INVESTIGATION OF PARASITIC DISEASES IN LIVER OF CATTLE AT DINAJPUR DISTRICT

A THESIS

BY

UMME NUZHATUL ZANNAT

SEMESTER: MARCH – AUGUST/ 2011
REGISTRATION NO.: 1005032
SESSION: 2010-2011

MASTER OF SCIENCE (M. S.)

IN

PATHOLOGY

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

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February, 2012

DEDICATED

TO
MY

BELOVED PARENTS

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ABSTRACTS

The study was conducted in different slaughter house to find out the incidence of different diseases affecting the liver of cattle at Dinajpur district of Bangladesh. The investigation was carried out from March, 2011 to December, 2011. 55 cattle liver collected freshly from different slaughter house. Livers exhibiting gross alterations were cleaned with normal neutral saline and the changes were recorded. The lesions containing tissue samples were preserved in 10% formalin for fixation and subjected for histopathology. The incidence of fascioliasis shown highest 36 (65.45%) among 55 diseased livers examined, followed hydatidosis 11 (20.00%), Cirrhosis 5 (9.1%), abscess 2 (3.63%), and mixed infection 3 (5.45%). Among the investigation it was found that the prevalence of disease is higher in female than the male such the percentage found as 52.77% for female and 47.22% for male animal in case of fascioliasis, 45.45% male and 54.54% female for hydatidosis, 60% for female and 40% for male in case of cirrhosis, 100% female in case of abscess.

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INTRODUCTION

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INTRODUCTION

Cattle liver is an excellent source of high biological quality protein and vitamins, and is valuable for its anti-anaemic factors. It is also indespensible accessory organ of digestion and normal assimilation.

The liver lies almost entirely to the right of the median. Its long axis is directed obliquely downward and forward, about parallel with the median plane corresponds to the curvature of the right portion of the diaphragm. It is red-brown in colour and is rather friable. The weight is about 3-5Kg in adult cow.

This organ comprises three body system and two blood supplies with in one frame work. Blood reaches the liver via the hepatic artery and the portal vein. Inter lobular veins derived from the portal vein surround the lobule and blood passes from these through the sinusoids to the center of the lobule. This blood is mixed with hepatic blood derived from branches of the hepatic artery accompanying the portal vein so that blood reaching the centre of the lobule is not well oxygenated. The lobule is drained by the inter lobular branches of hepatic vein. Within the lobule there are two other fluid currents; bile moves from the lobule via bile canaliculi to bile ducts and tissue fluids move between the cells of the liver. Alterations in the movements of these fluids alter the functional efficiency of the liver.

Parasitism is one of the main constraints limiting livestock productions. Mortality of animals from parasitic diseases may not be alarming at times but

their direct effects in terms of reduced milk, meat, wool, hide production, infertility and loss of stamina of working animals are considerably greater (Baker & Muller, 1988).

In Bangladesh, cattle play an important role in domestic economy and trade. This species is useful in terms of draft power, high ecological potential on productivity and biological performances (Latif, 1994). On the contrary, the cattle of Bangladesh have been recognized to possess low reproductive performance (Alam and Ghosh, 1991).

Nagaraja (2007) reported that liver abscess have a major economic impact on the feedlot industry because of liver condemnation and reduced animal performance and carcass yield.

The liver acts as a storage organ for glycogen, fat, and some vitamins. It is also responsible for the synthesis of plasma protein, including fibrinogen,prothrombin and heparin. Biled, formed in the liver, is required for the absorption of fats, calcium, and fat soluble vitamins from the intestine. It also serves as the central organ of metabolism for fats, carbohydrate and proteins and it is an important centre for detoxification. The liver of cattle affected by various types of parasitic diseases such as Fascioliasis, Hydatidosis etc. It is believed that a large number of cattle liver is wasted each year due to affection from various parasitic diseases. Such a loss of valuable food item is of significant economic importance to this country, which is critically deficient in food especially of animal protein. Immidiate steps should be taken to prevent and control of such diseases by proper investigation.

The present research work on the pathological study of bovine liver was, undertaken with the following objects.

(a) To study the different disease conditions alterations of the liver of cattle caused by various diseases or conditions

(b) To study the gross and histopathological alterations occurring in different diseased conditions

(c) To identify the parasites affecting the liver found during the course of study.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

In this study, available and relevant literatures are reviewed with special emphasized on the gross and histopathological changes of collected liver. In the context of liver diseases through analysis of information gained from a careful history, physical examination and appropriately selected clinical and histopathological examination will provide a firm basis for correct concepts of the affiliated diseases. As a whole, it is needless to say quite a good number of literatures are available on various aspects of functional anomalies of this important organ during the last several decades and the avalanche of literatures has become difficult to analyze; so, the present review was therefore conceived as a short and critical guide to the part of this study.

Bhuyan, (1970) started that the percentages of paralyzed livers of cattle, Buffalos, goats and sheep were 71.55, 100, 30 and 14.6 respectively. He estimated that a total loss of protein from condemned livers in the East Pakistan (Now Bangladesh) during the year 1969 was 38, 90, 826 Ibs and its economic loss was Rs.50, 34,625.00.

Pullan *et al.*, (1970); studied the pathogenesis of *Fasciolia hepatica* infection in sheep. The infection was occurring due to presence of 5500 to 12000 metacercariae. In the more acute syndrome, death occurs 7 to 9 weeks after infection, with more than 1400 immature flukes in the liver parenchyma but more in the bile ducts. In less acute form, death occurred 9 to 15 weeks after infection with between 500 to 2300 flukes in liver, mostly of the bile ducts. In

those hosts which survived for more than 12 weeks, the flukes were immature, the gall bladder was very dark usually contained blood clots and the liver was more fibrotic.

Maliki and Mohiyuddin, (1970) described the obliterating endophlebitis of hepatic veins affecting the portal circulation is an infrequent condition most commonly caused by thrombosis. Chronic occlusion, relatively more common, is a complication of intrahepatic disease compatible with survival for months. Literature in animal pathology reveled very reports and the disease is found affecting uterine veins, the supramammary veins in suppurative bronchopneumonia or tuberculosis as reported by Runnels *et al.*, (1965). Hence a case of obliterating endophlebitis in a buffalo involving portal circulatory system has been reported.

Grossly Liver had multiple nodulation, hard and shrunken, stimulating had nailed appearance. It was congested and hemorrhagic in the unaffected areas. Once incision, the liver was quite tough and rubber like.

Enormous amounts of connective tissue were observed in the wall of the exposed portal vessels narrowing the lumen which contained yellowish red dirty fluid. No calcification or grittiness of the organ was observed. Histologically- the section reveled hyperplastic, proliferative and infiltrative changes. The infiltration preliminary consisted of mononuclear leucocytes, lymphocytes and giant cells, diffusely scattered in the abundant process was most intense in the media and adventitia and varied in character with the stages of its development.

Several of the portal veins were converted into fibrous cords which appeared spongy vascular structure due to canalization. The proliferation was chiefly

due to the leucocytes in early stages of the development had round or oval and faintly stained nuclei with less chromatin granules or materials, while the matured cells were smaller in size with deeply stained nuclei due to compact chromatic material. The normal architecture of the hepatic parenchyma was disturbed and distorted with lobulation atrophy and other degenerative changes. All the major vessels such as the portal veins and even the arteries were thickened.

Malik and Prakash, (1972) studied the structure of liver parenchyma, and according to them it was similar in the two species. Buffalo liver had a thinner capsule than that of cattle. The area of the hepatic lobule and central vein and the size of hepatic cells were slightly larger in buffaloes than in cattle but this differences were statistically significant.

Smith *et al.*, (1972); identified the immature flukes spent about six weeks in the liver parenchyma, causing damage to liver and the larvae lie in a pool of blood fibrin and cellular debris. In that case, neutrophil, eosinophils and lymphocytes became the part of the infiltrate and in older lesions, macrophages and epitheloid cells became increasingly numerous. Later the biliary epithelium became proliferated partial or complete occlusion of bile ducts and wall of the bile ducts become fibrosis in the perilobular connective tissue.

According to them livers containing several or many minute yellowish foci of necrosis, 1 or 2 mm. in diameter scattered without grossly apparent relation to the lobular architecture and occurring most often in well fattened young cattle, were the so dust livers. They consisted of collections of hepatic epithelial cells in a state of coagulative necrosis or in the process of

disappearing, mingled with or surrounded by a thin sprinkling of neutrophils and lymphocytes. All saw dust livers did not become abscesses.

They reported the hydatid cysts had the tendency to produce endogenous daughter cysts which displaced the vital tissues. The growth of cysts was slow and was encapsulated by dense fibrous tissue of the host. The larva had an outer dense laminated wall without nuclei which enclosed a germinal layer and from the brood capsules arose.

They also stated that "Sawdust livers" and Telangiectasis were often concurrent and probably predisposed to the hepatic abscesses. They cited that the incidence of abscesses approximated 5 percent of the cattle slaughtered throughout the United States, reached 10 percent in the Rocky Mountain area and 100 percent in some shipment from certain-feedlots. The numbers of abscesses in a liver typically were between 3 to 8 and their size was from 2 or 5 cm. in diameter. They stated that the early lesion was one of coagulative necrosis and the true abscesses consisting of encapsulated pus represented older processes. They concluded that the abscesses were at first necrotic areas and later, chronic abscesses with the granulomatous reticulo-endothelial type of reaction.

