INVESTIGATION OF HEPATOPATHOLOGICAL DISORDER OF CATTLE AT DINAJPUR DISTRICT

A THESIS

BY

POLASH BASAK

SEMESTER: MARCH – AUGUST/ 2010
REGISTRATION NO.: 0905081
SESSION: 2009-2010

MASTER OF SCIENCE (M. S.)

IN

PATHOLOGY

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

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Submitted to the
Department of Pathology and Parasitology
Faculty of Veterinary and Animal Science
Hajee Mohammad Danesh Science and Technology University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE (M. S.)

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AUGUST, 2010

DEDICATED TO

MY

Acknowledgement

All praises are to almighty God Who enables the author to complete the research work successfully and to submit the thesis leading to Master of Science (MS) degree in Pathology.

The author expresses heartfelt respect, gratitude and sincere appreciation to his research supervisor Dr. S. M. Harun-ur-Rashid, Associate Professor, Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, for his scholastic guidance, supervision, constructive criticisms and constant inspiration during the entire period of the study as well as the research work.

Immense indebtedness, heartfelt gratitude and sincere appreciation are extended to author's co-supervisor Dr. Md. Nazrul Islam, Assistant Professor, Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, for his valuable advice, exclusive suggestions and provisions of facilities and supports needed to complete this research work.

Profound respect and gratitude are expressed by the author to Dr. Sheikh Arafatur Rahman, Lecturer, Department of Pathology and Parasitology (HSTU) for co-operation extended during the course of the study.

Thanks are also extended to Mamun, Mominul, Rozina, Salam, Reba, Abu sayed for their encouraging attitude in the study period. The author expresses his thanks to all technicians and office staffs of the Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their technical assistance during the research.

The author expresses gratefulness and thanks to the butcher man which helps to giving me the information of slaughtered animal and also help in collection of cattle liver.

The author wishes to express his sincere indebtedness to his parents for their blessing and encouragement throughout the course of the study.

Thanks and appreciations are also extended to the author's friends and well wishers.

The author

August, 2010

ABSTRACTS

The purpose of this study was to find out the incidence of different diseases affecting the liver of cattle in Dinajpur District of Bangladesh. The investigation carried out in the different Butcher house. The investigation was conducted from 1st July, 2009 to 30th June, 2010. On gross and microscopic examination of 52 disease livers that are collected freshly from different slaughter house were subjected for different disease condition. Livers exhibiting gross alterations were cleaned with normal neutral saline and the changes were recorded. The lesions containing tissue samples were collected in 10% formalin for fixation. The incidence of fascioliasis shown highest 34 (65.38%) among 52 diseased livers examined, followed hydatidosis 13 (25%), Cirrhosis 5 (9.62%), abscess 2 (3.85%), paramphistomiasis 1 (1.92%) and mixed infection 3 (5.77%). Among the investigation it was found that the prevalence of disease is higher in female than the male such the percentage found as 52.94% for female and 47.06% for male animal in case of fascioliasis, 61.54% female and 38.46% male for hydatidosis, 60% for female and 40% for male in case of cirrhosis, 100% female in case of abscess, 100% male in case of paramphistomiasis.

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ABBREVIATION AND SYMBOLS

^0C	: Degree celcius
%	: Percentage
et al	: And his associates
etc.	: Etectera
Fig	: Figure
g	: Gram
cm.	: Centemetre
hr	: Hour
H & E	: Hematoxylin and Eosin
HSTU	: Hajee Mohammad Danesh Science and Technology University.
lbs	: Pounds
min	: Minute
ml	: Milliliter
mm	: Millimeter
MS	: Master of Science
No.	: Number
Sec	: Second
UK	: United Kingdom
USA	: United States of America
WHO	: World Health Organization

CHAPTER- I

INTRODUCTION

CHAPTER I

INTRODUCTION

Cattle is an important domesticated livestock in Bangladesh, plays an important role to supplying milk and meat of high caloric value to meet up our protein requirements, promote agricultural production by voiding of large quantities of dung and urine, by earning substantial amount of foreign exchange from exported skin and bones (byproducts) and by saving our lands at least partially from pollution of artificial fertilizer by supplying natural manure. The total population of cattle in our country is about 22.87 million in the year 2007 (Department of Livestock Services).

Liver is the largest solid gland of the body. In fresh condition it is reddish in colour. The weight is about 3-5 kg in an adult cow. It is placed in the right side of the abdominal cavity in an oblique downward and forward direction. The gland extends from lumbocostal angle to the level of 7th to 8th rib and is kept attached in the abdomen with five ligaments. It presents two surfaces and four borders. The liver acts as a storage organ for glycogen, fat and some vitamins. It is also responsible for the synthesis of plasma proteins including fibrinogen, prothombin and heparin, bile 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, carbohydrates and proteins and it is an important centre for detoxification.

Liver is one of the important and vital organs of the body of animals. Like other animals, liver of cattle is also susceptible to various disease or conditions, which can lead to malfunction or nonfunction of it and is commonly manifested by sings of jaundice. Bile secretion or detoxification, deposition of carbohydrate, fats, vitamins, some minerals, production of plasma proteins, erythropoiesis in some situations etc, are partly or wholly impaired by affections of liver caused by some etiologic agents such as Fascioliasis, hydatidosis, cirrhosis, hepatic ulcers, abscessation, calcification etc. are the common conditions of the liver in which the livers are condemned for human consumption. Not only that, these above conditions may also lead to a fatal situation of the animal and if it so happens, the totals economic loss of the owner of cattle has occurred.

Among many parasitic problems of farm animals, fascioliasis is a major disease, which imposes direct and indirect economic impact on livestock production, particularly of sheep and cattle. Hammond 1990; Saleha 1991 Menkir *et al.*, 2007; Swai and Ulicky 2009ssss state that the total economic loss incurred as a result of condemnation of infected livers due to all causes and fascioliasis was approximately 2,096 US $ and 1,780 US $ respectively. Losses due to weight loss were estimated to be 5,943 US $.

Anwar-Ul-Haq (1994) reported an Annual national loss of 26.5 million rupees was estimated due to condemnation of hydatid infected organs during the year 1990. Almost half of the total economic loss was being born by Sindh followed by Punjab 13.3 and 7.3 million rupees.

According to Jampolsy (1979), the economic loss caused by hydatidosis in a slaughter house in 10 years time in the USSR (previous) amounted to 273000 rubbles.

As liver is one of the vital organs of the body, any damage to the liver parenchyma may lead to impairment of its normal functions which consequently followed by illness of the animals or susceptibility infections which may, even cause death of the animals. Such a considerable economic loss from the condemned livers can hardly be sustained by a poor nation like ours. These losses can be minimized by prevention and control of such diseases affecting the livers after proper investigation. But, so far no systemic investigation on the pathology of cattle liver and pathogenesis of conditions that affects liver has been carried out in our country.

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

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

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

(c) To determine the diseases most commonly and seriously affecting the livers of cattle

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

Goal:

- Study of hepatopathological conditions encountered in bovine animals.

CHAPTER-II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Among various types of abundant publications a careful selection in respect of their significance, is necessary and from this point of view, following veterinary medical topics comprehensively compiled and distilled to provide the best source of current practical information regarding pathological conditions of the liver of cattle. 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.

Sundoram and Natarojan (1960) examined 547 carcasses of zebu cattle and 706 buffaloes at the city of madras abattoir, which reveled that the respective incidence of hydatid disease was 13.5% and 11.61%. The localization of the cyst was in the following decreasing order: lung, liver and spleen. The percentage of different forms of cyst was, in zebu cattle and buffaloes respectively, unilocular cysts 96.36% and 95.31% with multiloculated cysts 0.18% and 0.86%, degenerated cysts 2.86% and 3.75%, sterile cysts 81.43% and 87.03%, fertile cysts 18.57% and 12.96%.

The authors want to describe the physical appearance of the fertile and sterile cysts.

Dawes (1963a) worked on various stages which was brought about in the epithelial lining of the bile duct while the young *Fasciola hepatica* were moving through the hepatic parenchyma before reaching the bile duct; chemical agencies and inflammatory process were probably responsible for these changes. Minute cytoplasmic blebs at the free margin of the epithelial lining cells seemed to be the first indication of what become an intense hyperplasia of the tubular outgrowths and an associated fibrosis in the underline connective tissue. When the flukes invade the bile duct their spine and suckers quickly abrade the superficial cells and they feed on the tissue debris so produced.

