flukes as compared to *F. hepatica*, which may be attributed to the longer duration of migration in hepatic parenchyma, larger size and spines present all over the tegument of the former species (Ogunrinade, and Anosa, 1981).

2.4 Pathology

The disease fascioliasis is by most authors clinically described to occur in three phases, viz., acute, sub-acute and the chronic. The acute phase commences between 2-6 weeks after ingestion of substantial number of metacercariae and the species involved. In *F. hepatica* it may be 2000 metacercariae for sheep while for *F. gigantica* 300 metacercariae can produce similar condition. The acute phase is characterized by severe haemorrhage caused by the migrating juvenile flukes in the hepatic tissue rupturing the blood vessels. The liver parenchyma, particularly the ventral lobe associated with gall bladder, is severely damaged assuming an uneven surface covered with blood clots. In late stage between 11-25 weeks of infection depending on the species of parasite and host involved, the animal squats in a specific posture on its right abdomen resting the ground and head turned to opposite direction. The animal disinclines to stand or move and feed. Between 3-7 days the condition worsens and the animal lays flat with extended body, blood froth may ooze out through mouth and nostrils before death. At necropsy, the liver is enlarged, haemorrhagic covered with fibrous clots and necrotic tunnels with migrating flukes. The ventral lobe is covered with fibrous exudates and from sub capsular haemorrhage the blood stained fluid pass into abdominal cavity with ascitis. In small ruminants multiple adhesions of adjacent organs with liver are present. Sheep may die suddenly without exhibiting any clinical manifestations, or following weakness, anorexia and pain. (Freeland, 1976).

The subacute form of disease results when the host ingests moderate doses of metacercariae over a longer period and there in influx of different migratory stages of flukes in the liver. While some have reached bile ducts and caused cholangitis other are still migrating tissues like that of acute disease but of less severity. Liver shows enlargement and haemorrhagic tracks all over the surface and in substance. Rupture of sub capsular haemorrhage is rare. The animals show weight loss and there is accumulation of fluid in the abdomen causing ascites with submandibular and facial oedema. Other clinical manifestations include anaemia, hypoalbuminaemia, eosinophilia and elevated alanine aminotransferase (ALT) and aspartate tgransaminase (ASL) serum levels. Animals, particularly sheep harbouring *Clostridium novyi* in liver, after invasion with juvenile fluke can lead to necrotic *hepatitis* called 'black disease' with fatal consequences. Chronic fascioliasis is manifestation caused by infection with moderate number of metacercariae. It is the most common form of infection in animals and also man. Liver pathology consists of progressive biliary cirrhosis; bile ducts are prominent, thicken, fibrous and may be calcified. Fibrosis is as a result of repair of migratory tracks and cholangitis. Bile ducts containing flukes are dilated, filled with fluke eggs, blood and tissue cells. The fluke spines embed in the epithelium resulting in hyperplasia. In later stage, encrustation of calcium form complete cast of bile duct. At necropsy the liver is pale and hard with irregular outline. The liver pathology is characterised by hepatic fibrosis and hyperplastic cholangitis. There is progressive loss of condition. Anaemia and hypoalbuminaemia results in submandibular oedema and ascites. Anaemia is hypochromic and macrocytic accompanied with eosinophilia. Fasciola eggs are demonstrated in faeces. In milder infections the disease may go un-noticed but effect of production could be significant due to inappetance and effect on post absorptive metabolism of protein, carbohydrate and minerals. Acute Fasciolosis causes huge economic loses as directly or indirectly in terms of anemia due to its ability to suck blood to the extent of 0.2-0.5 ml per day and decrease in the total proteins especially albumin (Soulsby, 1987).

2.5 Immunology

A number of studies indicated the mechanism of resistance at the gut wall is thymus-independent and that non-specific and hypersensitivity reactions may play a role. The juvenile flukes migrating through intestinal wall and peritoneum induce infiltration of eosinophils, IgG1 and IgG2 antibodies around the parasites. Protection to *F. hepatica* in rats has also been associated with accumulation of eosinophils and IgE sensitised cells in the gut wall and juvenile antigen specific IgG1, induced in early infection (Tliba *et al.*, 2000).

Contrarily, sheep seems to show no resistance to reinfection with *F. hepatica* despite of large infiltration of white blood cells in liver and production of antibodies to the parasite and fibrosis in the liver8. In cattle, elimination of primary *F. hepatica* infection at 20-28 weeks coincided with highest level of homocytotropic antibodies whereas drug abbreviated *F. hepatica* infection induced resistance resulting in insignificant liver fibrosis (Hoyle *et al.*, 2003).

