

**PATHOLOGICAL INVESTIGATION OF LIVER DISEASE
IN GOAT, A SLAUGHTER HOUSE STUDY IN RANGPUR
SADAR**

**A THESIS
BY**

MOST. AINUNNAHAR
REGISTRATON NO.: 1605472
SEMESTER: January-June/2018
SESSION: 2016-2017

**MASTER OF SCIENCE (M.S.)
IN
PATHOLOGY**



**DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR**

JUNE, 2018

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JULY, 2018

Dedicated to
My
Beloved Parents

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The author

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ABSTRACT

This study was conducted to investigate the different pathological condition of liver of goat at Rangpur Sadar of Rangpur district in Bangladesh. Cases were recorded with respect to age and sex from different slaughter house of study area from December, 2017 to May, 2018 and diagnosis was made on the basis of histopathological examination. A total of 310 liver were examined of which 38 were infected. Overall prevalence of liver diseases was (12.26%) and highest prevalence was observed in Liver abscess (2.9%) followed by Hepatitis (2.58%), Fascioliasis (2.25%), Tuberculosis (1.94%), Cirrhosis (1.29%) and Hydatidosis (1.29%).

Female goats were more susceptible (55.26%) than male (44.73%). The prevalence was varied according to age and highest was in adult (68.42%) than young (31.58%). Most of the affected lesions were produced by the suspected liver were examined microscopically. These results indicate that, the prevalence of various liver diseases in the goats was higher at Rangpur Sadar. Thus there is a need for an appropriate control measures in order to prevent and minimize the loss caused by such diseases.

Key Word: Prevalence, liver diseases, Gross and Histopathology.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	=	Percentage
>	=	More than
<		Less than
&	=	and
°C	=	Degree Centigrade
CONTD.	=	Continued
DLS	=	Directorate of Livestock Services
et al.	=	and other
ed.	=	Edition
FAO	=	Food and Agricultural Organization
Fig.	=	Figure
H & E	=	Hematoxylin and Eosin
ml	=	Mililiter
No.	=	Number

CHAPTER-I INTRODUCTION

Animal agriculture, particularly cattle, poultry and goat sub-sectors is contributing significantly to Bangladesh economy. Milk, beef, eggs, poultry meat and goat meat (chevon) are sources of animal protein that are in great demand and their demand will continue to increase with increasing population. Goat is one of the most important livestock species in Bangladesh and particularly useful for low-income farmers, landless labors and distress women, who can't effort to rear cattle, hence, goat is called "the cow of poor people". Goat keeping is related to the rural poor and landless specially women (Chowdhury, 2002). Most of the goats reared are Black Bengal, reputed for their prolificacy, fertility, early sexual maturity and adapted to hot humid climate. There are also Jamunapari breed and some other crossbreds. The contribution of livestock in Gross Domestic Product (GDP) during 2017-2018 was 1.54% while the share of livestock in Agricultural GDP during the same time was 13.62%. Goat occupies a significant position as an animal genetic resource in the pre-dominantly agro-based farming system in Bangladesh (Hossain, 2006). About 261.00 lakh goats are distributed throughout the country (DLS). The present production systems of goats are in scavenging by small holder farmers with 2-5 goats per farmer. The contribution of goat for livelihood improvement and food security is well established in Bangladesh. Bangladesh there in total foreign exchange earning accounts for about 6.2 % in which share of goat is enormous (Alam, 2015).

There exists a variety of problems in goat rearing of Bangladesh such as insufficient pasture land, lack of technical expert, insufficient supply of vaccine, lack of epidemiologic study and shortage of government employee in the field level and various diseases of different systems of animals. Lack of proper care and overall faulty husbandry practices are also responsible for higher goat mortality in the prevailing production system (Husain *et al.*, 1993). Among various problems, diseases play an important role interfering with the development of goat production in our country. The occurrence of diseases is an important factor which influences the productivity and economy of goat farming. Goats suffer from many animal diseases and some of these are common with other livestock species, while a few are specific to goats only. Diseases in goats result in mortality and morbidity losses, resulting in lower production. Several studies (Paliwal *et*

al., 1991;) have shown that on an average 20 % of kids and 10 % of adult goats die each year. Liver is considered the most important organ for animal health production and reproduction. Many of the metabolic activities of the body occurred in the liver. Liver infection is an important disease that affects all kinds of meat producing animals, this lead to great losses to live-stock production and national income due to condemnation of great numbers of livers in the slaughter houses (Foster and Woods, 1970 and Tomate, 1973). Liver is involved in many metabolic disorders and parasitic diseases (Saleha, A.A., 1991). It may harbor pathogens (Fetcher, A., 1983) which are dangerous for human consumption when passed with localized or mild infection. However, the most common liver lesions which are harmful to human health are fasciola (FA), liver abscess (LA) hydated cyts (HC) etc. (Mellau, L.S.B., *et al.* 2010). FA is one of the most important parasitic diseases of livestock in the tropical and subtropical areas (Solusb, E.J.L., 1986) with an enormous economical impact on livestock production (Max, R.A., *et al.* 2006).

Different studies have been carried out in various parts in the world and also in Bangladesh, but limited attempt have been taken to study the prevalence of liver diseases in Rangpur district. However, over the last some decades, there are tremendous changes in the climates, life styles of people and husbandry practices of livestock all over the Bangladesh. Considering these facts, present study was undertaken to investigate the pathological condition of liver of goat, along with following objectives:

- To investigate the overall prevalence of liver diseases of goat at Rangpur sadar.
- To determine the gross and histopathological changes in affected liver of slaughtered goat.

CHAPTER-II

REVIEW OF LITERATURE

In this study, obtainable and related literatures are overlook with the emphasized on the prevalence of different liver diseases of goat with different parameters of breed, sex, age, season is reviewed here under the following subheadings.

2.1. Prevalence of liver diseases with parameters

Elias Gezahegn, et al. (2017). Out of 768 sheep and goats slaughtered 67.7% of liver revealed total condemnation. The major cause of these liver condemnations are due to *Cysticercus tenuicollis* (11.5%), Calcification (20.31%), Cloudiness (20.57%), Cirrhosis (5.08%), Hepatitis (5.9%), Fatty degeneration (2.99%), *Steilesia hepatica* (3.65%), Adhesion (7.42%), and Hydatidosis (0.13%). Except in *C. tenuicollis*, calcification and cloudiness ($p < 0.05$) which has a significance difference were observed between sheep and goats, insignificance difference ($p > 0.05$) were recorded between sheep and goats in other lesions. Concerning the origin of the animal significance difference ($p < 0.05$) in cloudy lesion were observed on sheep but insignificant difference observed in another lesion. Similarly, significance difference were observed in *C. tenuicollis*, Calcification, and fatty degeneration ($p < 0.05$), and insignificance difference was observed in other lesions in goats.