Stenius (1963) stated that *Fasciola hepatica* caused dilation of the bile ducts in the left lobe of the liver and thickening of their walls in cattle slaughtered in Helsinki. A brownish exudates and a number of mature *Fasciola hepatica* were found in the lumen. Microscopically the epithelial layer of the bile ducts were seen to the partially disintegrated, but simultaneous proliferation of epithelial cells occurred. Thickening of the bile ducts was the result of connective tissue proliferation.

With special reference to the brilliant cells of **Hamazaki and Shirai 1963)** studied the histopathology of bovine liver affected by *Fasciola hepatica* and observed the above mentioned cells deriving from sooth muscle fibers of the bile ducts skeleton and interlobular septa, infected with *Fasciola hepatica*. According to him these cells were involved with initial response to the pathological changes and increased gradually in number.

Runnells *et al.*, (1965) recorded that the young hepatic abscesses were sharply defined, light yellow, greenish-yellow, or dirty grey foci with pulpy or creamy centres. They were odourless of foul smelling. The old abscesses varied in size, were encapsulated and calcified. Microscopically the abscesses begun as merely collection of leukocytes, later, each abscess was surrounded by an inflammatory zone and a connective tissue capsule surrounded by a margin of leukocytes. In young abscesses numerous bacteria were present in the pus but in older one there were few or none bacteria.

Tsvetavera and Gumensehikova (1965) elucidated the pathology and histochemestry of the liver of cattle with acute and chronic fascioliasis. The acute cases were characterized by traumatic lesions accompanied by depletion of glycogen in the liver cells, which was regarded as an important stage in pathogenesis and in sensitization of the animal. In mild infection traumatic lesions were still present, but reparative processes were at work. The cholangitis was catarrhal and desquamative in character. The glycogen portion of damage portions of liver was decreased but some still remain in unaltered position. In chronic cases there was cirrhosis of the parenchyma and biliary lesions were represented by chronic cholangitis.

Ross and Dow (1966) reported enlarged, dark and congested liver infected with experimental fascioliasis in young calves. In acute cases, death was observed. Numerous venous thrombi were revealed during histopathological study. Large number of immature parasites was also found in the liver.

Ross (1966) examined 900 cattle of which 80% were found to be infected. Immature flukes were found from June to April with peak

levels between Octobers to December but the highest adult burdens were observed between Januarys to March. He found the developing liver flukes produced constant microscopic lesions in the liver of Cattle between 3rd and 6th week post infection. Red superficial tract was produced and from the 7th to 9th week localized red hemorrhagic plaque lesions developed prior to entry to the flukes to the bile ducts. On entry to the bile ducts, fibrosis of the duct wall and acinar hyperplasia results with recognizable calcification in the wall. He added that the inhibited flukes were found to survive in the plaque lesions in the parenchyma for as long as one year.

According to **Asdrubali and Mughetti (1966)** changes in gall bladder of cattle suffering from sub-acute and chronic fascioliasis were characterized by hyperplasia of the tubuloalveolar glands, increases in the number of enterochromaffin cells and of the large cells containing eosinophilic granules.

During epidemiological investigation of fascioliasis in pigs **Ashizawa *et al.*, (1966)** recognized chronic cholangitis, chronic interstitial hepatitis and traumatic damage caused by the migration of the young flukes. The later two types of damages were mild. They also reported the frequent affection of the left lobe than the right lobes; which suggested that the portal vein is the main route for the migration of young flukes from the intestine. Cholangitis compared to that of ruminants were observed.

Detail gross and microscopic hepatic changes were studied by **Dow *et al.*, (1967)** with experimental *Fasciola hepatica* infection in calf liver which was disclosed by the presence of migratory flukes into the bile ducts, resulted in proliferation of the epithelium producing a glandular mucosa. Destruction of the lining which become fibrosed and then

calcified due to the activity of the fluke was also recorded. According to the authors fibrosis in the liver in chronic fascioliasis may result from (i) the parenchymal damage caused by migratory flukes and cause damage in the portal tracts, following the entry of the fluke into the bile ducts.

Dow *et al.*, (1968) described the histopathological appearance in experimental infection with *Fasciola hepatica* in lamb. Two phases of parenchymal migration of the parasite such as free migrating and localized phase of migration before entry into the bile ducts were observed. Hepatic cells regeneration were indicated but hepatic fibrosis were minimally reported. The localized phase of migration was associated with a unique peripheral palisade of giant cells in the flukes in the bile ducts produce fibrosis of duct walls. However, remained pliable and expanded to accommodate the parasite and calcification was never observed.

Sheikh and Hussain (1968), examined 210 animals at abattoir in Lahore, West Pakistan, During February to august 1967 revealed *Echinococcus granulosus* cysts in 52 of 148 buffaloes (35%) and 17 of 62 cattle (27%). Using various cysts fractions as antigen, casoni's skin test failed to give any specific reaction in the cattle and buffaloes.

The gross and histopathology o the bovine liver due to fascioliasis was studied by **Irfan and Lee (1968)**. They found areas of hemorrhagic tracts caused by the migrating flukes which were infiltrated by eosinophils. Some of the bile ducts show hyperplasia and increased secretory activity. In many areas cirrhosis accompanied by pressure atrophy, fatty change and necrosis of the parenchymatous cells were reported.

Deposition of hemosiderin and in some cases nodules of mononuclear cells was also observed.

Sengupta and Iyer (1968) studied the changes in advanced stages of *Fasciola gigantica* infection in the buffalo livers which were characterized by thickening of the portal triads with fibrous tissues extending into the neighboring liver parenchyma, and resulting in monolobular or perilobular cirrhosis. The hepatic arterioles exhibited median hypertrophy with sub-endothelial proliferation of connective tissue. The bile ducts revealed hyperplastic changes of the lining epithelial cells, which at times assumed papillomatous proportion. Most o the ducts were, however devoid of the lining epithelial cells as a result of the movement of flukes with in the ducts. Numerous eggs were seen in the bile ducts and in the parenchyma the granulomatous lesions due to eggs were reported.

Cameron and Webster (1969) described the histogenesis of the hydatid cyst on the livers of bovines, pigs, sheep, deer and equines which followed a generally similar pattern in all species. According to them, the hydatid cyst composed of 3 layers. (i) The living cyst or nucleated layer was a thin walled single layered cell membrane enclosing a slightly clear sterile fluid and budding inwards as brood capsules. (ii) The laminated layer was a tough elastic layer and consisted of chitin or keratin related material and calcium. It increased with age and prevented the structure of bacteria. (iii) The adventitia- the growing cyst caused a pressure atrophy of the adjacent liver tissue and irritation of the metabolites caused the formation of toxic epitheloid giant cell zone in which there was a heavy reduction of eosinophils. The cells

radiated from the cyst and had some degree of cloudy swelling of actual necrosis. The zone gradually subsided and formed fibroblast cells.

According to **Rahko (1969),** the changes of natural Fasciola hepatica infection in cattle included frequent thrombosis and haemorrhages, extensive disintegration and coagulative necrosis of hepatic cells and abundant infiltration of inflammatory cells.

According to **Bhuyan (1970),** 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 then 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 hab 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 have been 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.

According to **Smith *et al.*, (1972)** 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.

According to **Magzoub and Kasim (1978)** 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.

According to **Jampolsy (1979)** 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 was 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.

According to **Gupta (1981)** 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 are 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), from February 1978 to February 1981 in Bangladesh, examined 526 cattle, 315 buffaloes, 178 sheep and 245 goats at slaughter houses of Dhaka, Narayanganj, Munshiganj, Joydevpur, and Savar, 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.

A survey of 361 cattle and 254 buffaloes was made by **Upadhyay and Sahai (1986)** 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 are available in large numbers during June-September, leading to high incidence of infection during October to November.

Histochemical studies by **Upadhyay *et al.*, (1987)** 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 cxplanatum* 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.

Histopathological study of **Swarup and Pachauri (1987)** showed 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 are 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) study 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.

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-YeonSoo *et al.*, (2001) survey 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 and Vural (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%.

Nagarajan *et al.*, (2007) determine 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.

Nagaraja and Lechtenberg (2007) described that liver abscesses in feedlot cattle result from aggressive grain-feeding programmes and are influenced by a number of dietary and management factors. They have a major economic impact on the feedlot industry because of liver condemnation and reduced animal performance and carcass yield. Ruminal lesions resulting from acidosis usually are accepted as the predisposing factors. Generally, control of liver abscesses in feedlot cattle has depended on the use of tylosin, which reduces abscess incidence by 40-70%.

Khan *et al.*, (2008) study reports 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 and Kakarsulemankhel (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 ($P < 0.05$, $\chi^2 = 4.36$) 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 ($P < 0.05$).