Haroun and Hussein, (1975) were made an investigation of the pathological, haematological and biochemical aspects of naturally occurring bovine fascioliasis in the Sudan. 228 animals infected with *Fasciola gigantica* and 25 non-infected controls were used in the study. The infected cattle revealed emaciation, typical liver pathology, and, occasionally, lesions in the lung and the pancreas.

Runnels *et al.*, (1976); recorded that the young hepatic abscesses were sharply defined, light yellow, greenish yellow or dirty gray foci with pulpy or creamy centers. They were odorless or foul smelling. The old abscesses varied in size, were encapsulated and calcified.

Elmossalami and Darweish, (1977) reported that *Fasciola hepatica* predisposed to bacterial infection. In the abattoir in Cairo, Egypt, they examined 15 normal sheep livers and 66.7 percent were found infected with various bacteria. 30 livers containing *Fasciola hepatica* were examined and all were infected with bacteria.

Uzoukwu and Ikeme, (1978) reported hepatic changes in natural *Fasciola gigantica* infections of the Fulani zebu cattle. Histopathologically, they observed 3 types of chronic reaction: numerous small abscesses containing Fasciola larvae or eggs with cellular reactions and calcifications; marked inter lobular fibrosis, proliferation of bile ducts and peribiliary fibrosis in absence of parasites; and nodular growths consisting of hyperplastic bile ductules lacking secretary ability.

Magzoub and Kasim, (1978) according to the prevalence of fasioliasis was highest when rainfall was higher and irrigation scheme were more developed. **Salo *et al.*, (1978);** reported that the main source of fascioliasis in dairy cattle was rice straw and they found hepatic abscesses in all cases in acute fascioliasis in dairy cattle.

Verma, (1978) reported that 6.88% of the animals slaughtered in bareilly, India, harbored hydatid cysts. 130 of 928 buffaloes, 18 of 346 sheep, 11 of 362 goats were found to be harbor fertile hydatid cyst and most of the cysts were recovered from the lungs.

Jampolsy, (1979) identified that the economic losses caused by hydatidosis in a slaughter house in 10 years time in the USSR (previous) amounted was 273000 roubles.

Schillhorn van Veen, Folaranmi *et al.*, (1979); examined one thousand and twenty-four cattle, 550 sheep and 1,748 goats slaughtered in a rural slaughter slab during 1973 to 1975 were examined for evidence of liver fluke infections. The prevalence rate of *Fasciola gigantica* and *Dicrocoelium hospes* infections were respectively 65.4% and 56.0% in cattle, 40.8 and 13.1% in sheep and 17.6 and 5.2% in goats. Other trematodes detected were *Schistosoma bovis* and paramphistomes. The seasonal incidence of *F. gigantica* as well as of *D. hospes* was highest during and directly after the rainy season. The lower prevalence rate of *F. gigantica*, especially in the younger animals.

Al-Abbassy *et al.*, (1980); examined slaughtered sheep, goat, cattle and camels at Baghdad abattoir, Iraq for hydatid cysts and recorded 20% of camels, 5.9% of sheep and 4.9% of cattle were positive. They reported that, of 450 goats, 23 (5.1%) were infected among which (2%) had the fertile cysts and the cysts were mainly found in the liver.

Wensvoort *et al.*, (1980); stated that in chronic bovine fascioliasis marked perilobular fibrosis was present 8 weeks after infections, increased severity upto 12 weeks and after 20 weeks it decreases simultaneously, an infiltration with eosinophils and edema appeared in the portal tracts. There was proliferation of biliary epithelium followed by distinct cholangioles.

Prasad and Mondol, (1980) studied on hydatidosis at Patna and Ranchi in India. Of 1480 goats killed 102 (6.98) had hydatid cysts. Males were more

frequently infected (6.33) than females (0.95). Among them 18.8% cysts occurred in liver and 71.9% of the cysts were fertile.

Gupta, (1981) studied the liver was grossly enlarged, yellowish and had rounded edges. Microscopically the liver showed portal hepatitis, fatty changes and large eosinophilic intracytoplasmic inclusion bodies in the hepatocytes. The inclusion bodies were surrounded by halo and were stained bright red with Shorr's triple stain.

Shaba *et al.*, (1983); examined livers of 4020 domestic animals from south west Iran indicatated that 20.5% of the animals were infected with *F. gigantica* and/ or *F. hepatica*. The infection rates 91.5% for buffaloes, 49.2% for cows, 295 for sheep and 11.25 for goats.

Shaikh *et al.*, (1983); examined 526 cattle, 315 buffaloes, 178 sheep and 245 goats at slaughter houses of Dhaka, Narayanganj, Munshiganj, Joydevpur, and Savar, from February 1978 to February 1981 in Bangladesh. Fasciola gigantica was found in 59.3%, 78.7%, 27% and 18% respectively. Echinococcus (hydatid cysts) in 14.8%, 41.6%, 14.0% and 0.8% and Coenurus cerebralis in 0.6% of buffaloes and 4.1% of goats numbers during June-September, leading to high incidence of infection during October to November.

Yaman *et al.*, (1985); state that the prevalence of hydatid disease was investigated in 704 sheep, 391 goats and 280 cattle slaughtered at two abattoirs in North Jordan. The infection rates for these animals were 4.0, 3.6, 11.4 and 8.8%, respectively. In general, older animals had higher infection rates than younger ones. The percentage of infected animals that had fertile cysts was 66.7% in camels, 34.3% in cattle, 28.6% in goats and 7.1% in

sheep. Out of 143 single cysts recovered from cattle, 52.4% were fertile; 29.2% of cysts in goats and 8.0% of those in sheep were fertile. However, infected sheep had more fertile multiple cysts than other animals.

Upadhyay and Sahai, (1986) done a survey of 361 cattle and 254 buffaloes at Ranchi, India which revealed 53.5% (136) of the buffaloes to be infected by *Gigancotyle explanatum* mostly in the liver and bile ducts. No cattle were infected. A that this was directly related to the life cycle and availability of the intermediate host snail, *Gyraulus convexiusculus.* Sails were available in large numbers during June-September, leading to high incidence of infection during October to November.

Upadhyay *et al.*, (1987); Histochemical studies was done and revealed marked depletion in polysaccharide complex substance, glycogen and acid mucosubstance in the hepatic cells of the liver of buffaloes (Bubalus bulabis) infected with *Gigantocotyle explanatum,* where as these substance showed strong possibility in different portions of flukes and also in epithelial cells of the bile ducts. On the other hand, histopathological alterations were mainly confined to the bile ducts. In cut sections, the parasites were seen in the lumen of the ducts and the epithelial cells were showing hyperplasia and hypertrophy. There was also peribiliary cirrhosis, particularly around the affected bile ducts. Parasites were lying free in the lumen of the bile ducts or were firmly attached to the mucosa. Infected livers were enlarged, pale and hard studies with hemorrhagic spots, the bile ducts were congested with papillary projections of the mucosa. Moderate mononuclear cells infiltration of the lamina propria and hyperplasia and hypertrophy were evident in the bile ducts and there was severe peribiliary cirrhosis. The portal tract also showed monocytic infiltration.

Swarup *et al.*, (1987); studies on the revalence and clinicopathology of naturally infected *Fasciola gigantica* and *Gigantocotyl explanatum* in buffaloes which showed 61.78% infection with either of the two or both type of parasites. Highest infection rate was observed for *Fasciola gigantica* (29.26%) and lowest for *Gigantocotyl explanatum* (19.51). The histopathological changes in liver associated with 2 types of infections have been described. *Fasciola gigantica* infection was more harmful than *Gigantocotyl explanatum* infection in livers of buffaloes.

Swarup and Pachauri ,(1987); showed histopathological study and revealed that the *Fasciola gigantica* infected organs showed typical lesions of early chronic stage of infection, most important being disruption of hepatic cells loss of normal architecture of liver parenchyma, cellular infiltration and fibrotic and hyperplastic changes in the bile ducts. The infected livers had significantly low fat and high protein content without any significant changes in ash and energy contents. Pas positive substances were extensively depleted in infected liver parenchyma, evenly distributed in non infected hepatic cells and abundant in parasitic organs. The alkaline phoshatase activity in the infected livers was comparatively low. No changes in lipid distribution could be demonstrated in the liver section from infected and control buffaloes.

Hammond, (1990) worked on different helminthes disease and stated that among many parasitic problems of farm animals, fascioliasis is a major disease, which imposes direct and indirect economic impact on livestock production which causes most economic losses of the livestock industry.

Saleha, (1991) stated that the common causative agents were Fasciola hepatica and F. gigantica which require various species of Lymnaea, fresh water snails, as their intermediate hosts. The epidemiology of the disease and

its prevalence in Malaysia is mentioned briefly. The disease causes considerable impact on the economy of the livestock industry.

Dhote *et al.*, (1992); studied the hepatic lesions in cattle. Various pathological lesions encountered (67/133, 50.4%), were hydatidosis (23.3%), abscess (6%), schistosomiasis (0.8%) hemorrhage (8.3%), Granular degenerative changes (3.8%), cirrhosis (5.3%), fatty changes (1.5%) and necrosis (1.5%). In similar studies the occurrence of pathological conditions was found to be 22.4% and 29.0% by earlier workers. Among the various lesions observed hydatidosis was most common (23.30%) even then its occurrence was lower than those reported earlier.