Study knowledge about prevalence, diagnosis, treatment and control of Fascioliasis has been reviewed. This article evaluates more recent work along with previous studies. The fecal egg count, signs / symptoms and specific antibodies in serum were the only diagnostic tools in the past however now for detection of *F. hepatica* specific copro-antigen has been developed and commercialized. An indirect enzyme linked immunosorbent, assay (IEA) allows an early diagnosis. The tracer animals and snail studies have widened the existing knowledge. The treatment has been carried out mainly with Fasciolicides (Albendazole, Oxyclozanide and Triclabendazole), however resistance has been developed. Most of the recommended treatments are not feasible. Seasonal deworming is essential. Pasture management by creating bio competent environment with snail predators can be very effective in reducing the rate of incidence and controlling the problem. Vaccine is there but is not frequently used due to incompatible immune response. The studies on reduction in milk yield due to Fascioliasis are still lacking and require extensive research/ investigations (Rana *et al.*, 2014).

Suggesting protective immunological response reducing migration of flukes into the hepatic tissue and bile ducts. Further studies have suggested a correlation between the IgG2 response to fluke proteinases (cathepsin L) vaccine and protection in cattle, whereas the IgG1 response was correlated with susceptibility and positively regulated by IL4. The elevated parasite-specific IgG1 but low IgG2 levels in infected cattle is consistently observed (Estes and Brown, 2002).

Fasciola evade the host immune attack by frequently sloughing the tegumental associated glycocalyx layer and target of host antibody-mediated eosinophils, neutrophils or macrophages attacks (Piedrafita *et al.*, 2001).

Other Fasciola isoenzymes viz., cysteine proteases (cathepsin L and B) are considered to play main role in tissue invasion and immune evasion (Mulcahy and Dalton, 2001). The cathepsin Ls can 1) degrade both extra cellular matrix (fibrillar types I and II collagen) and basement membrane (type IV collagen) aiding in parasite tissue invasions; 2) degrade haemoglobulins rendering it to the parasite for nutritional purposes and help in parasite survival, and 3) cleave immunoglobulins in the hinge region (Berasain *et al.*, 2000) preventing antibody mediated attachment of eosinophils, neutrophils and macrophages. Recently secretory products of Fasciola rich in cathepsins has been shown to suppress T cell proliferation in sheep in vitro (Prowse *et al.*, 2002).

2.6 Diagnosis

The diagnosis of Fasciolosis is based on the detection of eggs in feces or *F. hepatica* specific antibodies in serum. Recently, a method based on detection of *F. hepatica* specific copro-antigen has been developed and commercialized (Mezo *et al*., 2004). The sensitivity (Se) and specificity (Sp) of these tests have been determined after experimental infection (Cornelissen *et al*., 2001) or by using two distinct populations, a positive population selected from an enzootic area and a negative population from a fluke free area. (Ibarra *et al*., 1998; Mezo *et al*., 2004).

The use of ELISA kit manufactured by Institut – Pourquier which employs the “f2” antigen is capable of detecting antibodies of fasciola hence it allows early detection of the disease as fluke specific antibodies can be detected in the serum of most animals by 14 days post infection (Hillyer, 1999; Castro *et al*., 2000; Kaplan, 2001; Reichel, 2002).

During the migration of liver flukes through the liver parenchyma (7-35 days after infection), the immature flukes released antigens (which stimulate the production of antibodies) which are detectable in the serum but once the flukes reached the bile ducts, antigenic release ends and antigens are no longer present in sera hence the demonstration of circulating antigens in sera definitely allows the early detection of live immature flukes (Sanchez *et al*., 2001). PCR is also used in the identification of intermediate/hybrids between F. hepatica and F. gigantica (Li *et al*., 2009). PCR has been used for the identification of F. hepatica making it possible to determine resistance markers for diagnosis (Fairweather, 2005).

2.7 Treatment

Several drugs are effective for fascioliasis, both in humans and in domestic animals. The drug of choice in the treatment of fasciolosis is triclabendazole, a member of the benzimidazole family of anthelmintics (Savioli *et al.*, 1999). The drug works by preventing the polymerization of the molecule tubulin into the cytoskeletal structures, microtubules. Resistance of *F. hepatica* to triclabendazole has been recorded in Australia in 1995 (Overend and Bowen, 1995) and Ireland in 1998 (Mulcahy and Dalton, 1998). Praziquantel treatment is ineffective (Schubert and Phetsouvanh, 1990). There are case reports of nitazoxanide being successfully used in human fasciolosis treatment in Mexico (Rossignol *et al.*, 1998).

2.8 Prevention and Control

Hygienic feeding habits are the best preventive measures for keeping parasitic infections. So is true for fascioliasis. Besides promoting public health awareness through education in endemic areas are proving to be increasingly effective in prevention of fascioliasis diseases these days. So far fascioliasis is concerned, avoiding of watercress plants, water chestnuts and vegetation grown in aquatic environment, consuming raw has far reaching consequences in endemic areas. Thorough washing of edible aquatic vegetation with running water can get rid of metacercariae to great extent and treating vegetation with 1% citric acid, 1% acetic acid (12% commercial vinegar), liquid soap (1.2%) or potassium permanganate solution (24 ppm) kills and detaches all metacercariae (Pan American Health Organization, 2003).