Swai, E. S. and Ulicky, E. (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.

CHAPTER- III

MATERIALS AND METHODS

CHAPTER III

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3.1 Experimental animal

The experimental animals of this study were cattle which were 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 town for detailed pathological examinations.

3.3 Research duration

A total of 52 cattle liver were examined for gross pathological abnormalities during the period from 1st July, 2009 to 30 June, 2010 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 52 livers along with gall bladders 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

During necropsy, various organs having gross lesions were collected 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 deparaffinized 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.

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

On gross and microscopic examination of 52 disease livers that are collected freshly from different slaughter house are subjected for different disease condition. The liver shows abnormality in its parietal and visceral surface grossly and on microscopic examination there found different obliteration of its normal architecture. The disease or disorders are shown in Table 1. The incidence of fascioliasis shown highest 34 (65.38%) among 52 diseased livers examined, followed hydatidosis 13 (25%), Cirrhosis 5 (9.62%), abscess 2 (3.85%), paramphistomiasis 1 (1.92%) and mixed infection 3 (5.77%).

4.1 Fascioliasis

Among 52 diseased livers examined, 34 (65.38%) were found to be affected with liver fluke, *Fasciola gigantica.* Of these 34 Fasciola affected livers 18(52.94%) belonged to female and 16(47.06%) 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

In acute form, the liver were slightly swollen or enlarged with rounded edges and the colour become paler than normal chocolate

colour. The age of these animals ranged from two year to eight years. The capsule was more or less thick, opaque and rough. 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. The surfaces of the liver, in some instances exhibited few depressed areas which on cutting open, appeared as tracks or tunnels filled with bloody exudates. These tunnels were produced due to migration of the parasites, there also recorded pipe stem appearance of the liver caused by the migration of the parasites (Fig. 2) Immature flukes were present in this tunnels in some cases. After opening the bile ducts, few immature or no flukes were founds.

In the chronic form livers were greatly enlarged, hard in consistency and their edges become round. 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 show calcified mass (Fig-3). The calcified mass become fibrosed which were tough in consistency and hard to cut and during cutting there produce gritty sound. The left lobe was uneven on the both parietal and visceral surfaces and displayed much more thickening than the right. The bile ducts were greatly dilated and on cutting were found to the packed up with flukes. The gall bladder was very much distended with bile and hepatic lymphnodes were enlarged.

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 Histopathologic changes

In acute cases, the histopathological changes were found mainly in the portal area. The grossly visible spot or track was represented by the presence of numerous lymphocytes and monocytes in the parenchyma of liver (Fig-4) and accompanied by hemorrhage and oedema. In the zone of reaction the blood vessels were highly congested or ruptured. In some sections, the centre of the track contained a mass of degenerating neutrophils which were thought to be the part of a migrating immature flukes. Due to migration there destruction of the hepatic cord, extra vascular erythrocytes are seen (Fig-5). Dissolution of many hepatocytes, hepatic necrosis along with infiltration with reactive cells and found total architectural destruction of the hepatic parenchyma (Fig-6).

In chronic fascioliasis the hemorrhagic tracks or tunnels were represented by the areas infiltrated with fibroblasts, neutrophils, lymphocytes and mononuclear cells. In many sections, the lobular architectures were found to be distorted (Fig-5) by heavy accumulation of lymphocytes and proliferation of fibrous connective tissue in the portal area. In some cases, fibrous connective tissue penetrated into the parenchyma of the lobules. Many portal triads were observed to be closer to each other, apparently due to fibrosis and destruction of the normal lobular architecture.

4.2 Hydatidosis

Thirteen (13) of disease livers (25%) were found to be affected with hydatid cyst. Among them eight (61.54%) were from female and five (38.46%) from male cattle. Two liver affected with hydatidosis were also affected with fascioliasis.

4.2.1 Gross changes

The cysts were found both of the parietal and visceral surfaces of the left, right and caudate lobe. The maximum number of the cysts in a liver was twenty five and the minimum being one (Fig- 7). Most of the cysts were situated superficially just beneath the capsule, mainly at the dorsal part of the right lobe. The size of the cyst ranges from 0.5.0 to 15.0 cm. in diameter (Fig-8). The cystic fluid was invariably clear and its volume ranges from 1 to 500 ml. Numerous brood capsules originating from the inner most layer of the cyst were observed to float as hydatid sand in the cystic fluid.

The colour of the livers was white glistening in some cases but exhibit almost normal colour. Wherever, there were heavy infections with hydatid cyst, the liver was very soft and upon pressure it was fluctuated (Fig-9). Some dark haemorrhagic spots were noted in the parenchyma of the liver adjacent to the cyst. 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.

4.2.2 Histopathologic changes

Around the hydatid cyst, there was marked cellular reaction characterized by proliferation of fibroblasts, infiltration of mononuclear cells and eosinophils. The hepatic cells around the cysts showed degeneration and necrosis (fig-10) along with flatten nucleus due to the cystic pressure with accumulation of reactive cells in the necrotic areas. The blood vessels including the central veins were congested. The sinusoids were dilated and engaged with blood. Due to pressure of the developing cyst there present a clear zone of demarcation, pressure necrosis, obliteration of the hepatic parenchyma and destruction of the tissue (Fig-11). The normal architecture of the lobules around the cyst was distorted in most of the cases. The inner most nucleated layer containing one layer of cells was seen to project inwards to form blood capsule. The middle laminated stained dark reddish due to presence of chitin or keratin. The outer fibrous adventitis was seen to be infiltrated with eosinophils, macrophages.

4.3 Paramphistomiasis

Only one (1.92%) of the diseased liver was found affected with paramphistomiasis. The affected liver belongs from male cattle.

4.3.1 Gross changes

Grossly the affected livers were turgid and round edged. Dirty fibrin strands were found attached to the medial surface of the right lobe near the portal hepatitis. Large bile ducts were very much prominent and hard with fibrosis.

On cutting open the early lesions were characterized by the prevalence of button shaped areas, with grooves to the periphery. Obstructions of

the bile ducts were found due to large number of parasites (Fig-12) in the duct's lumen. The measurement of the fluke on scale show near about 1 cm. in diameter (Fig-13). Scattered pin point hemorrhages and erosions were commonly observed on the luminal border of the large bile ducts. The entire luminal border of the large bile ducts were covered by thick catarrhal exudates admixed with dirty bile and large number of flesh colored or whitish mature paramphistomes were found attached to the luminal border. They formed individual colony, where they were attached by their oral suckers (Fig-14). In advanced cases numerous small round oblong seeds resembling the size of lentils were found, over the luminal border, where they were attached in the bile ducts. No significant changes were observed in the parenchyma and blood vessels grossly.

4.3.2 Histopathologic changes

Microscopically the luminal areas where the parasites were attached showed edema and thickened and the area of attachedment were elevated. There were slight cellular reactions, represented by the infiltration of neutrophils and mononuclear cells. The peripheral border of the bile duct was very much thickened with the proliferation of fibrous tissue. Numerous new bile ducts with hyper plastic changes in its luminal border were demonstrated also in these areas. Over the hyper plastic changes, thick area of fibrous covering was found. Tortuous hemorrhagic and edematous tunnels were frequently noticed in the thick fibrous covering over the hyper plastic areas, which might be due to its migration.

Sinusoids were dilated but empty and the proliferation of fibrous tissue and slight infiltration of round cells were found around the central

veins occasionally. Central vein in some cases were dilated and infiltration of reactive cells (fig-15) along with destruction of hepatocytes. Parenchymal nuclei were pyknotic and cytoplasmolysis were evident adjacent to the affected bile ducts.

4.4 Cirrhosis

A total of 5 cases (9.62%) showed partial or complete cirrhosis. Among them three of female and two of male cattle were affected with cirrhosis.

4.4.1 Gross changes

Cirrhotic liver has hard and leathery in consistency. It was tough to cut with scissors (Fig-16). In most of the cases adult flukes were recovered. The liver was contracted in many cases sinks into water. It was observed that the migration of only one or two adult liver fluke could initiate liver cirrhosis. The color of the liver was pale or whitish gray. 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.

4.4.2 Histopathological changes

Considerable proliferation of fibrous connective tissue was marked mainly in the portal area. There were a few lymphocytic infiltrations in the fibrous strands (Fig-17). 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.

4.5 Abscess

Abscess was found in only two cases (3.85%). Abscess liver was mainly found in female cattle.

4.5.1 Gross changes

The gross pathological changes were characterized by the presence of whitish or yellowish foci (Fig-18) on the surface of the liver. The size of the abscess varied from 0.5 to 1.5 cm in diameter and the number of the foci varied from 1-3. One of the containing liver pyogenic lesions was also associated with fascioliasis. The foci of abscess were odorless.