Islam K. M. et al. (2016). A total of 2000 Black Bengal goat livers were examined, of which 202(10.10%) livers were found to be affected with fascioliosis. Prevalence of fascioliosis was significantly higher in young goats (15.58 %) than in adult (9.59%) and female goats (13.10%) were more susceptible than male goats (7.10%). Seasonal prevalence of fascioliosis was also studied. Highest prevalence (16.51 %) was recorded during rainy season and lowest prevalence (4.70%) was recorded during summer season.

Tatiane C. F. et al. (2016). One hundred and ninety livers condemned due to chronic disease (fibrosis) were evaluated in a bovine slaughterhouse over 12 months. Hepatic lymph nodes were also examined while still attached to livers. The major macroscopic lesion observed in the livers was moderate to severe atrophy of the left lobe associated with compensatory hypertrophy of the right lobe.

Danbirni, S. *et al.* (2016). A study of 12,429 slaughtered goats show the prevalence of tuberculosis-like lesions. The lesions were counted, visually examined and palpated during post mortem meat inspection. Of the 12,429 goats slaughtered and examined, tuberculosis-like lesions were observed in four (4) slaughtered goats with a prevalence of 0.03

Khan S.A, *et al.* (2015). Two hundred and fifty liver samples along with gall bladder and bile ducts show prevalence of liver fluke infestation was recorded as 30% in young and adult and 32% in aged sheep. *Fasciola hepatica* was found 50% and *Fasciola gigantica* 10% in case of young whereas in adults 20% with *fasciola hepatica* and 14% with *Fasciolagigantica* 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.

Mazedul Islam *et al.* (2015). A total 26443 goat were slaughtered of which 1010 were found to be affected with Fascioliasis. The overall prevalence of *Fasciola* infection among slaughtered goat was 3.82%. The highest prevalence (5.6%) was found in July, 2014. Among the seasons there was no significant variation ($P>0.05$) in the prevalence of Fascioliasis where the prevalence was 4.01%, 3.8% and 3.65% in winter, summer and rainy season respectively. Regarding sex, prevalence in male and female goat is 3.79% and 3.85% respectively ($P>0.05$). Among the two regions, Region B had the significantly ($P<0.01$) higher prevalence of Fascioliasis (19.19%) than region A (3.009%). On economic basis, during the study year it was estimated that there was 3.82% liver loss due to condemnation which amounted BDT 184981.5 or US\$ 2374.9 economical loss per year.

Okoye, *et al.* (2015). The livers of slaughtered cattle were examined by visualization, palpation and incision. Macroscopically, some of the infected livers appeared to be slightly swollen with pale color at the round edges, while some appeared greatly swollen, with a few small irregular whitish areas indicating fibrosis over the parietal surface. In some cases, the capsule was thick and rough with whitish or reddish discoloration and parenchyma was hard due to fibrous tissue. Fibrosis of the bile ducts with numerous small

and large patches scattered over the parietal surface and the pipe stem appearance of the liver were noticed.

R. Godara *et al.* (2014). Record That the prevalence of hydatidosis was carried out in slaughtered goats ($n = 177$) by liver and lung examination. The prevalence rate was 19.8 %. A total of 14.1 % goats had cysts in both the livers and lungs while 2.3 and 3.4 % goats had cysts in the livers or lungs, respectively. A total of 9.1 % goats had fertile cysts. The adult goats (>4 years) had a significantly higher ($p = 0.01$) prevalence rate as compared to the young goats (<2 years). Sex had no significant effect on the prevalence of hydatidosis in goats.

Dinaol B.K *et al.* (2014). In this cross sectional study a total of 284 ruminants (77 cattle, 99 sheep and 108 goats). On the basis of gross pathological examination study animals were grouped into three: group-A (78.87%) showed no fasciola spp. and no visible gross lesion (taken as control groups), group-B (12.32%) confirmed with fasciola and fasciola indicative lesion and, group-C (8.80%) were co-infected (fasciola presence, fasciola indicative lesion and other lesions).

M. Sevinc, *et al.*, (2014). The purpose of this investigation was to establish any changes that may occur in liver function in dairy cattle with fatty liver. The liver fat content was determined in all cows histologically. Cows with fatty liver were grouped according to fatty liver fat content as cows with mild, moderate and severe hepatosteatosis. Some chemical parameters (bile acid, AST, ALT, GGT, ALP, CPK, glucose, total protein, albumin, globulin, total bilirubin and urea) were measured. There was a significant increase ($p < 0.001$) in GGT, CPK and AST activities in cows with severe fatty liver than controls. The serum bile acid and urea concentrations were significantly higher ($p < 0.001$) in cows with severe and moderate fatty liver than controls. The bile acid and albumin levels were also significantly different between severe and moderate fatty liver groups. In conclusion, bile acid, GGT, AST and albumin parameters seem to be helpful for liver function in cows with fatty liver. However, because of considerable variation of these results they should be interpreted with caution.

Suhair S.M. *et al.* (2013). In this retrospective study it was found that both cattle and sheep suffered from fascioliasis (FA), liver cirrhosis (LC), liver abscess (LA), calcification (LCA), hydated cyst (HC), fatty change (FG) and ictrus (IC). The

prevalence of FA, LC, LA, LCa, HC,FG and IC in cattle was 91%, 1.76%,2.80%,2.26%,1.05%,0.03% and 0.40 %respectively ,while in sheep it was 0.19%,3.39%, 77.75%,11.38%,3.45% 0.83% and 3.07% respectively. The incidence of FA and LC were significantly higher in cattle compared to sheep and that of LA and LCa ,FG and IC were significantly higher in sheep compared to cattle . In cattle, FA prevalence was mostly higher during winter compared to summer and autumn.

Hanaa A. E. et al. (2012) conducted the study of seventy-five liver samples of (55 adult sheep and 20 adult goats) and 26.7% of them showed parasitic infestation. With a rate, 11.8% *fasciolosis* (4 sheep, 5 goat), as well as 10.6 % *C.tenuicollis* (5 sheep, 3 goat). Histopathologically, liver tissues with fascioliasis showed 2.6 % acute hepatitis, 9% chronic catarrhal cholangio-hepatitis with hyperplastic biliary epithelium including granulome formation in 4 % of them. Biliary epithelium was greatly hyperplastic forming papillomatous projections with goblet cell hyperplasia. While liver Sections infested with *C. tenuicollis* revealed presence of 2.6% acute hepatitis with cyst formation, 8% chronic cholangio-hepatitis. Out of 21(26.7%) parasitically infested animals.

Hossain M.M. et al. (2011). In the study 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 ($P \leq 0.05$) indifferent age groups and sex of the animals. The study indicates that *Fasciola* infection in Black Bengal goats associated with age and sex of the animals; and seasons of the year.