Bettini and Marcato, (1992) studied of 66 primary hepatic tumours was carried out on cattle. These consisted almost entirely of adult females. Fifty hepatocellular tumours (10 adenomas and 40 carcinomas), 10 cholangiocellular tumours (three adenomas and seven carcinomas), two cavernous haemangiomas, two haemangioendothelial sarcomas, one fibroma and one Schwannoma were diagnosed. The 50 hepatocellular tumours were classified into adenomas (20 per cent) and carcinomas (80 per cent), both prevalent in the solitary macro nodular form. The hepatocellular carcinomas were divided into six groups based on their distinctive histological arrangement and the morphology of the neoplastic cells: trabecular (55 per cent), pseudo glandular (10 per cent), solid (12 per cent), scirrhous (12 per cent), pleomorphic (7 per cent) and fibro lamellar (2 per cent). Twenty-seven of 50 hepatocellular tumours (54 per cent) were associated with features of blood-filled lakes resembling telangiectasis within the neoplastic tissue.

Anwar-Ul-Haq, (1994) The study under report revealed investigations on economic losses, biometry and chemical composition of hydatid cyst in

cattle. Five thousand cattle of either sex were examined at slaughter in Faisalabad abattoir during 1988 to 1990. A prevalence of hydatidosis in 35 percent cattle was recorded. The hydatid cyst was found in various organs i.e. liver, lungs, spleen, heart and kidneys. Liver contained 25.31 percent cystic burden while lungs and spleen were having 47.31 and 1.83 percent of cysts, respectively.

Johnson *et al.*, (1999); examination of 675 caprine livers from a slaughterhouse in the Greater Muscat area in the Sultanate of Oman 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%). Although the cause for the hepatic lipidosis was not determined it appeared to be a reversible condition based on the absence of degenerative nuclei within the affected hepatocytes. Conditions observed in only a single liver were subcapsular (non-parasitic) cysts, focal necrosis, micronodular cirrhosis, extensive bile duct proliferation and diffuse haemorrhage.

Masuduzzaman *et al.*, (1999) In PM examination of 2460 slaughtered cattle in different abattoirs of Chittagong city and Natore town during the period from July 1996 to June 1998, a total of only 62 (2.52%) cases of hepatic abscess were recorded. Histopathological findings are briefly described.

Anwar *et al.*, (2000); was studied the prevalence and biometry of hydatidosis in 5000 cattle of either sex. Magnitude of prevalence was 35.0 %. The

hydatid cysts were found in liver (25.31 %), lungs (47.31 %), and spleen (1.83 %). Mixed infection of hydatid cysts was found both in male (23.72 %) and female (26.18 %) cattle in liver, lungs and spleen. Lungs were the most commonly infected organ both in males (29.32%) and females (49.55%). Irrespective of sex, lungs had the highest fertility rate (76.93%) with the highest number and largest size of cysts.

Bostelmann *et al.*, (2000); described the hepatic lesions caused by *Fasciola hepatica* was conducted in 3 domestic ruminants: cattle, buffaloes and sheep. Liver samples were processed by routine histological methods. The staining procedures included haematoxylin-eosine, Schorr (triple staining), Mallory (triple staining) and toluidine blue. The most frequent lesions found were the following: parasite sections inside the biliary ducts, portal system fibrosis and biliatry duct hyperplasia. Lymphocytes, plasmocytes, eosinophils and neutrophils were found in cattle and buffaloes, characteristic of a chronic disease. The presence of eosinophils in sheep was characteristic of the initial phase of chronic fascioliasis.

Kim-Yeon Soo *et al.*, (2000); was surveyed of fascioliasis of Korean native cattle raised in specialized commercial breeding farms and local farms in the Kangwon province of Korea was conducted from November to December 1996 using both intradermal tests and fecal sedimentation techniques. Fecal samples were collected from cattle found to be fascioliasis-positive based on the intradermal test. Liver tissues were randomly collected from an abattoir for histopathological examination of liver fluke infection. Among the 60 faecal samples from positive cattle, eggs of Fasciola hepatica were found in 51 (85.0%) on the basis of the sedimentation technique. According to the cattle raising areas, 26.7 (20/75 head) and 29.4% (40/136 head) of cattle in Wonju and Wheongsung, respectively, tested positive in the intradermal test.

On the basis of age, 7 out of 100, 41 of 93, and 12 of 18 head in the 1-3, 4-6 and 7-10 years age groups, respectively, gave positive results in the intradermal test. Overall infection rates with fluke larvae in the slaughtered cattle at an abattoir in Wonju was 24.7% (37/150 head). Based on histopathology, liver lesions such as inflammation; infiltration with eosinophils, polymorphonuclear cells, mononuclear cells and multinucleated giant cells; proliferation of connective tissues; calcification and abscess formation, were observed.

Atasever *et al.*, (2002) worked on 3470 livers of beef cattle slaughtered by the Ankara Meat and Fish Company was inspected. In these animals, 82 livers had telangiectasis, and the incidence of lesions was 2.3%. They were characterized grossly by single or multiple red-brown foci, generally 1 to 5 mm in diameter, and occasionally larger. They were located on the parietal surface of the lobus dexter or rarely scattered throughout the organ. Microscopically, dilated sinusoids filled with erythrocytes were observed in initial lesions; the distortion, atrophy and necrosis of hepatocytic cordons were seen in advanced cases. There was no correlation between liver abscesses and telangiectasis.

Sevinc and Basoglu, (2002) measure the lipid content in liver biopsies for the determination of the presence and degree of the fatty liver in dairy cows with periparturient diseases. 118 cows were included in the study, of which 25, 23, 25, 23, 10, and 12 had parturient paresis, spontaneous ketosis, abomasum displacement, retained placenta, mastitis, and metritis, respectively. Liver biopsies were performed in the right 11th to 12th intercostal space. Histopathological examinations showed that cows with milk fever, ketosis, abomasal displacement, retained placenta, mastitis, and endometritis also suffer from fatty infiltration of varying degrees, indicating

that these diseases are associated with fatty liver. The incidence of fatty liver was 76% in cows with milk fever. Moderate to severe fat infiltration was observed in all ketotic cows except one. Varying degrees of fatty infiltration was found in 21 cows with displaced abomasum. The incidence of fatty liver in the studied cases was 91.5%.

Velusamy *et al.*, (2002); The pathomorphological changes in the liver of calves at 26 weeks experimentally infected with 100-400 viable metacercariae of Fasciola gigantica. Submandibular swelling, loss of subcutaneous fat, ascites and clay pipe stem type bile ducts containing intact or degenerated flukes were observed. The liver was enlarged and firm in consistency. Neither migratory tracts nor haemorrhages were observed. Histopathologically, the liver exhibited portal as well as intralobular cirrhosis forming psedulobulations, and cellular infiltration comprising large mononuclear cells and eosinophils and proliferation of bile ducts. Hypertrophy and hyperplasia of Kupffer cells were also seen. In bile ducts, hyperplasia, regeneration of the epithelium, calcium deposition and intense infiltration of eosinophils, lymphocytes and mononuclear cells along with extensive fibrosis were evident.

Bobe *et al.*, (2004); observed Fatty liver (i.e., hepatic lipidosis) was a major metabolic disorder of many dairy cows in early lactation and was associated with decreased health status and reproductive performance. In severe cases, milk production and feed intake was decreased. Therefore, a practical preventative or an efficacious treatment of fatty liver could save millions of dollars yearly in treatment, replacement, and production losses for dairy farmers. Fatty liver develops when the hepatic uptake of lipids exceeds the oxidation and secretion of lipids by the liver, which usually is preceded by high concentrations of plasma NEFA mobilized from adipose tissue. Excess

lipids are stored as triacylglycerol in the liver and are associated with decreased metabolic functions of the liver. Liver can be categorized into normal liver or mild, moderate, or severe fatty liver; the latter can be subdivided further into nonencephalopathic severe fatty liver and hepatic encephalopathy. Insufficient or unbalanced dietary intake, obesity, and elevated oestrogen concentrations are involved in the etiology of fatty liver, which is associated with greater incidence of dystocia, diseases, infections, and inflammations. Because even mild fatty liver is associated with decreased health status and reproductive performance of dairy cows, prevention of fatty liver by supplying cows with sufficient nutrients and a clean and health-promoting environment in the peripartal period would reduce production losses of cows more than would any treatment of fatty liver. This, however, might not be enough for cows that are obese or do not eat well, had calving difficulties or twins, have metabolic or infectious diseases, or are in severe negative energy balance because of high milk production immediately after calving. Potential and commonly used preventatives, as well as treatments, are discussed in the review. Currently, detection of fatty liver is possible only by minor surgery. Ultrasonic techniques offer a potential tool to noninvasively detect fatty liver. Future gene-array and proteomic studies may provide means to detect early molecular events in the etiology of fatty liver plus their connection with immune function and reproductive performance so that more effective treatments and preventatives of fatty liver can be developed. Such advances hopefully will make fatty liver a problem of the past.

Berning and Daugschies,(2005) was surveyed between 1 July 1998 and 23 of July 1999, a total of 32 623 cattle were slaughtered at the abattoir of the Premium Fleisch AG Zeven in Lower Saxony, Germany. 31 464 of these (originating from 23 districts) were evaluated for visible infections with Fasciola hepatica. Rotenburg (Wumme) was the only district to continuously

carry out a regional control programme against fascioliasis. In this district, the prevalence of F. hepatica in all animals was 0.67%. In the other districts, 1.43% of the animals were positive for F. hepatica. The infected animals showed a reduced performance. Compared to non-infected animals, the muscle mass and carcass weight of animals suffering from fascioliasis were reduced. Although control measures against F. hepatica can not totally prevent these unwanted effects, it is questionable whether regional control programmes are economically justified in districts with a low overall prevalence. The cost-benefit ratio could be improved if these actions would be restricted to areas or production units with a high risk. These may be identified by registration and documentation of the respective slaughterhouse data.