4.5.2 Histopathological changes

Histopathological changes were characterized by the presence of polymorpho nuclear leukocytes at the centre surrounded by a thin fibrous capsule. Fibrous thickening of the liver capsule and moderate infiltration lymphocytes, mononuclear cells and pus cell were characterized and destruction of the hepatocytes with obliteration of hepatic cords. (Fig-19).

4.6 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.

4.6.1 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.

4.6.2 Histopathologic changes

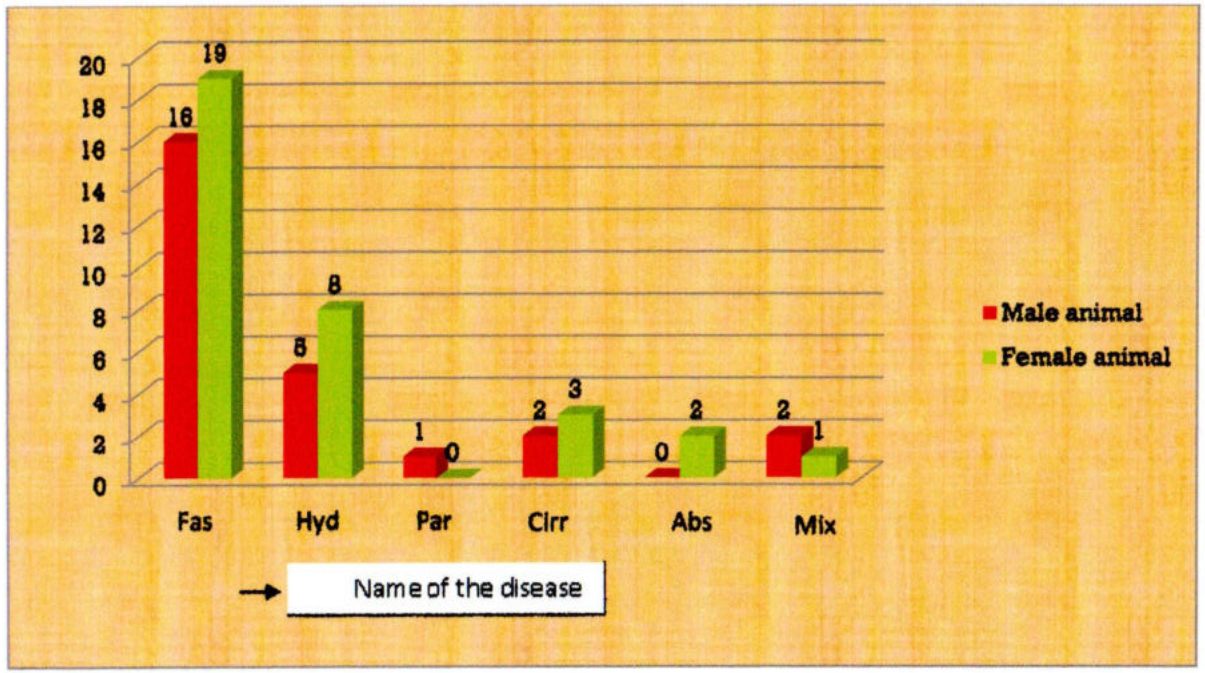
Microscopically, these areas were containing a large reserve of blood circumscribed by fibrous connective tissue. (fig.17).The parenchyma and portal triads around these areas were fibrosed and infiltrated with eosinophils and mononuclear cells. Cellular infiltrations with some fibrous connective tissue (fig-20) 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 disease found during the investigation period from July 2009 to June 2010.

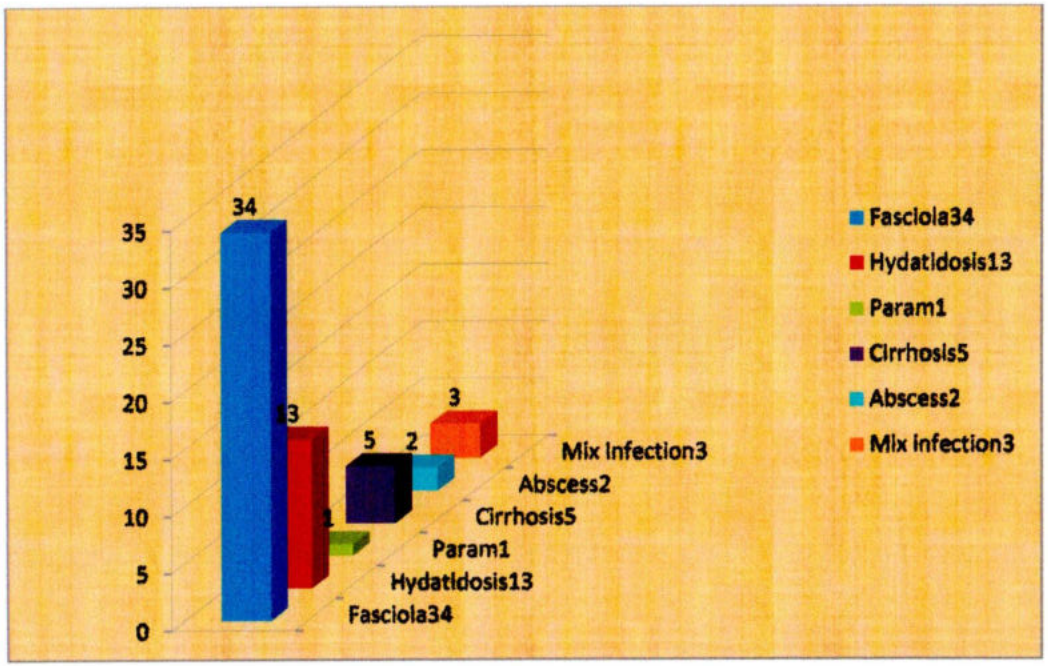
Disease /Disorder	No of Diseased liver examined	Number affected	% of Infection	Remarks
Fascioliasis	52	34	65.38	3 cases associated with Hydatidosis & Cirrhosis.
Hydatidosis	52	13	25.00	2 cases associated with Fascioliasis
Paramphistomiasis	52	1	1.92	_
Cirrhosis	52	5	9.62	1 cases associated with Fascioliasis
Abscess	52	2	3.85	_
Mixed Infection	52	3	5.77	Associated with Fascioliasis, Hydatidosis, Cirrhosis
Total pathological condition	58	58		

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

Disease /Condition	No. of animals affected	No. of male affected	No. of female affected
Fascioliasis	34	16	18
Hydatidosis	13	5	8
Paramphistomiasis	1	1	_
Cirrhosis	5	2	3
Abscess	2	_	2
Mixed Infection	3	2	1
Total Pathological condition	58	26	32



Graph 1 - Graphically representation the ratio of male and female animal in different Disease condition.



Graph 2 - Graphically representation of different disease condition.

Fig. 1: Liver of cattle with numerous swelling and round shape of the organ with few nodulations on the surface due to fascioliasis.

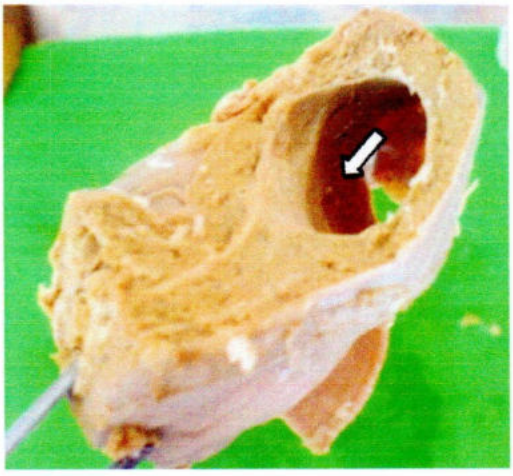


Fig. 2: On cut section Cattle liver showing migratory tract due to fascioliasis

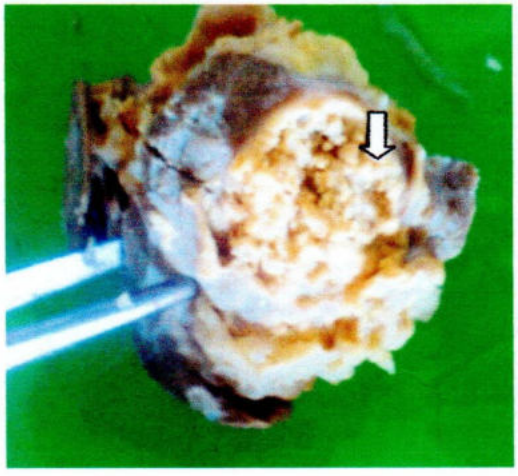


Fig. 3: Liver shows calcified mass.