L.S.B. Mellau, et al. (2010). In this Study a total of 115186 cattle, 61551 sheep and 37850 goats were slaughtered and 18829 (16.3%), 10515 (17.1%) and 7011 (18.5%) livers of cattle, sheep and goats respectively were condemned due to 11 diseases/conditions namely fasciolosis, stilesiosis, hydatidosis, calcified cysts, abscess, *Cysticercus tenuicollis* infection, telangiectasis, hepatitis, fatty degeneration, melanosis and liver cirrhosis. Of the slaughtered cattle, in sheep and goats a significantly ($p < 0.05$) higher prevalence of fasciolosis was recorded in cattle liver (8.6%) than sheep (3.1%) and goats (3.1%). Collectively, fasciolosis was the leading cause of liver condemnation and was responsible for 52.6, 18.2 and 16.9% of total liver condemnations in cattle, sheep and

goats respectively. Highest percent of liver condemnations were observed during the rain season (March to June).

S. Talukder, et al. (2010). In this study, livers of male and female goats were collected randomly from slaughter house during a period of 1 (one) year (November, 2007 to October, 2008). A total of 325 Black Bengal goat livers were examined, of which 70 livers were found infected with *Fasciola gigantica*. The common histopathological changes found in this study were the migratory tract with lymphocytic infiltration, atrophy, necrosis and fatty changes in the liver. The study revealed that age of the animal has significant effect on Fascioliasis in goats. Significantly ($P < 0.01$) highest infection rate was found in 2-2.5 years old animals (50%). The sex of the animal was also found as important determinants for Fascioliasis. In this study out of 219 male goat livers 30 (13.70%) were infected with *Fasciola*.

Sohair, et al. (2009). Out of 56 bovine liver samples, 17 (30.4%) were apparently infected with acute suppurative hepatitis while 39 (69.6%) out of 56 cases appeared to be infected with chronic hepatitis. A total of 35 bacterial isolates (18 anaerobic and 17 facultative anaerobic) were recovered from acute suppurative hepatitis specimens. *Fusobacterium necrophorum* was the most predominant anaerobic isolates (7 isolates) and *Arcanobacterium pyogenes* was the most frequently isolated facultative anaerobic organisms (6 isolates). While, polymicrobial isolation were detected in 13 (76.5%) instances. In case of chronic hepatitis *Clostridium perfringens* was the most predominant isolated anaerobe (26 isolates), *Escherichia coli* was the most frequently isolated facultative anaerobe (17 isolates) and Polymicrobial isolation were detected in 34 (87.2%) instances. A total 31 *C. perfringens* isolates were tested for its toxin by using dermonecrotic reaction in guinea pig. *C. perfringens* type A represented 40% and 65.4% while, type D represented 60% and 23.1% for acute and chronic hepatitis, respectively.

Khaled M.A. et al., (2008). A total of 1432 slaughtered goats were examined and the most prevalent problems were gastrointestinal parasitism (45%), caseous lymphadenitis (19%), *Oestrus ovis* (8.1%), liver abscesses (19%), hydatid cysts (11%), mange due to *Sarcoptes* sp. and *Psoroptes* sp. (28.5%) and accumulation of plastic foreign bodies in the rumen (14.8%). Bacterial pneumonia were detected in (6.6%) while verminous pneumonia in 4.7% of slaughtered goats.

F. Ahmedullah, et al. (2007). Livers (n = 80) of slaughtered adult buffaloes were examined and amphistomiasis was found in 31.25% and *Fasciola gigantica* infection was in 22.5% cases. Hydatidosis (2.5%), abscesses (3.75%), and haemorrhages (2.5%) were found in the liver. Histopathologically, cirrhosis was found in 31.25% cases. Nodular hepatitis 7.5%, granulomatous hepatitis 5% and parasitic cholecystitis 15% were also recorded during the investigation. Survey showed that the severity of infection with *G. explanatum* 31.25% and *F. gigantica* 22.5% and cirrhosis 31.25% were of highest percentage.

Eugene H. et al. (1999). Examination of 675 caprine livers from a slaughterhouse revealed that 63 (9.3%) exhibited gross pathological changes leading to condemnation of this organ. Forty of these livers (71.4%) exhibited one major abnormality, whereas the remaining 28.6% had two or more lesions. The most frequently occurring disorder was diffuse hepatic lipidosis (4.0%), followed by bacterial associated abscesses (2.4%), cysticercosis (1.9%), and eosinophilic granulomata (1%).

E.H. Johnson, et al. (1999). Livers from 36 of 684 (5.3%) apparently healthy goats examined exhibited gross pathological findings characterized by extremely pale, friable, fatty livers encompassing the entire organ. Histopathologically, diffuse hepatic lipidosis and occasional bile duct proliferation. Periodic acid Schiff-positive, diastase-resistant pigment was observed in the macrophages lining the sinusoids. These histopathological lesions were consistent with those characteristic of ovine white liver disease. Cobalt analysis revealed that normal livers had six times more cobalt and a 3-fold less fat content than those measured in the fatty livers.

R. G.Helman. et al. (1995). Found that in hepatic fatty cirrhosis (HFC) 500 2-6-year-old Rambouillet ewe sheep developed lesions of HFC. Grossly, changes first began in the subcapsular hepatic parenchyma along the porta hepatis and spread peripherally until, in the final stages of the disease, approximately 80% of the liver was affected. Ascites, hydropericardium, and acquired hepatic vascular shunts were present in sheep with severe HFC.

María E. P. et al. (1995). The study represents a review of case records and laboratory data of 84 horses presented with acute or chronic liver disease. 40 horses (48%) had serum protein concentrations above the maximum reference value (7.7 g/dL). The

increase in serum protein concentration was associated with hyperglobulinemia ($P= .00005$, $R^2= .80$). Only 13% (11/84) of the horses had serum albumin concentrations below the minimum reference range (2.5 g/dL), and hypoproteinemia was found in only 1 of these horses. Of these, 18% (9/51) of the horses with chronic liver disease and 6% (2/33) of the horses with acute liver disease had albumin concentrations below the minimum reference value. Globulin concentrations in 64% of the horses (54/84) were above the maximum reference value (4.0 g/dL).

J. Santa Rosa, *et al.* (1989). Post-mortem examination of 658 goats showed that 17 (2.5%) exhibited hepatic abscesses. The following bacteria were isolated: *Corynebacterium pseudotuberculosis* (58.9%), *Escherichia coli* (11.8%), *Corynebacterium sp.* (11.8%), *Pasteurella haemolytica* (5.9%), *Proteus sp.* (5.9%) and *Staphylococcus aureus* (5.9%). Hepatic abscesses occurred more frequently in adult animals. Eleven of the 17 goats (64.7%) were older than 12 months of age. Four goats (23.5%) were 12 months of age and only two (11.8%) were less than 1 year of age. All of the goats with hepatic abscesses were in poor nutritional condition and the abscesses were invariably associated with accompanying pathological disorders at other body sites.

P. J. Mitchell, *et al.*, (1982). Record that outbreak of ovine white liver disease (WLD) on 7 farms were investigated. Most occurred in late spring and mainly affected lambs 3 to 6 months old, with a morbidity of 20 to 100% and mortality of 8 to 15%. Clinically affected lambs showed illthrift, emaciation and bilateral, serous, ocular discharge. Clinical pathology showed mild anaemia, elevated serum liver enzymes (GGT, OCT, AST) and low levels of serum vitamin B12.