Nagarajan *et al.*, (2007); was determined the incidence, severity and clinical significance of fatty liver syndrome in cattle. Fifty cattle and buffaloes brought for treatment at the large animal medical unit of Madras Veterinary College Hospital with a history of recent calving, inappetence and reduction in milk yield were included in the study. The animals showed decreased milk production (30 animals), primary ketosis (8 animals), chronic indigestion (6 animals) and blood parasitism (6 animals). Liver biopsy was performed in all animals. Of the 50 cases examined, histopathological changes such as fatty liver, diffuse hydropic degeneration, focal hydropic degeneration and focal necrosis were observed in 16, 42, 36 and 6% of the animals. Fatty liver was classified depending on the histopathological findings in the biopsy material into mild (0-20%), moderate (20-40%) and severe (more than 40%).

Ahmedullah, *et al.*, (2007); worked on Livers (n = 80) of slaughtered adult buffaloes were examined for pathological changes during the period from July 2006 to March 2007 in two slaughter houses of Barisal district. Livers

exhibiting gross alterations were cleaned with normal neutral saline and the changes were recorded. The lesions containing tissue samples were collected in 10% buffered formalin for fixation. Grossly, *Gigantocotyle explanatum* infection (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.

Marcos and Machicado,(2007) focused on the development of fibrosis of the liver of cattle with *Fasciola hepatica* infection, correlating with the intensity of infection. Animals with an established diagnosis of chronic *F. hepatica* infection were identified in a slaughterhouse in Lima, Peru. The study included 24 fresh cattle livers from infected animals and two uninfected controls. Tissues were stored at 4 degrees C for approximately 8 h after which they were brought to a necropsy room and examined. Between 9 and 12 biopsies were randomly obtained from each liver. Histological staining of formalin-fixed liver sections with haematoxylin and eosin (H & E) and Masson's trichrome were performed. Liver samples were examined using a pathology protocol that included 30 items. Histopathologically, 16 out of 30 liver specimens (67.6%) showed diffuse fibrotic lesions (cirrhosis) with a mean number of *Fasciola* of 116+or-30 (range 4-435). Pathological data were matched to number of adult parasites and presence of cirrhosis after being reviewed by two independent pathologists.

Dore *et al.*, (2007); the characteristic clinical manifestations, clinicopathologic findings, treatment, and outcome of dairy cattle with liver abscess are poorly defined. Animals: The study included 18 Holstein cows with liver abscesses. A retrospective study of medical records was performed. Cattle with liver abscess were identified by ultrasound examination or exploratory laparotomy. The most common reason for examination was anorexia (14/18). Five cows had fever, 5 were bradycardic, and 5 were tachycardic. Peritonitis (n=6) and vagal indigestion (n=4) were the most frequently associated diseases. Neutrophilia (n=14), hyperfibrinogenemia (n=11), and high serum globulin concentration (n=10) were characteristic of chronic inflammation. Evidence of liver disease on serum biochemistry profile was uncommon. The most common bacterium isolated from the abscess was Arcanobacterium pyogenes (n=4). Anaerobic bacteria were isolated frequently (n=7). There were 6 polymicrobial isolates, with both aerobic and anaerobic bacteria, out of 8 positive samples. Medical treatment was successful in 5 of 7 cattle. Five cows were euthanized and postmortem examination revealed 2 cattle with thrombosis of caudal vena cava. Holstein dairy cattle affected by liver abscess exhibit no pathognomonic clinical signs. Clinicopathologic findings were often consistent with a chronic active inflammation. Liver abscesses should be included in the differential diagnosis in cattle with a chronic inflammatory process, cranial peritonitis, or vagal indigestion. Prolonged treatment with antimicrobials might be successful.

Rissi *et al.*, (2007); reported on the epidemiology, clinical signs, pathology, and differential diagnosis of acute hepatic disease among Brazilian cattle caused by poisonous plants was discussed.

Johnson *et al.*, (2008) A 15-year-old Limousin-cross cow was presented for examination with neurological signs and serum biochemical changes

consistent with liver disease. Necropsy revealed enlargement of the liver with multifocal firm, depressed, pale, circumscribed lesions throughout the parenchyma. Within the gallbladder there were exophytic and villiform mucosal masses. Microscopically, hepatic structure was displaced by neoplastic cells forming trabeculae, nests and rosettes. There was transmural infiltration of the gallbladder by similar cells. The histological pattern of growth of the neoplastic cells, the presence of silver-stained cytoplasmic granules within these cells and the immunohistochemical demonstration of chromogranin A supported the diagnosis of neuroendocrine carcinoma. Bovine liver and gallbladder neuroendocrine carcinomas are rare and this is the first detailed documentation of the disease in the United Kingdom.

Khan *et al.*, (2008); Studied the prevalence, effects of treatment and cost benefit analysis of bovine fasciolosis in five districts of Punjab Province viz Sargodha, Jhang, Muzaffargarh, Lodhran and Layyah. From each of the five districts, 80 animals were selected and fortnightly screened through standard coprological procedures for a period of one year for the presence of eggs of Fasciola species. Of 4800 faecal samples analyzed, 1222 (25.46%) were found positive for fasciolosis. The occurrence of *Fasciola* (*F.*) *gigantica* (22.40%) was higher ($P < 0.05$) than *F. hepatica* (3.06%). Highest month-wise prevalence ($P < 0.05$) of fasciolosis was found in winter (39.08%) followed in decreasing order by spring (29.50%), autumn (20.33%) and summer (12.92%). District-wise prevalence of fasciolosis was highest ($P < 0.05$) in Sargodha (40.31%) and lowest in Layyah (11.77%) while other districts were having intermediate values of prevalence of fasciolosis. Species-wise prevalence of fasciolosis was found higher ($P < 0.05$) in buffaloes (30.50%) as compared to cattle (20.42%).

Kakar *et al.*, (2008) Investigated that the Prevalence of endo and ecto-parasites in cows and buffaloes were in Quetta city of Pakistan. A total of 396 livers and gall bladders of cows and 340 of buffaloes were selected randomly. Overall prevalence of liver parasites in cows and buffaloes was 45.70 and 37.05%, respectively. The species found in livers of cows were: *Fasciola hepatica* (16.16%), *Fasciola gigantica* (12.37%), *Paramphistomum explanatum* (7.82%) and mixed infections (9.34%). The corresponding values for buffaloes were 11.47, 13.52, 5.58 and 6.47%.

Mwabonimana *et al.*, (2008); was to establish the prevalence and economic significance of fasciolosis in cattle slaughtered at Arusha abattoir in Tanzania. A 3-year database (2005-2008) from the abattoir was retrieved and analysed. In addition, meat inspection was carried out for one month (July 2008) with focus on Fasciola infection and its associated economic loss due to liver condemnation. Results from the retrospective study revealed that 8302 (6.7%) livers out of 123790 examined livers were condemned due to fasciolosis. Analysis of primary data (meat inspection) showed that 150 of 469 cattle livers condemned were due to fasciolosis, a relative condemnation rate of 32.0% per month. Based on the current local price of liver, the economic loss per month due to liver condemnation was estimated at Tanzania shillings (TZS) 1,800,000/- (approximately US $1,500), which summed to TZS 21,600,000/- (US $18,000) per annum.

Regassa *et al.*, (2009); assess the status of cystic hydatidosis in cattle slaughtered at Hawassa Municipal abattoir. Out of the total 632 cattle examined visually and manually (palpation and incision), 333 (52.69%) were found harboring hydatid cysts. A significantly higher infection was detected in older cattle than young. Of the total 333 infected, 123 (36.9%) had hydatid cysts only in the lung, 23 (6.9%) in the liver, 12 (3.6%) in the spleen, five (1.5%) in the heart, and three (0.9%) in the kidney while the rest 167 (50.2%)

had multiple organ infections. Of the 530 viscera harboring hydatid cysts, the highest (52.83%) was lung followed by liver (34.15%), spleen (9.06%), heart (3.39%), and kidney (0.56%). Size assessment made on 874 cysts indicated that 308 (35.3%) were small, 251 (28.7%) medium, 89 (10.2%) large, and 226 (25.9%) were calcified. The distribution of characterized cysts in different organs based on their size was found to be statistically significant.

Swai, and Ulicky, (2009) A survey was carried out in Hai town abattoir during the period of August 2004 to August 2005 (13 months) in order to estimate prevalence and economic importance of bovine fascioliasis in slaughtered cattle. Prevalence of fascioliasis was calculated as the number found infected with *Fasciola*, expressed as the percentage of the total number of cattle slaughtered. A total of 2114 cattle originating from Weruweru–secondary market and local livestock traders were examined. Three hundred and forty one livers (16.1%) of these cattle were condemned for various reasons. Two hundred ninety seven (14.05%) were infected with Fasciola. The monthly incidence was highest during the period immediately before and after onset of the short rain.