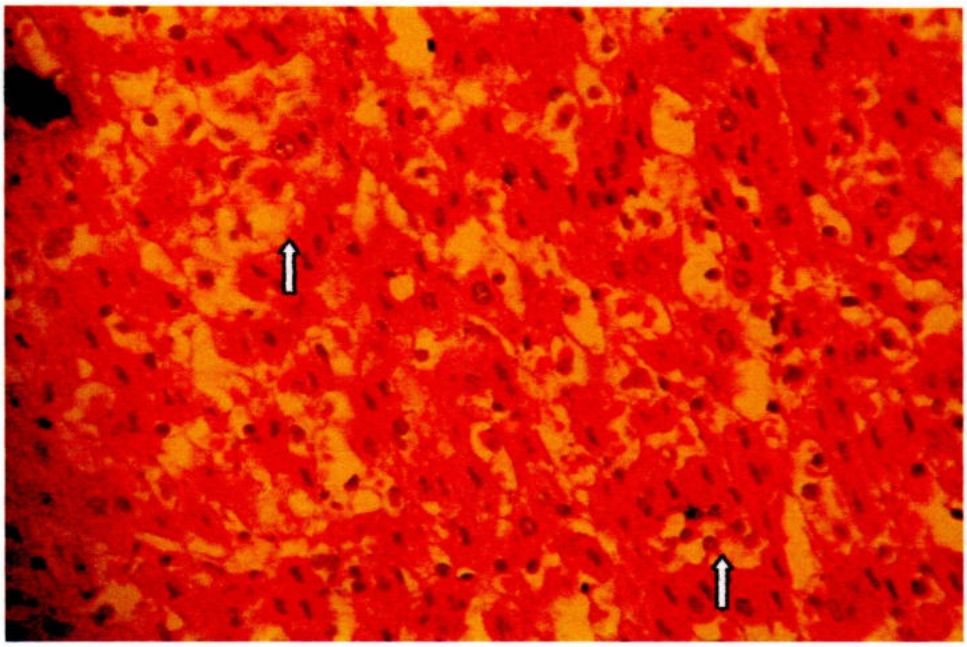


Fig. 4: Accumulation of lymphocytes and monocytes in the parenchyma of liver (X 40).

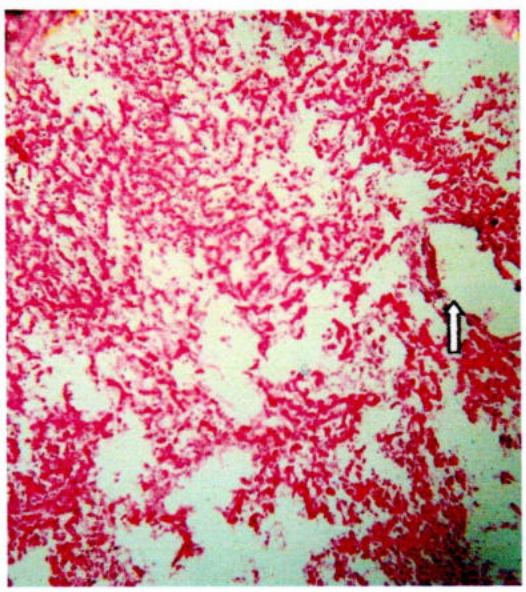


Fig.5: Destruction of hepatocytes and extravascular erythrocytes (X10)

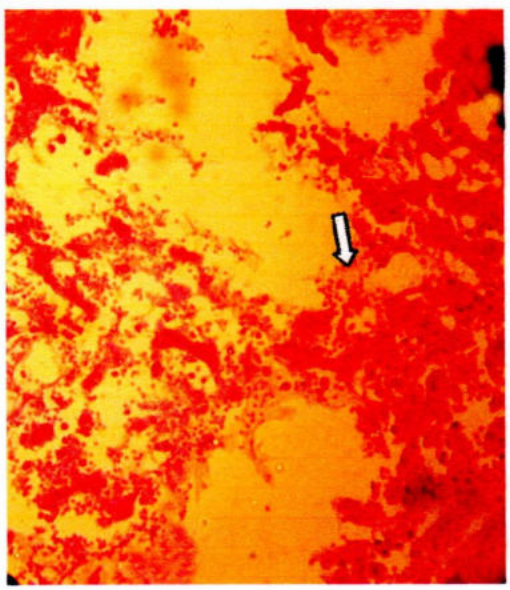


Fig.6: Architectural destruction of hepatic parenchyma (X40) due to fascioliasis.

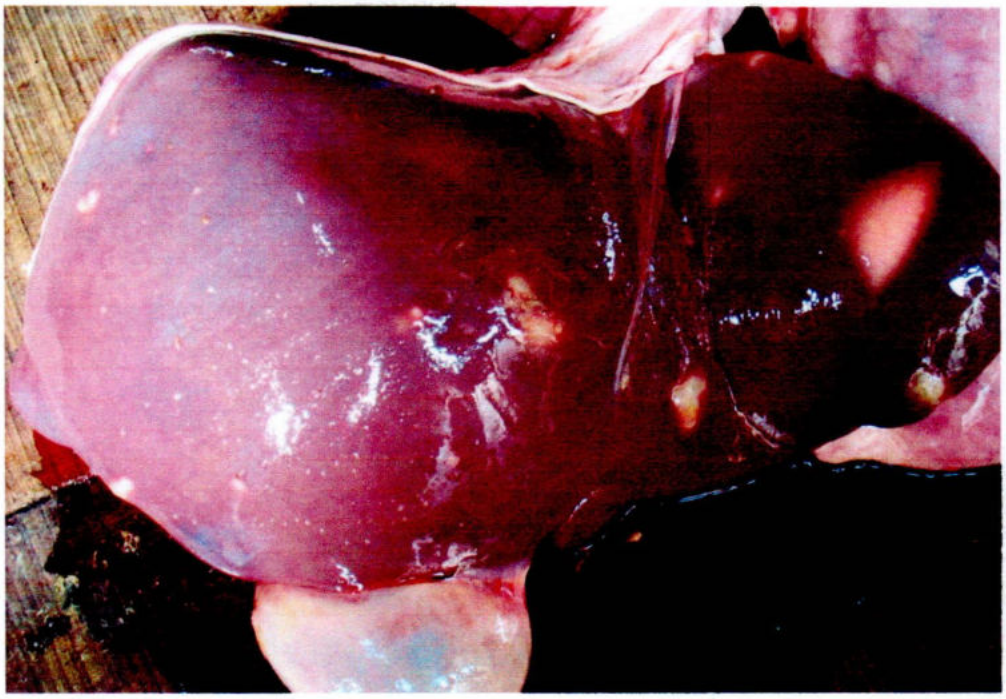


Fig. 7: Liver of cattle showing hydatid cysts within the liver parenchyma.