In country like Bangladesh and elsewhere too, providing animals' clean water to drink and prevent these enter into natural water reservoirs infested with snails shall greatly reduce the risk of acquiring fascioliasis. Ensiling of animal feed such as paddy straw likely to be infested with metacercariae kills not only the deposited metacercariae (Gupta & Kamra, 1987). But also increase the digestibility and nutritive quality of the feed. Elimination of snails from environment shall break the life cycle of the parasite. This can be achieved by application of molluscicides like copper sulphate, bayluside, trifenmorf with caution because this is not always possible due to environmental objections. Cleaning of ponds, ditches, canals etc., of unwanted aquatic vegetation can drastically reduce the mollusc population and almost eliminate all the molluscan eggs. Plant origin molluscicides, which degrade into simpler components within short period of application, may also be fruitful (Gupta *et al.*, 1988).

Biological methods using predators (carnivore fishes, ducks, etc.), parasites of snails can also be used effectively. The other measure for control of parasitic infections is vaccination. Since success achieved in development of vaccine against the helminth Dictyocaulus viviparous, the lung worm of cattle, in 1960s, researchers have been engaged to explore the field with other helminth parasites also. The approach for vaccine development against fascioliasis has not been much different so far. Initial trials consisted of vaccine using radiation-attenuated metacercariae followed by crude somatic parasite extracts and mixtures of secreted parasite proteins (Haroun & Hillyer, 1986).

Since then a number of parasite proteins identified and characterized from both *F. hepatica* and *F. gigantica* have been tested in vaccine trials with partial success in different laboratory and natural animal hosts. The current vaccine strategy implies the immunological mechanism by which the parasite metabolic products (enzymes) variously termed as ‘defined, natural and conventional’ antigens aiding in invasion, survival and evasion of host immune responses are neutralized rendering the parasite helpless. Such parasite proteolytic enzyme agents known as defined molecular candidate vaccine agents are being looked forward against Fasciola with bright prospects. The basic concept is that if such vaccination is successful it can reduce parasite transmission enormously. Lastly but not the least, prevention of human infection by avoiding consuming metacercariae is much discussed above. Measures are many but to follow these require patience and concern. Otherwise, human infection is like meeting a fatal accident as a result of careless driving. Treatment of animals with broad-spectrum fasciolicide would prevent contamination of environment with parasite eggs and subsequently infection of intermediate snail host and contamination of environment with metacercariae. On the basis of strategies used to identify these defined candidate vaccine molecules of *F. hepatica* and *F. gigantica*, three prototype antigens have been identified These are: (1) The fatty acid binding proteins (FABP), a cross protective antigen of *F. hepatica* recognized by cross reactive antibodies raised against the trematode Schistosoma mansoni, rendering cross protection against S. mansoni. (2) The glutathione stransferase (GST), a Fasciola molecule homologous to antigen previously shown to protect animals against S. mansoni and S. japonicum infection. (3) Cathepsin-L, the essential Fasciola molecules, constituting of cysteine proteinases and predicted to perform functions essential for parasite growth and survival. Under this category, *F. hepatica* cysteine proteinase recognized by the host post-infection inducing high levels of protection of up to 70% in sheep vaccinated with the purified cystiene proteinases (Spithill *et al.*, 1997).

CHAPTER III

MATERIALS AND METHODS

3.1 Study area and duration

The study was conducted for a period of six months starting for July to December, 2018. This study was designed to focus on the prevalence of fascioliasis in small ruminant at sadar upazilla of Dinajpur. The experimental plan was prepared in the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammed Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh. During the investigation a total of 106 sample (80 goats and 26 sheeps) were collected from different areas of sadar upazila of Dinajpur district (50°37′ N. latitude and 88°39´ E longitude on the eastern bank of the river Punarvhaba), Bangladesh.