A.W.M. Shamsul Islam. (1982). Four hundred and thirty-nine buffaloes of different age groups in Mymensingh, Bangladesh, were examined and 42.36% were found to be infected with hydatidosis. The infection was recorded in the liver 28.47%, lungs 32.80%, spleen 3.22%, heart 2.15%, kidney 1.08%, omentum 0.54%, and both liver and lungs 31.72%. The weak (78.40% and 81.25%), medium (15.20% and 11.11%) and heavy (6.40% and 7.64%) infections were recorded in the liver and lungs, respectively. Cysts were fertile in 10.34% of cases, sterile in 66.12%, calcified in 12.38%, suppurative in 7.76%, and undeveloped in 3.40%. The lowest (8.88%) and the highest (68.47%) infections were recorded in 03 and 9-year old buffaloes and above.

CHAPTER III

MATERIALS AND METHODS

3.1 Study Area, Time and Experimental Animal

This study was conducted in Rangpur sadar at Rangpur District of Bangladesh. Laboratory analysis of collected liver samples were carried out at the Department of Pathology, under the Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh for the period from December 2017 to May 2018. All the studied animals were Different breeds of goat, which were purchased by the butchers from different area of Rangpur sadar, reared in rural husbandry practices. Both male and female goats of two different age groups such as young (<1.5 years) and adult (≥ 1.5 years) were considered.

3.2 Study population

A total of 310 goats were slaughtered over six months and examined. The data of the gross lesions were collected.

3.3 Examination of Liver and Collection of Samples

Post-mortem examinations of slaughtered goats were carried out and livers sample were closely examined for gross pathology.

3.4 Laboratory Procedure

During collection of sample, affected goat livers were subjected to thorough investigation and those showing evidence of infection were collected and processed for histopathology. Formalin fixed liver tissues were processed, embedded in paraffin wax, cut in appropriate thickness and stained with hematoxylin and eosin as per standard methods described by Luna (1968).

3.4.1 Equipment and appliances for necropsy:

- Goat (Liver)
- Scissors
- Forceps
- Gloves

- Musk
- Scalpel
- Knife
- A pair of shears
- 10% formalin

3.4.2 Equipment and appliances for histopathology:

- Samples (Liver)
- 10% formalin
- Chloroform
- Paraffin
- Alcohol
- Tap water
- Xylene
- Hematoxylin and Eosin stain
- Distilled water
- Clean slides
- Cover slips
- Mounting media (DPX)
- Microscope

3.4.3 Cleaning & sterilization of required glassware

Test tubes, glass tubes, glass slides, cover slips, beakers, pipettes, reagent bottles, glass bottle, spirit lamp, measuring cylinders etc. were used in this study. The conical flask, measuring cylinder, beakers, glass slides, cover slip, for slide preparation for histopathological study and staining of organisms after smear and pipettes, reagent bottle, glass tubes for different biochemical tests. New and previously used glassware were collected and dipped in 2% sodium hypochlorite solution and left there until cleaned. After overnight soaking in a household dishwashing detergent solution, the glassware were cleaned by brushing and washed thoroughly in running tap water and rinsed three times in distilled water. The cleaned glass wares were then dried on a bench at room temperature or in an oven at 50-70°C.

3.4.4 Tissue processing

1. Collection of Tissue: During tissue collection, the following points were taken into consideration-

- The tissues were collected in condition as fresh as possible.
- Normal and diseased tissues were collected side by side.
- The thickness of the tissue were as less as possible (5-7 μm approximately).

The following tissues were collected from the suspected goats with internal parasitic infection in the Histopathology Laboratory of Department of Pathology and Parasitology, HSTIJ, Dinajpur, for histopathological examination.

- Liver

2. Fixation: Fixative (10% Formalin) was added by 10 folds of the tissue size and weight.

It is important to consider that a fixative should not be too toxic to its handler, and it should not damage the tissue being preserved.

3. Washing: The tissues were trimmed into a thin section and washed over night in running tap water to remove formalin.

4. Dehydration: Dehydration by ascending ethanol series to prevent shrinkage of cells as per following schedule

- 50% alcohol- 1 hour
- 70% alcohol- 1 hour
- 80% alcohol- 1 hour
- 95% alcohol- 1 hour

5. Cleaning: Cleaning by chloroform for 3 hours to remove ethanol (1 and half hour in each of 2 chloroform jar)

6. Impregnation: Impregnation was done in melted paraffin (56-60°C) for 3 hours.

7. Embedding: Paraffin blocks containing tissue pieces were made using templates and melted paraffin.

8. Sectioning: Then the tissues were sectioned with a microtome at 5-6µm thickness. The section were allowed to spread on luke warm water bath (40-45°C) and taken on a glass slide. A small amount of gelatin was added to water bath for better adhesion of the section to the slide. The slides containing section were air dried and stored in cool place until staining.

3.4.5 Routine Hematoxylin and Eosin staining

Preparation of Ehrlich's Hematoxylin solution

➤ Hematoxylin crystal	4.0gm
➤ Alcohol, 95%	200ml
➤ Ammonium or potassium alum	6gm
➤ Distilled water	200ml
➤ Glycerine	200ml
➤ Glacial acetic acid	20nil

Hematoxylin is dissolved in the alcohol and the alum is dissolve in distilled water and mixed thoroughly. After these are in complete solution the glycerin and acetic acid are added.

Preparation of eosin solution

➤ Eosin Y, water soluble	1.0gm
➤ Distilled water	20 ml
➤ Dissolved and added 95% alcohol	80 ml

Eosin was dissolved in water and then 80 ml of 95% alcohol was added.

Working eosin solution

➤ Eosin stock solution	1 part
➤ Alcohol, 80%	3 parts

0.5ml of glacial acetic acid was added to 100ml of working eosin solution just before use.