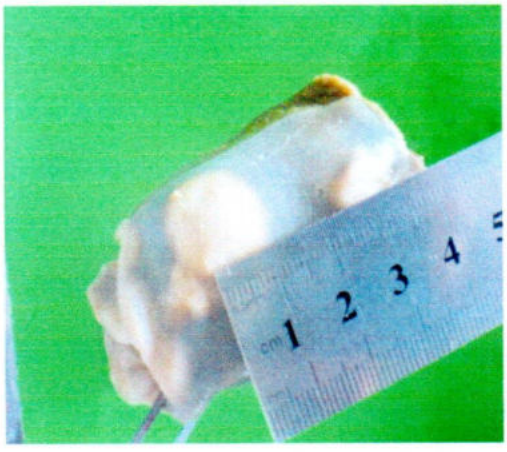


Fig. 8: Measure the size of the hydatidosis

Fig. 9: Pits on pressure due to hydatidosis

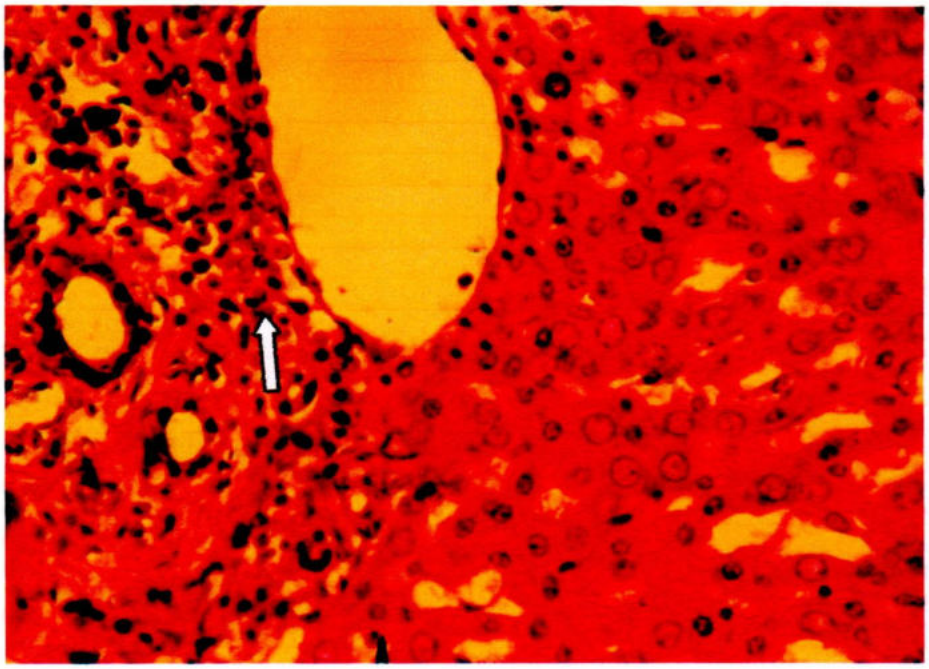


Fig.10: Hepatocytic necrosis due to cystic pressure with subsequent accumulation of reactive cells in the necrotic areas. (x40).

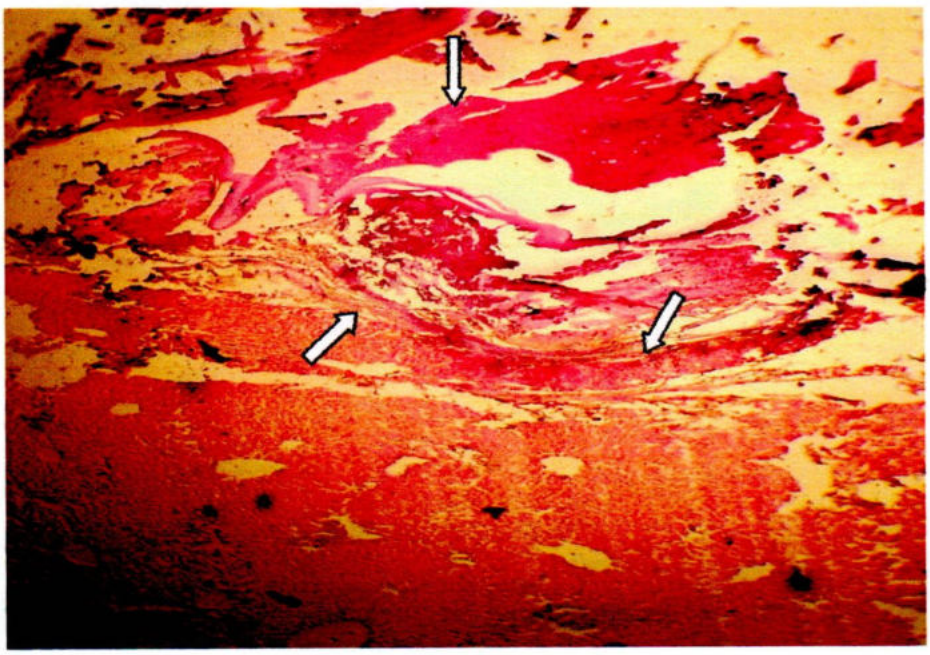


Fig. 11: Pink coloured calcium salt, Zone of inflammation and obliteration of the hepatic parenchyma(X 4).

Fig.12: Obstruction of bile ducts found in along with adherence with parasites.

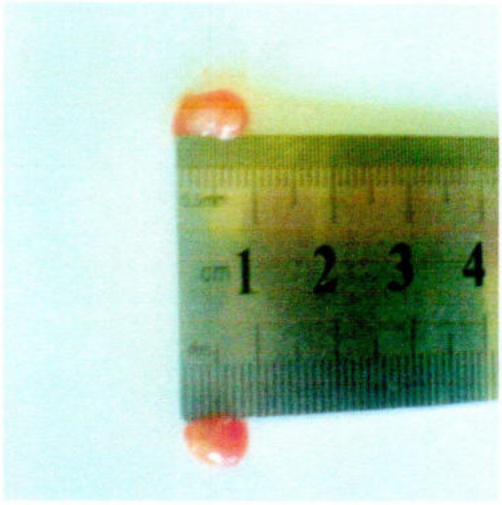


Fig-13. *Paramphistomum sp.* measure in Scale.

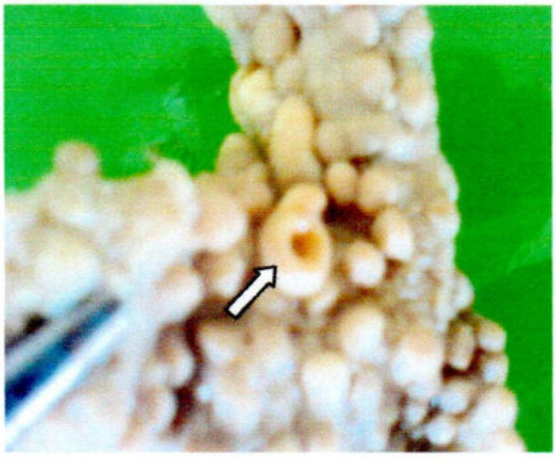


Fig-14 Adult fluke attach in the bile duct with oral sucker

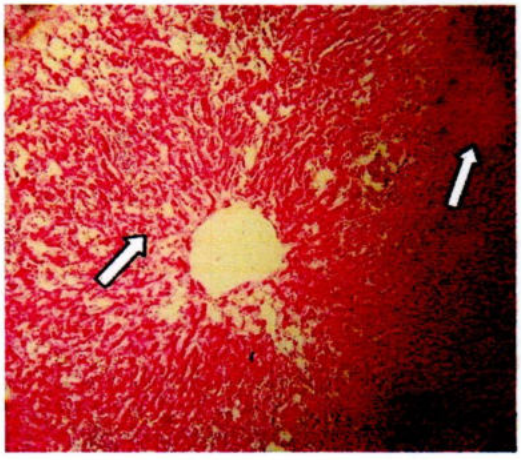


Fig -15 Dilated central vein along with diffused destruction of hepatocytes from the hepatic cords and infiltration of reactive cell (X10)