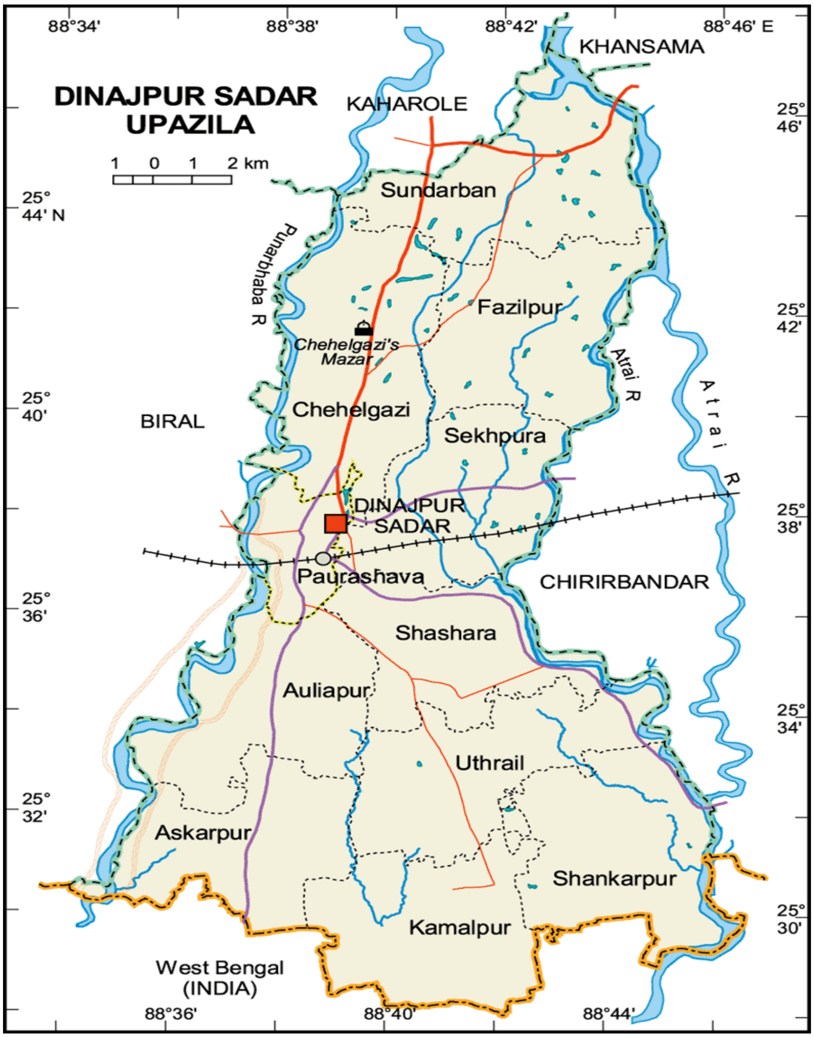

Figure 2: Map of the Area study

3.2 The major work of the present study

- Collection of faecal sample directly from the rectum by using fingertips in selected goat and sheep at the area of the study
- Preservation and immediate shipment of collected samples to the Laboratory.
- Investigation of the prevalence of fascioliasis in small ruminant in relation to age**,** Sex, and nutritional status.

3.3 Test material

- Protective apron, gloves and mask.
- Fresh faecal sample from rectum.
- Vial
- Mark pen
- 10%Formalin
- Note Book
- Pen
- Sodium chloride
- Water or Flotation Solution
- Beakers containers
- Pasteur pipettes
- Test tubes
- Centrifuge Machine
- Slides
- Coverslip
- Microscope

3.4 Selection of animal

A total of 106 faecal sample including 80 goat and 26 sheep were collected purposefully from kamalpur,shankarpur,askarpur,uthrail,auliapur,shashara,fazilpur,shekhpura. Those animals were considered for the present study as study population. Study population was divided into three age groups i.e. young (6 month-1year); adult (1-2 years); old (2- above years) on the basis of owner record and dental formula. Their sex was divided into (Male and Female) and also their nutritional status was divided into two healthy or poor on the basis of (clinical observations).

3.5 History, Physical and Clinical Examination

History along with other necessary information was taken from individual farmers by cross questioning. History included Age, sex, breed, clinical signs and location of small ruminant and previous history of fascioliasis. Physical examination was done for each sheep and goat in the study area. Simultaneously faecal sample was collected directly from the rectum.

3.6 Collection and examination of faeces

About 5-10 grams of faeces were collected from each animal. Collected samples were transferred to a vial containing 10% formalin and labeled properly. The faecal sample was examined using standard direct smear, floatation and sedimentation methods

The direct smear technique was done for examination of egg of fasciola and it was conducted by mixing a drop of water with bit of faeces using an applicator stick on glass microscopic slide covered with cover slip and examined at low and high power.

In floatation technique faeces were mixed thoroughly in a saturated sodium chloride solution and strained through a wire sieve into a test-tube. Further saline solution was added until a positive convex meniscus appeared and a coverslip applied immediately. It was allowed to stand for 8 to 10 min. The coverslip was then removed, applied to a glass slide and examined under a microscope using a 10X objective and high power.

In sedimentation technique faeces were mixed thoroughly with water in a beaker, strained through a wire sieve into a beaker and the mixture left to stand for 4 min. The supernatant was poured off, and then the procedure was repeated 2 to 3 times until the supernatant was clear. The sediment was then poured into a petridish and then the sediment was examined under the microscope using a 10X objective.