Raji *et al.*, (2010) described that Disease prevalence in ruminants constitutes a serious impediment to livestock production in Nigeria. Records of common cattle diseases prevalent in Zaria were pertinent for effective control programmes. Knowledge of the extent to which the public is exposed to zoonotic diseases through meat consumption is useful in preventive medicine. The slaughter house in Zango-Zaria was visited between January and September, 2008 to obtain information on pathological conditions found in cattle slaughtered during the same period. A total of 7812 cattle were examined. Fascioliosis constituted 23. 41%, haemonchosis 11.61%, pericarditis 17.06%, pneumonia 8.79%, liver cirrhosis 10.41%,

fracture6.50%, pulmonary emphysema 4.71% and abscesses in liver, lungs and kidneys 4.55% of the pathological conditions noted. Seasonality trend of some conditions was noted, that is streptothricosis was highest in July, abscesses in September, liver cirrhosis in April and emaciation and haemonchosis , pneumonia and pericarditis occurred mostly in June. Of the 5758 organs having lesions, 598 whole organs were totally condemned. The number of carcasses partially condemned was 5160. The results highlight the need for improved meat inspection practices at abattoirs and awareness of butchers and cattle traders.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental animal

The experimental animals of this study were cattle which submitted for slaughtering in the slaughter house.

3.2 Experimental area

The investigation was carried out in the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. Livers of cattle were collected from different slaughter houses of Dinajpur for detailed pathological examinations.

3.3 Research duration

A total of 55 cattle liver was examined for gross pathological abnormalities during the period from January, 2011 to December, 2011 and brought for diagnosis to the laboratory of the Department of Pathology and Parasitology in HSTU.

3.4 Pathological examination

3.4.1 Macroscopic examination

A total of 55 livers showing grossly visible abnormalities were collected for detailed pathological studies. All the macroscopic changes were noted. Incisions were given to the affected area to study the nature of the macroscopic lesions, whenever needed. Presence of any cyst with its size and location in the liver was also recorded. The blood vessels and biliary ducts were opened with the help of scissors and forceps and the liver flukes were

collected. The liver was then cut into small slices and the slices were squeezed and macerated in normal saline. After careful removal of larger debris's the smaller ones was washed several times with normal saline until the supernatant fluid was clear. The sediment was then examined thoroughly for parasites, sometimes with the aid of a magnifying glass. The collected trematodes were washed in normal saline and distilled water for several times and preserved in glycerin alcohol solution which was composed 95 parts of 70 percent ethyl alcohol and 5 parts of glycerine. The parasites were identified in the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU).

3.4.2 Histopathological study

Various organs having gross lesions were collected from slaughter house then preserved at 10% formalin, after that processed, sectioned and stained for histopathological studies following a standard procedure **(Luna, 1968).**

3.4.2.1 .Materials required for histopathology

Equipment and appliances:

- Samples (Liver)
- 10% formalin
- Chloroform
- Paraffin
- Alcohol
- Tape water
- Microtome
- Xylene
- Hematoxylin and Eosin stain

- Distilled water
- Clean slides
- Cover slips
- Mounting media (DPX).
- Microscope

3.4.2.2. Processing of tissue for histopathology

1. Collection of tissue and Processing

During tissue collection the following point were taken into consideration-

The tissues were collected in conditions as fresh as possible. Normal and diseased tissues were collected side by side. The thickness of the tissues were as less as possible (5mm approximately).

The tissues (liver) were collected from the Cattle in the Histopathology Laboratory of Department of Pathology and Parasitology, HSTU, Dinajpur.

2. Fixation: 10% formalin was added in the plastic container. (10 folds of the tissue size and weight) and fixed for 3-5 days.

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

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

- ❖ 50% alcohol – one hour
- ❖ 70% alcohol – one hour
- ❖ 80% alcohol – one hour
- ❖ 95% alcohol – one hour
- ❖ Absolute alcohol – three changes (one hour for each changes.)

5. Cleaning: the tissues were cleaned in chloroform for 3 hours to remove ethanol (1 and half hr in each, two changes).

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 molten paraffin

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

3.4. 2.3. Routine Hematoxylin and Eosin staining procedure

- **Preparation of Ehrlich's Hematoxylin solution**

Hematoxylin crystals	4.0 g
Alcohol, 95%	200.0 ml
Ammonium or potassium alum	6.0 g
Distilled water	200.0 ml
Glycerine	200.0 ml
Glacial acetic acid	20.0 ml

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**

1% stock alcoholic eosin

Eosin Y, water soluble	1 g
Distilled water	20 ml
95% alcohol	80 ml

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

Working eosin solution

Eosin stock solution	1part
Alcohol, 80%	3 parts

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

3.4.2.4 Staining protocol

The sectioned tissues were stained as described bellow:

- The sectioned tissues were deparafinized in three changes of xyline (three minutes in each)
- Then the sectioned tissues were rehydrated through descending grades of alcohol as per following schedule.
  - Absolute alcohol – three changes (three minutes for each)
  - 95% alcohol - two minutes
  - 80% alcohol - two minutes
  - 70% alcohol - two minutes
  - Dipping with distilled water for 10 minutes.
- The tissues were stained with Harris hematoxylin for 2-10 minutes.
- Washed in running tap water for 10-15 minutes.
- Then the tissues were dipped in ammonia water (few dips).
- Stained with eosin for one minute.
- Differentiated and dehydrated in ascending grade of alcohol.
  - 95% alcohol – three changes (2-4 dips for each.)
  - Absolute alcohol – three changes (2-3 minutes for each)
- Cleaned in xyline: three changes (five minutes each).
- Tissues were mounted with cover slip by using DPX

The slides were dried at room temperature and examined under a low (10X) and high (40X, 100X) power objectives.

EXPERIMENTAL DESIGN

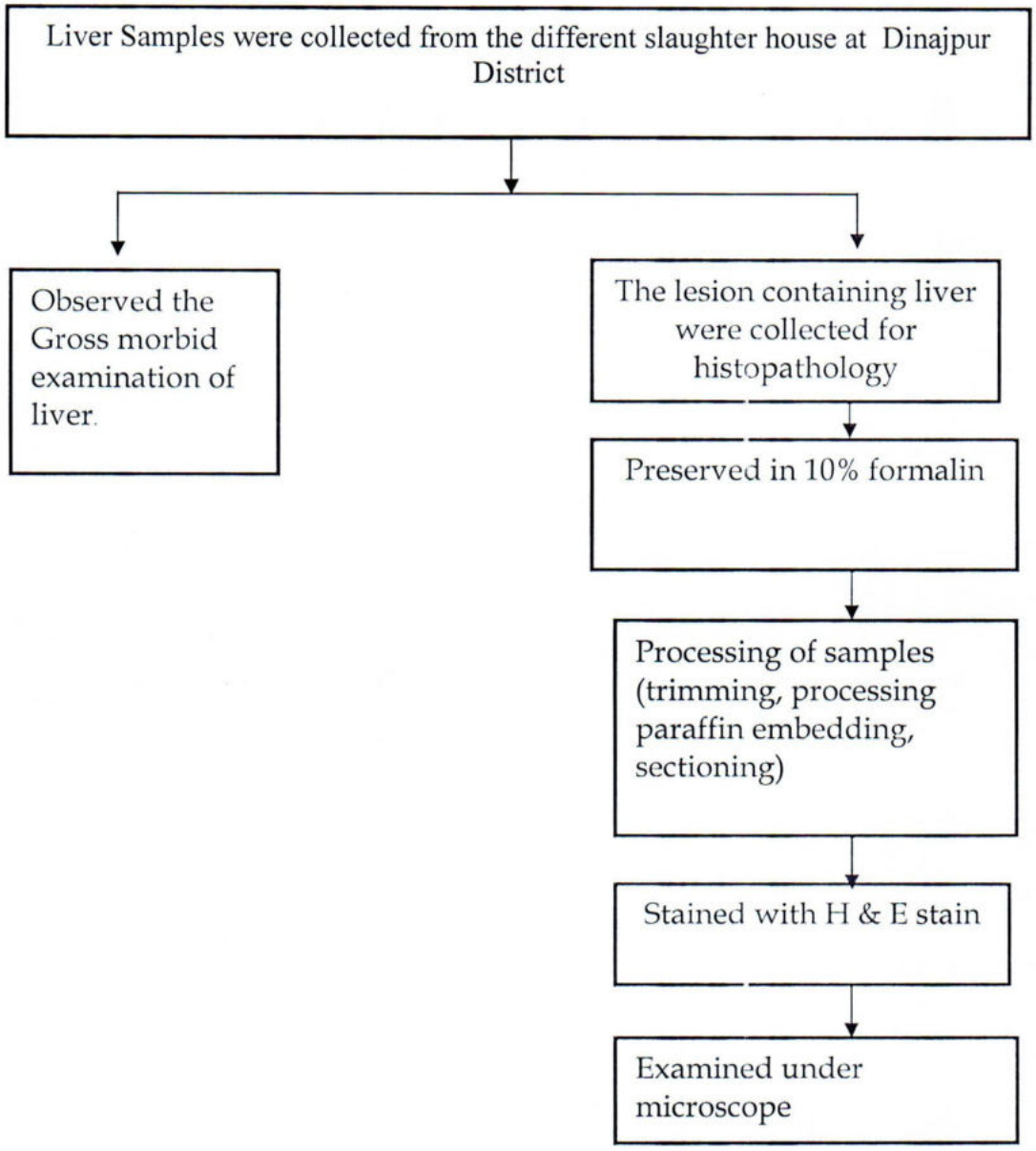

Figure: Flow diagram of the experiment

CHAPTER 4

RESULTS

CHAPTER 4

RESULTS

Gross study was performed during collection of samples from slaughterhouses primarily and then during trimming of the samples for histopathology. 55 diseased livers were collected from different slaughter house in different diseases condition for gross and microscopic examination. The liver shows abnormality in its parietal and visceral surface grossly and on microscopic examination there found different obliteration of its normal architecture. The prevalence of gross and histopathological conditions of the cattle livers are presented in Table 1.