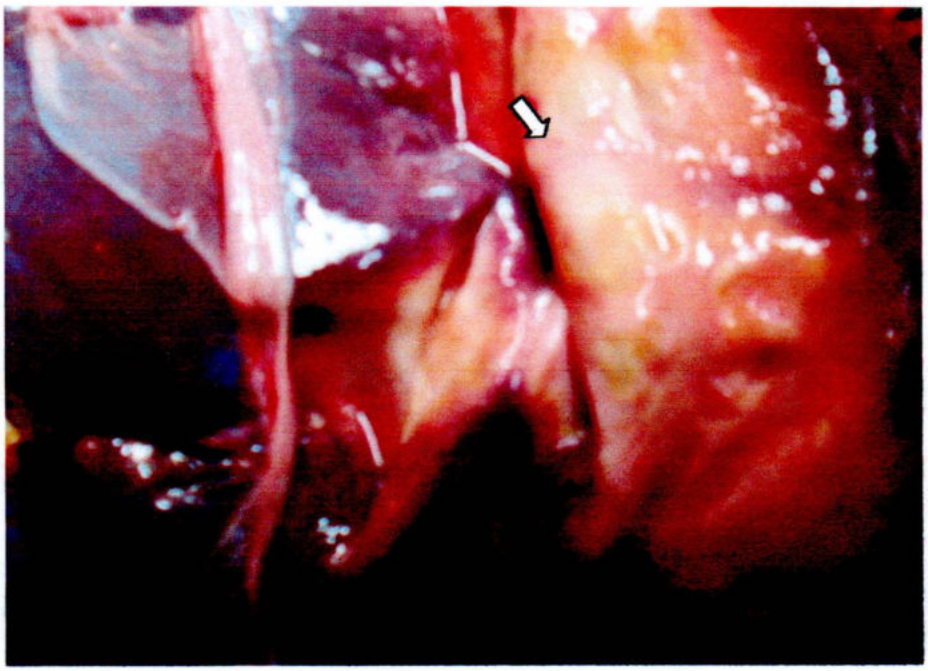


Fig. 16: Cirrhosis in the liver

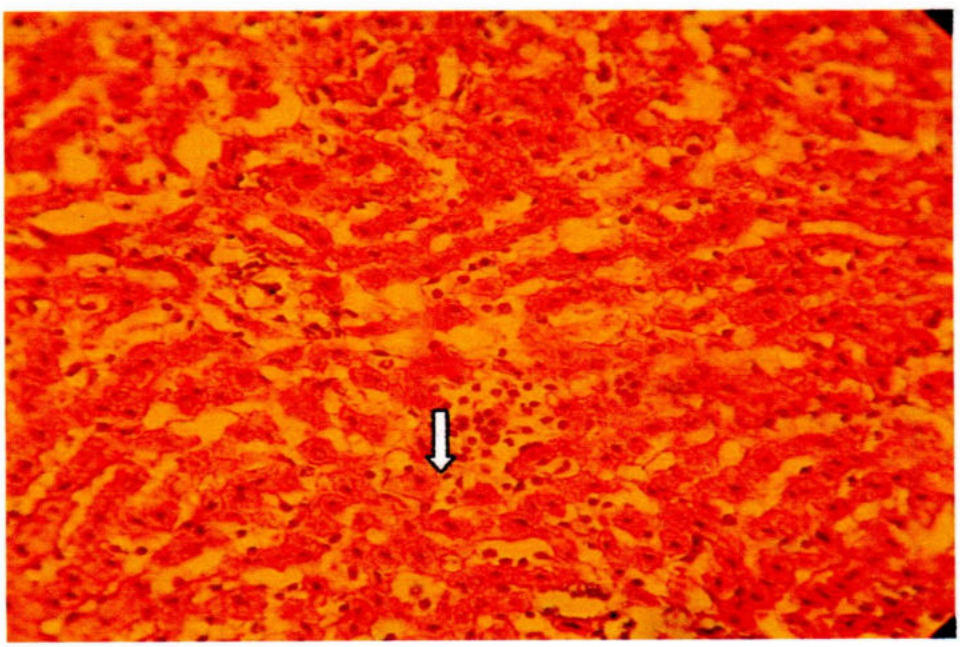


Fig. 17: Fibrous connective tissue along with lymphocyte and mononuclear infiltration in cirrhosis (X 40).

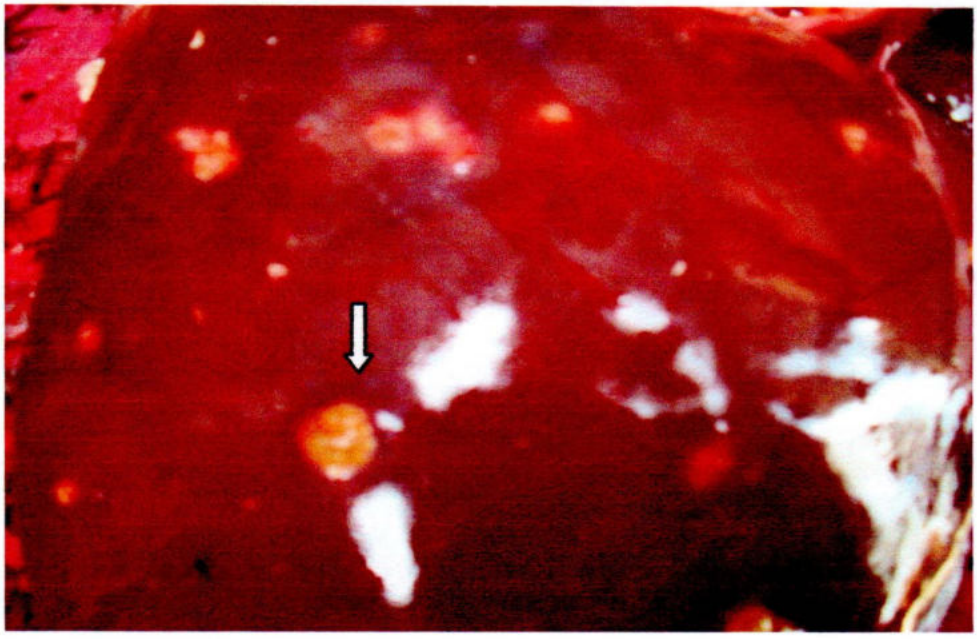


Fig. 18: Yellowish dot like foci represented of hepatic abscess.

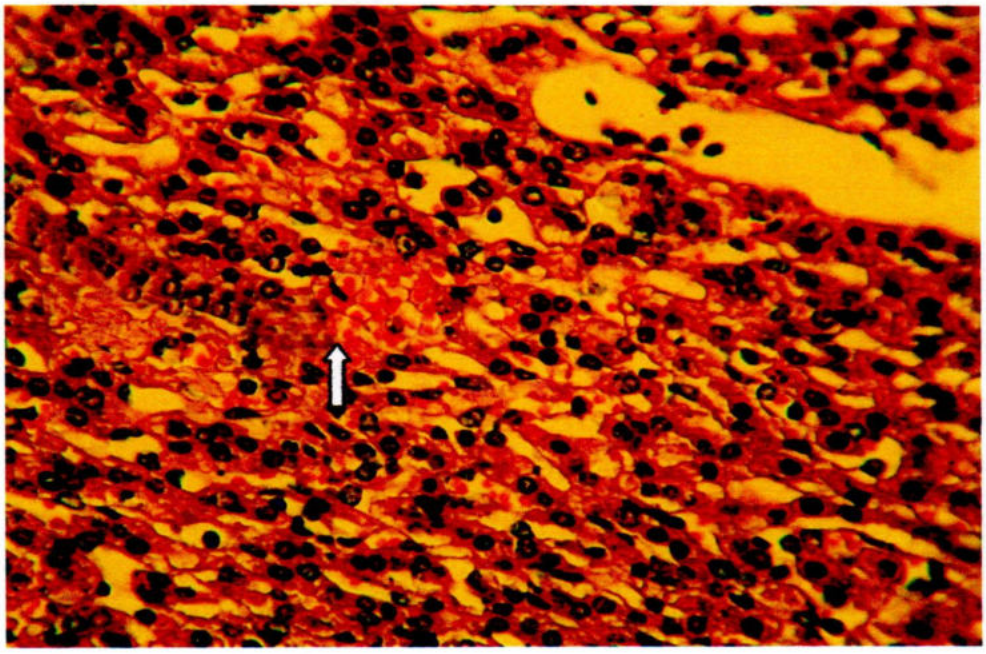


Fig.19: Hepatocytic destruction with obliteration of hepatic cords (X 40).

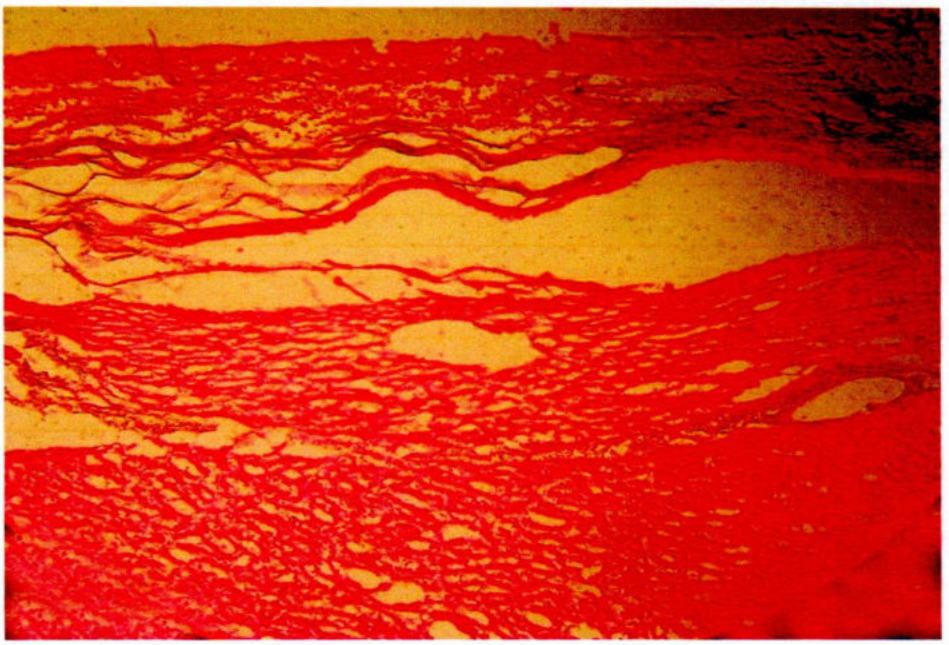


Fig. 20: Cellular infiltration with some fibrous connective tissue (X 10).