Procedure of Routine Hematoxylin and Eosin Staining

1. Deparaffinization in Xylene: The sectioned tissue were deparaffinized in three changes of xylene (three minutes in each change)

2. Rehydration in descending grades of alcohol

- Absolute alcohol — three changes (three minutes for each change)
- 95% alcohol - two minutes
- 80% alcohol - two minutes
- 70% alcohol - two minutes

3. Dipping with distilled water for 10 minutes.

4. The tissue were stained with Ehrlich's Hematoxylin for 2 — 10 minutes.

5. Washing in running tap water (10-15 min)

6. Dipping in lithium carbonate (few dips)

7. Staining in Eosin solution for 1 mm.

8. Dehydration in ascending grades of alcohol as following:

- 95% alcohol — three changes (2-4 dips for each change)
- Absolute alcohol — three changes (2-3 minutes for each change)

9. Cleaned in Xylene: three changes (five minutes for each change),

10. Tissues are mounted with cover slip by using suitable mounting media (DPX) or Canada Balsam.

11. Observation of the tissues under microscope using low and high magnification.

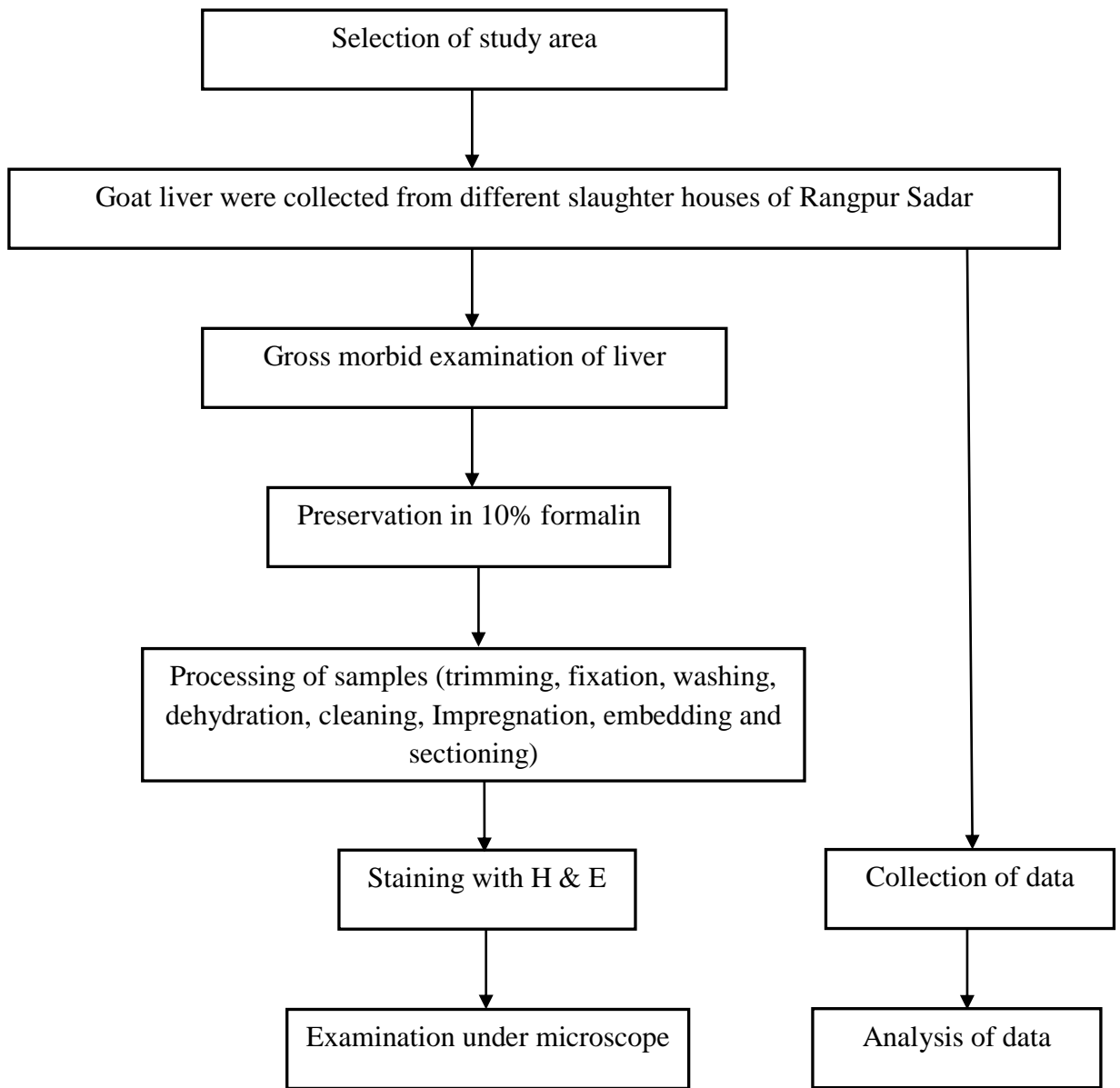
3.5 Statistical Analysis

Variations on the prevalence of diseases in different age groups, and sex were analyzed by logistic regression using statistical software SPSS (Version 20).

3.6 Photography

The histopathological slides of endoparasite affected organs were placed under microscope (Leica, Germany) and the respective microphotographs were taken directly by a digital camera (SAMSUNG ST56, 16.1 MEGAPIXEL, CHAINA) using both low and high objectives (X4, X10). The photograph were then placed in computer, image selection and magnification were further modified and placed in the thesis for better illustration of the results.

3.7 Experimental Design



Flow diagram of the experiment

CHAPTER IV

Result

4.1 The Overall Prevalence

In the present study, a total of 310 of slaughtered goats were examined of which 38 infected with liver diseases. The overall prevalence of liver diseases was 12.26%. The highest prevalence was found in Liver abscess (2.9%) followed by Hepatitis (2.58%), Fascioliasis (2.25%), Tuberculosis (1.94%), Cirrhosis (1.29%), Hydatidosis (1.29%).

Table 1: The prevalence of different liver diseases

Liver diseases	Total No. of goat examined	No. of affected liver	No. of positive cases	Prevalence (%)
(i) Fascioliasis	310	38	7	2.25
(ii) Cirrhosis			4	1.29
(iii) Tuberculosis			6	1.94
(iv) Hydatidosis			4	1.29
(v) Liver abscess			9	2.9
(vi) Hepatitis			8	2.58

n= Number of animals

4.2 Sex wise prevalence of different liver diseases

In this study, it was detected that the overall prevalence of infections was significantly higher in females (55.26%) than male (44.73%). Females were 1.24 times more susceptible than males. In case of Fascioliasis female (19.08%) were commonly affected than male (17.64%). In Cirrhosis prevalence was relatively higher in male (11.76%) than female (9.52%). In Tuberculosis, is frequently higher in female (19.08%) than male (17.64%). In Hydatidosis, female (9.52%) were more susceptible than male (11.76%). In liver abscess, male (29.4%) were more susceptible than female (19.08%) where in Hepatitis, male (11.76%) were less affected than female (23.81%).

Table 2: Sex wise prevalence of different liver diseases

Liver diseases	Male n = 17 (44.73%)		Female n = 21 (55.26%)		level of significance
	No. of affected	Affected (%)	No. of affected	%of affected	
Fascioliasis	3	17.64	4	19.08	NS
Cirrhosis	2	11.76	2	9.52	NS
Tuberculosis	3	17.64	4	19.08	NS
Hydatidosis	2	11.76	2	9.52	NS
Liver abscess	5	29.4	4	19.08	NS
Hepatitis	2	11.76	5	23.81	NS
Chi-square value (P value)	1.276 (0.937)				

n= Number of animals, NS= Not significant

4.3 Age wise prevalence of different liver diseases

In this study, all the goats were group in two. One group, G1 (< 1.5 year) and other group, G2 (>1.5 year). In case of Fascioliasis, prevalence is higher in G2 (>1.5 year), and other group G1 (< 1.5 year). In case of Fascioliasis, prevalence is higher in G2 (19.23%) group than G1(16.66). In Cirrhosis prevalence was relatively higher in G2 (11.53%) than G1 (8.33%). In Tuberculosis, is frequently higher in G2 (11.63%) than G1 (8.33%). In Hydatidosis, G1 (15.38%) were more susceptible than G2 (15.38%). In liver abscess, G1 (25 %) were more susceptible than G2 (23.08%) where in Hepatitis, G1 (25%) were less affected than G2 (19.23%).