The incidence of fascioliasis shown highest 36(65.45%) among 55 diseased livers examined, followed by hydatidosis 11(20.00%), Cirrhosis 5 (9.1%), abscess 2 (3.63%), and mixed infection 3 (5.45%), respectively.

4.1 Fascioliasis

Fascioliasis was found in 36 cases (65.45%) among 55 affected liver with fluke *Fasciola gigantica* . Of these 36 *Fasciola* affected livers 19 (52.77%) belonged to female and 17(47.22%) belong to male cattle. Three livers affected with fascioliasis exhibited mixed infection with either of hydatidosis or cirrhosis. Two forms of fascioliasis were recorded during these investigation namely sub-acute and chronic infections.

4.1.1 Gross changes

Grossly the lesions were manifested in acute and chronic forms.

In acute form, the affected liver were slightly swollen and appeared pale with round edge. The capsule was more or less thick, opaque, and rough with whitish or reddish discolouration on the visceral surface. A thick band of whitish or blood stained fibrin was found attached to the wall of visceral surface. Light hemorrhagic spots or elongated tracks, with numerous swelling and round shapes of the organ and soft in consistency (Fig.1) were visible mainly at the parietal surface of the right lobe.

In the chronic cases numerous Fasciola were founded in affected liver and the liver was greatly enlarged with presence of a few small irregular whitish areas indicating fibrosis scattered over the parietal surface(Fig-2). The lobes were not distinct which presented a round shape of the liver. The colour of the liver was very pale; the capsule was thick, opaque and rough, closely adhered to the parenchyma. Shreds of whitish fibrin were found to the ventral surface of the liver. The liver as a whole become flabby and easy to cut, sometime due to migration of liver or death of the fluke, there causes necrosis and due to necrosis the part of the liver become harden and showed calcification and there also recorded pipe stem appearance of the liver(Fig-3). The calcified tissue become fibrosed which were tough in consistency and hard to cut and during cutting there produce gritty sound. In some chronic cases, some livers reduced in size with irregular and granular appearance. In some cases, hepatic nodules of different sizes and shapes separated by invading tracks of fibrous connective tissue were distinctly seen. Such livers were lathery in consistency and did not cut easily. The capsule was thick and opaque. The periphery of the caudate and left lobe appeared to be thin and whitish due to fibrosis.

4.1.2 Histopathological changes

Histopathologically, the parasitic (Fasciola sp) section was found(Fig-4) .The parasite pressure the surrounding tissue.As a result hepatocyte And hepatic lobules were destroyed and destroyed tissue were replaced by the proliferation of fibrous connective tissue. There were found total architectural destruction of hepatic parenchyma and infiltration of reactive cells(Fig-5).

4.2 Hydatidosis

Among 55 diseased liver examined, eleven(20.00%) of disease livers were found to be affected with hydatid cyst where five (45.45%) were from male and six (54.54%) from female cattle. Two liver affected with hydatidosis were also affected with fascioliasis.

4.2.1 Gross changes

Grossly the cyst wall was thick and contains gelatinous yellow soft material without any specific organisms(Fig-6). The cyst were found both of the parietal and visceral surface of the left, right and caudate lobe. The maximum number of the cyst in a liver was 25 and the minimum being one. They were identified as hydatid cyst and were measured between 0.5 to 15 cm in their greatest diamensions. Cysts had fairly thick cuticle, with dark hemorrhagic spots in the parenchyma adjacent to the outer cuticle. The cystic fluid volume ranges from 1to 500ml.

Numerous blood capsules originating from the inner most layer of the cyst were observed to float as hydatid sand in the cystic fluid. Wherever, there were heavy infections with hydatid cyst, the liver was very soft and upon pressure it was fluctuated. Some dark haemorrhagic spots were noted in the

parenchyma of the liver (Fig-7). The parenchyma around the cyst seemed to be hard and fleshy. The liver capsule becomes thicker over the cyst in relation to that of other part.

4.3 Cirrhosis

A total of 5 cases (9.1%) showed partial or complete cirrhosis. Among them three of female and two of male cattle were affected with cirrhosis.

4.3.1 Gross changes

Macroscopically, the surface of liver was irregular, the consistency was hard and the colour of the liver was pale or whitish gray. It was tough to cut with scissors (Fig-3) and presence of adult fluke in the liver. It was observed that the migration of only one or two adult liver fluke could initiate liver cirrhosis. The capsule was wrinkled. Sometime there was found whitish spots, slightly depressed were focally distributed in the parenchyma of the liver. The spots were hard and firm, tough to cut through incision and on pressure nothing was found to come out from the spots. In many cases, the was sinked into water.

4.3.2 Histopathological changes

On microscopic observation, there were presence of fibrosis, or the proliferation of connective tissue mainly in the portal area. There were a few lymphocytic infiltrations in the fibrous strands (Fig-8). The portal vein becomes dilated and irregular in shape. The proliferating fibrous tissue progress to the interlobular spaces, as a result the individual lobules were squeezed. Due to squeeze the haemorrhagic dot are found in the lobular space. Degenerative changes of the hepatic cells were also found as necrotic foci and there were found infiltration of lymphocytes and mononuclear cells.

4.4 Abscess

Abscess was found in only two cases (3.63%). Abscess liver was mainly found in female cattle.

4.4.1 Gross changes

Grossly the affected livers were characterized by the presence of whitish or yellowish foci (Fig-9) on the surface of the liver and the number of the foci varied from 1-3. The foci of abscess were odorless. The size of the abscess varied from 0.5 to 1.5 cm in diameter and the affected livers were also associated with fascioliasis.

4.4.2 Histopathological changes

Liver abscesses have a necrotic center containing leukocytes, hepatocytes and cellular debris. The area surrounding the necrotic center contains macrophages and multinucleated giant cells. The next layer contains plasma cells, degenerating hepatocytes, immature fibroblasts, neutrophils, macrophages, and immature collagen strands. The capsule consists of fibrous connective tissue. (Fig-10)

4.5 Mixed infection

Mixed infection was evident by the presence of two or more diseases of which three livers were affected with Fascioliasis, Hydatodiasis and Cirrhosis. Along with Fasciola there found two livers with Hydatidosis and one liver with Cirrhosis.

4.5.1 Gross changes

Grossly all the changes observed in mixed infection were similar to the lesions described earlier in the individual disease. Apart from these two to three nodular structures, 4-5 cm. in diameter were found on the medial

surface of the left lobe along with the course of bile ducts and blood vessels. On incising, the nodules showed laminated areas with caseous mass which was yellowish in colour. Sometime there found calcified mass in the liver due to extensive migration of flukes. The calcified mass is hard to cut and gritty sound occurs due to cut (Fig-3).

4.5.2 Histopathologic changes

Microscopically, these areas were containing a large reserve of blood circumscribed by fibrous connective tissue.The parenchyma and portal triads around these areas were fibrosed and infiltrated with eosinophils and mononuclear cells. Cellular infiltrations with some fibrous connective tissue are seen. In some cases irregular tunnels or tracts originating from the portal area and also from the central vein extended to the parenchyma causing destruction of hepatic cells. These tunnels and tracts were surrounded by slight fibrosis and might be due to migration of parasite calcification was found in the hyperplastic bile ducts epithelium.

Table 1. Number of diseases found during the investigation period from March, 2011 to December, 2011.

Diseases /Disorders	No of Diseased liver examined	Number affected	% of Infection	Remarks
Fascioliasis	55	36	65.45	3 cases associated with Hydatidosis & Cirrhosis.
Hydatidosis	55	11	20.00	2 cases associated with Fascioliasis
Cirrhosis	55	5	9.1	1 cases associated with Fascioliasis
Abscess	55	2	3.63	–
Mixed Infection	55	3	5.45	Associated with Fascioliasis, Hydatidosis, Cirrhosis
Total pathological condition		57		

Table 2. Showing distribution of the pathological conditions in relation to sex

Diseases/Conditions	No. of animals affected	No. of male affected	% of infection of male	No. of female affected	% of infection of female
Fascioliasis	36	17	47.22	19	52.77
Hydatidosis	11	5	45.45	6	54.54
Cirrhosis	5	2	40	3	60
Abscess	2	-	-	2	100
Mixed Infection	3	1	33.33	2	66.66
Total Pathological condition	57	25		32	

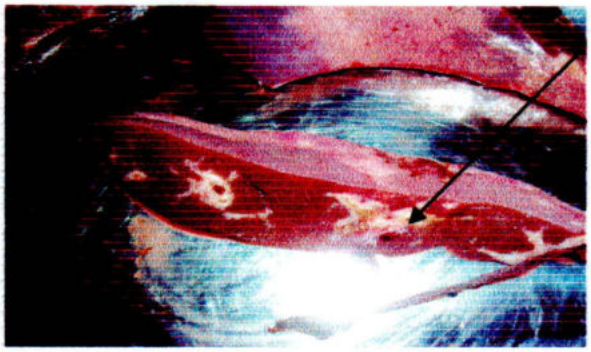

Fig 1: Thick band of whitish or blood stained fibrin was found attached to the wall of visceral surface