3.7 Experimental layout

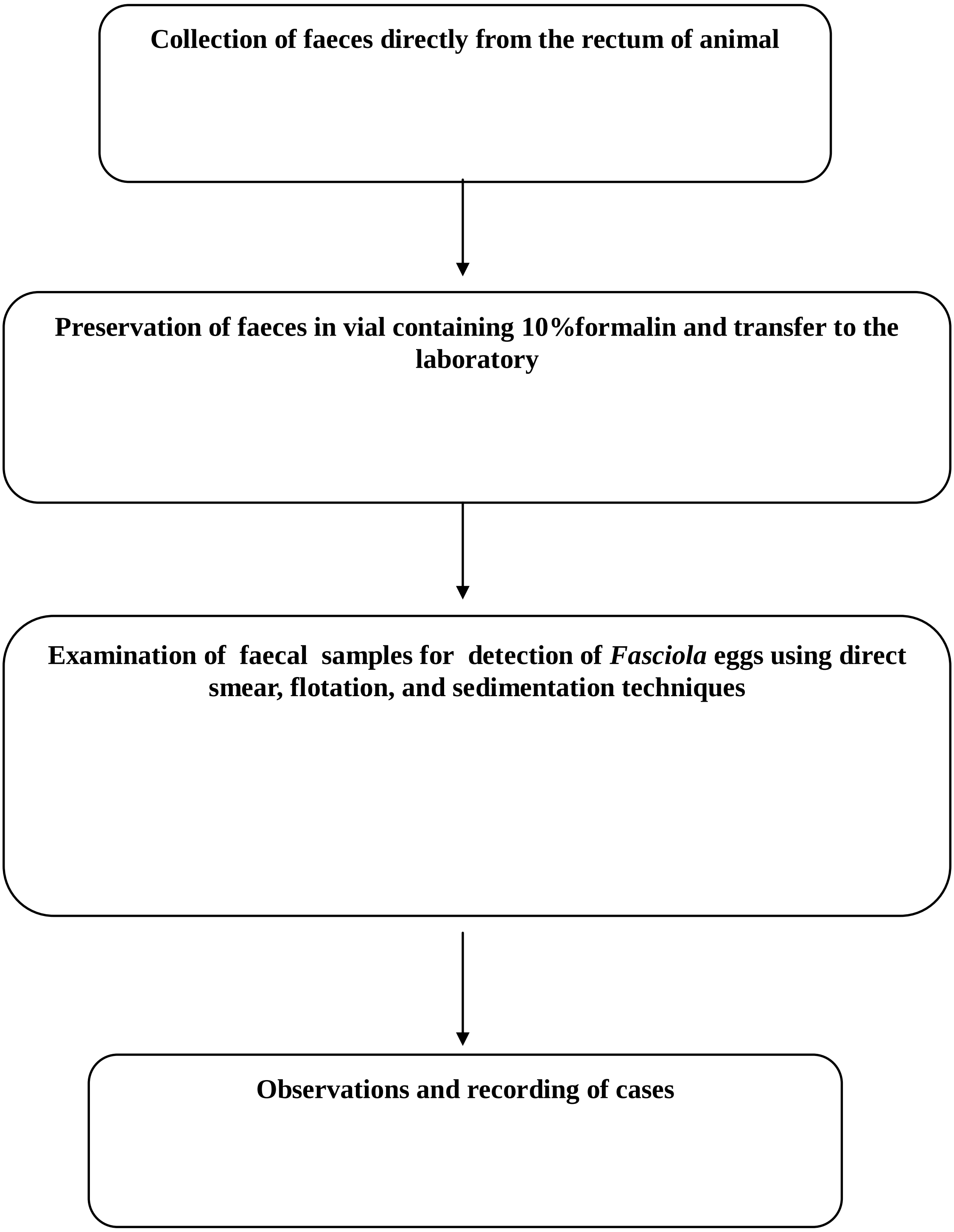

Figure 3: Experimental layout

Figure 4: Collection of faeces sample from the rectum of goat

Figure 5: Collecting feacal sample from the rectum of sheep

Figure 6: Preservation of faeces in the vial containing 10% formalin

Figure 7: Preparation of slide by direct smear method

Figure 8: Observation under microscope using various objectives

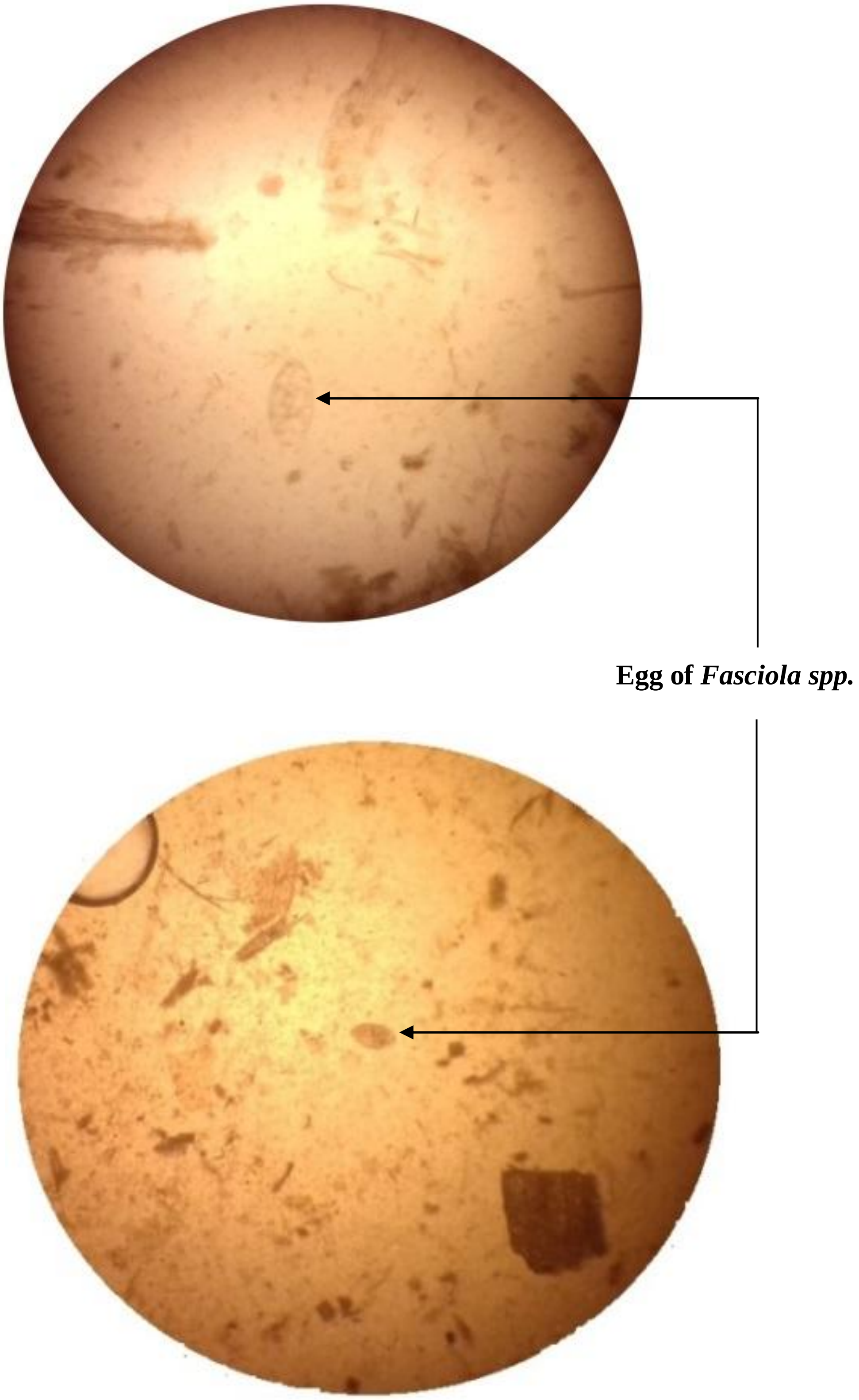

Figure 9: Egg of *Fasciola spp.*

3.8 Diagnosis of Fascioliasis

Tentative diagnosis was made on the basis of history, physical and clinical signs (depression, dullness, in appetite, rough hair coat, diarrhea, emaciation). Confirmatory diagnosis was made on the basis of faecal examination findings; Fasciola eggs were confirmed by the characteristics of oval shaped, eccentric morulla, operculum present, yellow brown in color (Valero *et al*., 2009). Presence of single eggs of *Fasciola sp*. in one microscopic focus during faecal examination was recorded as positive for fascioliasis.

3.9 Statistical Analysis

The data were recorded and analyzed statistically by using statistical software ‘SPSS’ (version 22). Chi–Square Test were performed and the results were expressed in percentage with P–value and significance was determined when $P<0.05$.

CHAPTER IV

RESULTS

4.1 Prevalence of fascioliasis in relation to species

A total of 106 faecal samples were examined (80 goat samples and 26 sheep samples), among 22 samples were positive. In the study, the overall prevalence was 20.75% and In goat the prevalence was 20% and In sheep the prevalence was 23.07%. It is found that species had no significant (P < 0.05) effect on the prevalence of fascioliasis in small ruminant.