Table 3: Age wise prevalence of different liver diseases

Liver diseases	G1=Age (<1.5 year) n=12 (31.58%)		G2=Age (>1.5 year) n=26 (68.42%)		level of significance
	No. of affected	Affected (%)	No. of affected	%of affected	
Fascioliasis	2	16.66	5	19.23	NS
Cirrhosis	1	8.33	3	11.53	NS
Tuberculosis	1	8.33	3	11.63	NS
Hydatidosis	2	16.66	4	15.38	NS
Liver abscess	3	25	6	23.08	NS
Hepatitis	3	25	5	19.23	NS
Chi-square value (P value)	0.341 (0.997)				

n= Number of animals, NS= Not significant

4.4 Gross changes

In fascioliasis the gross pathological changes of the liver in chronic fascioliasis were increased size of the organ due to inflammatory changes in the parenchyma and fibrosis of the bile ducts containing adult flukes. In acute form, the livers were slightly swollen or enlarged with rounded edges and the color became paler than normal with numerous small and large hemorrhagic patches scattered over the parietal surface of all the lobes (Fig. 5&7).

In Hydatidosis, hydatid cysts were fluid filled, some up to the size of oranges or grapefruits were founded in the livers.

In liver cirrhosis, grossly fibrotic mass present sometimes regenerating nodule or tubercle formation and fibroblastic capsule present. Yellowish color, small, firm, irregular surface.

In tuberculosis, nodule or tubercle formation. Granular mass present. The affected organ hard to cut and gritty sound occur due to calcification. (Fig. 4)

In liver Abscess, grossly, whitish foci on the surface of the liver were found. Size varied from 1 to 3 cm in diameter (Fig. 1-3).

In Hepatitis, hepatitis abscess found. Formation of large numbers of newly formed bile duct. Enlargement of liver. (Fig. 6)

4.5 Microscopic changes

In fascioliasis, there were multiple cross section and longitudinal section of adult worm, diffused fibrous connective tissue proliferation which bring pressure atrophy to the adjacent hepatic cells resulting hepatic chirrrosis with mononeuclear inflammatory and lymphocytic proliferation throughout the liver debris of worm in the intrahepatic bile duct with thickened blood vessel due to fibrosis (Fig. 8).

In hydatidosis, there were hydatid scolex in the central vein surrounded by fibrosis with severe scattered of inflammatory cells surrounding the fertile cyst and finally completely autolization of liver. (Fig. 10 &11)

In liver cirrhosis, degeneration and necrosis of hepatocytes, and replacement of liver parenchyma by fibrotic tissues and regenerative nodules, and loss of liver function. (Fig. 12 &13)

In tubercullasis, mass destruction of hepatic parenchyma with fibroblastic proliferation. Huge infiltration of reactive cells and nodule formation histopathologically characterized by central granular caseous masses surrounded by fibroblastic capsule with dilated capillaries.

In liver Abscess, destruction of hepatic tissue contain necrotic matter or pus or pus forming bacteria huge reactive call infiltration.

In Hepatitis, necrosis and degeneration with presence of multiple variable sized abscesses in the hepatic parenchyma consisted of homogenous structureless mass of necrotic cells surrounded by heavy aggregations of inflammatory cells mainly neutrophils, histiocytes and lymphocytes (Fig. 9 &14).



Fig. 1. Liver showing abscess



Fig. 2. Liver showing calcification and abscess

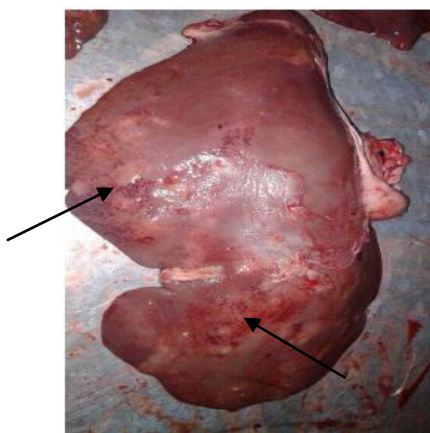


Fig.3. Liver showing, haemorrhage, multifocal abscesses and calcification



Fig. 4. Liver showing Tuberculosis



Fig.5. Fascioliasis affected liver



Fig. 6. Liver showing Hepatitis

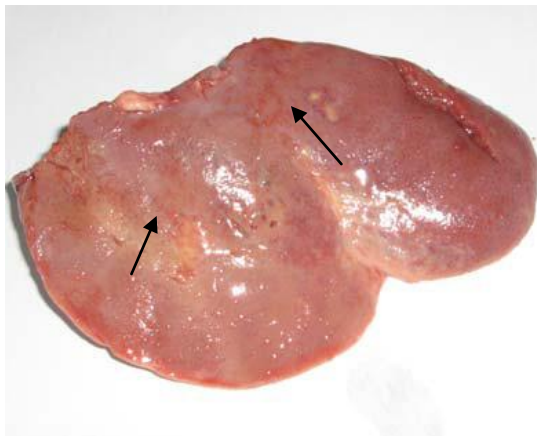


Fig. 7. Acute form of Fascioliasis in left and right lobe of liver

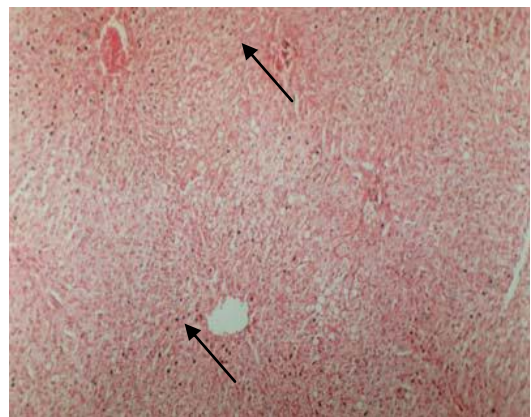


Fig. 8. Liver section showing atrophy, necrosis due to chronic Fascioliasis (10x)

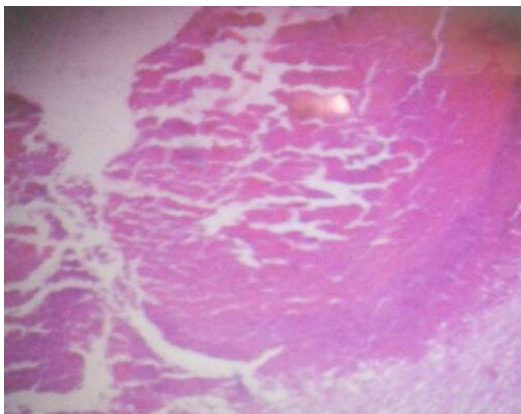


Fig.9. Liver section showing hepatic necrosis. (10x)