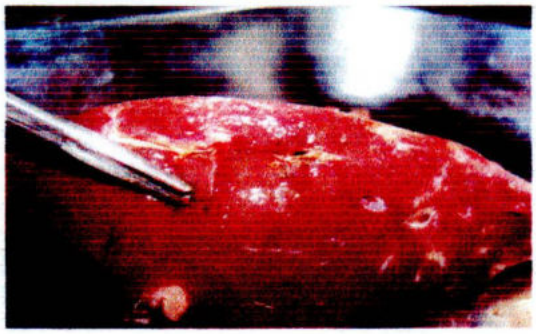

Fig.2: Liver of cattle with fascioliasis

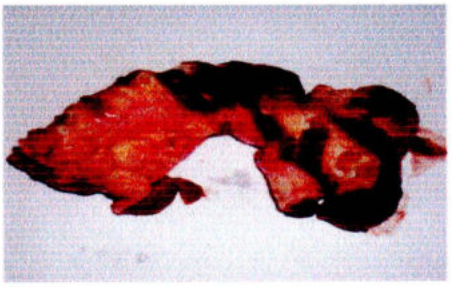

Fig. 3: Pipe stem liver due to fascioliasis

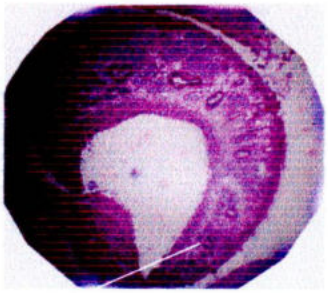

Fig.4: Cross section of fasciola(10x)

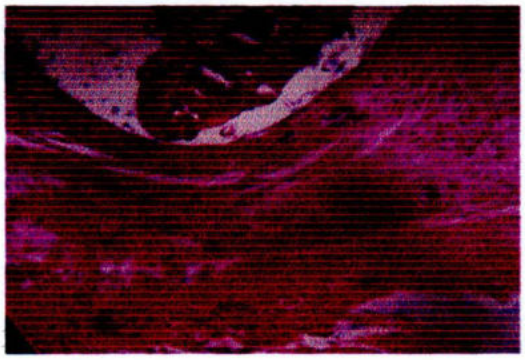

Fig.5:Proliferation of fibrous connective tissue in liver due to fascioliasis(10x)

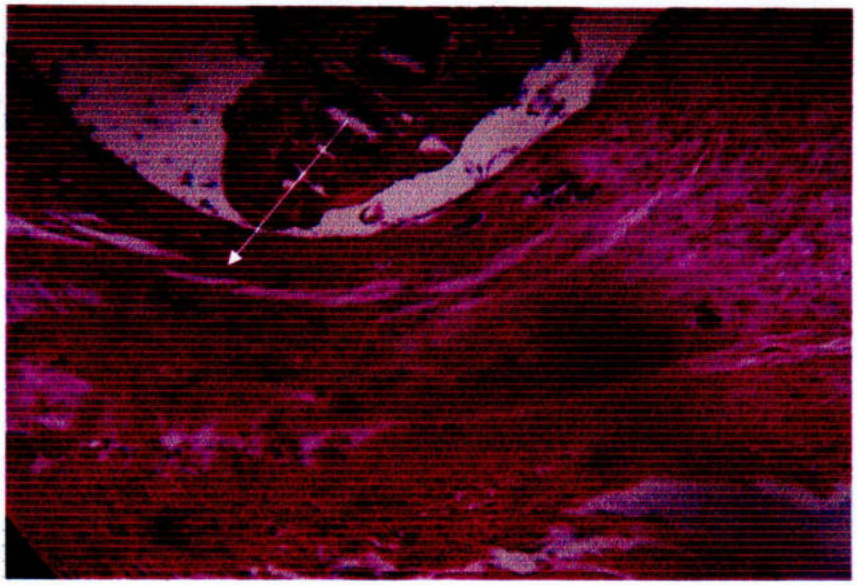

Fig.6: Hydatidosis in liver along with fascioliasis

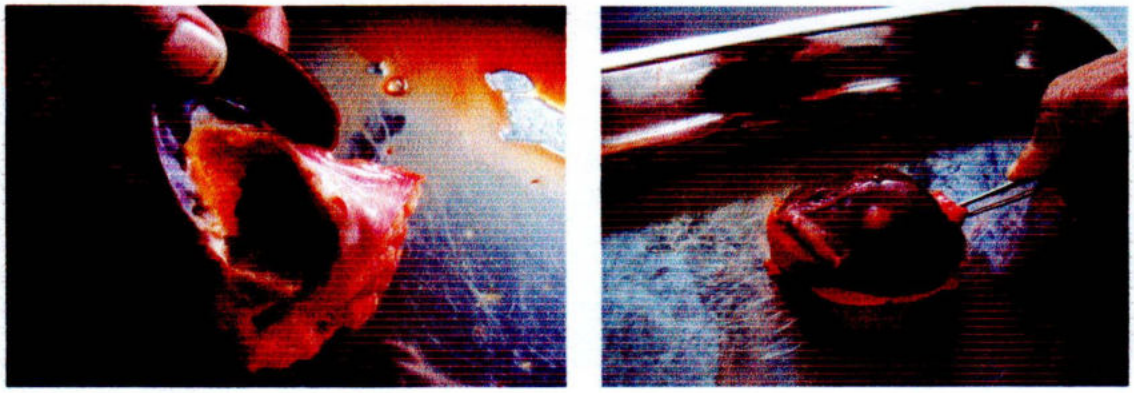

Fig.7: Dark haemorrhagic spot in the parenchyma of the liver

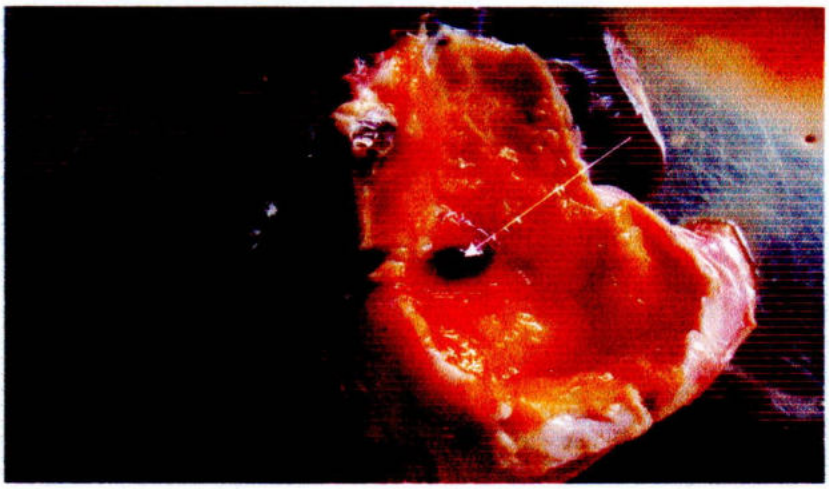

Fig.8:Architectural destructon of lobules in liver due to fascioliasis(4X)

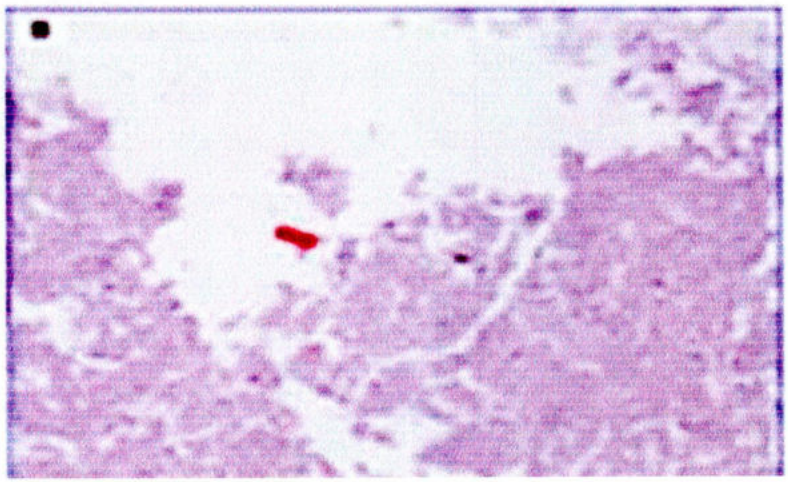

Fig.9: Abscess in liver

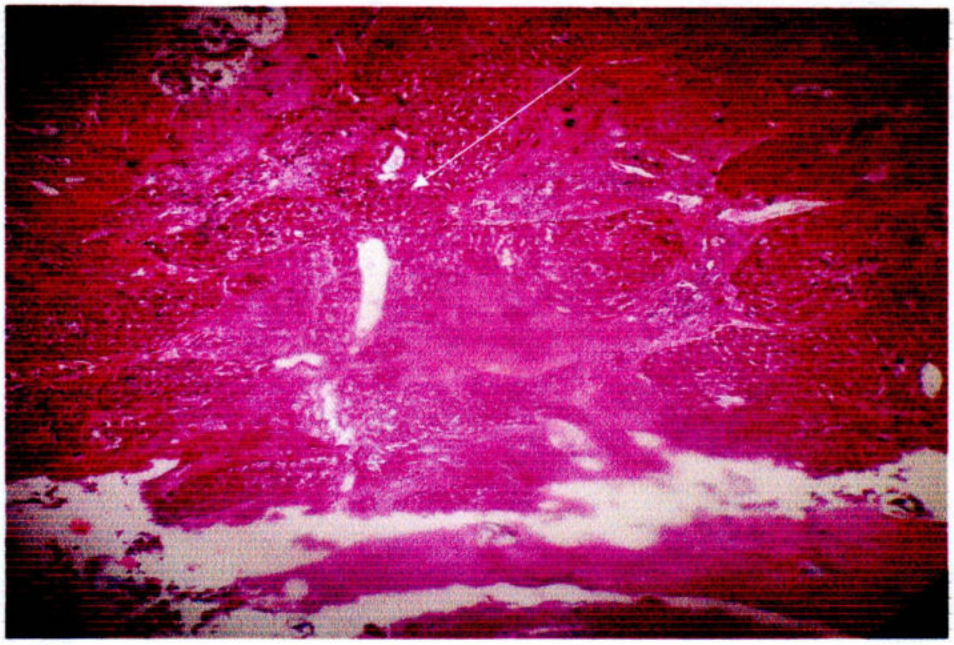

Fig.10: Liver abscesses have a necrotic center containing leukocytes, and cellular debris. (4X)

CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSSION

The research work was dealt with parasitic diseases in liver of cattle. This study was conducted at the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period from March, 2011 to December, 2011.