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSSION

The purpose of this study was to find out the incidence of different diseases affecting the liver of cattle in Dinajpur District of Bangladesh. The investigation carried out in the different slaughter house. The investigation was conducted from 1st July, 2009 to 30th June, 2010.

5.1 Fascioliasis

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. Phiri *et al.* (2005) 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.

Out of 52 examined livers, 34 (65.38%) were infected with fascioliasis. Studies on *Fasciola* infections in live animals, reported the prevalence of 56.5% in Arusha which is in northern Tanzania (Swai *et al.*, 2006). Examination of livers of 4620 domestic animals from south east Iraq indicated that 20.5% of all animals were infected with *Fasciola gigantica* and/or *Fasciola hepatica*. This has been reported in 1973 by Shaba *et al.* The infection rates were 91.4% for buffaloes, 49.2% for cows, 29.0% for sheep and 11.2% for goats. The gross and microscopic changes which

produced by *Fasciola gigantica* infection were almost similar with the earlier findings (Dow *et al.,* 1967, Soulsby 1982 and Dhote, *et al.* 1992). The hemorrhagic tracts on the parenchyma of liver 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 (Sengupta and Iyer, 1968). In cross section of liver there found pipe stem appearance of the liver caused by the migration of the parasites. Ansari-Lari and Moazzeni (2006) reported more or less same result on the prevalence of liver condemnations due to fascioliasis. Predominancy of the occurrence of fascioliasis as a single infection as seen in this study contradicts an earlier report By Trifonov (1980) who recorded a predominance of mixed infection in sheep liver in Bulgaria. Like Roberts (1968), Acosta-Fereira, *et al.,* (1980), Ross *et al.,* (1967), Dow *et al.,* (1967). And Sengupta and Iyer (1968), both acute and chronic forms of fascioliasis have been detected in the present study. The gross pathological changes in acute fascioliasis described by these authors may be summarized as light haemorrhagic spots or elongated tracked occurring on the surfaces of the liver.

The histopathological changes in chronic cases characterized by infiltration with lymphocytes and mononuclear cells and proliferation of fibroblasts in the area previously migrated by young flukes. Similar statement was made by Dow *et al.,* (1967) and Smith *et al.,* (1972). Heavy accumulation of lymphocytes and proliferation of fibrous connective tissues in the portal areas are seen. 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. Formation of blebs at the free margin of the epithelial cells undergoing hyperplasia was a common picture. Most advanced stages hyperplasia led to the formation of gland-like structures. The mucosa was often infiltrated with plasma cells and few eosinophils. Formation of new bile ducts and deposition of bile pigment in the tissue spaces were seen. These findings substantiated the observations of Stenius (1963), Dawes (1963) Acosta-Fereira *et al.*,(1980), Ross (1966), Ross *et al.*, (1967), Dow *et al.*, (1967) and Smith *et al.*,(1972). Similar to the finding of Dow *et al.*, (1967) and Rahko (1969), thrombi were found in the blood vessels. 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

Hydatidosis which bears zoonotic importance has been detected in thirteen livers. Among them eight (61.54%) were from female and five (38.46%) from male cattle. Here we found among 52 affected livers there found 25% liver (thirteen livers) affected. 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 of hydatid cysts varied from 0.5 to 20 cm. in their greatest dimension and the examined cysts were sterile. 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. Present study showed that the hydatid cyst consisted of 3 layers which were also reported in the observation of Cameron and Webster (1969). Moreila *et al.,* (1983) recode that 7.43% male and 12.35% female cattle were infected with hydrated cysts. The present study also revealed variation in male (38.46%) and female (61.54%) cattle, which is similar to Monirul (1993) that shows the variation in male (37.1%) and female (62.82%) buffaloes.

In three cases, the liver was found severely damaged with hydatid cyst. This was also found by Mamedov (1965). 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 Paramphistomiasis

This study suggested that the enormous presence of this parasite by their ascending migration through the common bile ducts from the duodenum, deserved to be entitled as one of the liver flukes. Actually the literatures on the pathological aspects of paramphistomiasis are very scanty. In this study it was found that the infection of paramphistomiasis was one (1.92%) cattle which are very low. This data is similar to Kakar *et al.,* (2008). During this study it was found that mature paramphistomiasis causing fibrous thickening and bile ductular hyperplasia of the affected bile ducts. The affected area was infiltrated

with eosinophils and huge mononuclear cells. The fibrous tissue proliferation in this case were collagen and elastic in type and no author has mentioned about the migratory tunnels in the proliferated bile ductular epithelium, observed in this study. Islam *et al.* (1992) reported 48.3% infestation rate of *Paramphistomum* species in water buffaloes in Bangladesh. This fluke was usually recovered from rumen, reticulum, duodenum, abomasum, small intestine, caecum, colon and large intestine. Paramphistomiasis is geographically widespread.

5.4 Cirrhosis

A total of 5 cases (9.62%) 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.

5.5 Abscess

It is believed that hepatic abscess observed is predisposed by the entrance and migration of immature flukes. Such as hypothelial statement has also been made by Salo *et al.*, (1978), Elmossalami and Darweish (1977) and Uzoukwu and Ikeme (1978).In this study abscess was found in only 3.85% cases, which is similar to that of Ahmedullah

et al., (2007). Grossly, whitish foci on the surface of the liver were found. Size varied from 0.5 to 1.5 cm in diameter. Polymorphonuclear leukocytes at the center surrounded by a thin fibrous capsule were seen microscopically. According to Runnels et al., (1976), hepatic abscess may or may not calcify. In the present study the hepatic abscess did not calcify.

5.6 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 VI

CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

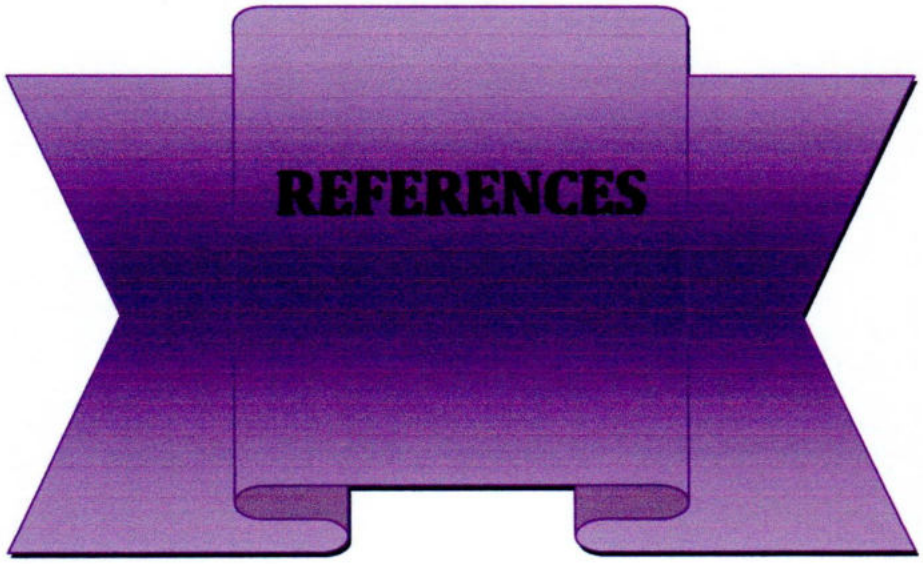
A total of 52 apparently abnormal cattle livers were collected from different abattoir in Dinajpur district for pathological investigations with a view to revealing various disease conditions affecting the liver of cattle.

In course of this study, 34(65.38%) were found to be affected with liver fluke, *Fasciola gigantica.* Of these 34 Fasciola affected livers 18(52.94%) belonged to female and 16(47.06%) belong to male cattle. 13 of disease livers (25%) were found to be affected with hydatid cyst. Among them eight (61.54%) were from female and five (38.46%) from male cattle. Only one (1.92%) of the diseased liver was found affected with paramphistomiasis. The affected liver belongs from male cattle. A total of 5 cases (9.62%) 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 (3.85%). 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 and paramphistomiasis.
2. Fascioliasis (*Fasciolia gigantica*) is greatly responsible for hepatic damage and demands immediate attention for taking control and eradication measures, where as the *Fasciolia gigantica* infection were also increased in rainy season. Other pathological conditions did not show any significant seasonal variation.
3. Female species were found to be frequently affected with hydatidosis than male species. Hydatidosis recorded in male and female species 38.46% and 61.54% respectively. Other pathological condition did not show any significant sex variation.
4. Mixed infections were represented by more than one type of infection, which was found increased with the advance of ages.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