Table 1: Species wise prevalence of fascioliasis

Species	Number of sample examined	Number of positive cases	Number of negative cases	Prevalence (%)	Chi-square value	P-value
Goat	80	16	64	20%	0.113	0.74 (NS)
sheep	26	6	20	23.07%		
Total	106	22	84	20.75%		

NS means no significant at 5% level of significance (P<0.05)

4.2 Prevalence of fascioliasis in relation to age

The highest prevalence of fascioliasis was observed in goat in the group ages among old (23.07%) followed by adults (20.58%) and young (15%). In sheep the highest prevalence of fascioliasis was observed in the group ages among old (25%) followed by adults (22.22%) and young (20%). It is found that age had no significant (P < 0.05) effect on the prevalence of fascioliasis in small ruminant.

Table 2: Age wise prevalence of fascioliasis

Species	Age group	Number of sample examined	Number of positive cases	Prevalence (%)	Chi-square value	p-value
Goat	0-1 year	20	3	15%	0.474	0.789(NS)
	1-2 years	34	7	20.58%		
	2 and above years	36	6	23.07%		
Sheep	0-1 year	5	1	20%	0.055	0.97(NS)
	1-2 years	9	2	22.22%		
	2 and above years	12	3	25%		

NS means nonsignificant at 5% of level of significance ($P<0.05$)

4.3 Prevalence of fascioliasis in relation to sex

In the present study the prevalence of fascioliasis of goat in female was higher (22.22%) than in males (17.14% %) and the prevalence of fascioliasis of sheep in female was higher (28.57%) than in males (16.67%). The present study revealed that the prevalence of fascioliasis was not significantly ($P<0.05$) affected by sex.

Table 3: Sex wise prevalence of fascioliasis

Species	Sex group	Number of sample examined	Number of positive cases	Prevalence (%)	Chi-square value	p- value
Goat	Male	35	6	17.14%	0.317	0.573(NS)
	Female	45	10	22.22%		
sheep	Male	12	2	16.67%	0.516	0.473(NS)
	Female	14	4	28.57%		

NS means non significant at 5% level of significance ($P<0.05$)

4.4 Prevalence of fascioliasis in relation to nutritional status.

In goat the prevalence of fascioliasis in poor group was higher (35.48%) than in healthy group(10.20%).and the prevalence of fascioliasis in sheep in poor group was higher (33.33%) than in healthy group (17.65%). The present study revealed that the prevalence of fascioliasis was significantly ($P<0.05$) affected by nutritional status in case of goat.

Table 4: Nutritional status wise prevalence of fascioliasis

Species	Nutritional status groups	Number of sample examined	Number of positive cases	Prevalence (%)	Chi-square value	p- value
Goat	Healthy	49	5	10.20%	7.584	00.006*
	poor	31	11	35.48%		
Sheep	Healthy	17	3	17.65%	0.816	0.366(NS)
	poor	9	3	33.33%		

***** means significant at 5% level of significance (P<0.05)

NS means no significant at 5% level of significance (P<0.05)

CHAPTER V

DISCUSSION

5.1 Fascioliasis associated with species

After examination of 106 faecal sample, 22 samples were positive. The result of the present study revealed that the overall prevalence of fascioliasis was 20.75%. In case of goat we examined 80 faecal sample and 16 samples were positive. In case of goat the prevalence was 20%. In case of sheep we examined 26 faecal sample and 6 samples were positive. In case of sheep the prevalence was 23.07%.

The prevalence was 16% in sheep and 6.6% in goat, respectively (Irfan *et al.*, 2016). The overall prevalence was 11.6% and the specific prevalence in the study was 14.6% in sheep and 8.8% in goat (Mulatu and Addis, 2011). The infection rate was recorded 32% in goat and in sheep 30.91% (Al-Mamun *et al.*, 2011). The prevalence rate was 20.75% in goat (Hossain *et al.*, 2011). The prevalence rate was 56.3% was in sheep (Asrat *et al.*, 2005). The prevalence was 12.70% in small ruminant (Mazyad and El-Nemr, 2002).

The result of the present study was nearly similar to the study of Irfan *et al*. (2016), Hossain *et al*. (2011) and Mulatu and Addis, (2011). But Al-Mamun *et al*. (2011), Asrat *et al*. (2005) and Mazyad and El-Nemr, (2002) were disagree with the present study results.

The variation with the findings of the present study was high and it might be due to location, use of anthelmintic, and duration of the study. Prevalence of fasciolosis is attributed by multi-factorial risk factors which comprise host, parasite and environmental effects. High-rainfall areas favour development and survival of both the intermediate host snail and the developmental stages of the parasite (Affroze *et al.*, 2013).

5.2 Fascioliasis associated with age

In the present study in both goat and sheep age was divided into three age groups i.e. young (6 month-1year), adult (1-2 years) and old (2 and above years). The prevalence of fascioliasis was observed in goat in the old (23.07%) followed by adults (20.58%) and young (15%). The prevalence of fascioliasis was observed in sheep in the old (25%) followed by adults (22.22%) and young (20%).