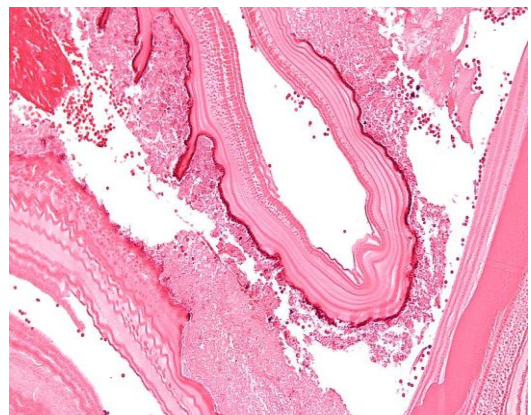


Fig. 10. Microscopic lesion of Hydatidosis (10x)

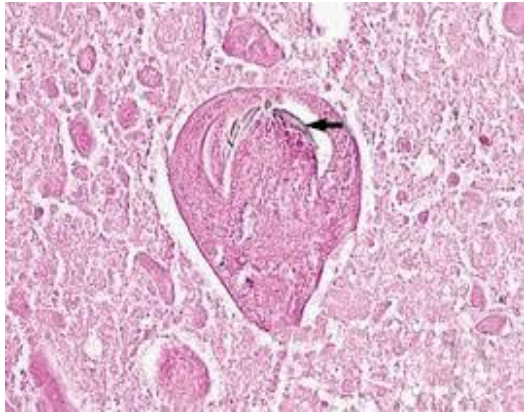


Fig. 11. Microscopic lesion of Hydatidosis (10x)

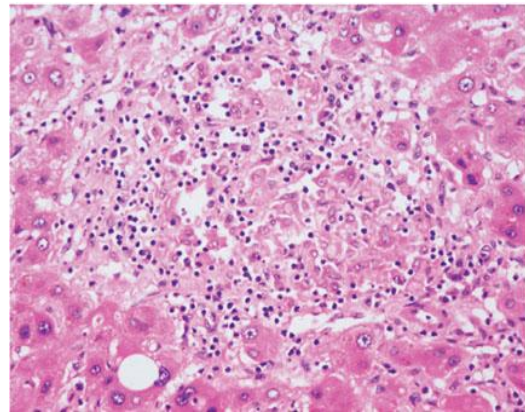


Fig. 12. Microscopic Lesion of Primary Biliary Cirrhosis (40x)

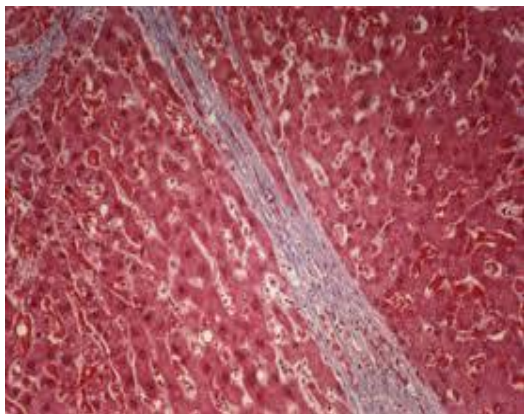


Fig. 13. Microscopic Lesion of Cirrhosis(10x)

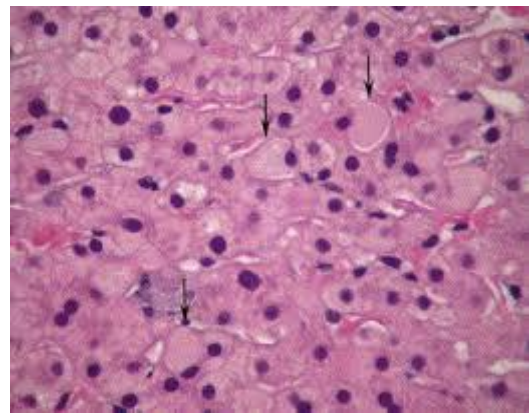


Fig. 14. Histopathology of Hepatitis

CHAPTER V

Discussion

During this study from December, 2017 to May, 2018 a total of 310 goat sample were collected throughout the period from the slaughter house at Rangpur sadar. The overall prevalence of liver diseases was 12.26%. This study found highest proportional prevalence rate in Liver abscess (2.9%) followed by Hepatitis (2.58%), Fascioliasis (2.25%), Tuberculosis (1.94%), Cirrhosis (1.29%) and Hydatidosis (1.29%). The result of recent study is more or less similar to Islam K.M (2016), Mazedul (2015), Suhair S.M (2013), Hanna (2012) and Hossain M.M (2011) who record prevalence of Fascioliasis (10.10%), (3.82%), (91%), (11.8%) and (20.75%) respectively. This variation might be due to shortage of low land, sample size, geographical distribution and other favorable condition. In case of Hydatidosis prevalence was (1.29%). This result more or less similar to R. Godara *et al.* (2014), Suhair S.M (2013), Khaled M. *et al.* (2008) who record the prevalence as (19.8%), (1.05%) and (11%) respectively. In case of tuberculosis the prevalence is (1.94%). This result is less than S Danbirni, *et al.* (2016) who record (0.03% to 0.05%). This result probably due to short study time comparing with this study. In case of Cirrhosis prevalence was (1.29%). This result more or less similar to Suhair S.M (2013) and R.G helman, *et al.*(1995), who record the prevalence as (1.76%) and (16%), respectively. In case of Hepatitis prevalence was (2.58%). This result more or less similar to Sohair, *et al.* (2009), who record the prevalence as (30.4%) with acute suppurative hepatitis while (69.6%) in chronic hepatitis. F. Ahmedullah, *et al.* (2007) who record the prevalence as nodular hepatitis 7.5%, granulomatous hepatitis 5% with chronic hepatitis. In case of Liver abscess prevalence is more or less similar to Suhair S.M (2013), Khaled M. *et al.* (2008), Eugene H.H. *et al.* (1999), F. Ahmedullah, *et al.* (2007), J. Santa Rosa, *et al.* (1989) who record the prevalence as (2.80%), (19%), (2.4%), (3.75%) and (2.5%) respectively. The findings of the present study are more or less similar in most cases with the earlier reports but the little more variations might be due to the differences in the sample size, period and place of study, collection method of samples, animals breed and categorization of infections, climatic and managemental factors.