5.1 Fascioliasis

The distribution of *Fasciola* is determined by that of its lymnaeid snail intermediate host. The parasite is generally limited to high rainfall and irrigation areas. *Fasciola* is an important parasite of cattle, but more particularly sheep and goats. Patent infections can develop in other wild and domestic animals and in humans. when eating water cress according to, Hutchinson *et al.,* (2003) . This study was focused on the development of fibrosis of the liver of cattle with Fasciola infection, correlating with the intensity of infection. For this purposes, Samples were collected randomly for both of the sexes of adult cattle, to record any sex variations in infection could be obtained in various diseases. There was no difference in prevalence between males and females. The same observation was also recorded by Bindernagel (1973). Therefore the seasonal variation could also be obtained in different parasitic diseases.

In this study 36(65.45%) of cattle livers were found to be affected with fascioliasis (Table 1) irrespective of seasonal variations and sex. But Ross (1966) and Henrikson and Pilegaard-Anderson (1980) showed occurrence of more adult flukes during the winter months. Phiri *et al.,* (2005) also reported seasonal variation of *Fasciola gigantica* and the pattern of distribution of *F.*

gigantica was significantly higher in areas of high rainfall than in those of relatively low rainfall. Sharp seasonal variation was found as described by (Soulsby, 1982) which might be due to the different climatic conditions in Bangladesh. Phiri *et al.,* (2005) studied prevalence and factors influencing occurrence of fascioliasis. Female animals got significantly higher percentage (52.77%) than male (47.22%) they reported. In some cases, the affected liver was slightly swollen and appeared pale in color with round edge, the capsule was thick, rough with whitish or reddish discoloration and fibrosis of the bile ducts which indicated subacute form of infection. Similar observations were made by Dawes (1963), Ross (1966) and Dow *et al.* (1967). Numerous small and large patches scattered over the parietal surface could be the indication of transperitoneal route of migration of young flukes. The damage of hepatic cells near these tracts might have resulted from feeding habit of these premature parasites (Kulkarni, 1982; Khalilov and Namosov, 1983). Some parts of bile ducts had cystic appearance due to dilatation. In some other cases, the liver was greatly enlarged with presence of a few small irregular whitish areas indicating fibrosis over the parietal surface and parenchyma was hard due to fibrous tissue which was thought to be due to healing of migratory tracts of immature parasites. In cross section of liver we also recorded pipe stem appearance of the liver caused by the migration of the parasites. Mahdi and Al-Baldawi (1987) and Ansari-Lari and Moazzeni (2006) also reported more or less same result on the prevalence of liver condemnations due to fascioliasis.

Microscopically, there were lymphocytic infiltrations. Considerable fibrous connective tissue proliferations were noted at the portal areas. In chronic cases, the areas of infiltration with lymphocytes and mononuclear cells and proliferation of fibroblasts represented the haemorrhagic tracts. Heavy accumulation of lymphocytes and proliferation of fibrous connective tissues

in the portal areas distorted lobular architectures. There were hyperplastic changes of the epithelial cells of the bile ducts with periductal connective tissue proliferation were found. In most advanced stages these hyperplastic bile ducts appeared like granular structures as recorded in the study of Upadhayay *et al.* (1987). Hepatic siderosis (Gupta, 1982) was also observed in this study. Wiedosari *et al.* (1991) studied comparative pathological study of hepatic changes induced by *Fasciola gigantica* and *Gigantocotyle explanatum* in Javanese thin-tailed sheep and reported pathological changes in the liver induced by *Fasciola gigantica* and *Gigantocotyle explanatum* were readily distinguishable from each other. In this study there were found calcification in the wall of bile ducts of the liver in chronic fascioliasis in cattle. Similarly, according to Ross, (1966)and Dow *et al.*, (1967) there found calcification in cattle, contrary to findings according to Simensen (1968) and Pullan *et al.*, (1970), says the bile ducts of the liver in sheep never calcified. Ross *et al.*, (1967) reported that there was no calcification in the pig liver in chronic fascioliasis. This difference in calcification might be due to species variation.

5.2 Hydatidosis

In the present study 20.00% livers were affected with hydatidosis but Sundaram and Natarajan (1960) recorded 11.61% cases. Sheikh and Hussain (1968) recorded that 35% buffaloes were infected with hydatid cysts in Bangladesh. Among them 5 (45.45%) were collected from female and 6 (54.54%) were collected from male cattle. This statement support Sheikh and Hussain (1968), who recorded that 35% buffaloes were infected with hydatid cysts in Bangladesh, But a higher prevalence of hydatidosis (60.6 %) has been reported from Sheikh *et al.*,(1983) found in Bangladesh. Higher prevalence has been attributed to a frequent contact between the intermediate

and the final host in this area. Multiple cysts found in most of the cases. Some affected livers were found with severe damage of the parenchyma. The size hydatid cysts varied from .5 to 15 cm. This finding supports the work of Soulsby (1969) but contradicts the work of Verster (1961) who found large number of fertile cysts in the liver of cattle. The parenchyma around the cyst was hard due to fibrous capsule. Around the hydatid cyst there was marked cellular reaction characterized by proliferation of fibroblasts, infiltration of mononuclear cells and eosinophils as reported earlier by Dhote *et al.* (1992).

5.3 Cirrhosis

A total of 5 cases (9.1%) showed partial or complete cirrhosis detected was almost equally distributed in male and female. Majority of the causes of cirrhosis were associated with fascioliasis. Therefore, it may be opined that fascioliasis is one of the most important causes of cirrhosis in cattle in this country. The liver was constricted in many cases. Numerous newly formed bile ducts in cirrhotic liver have been observed in the present study as described by previous researchers (Balasingam, 1962; Dawes, 1963; Gupta, 1983; Khalilov and Namosov, 1983). Considerable proliferation of fibrous connective tissue was marked mainly in the portal area. There was little lymphocytic infiltration of fibrous strands. Portal veins were dilated and irregular in shape.

5.4 Abscess

In these studys, 55 livers were collected from cattle where 2 livers were affected by abscess and the percentage is 3.63%. They were characterized grossly by single or multiple red-brown foci, generally .5 to 1.5 cm in diameter, and occasionally larger. They were located on the parietal surface of the organ. Microscopically, dilated sinusoids filled with erythrocytes were observed in initial lesions; the distortion, atrophy and necrosis of hepatocytic

cordons were seen in advanced cases. Atasever and Vural (2002). Polymorphonuclear leukocytes at the center surrounded by a thin fibrous capsule were seen microscopically. Similar histopathological changes have been reported in earlier studies (Runnels *et al.,* 1965; Uzoukwn and Ikeme, 1978). According to Runnels et al., (1976), hepatic abscess may or may not calcify. In the present study the hepatic abscess was founded.

5.5 Mixed Infection

Mixed infection was evident by the presence of two or more diseases of which three livers were affected with Fascioliasis and Hydatodiasis. Two livers with Fascioliasis and one liver with Cirrhosis were seen. The animal having hydatid cysts in livers contained remarkable low infection of *Fasciola gigantica.*

CHAPTER 6

SUMMARY AND CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

A pathological investigation on 55 apparently abnormal cattle livers were collected from different abattoir in Dinajpur district were carried out with a view to revealing various disease conditions affecting the liver of cattle.

In course of this study, 36(65.45%) were found to be affected with liver fluke, *Fasciola gigantica.* Of these 36 Fasciola affected livers 19(52.47%) belonged to female and 17(47.22%) belong to male cattle. 11 of disease livers (20.00%) were found to be affected with hydatid cyst. Among them five(45.45%) were from male and six (54.54%) from female cattle. A total of 5 cases (9.1%) showed partial or complete cirrhosis. Among them three of female and two of male cattle were affected with cirrhosis. Abscess was found in only two cases (3.63%). Abscess liver was mainly found in female cattle. Along with *Fasciola* two livers with Hydatidosis and one liver with Cirrhosis were found.

Both gross and histopathological features of each of the above disease have been described.

On the basis of the findings in this study the following conclusions may be drawn:

1. Livers of cattle are mostly affected with parasitic diseases such as fascioliasis, hydatidosis .
2. Fascioliasis (*Fasciolia gigantica*) is greatly responsible for hepatic damage and demands immediate attention for taking control and eradication measures, the *Fasciolia gigantica* infection were also

increased in rainy season. Other pathological conditions did not show any significant seasonal variation.

3. Mixed infections were represented by more than one type of infection, which was found increased with the advance of ages.

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