The prevalence of fascioliasis was higher in sheep in older animal (35%) compared to adult (30%) and young (30), (Khan *et al.*, 2015). The prevalence of fascioliasis was higher in goat in young animal (49.69%) compared to adult (28.79%), (Anjum *et al.*, 2014). In case of sheep prevalence was higher in adult (34.75%) than young (32.43%), (Chekol and Girma, 2018). In case of goat the prevalence was higher in old (20.83%) than young (16.27%), (Hossain *et al.*, 2011). In case of goat the prevalence was higher in young animal (33.33%) than adult (17.2%), (Njoku-Tony *et al.,* 2009). In case of sheep the prevalence of fascioliasis was higher in old (72.34%) compared to adult (52.55%) and young (66.6%), (Yonas *et al.,* 2018).

The result of the present study was nearly similar to the study of Khan *et al*. (2015), Chekol and Girma, (2018), Hossain *et al*. (2011) and Yonas *et al*. (2018). All of those agree that the old goat and sheep is higher prevalence than young and adult. But the results of Anjum *et al.* (2014), and Njoku-Tony *et al.* (2009) disagree with the present study.

The infection rate is higher in older animals could be due to physiological differences, such as stress, inadequate nutrition, infectious diseases their grazing habit close to submerge areas or lowest in younger age animals because of maternal immunity in younger animals (Ahmed *et al*., 2007).

5.3 Fascioliasis associated with sex

This study revealed that the prevalence of fascioliasis of goat in female was higher (22.22%) than in males (17.14%). The prevalence of fascioliasis of sheep was higher in female (28.57%) than in male (16.67%).

In case of sheep female recorded higher prevalence (66.66%) than males (33.33%), (Mukarim Abdurahaman *et al.*, 2019). The prevalence was higher in female (42.6%) than male (30.4%) in goat and in sheep prevalence was also higher in female (44.6%) than male (30.7%), (Isah, 2019). The prevalence of Fascioliasis among male sheep (13.4 %) was higher than that among females (13.2 %), (Ahmed *et al.*, 2007). The prevalence of fascioliasis in small ruminant was higher in male (62.3%) than female (60.1%), (Kiros *et al.*, 2016). The prevalence of fascioliasis is higher in female (39.15%) than male (19.96%) in goat, (Islam *et al.*, 2014).

The present study is similar to the findings of Mukarim *et al*. (2019), Isah, (2019) and Islam *et al*. (2014). All of those agree that the female is higher prevalence than male. But Ahmed *et al*. (2007) and Kiros *et al*. (2016) disagree with the present findings.

5.4 Fascioliasis associated with nutritional status

Nutritional status was divided in to two groups (healthy and poor). The prevalence of fascioliasis in poor animal was higher (35.48%) than in healthy (10.20%) in goat. In sheep prevalence was higher in poor animal (33.33%) than healthy (17.65%). The present study revealed that the prevalence of fascioliasis was significantly ($P<0.05$) affected by nutritional status in goat.

In case of sheep poor health animal recorded higher prevalence (36.5%) than healthy animal (27.03%), (Chekol and Girma, 2018). The prevalence of fasciolosis in small ruminant was found to be higher with poor body (76.9%) condition than those with medium (11.3%) and good body condition (6.4%), (Berhe *et al.*, 2017). The prevalence of Fascioliasis in small ruminant was higher in poor body condition animals (44.5%) than healthy animals (13.3%), (Bedada *et al.*, 2017). The prevalence of fasciolosis in small ruminant was found to be higher with poor body (41.2%) condition than those with medium (36%) and good body condition (25%), (Jemal, 2017).

The present study is similar to the findings of Chekol and Girma, (2018), Berhe *et al*. (2017). Bedada *et al*. (2017) and Jemal, (2017). All of those agree that the poor health animal show higher prevalence than healthy animal.

This could be due to stress associated with poor nutrition. Well-fed animals can with stand the harmful effects of parasites, can remain reasonably productive and may require less anthelmintic treatments when compared with under nourished animals (Bitew *et al.,* 2011).

CHAPTER V

CONCLUSION

In conclusion fascioliasis in small ruminant is one of the major constrains for small ruminant development in Bangladesh. The hot and humid climates in fact make this country a paradise for parasitic animals. So, the control measures should be taken by destruction of intermediate host (snail population). Avoiding low lying pastures have also significantly important for controlling fluke infections. The animals should be dewormed on time and take an account on management system. Prevalence study of fascioliasis is considered as tools for controlling parasitic infection. Although the present study results have some limitation because of low sample size and low duration of the study may lead to improper diagnosis. Further epidemiological study is strongly recommended. Despite of all constrains the present study findings will help researchers further epidemiological study of small animal fascioliasis.

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