In this study, it was detected that prevalence of liver diseases were insignificantly higher in females (55.26%) (at $P > 0.05$) than the male (44.70%). Females were 1.24 times more susceptible than males. In case of Fascioliasis female (19.08%) were commonly affected

than male (17.64%). This result agree with Islam K.M (2016), Mazedul (2015), Hossain M.M (2011), S. Talukder, *et al.* (2010). In Cirrhosis prevalence was relatively higher in male (11.76%) than female (9.52%). In Tuberculosis, is frequently higher in female (19.08%) than male (17.64%). In liver abscess, male (29.4%) were more susceptible than female (19.08%). In Hepatitis, male (11.76%) were less affected than female (23.81%). In case of Hydatidosis female (9.52%) were less affected than male (11.76%). R. Godara *et al.* (2014) record that sex had no significant effect on the prevalence of hydatidosis in goats. In most of the infections were found in female than male, the reasons for higher prevalence of infection in the females can't be explained exactly but it might be assumed that the 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).

It was revealed that age of the goats had insignificant effect on liver diseases Prevalence of liver diseases in goats was relatively higher in adult (>1.5 year), and other group young (< 1.5 year). In case of Fascioliasis, prevalence is higher in adult (19.23%) group than young (16.66%). The findings of the present study similar to Khan S.A *et al.* (2015), Hossain M.M *et al.* (2011), S. Taluckdar (2010). Islam K.M *et al.* (2016), record the prevalence of fascioliosis was significantly higher in young goats (15.58 %) than in adult (9.59%), which result disagree with this study. In Hydatidosis, young (16.66%) were more susceptible than adult (15.38%). The findings of the present study disagree with R. Godara *et al.* (2014) he found that adult goats (>4 years) had a significantly higher ($p = 0.01$) prevalence rate as compared to the young goats (<2 years). In cirrhosis, tuberculosis adults were insignificantly more susceptible than youngs, but in hepatitis youngs were more susceptible than adults. In case of liver abscess, prevalence is higher in young (25%) group than adult (23.08%). This result insignificantly agree with J. Santa Rosa, *et al.* (1989) who found prevalence of liver abscess more frequently in animals more than 1 years. This very difficult to found the exact occurrence of liver disease in different age group. But it might be due to exhausted immune system of adult and underdeveloped immune system of young.

In this study in fascioliasis the gross pathological changes of the liver in chronic fascioliasis were increased size of the organ due to inflammatory changes in the parenchyma and fibrosis of the bile ducts containing adult flukes. In acute form, the livers were slightly swollen or enlarged with rounded edges and the color became paler than normal with numerous small and large hemorrhagic patches scattered over the parietal surface of all the lobes. The findings of the present study similar to Khan S.A *et al.* (2015), S. Taluckdar (2010). Islam K.M *et al.* (2016). In Hydatidosis, Hydatid cysts were fluid filled cysts, some up to the size of oranges or grapefruits were founded in the lungs and livers and also in the peritoneal cavity. The findings of the present study similar to R. Godara *et al.* (2014). In liver cirrhosis grossly fibrotic mass present sometimes regenerating nodule or tubercle formation and fibroblastic capsule present. Yellowish color, small, firm, irregular surface. These findings similar with R.G helman *et al.* (1995), Suhair S.M (2013) and F. Ahmedullah, *et al.* (2007).. In tuberculosis nodule or tubercle formation. Granular mass present. The affected organ hard to cut and gritty sound occur due to calcification which is similar with S Danbirni, *et al.*(2016). In liver Abscess, Grossly, whitish foci on the surface of the liver were found. Size varied from 1 to 3 cm in diameter. The findings of the present study similar to Khalid *et al.* (2008) and F. Ahmedullah, *et al.* (2007). In Hepatitis, hepatic necrosis and degeneration with presence of multiple variable sized abscesses in the hepatic parenchyma consisted of homogenous structurless mass of necrotic cells. The biliary epithelium were hyperplastic with formation of large numbers of newly formed bile ductules. The findings of the present study similar to Sohair, *et al.* (2009) and F. Ahmedullah, *et al.* (2007).

In this study microscopically in fascioliasis, there were multiple cross section and longitudinal section of adult worm, diffused fibrous connective tissue proliferation which bring pressure atrophy to the adjacent hepatic cells resulting hepatic cirrhosis with mononeuclear inflammatory and lymphocytic proliferation throughout the liver debris of worm in the intrahepatic bile duct with thickened blood vessel due to fibrosis. The findings of the present study similar to Khan S.A *et al.* (2015), S. Taluckdar (2010). Islam K.M *et al.* (2016). In hydatidosis, microscopically there were hydatid scolex in the central vein surrounded by fibrosis with severe scattered of inflammatory cells surrounding the fertile cyst and finally completely autolization of liver. The findings of the present study similar to R Godara, *et al.* (2014), Khaled M. *et al.* (2008). The causes of liver cirrhosis are multifactorial but pathological characteristics are common to all cases of liver

cirrhosis, including degeneration and necrosis of hepatocytes, and replacement of liver parenchyma by fibrotic tissues and regenerative nodules, and loss of liver function. The findings of the present study similar to R.G Helman *et al.* (1995), Suhair S.M (2013). In tubercullasis microscopically destruction of hepatic parenchyma with fibroblastic proliferation. Huge infiltration of reactive cells and nodule formation with central granular caseous masses. This finding similar with S Danbirni, *et al.* (2016). In liver Abscess, microscopically destruction of hepatic tissue contain necrotic matter or pus or pus forming bacteria huge reactive call infiltration. The findings of the present study similar to Khaled M. *et al.* (2008), Suhair S.M (2013). In Hepatitis, microscopically granulomatous hepatitis of goat liver characterized by presence of RE cells, plasma cells, lymphocytes and there was also presence of haemosiderin pigment. Nodular appearance and infiltration of inflammatory cells were also found. The findings of the present study similar to Sohair, *et al.* (2009).

CHAPTER VI

CONCLUSIONS

The present pathological investigation and prevalence study on to the liver disease of goat at Rangpur Sadar in Rangpur district of Bangladesh was conducted in the Department of Pathology and Parasitology, HSTU, Dinajpur, during the period from December 2017 to May 2018. A total of 310 liver were examined of which 38 were infected and prevalence of liver diseases were made and the diseases were diagnosed on basis of laboratory diagnosis and pathological examination. Overall prevalence of liver diseases was (12.26%). The diagnosed diseases were Liver abscess (2.9%) followed by Hepatitis (2.58%), Fascioliasis (2.25%), Tuberculosis (1.94%), Cirrhosis (1.29%) and Hydatidosis (1.29%). Laboratory investigation was conducted by histopathological examination of suspected liver collected from slaughter houses. After review of the results obtained from this investigation, the following conclusions may be drawn:

1. Highly prevalent diseases are liver abscess, hepatitis, fascioliasis, tuberculosis.
2. Livers of female goats were found to be more prone to diseases than those of male goats.
3. Adult goats were more susceptible to diseases than young.

The prevalence of liver diseases studied by various examinations but it was difficult to find the source of individual infection, characterization of causal agents and figure out the histopathological lesions of all the diseases. Further studies should be done to find out the source, distinctive causal agent and histopathology of other infections more than the hepatic lesions and loss due to liver condemnation and diseases having public health importance to find out effective control strategies against specific infections.

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