PATHOLOGICAL STATUS OF FASCIOLIASIS OF BOVINE LIVER COLLECTED FROM SLAUGHTER HOUSE OF DINAJPUR DISTRICT OF BANGLADESH

A THESIS

BY

YONIS ABUKAR MOHAMED

SEMESTER: JANUARY - JUNE 2020

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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
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June, 2020 *The Author*

ABSTRACT

The pathological status of fascioliasis in cattle of Dinajpur city of Bangladesh was carried out from January to June, 2020 by clinical signs, pathological and coprological examinations. A total of 75 cattle (27 male and 48 female) were recorded as study population for the present study. Seven cattle were found with pathological conditions There was engorgement of bile duct with fibrosis and the percentage of fibrosis of fascioliasis was 10.6%. While 17.3% roughened and thick capsule with whitish or reddish discoloration throughout the capsule. Gross lesions of fascioliasis found in the liver were increase in the size of the organ were 14.6%.. The infected bile ducts were also filled with blackish brown exudates giving the pipe stem appearance of the liver was noticed 13.3%. In histopathological study of acute Fascioliasis, there was lot of hepatocytic alterations like swelling of individual hepatocytes by increase in size and characterized by opaque cytoplasm. And sometime there was fatty change in which clear vacuoles appeared in the cytoplasm with peripherally located nuclei. Microscopically, depending on the duration and intensity of the infection, various changes were observed in the structures of *Fasciola* infected cattle liver. In conclusion Gross lesions of fascioliasis found in the liver were increase in the size of the organ and also there was engorgement of bile the duct with fibrosis. The infected bile ducts were also filled with blackish brown exudates giving the pipe stem appearance of the liver was noticed. In some cases, there was roughened and thick capsule with whitish or reddish discoloration throughout the capsule. My recommendation to study on pathological investigation to find out gross and microscopic lesions of the disease. To carry out further studies on isolation, morphology and characterization of *Fasciola* sp. To develop good management practices like hygienic condition, vaccination program to minimize the risk of the disease.

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LIST OF ABBREVIATION AND SYMBOLS

GDP	:	Gross Domestic Product
FABP	:	Fatty acid binding proteins
GST	:	Glutathione stransferase
°F	:	Fahrenheit
ALT	:	Alanine aminotransferase
ASL	:	Aspartate tgransaminase
IEA	:	Indirect enzyme linked immunosorbent assay
SEA	:	Sandwich-enzyme linked immunosorbent assay
HSTU	:	Hajee Mohammad Danesh Science and Technology University
DPX	:	Mounting media
°C	:	Degree celcius
Ml	:	Milliliter
G	:	Gram

CHAPTER I

INTRODUCTION

Bangladesh, country of South Asia, located in the delta of the Padma Ramesh, R., & Sarin, M. M. (1992). And Jamuna rivers in the northeastern part of the Indian subcontinent and it is one of the most densely populated countries in the world with an estimated 1,033 people/km2 (United Nations, 2011). In Bangladesh, similar to human population density, livestock density is also highest (Cattle, goat, sheep and Buffalo) in the world with an estimated 145large ruminants/km^2 (BARC Bangladesh, 2010).

The total livestock population of Bangladesh is composed of 23.93 million cattle, 25.93 million goat, 1.48 million buffalo, 3.40 million sheep, 275.2 million chicken and 54.01 million duck (Campillo *et al.*, 2017). This density has been increasing every year. Statistics show that about 2.9% of national GDP is covered by the livestock sector, and its annual rate of growth is 5.5%. About 20% of the population of Bangladesh earns their livelihood through work associated with raising cattle and poultry (Education Commission (2015). In spite of a high density of livestock population, the country suffers from an acute shortage of livestock products like milk, meat and eggs. The shortage accounts for 85.9%, 88.1% and 70.7% for milk, meat and eggs, respectively (BAU 2015). However, if we desire to meet the increasing demand from domestic production, we will require an increase in production at the rate of 6 to 9 percent per year up to 2021(Leijenaar *et al.* 2015).

Livestock is an important component of the mixed farming system practiced in Bangladesh. The animals kept especially on a farm for economic benefits. Livestock constitute an important part of the wealth of a country, since in addition to draft power and leather; it provides manure, meat and milk to the vast majority of the people. Livestock resources necessarily encompass animal health care and welfare, quality production factors, and effective rearing to keep pace with expansion of entrepreneurship related to concerned industries. (Nidra Boti Khan 2017).

In addition, hides and skins, bones, offals, feathers, etc, help in earning foreign exchange. Livestock resources also play an important role in the sustenance of landless people (Ehsan, A. M., Geurden 2015). Livestock is an important sub-sector considered to be the backbone of agriculture. Parasitism is one of the most vulnerable causes of livestock diseases which are the major obstacle in the growth and development of animal health (Mahfooz *et al.* 2008).

Fasciolosis is a major problem that affects the productivity of livestock throughout the world (Bargues *et al.* 2019). It is caused by the genus *Fasciola* which is commonly referred to as liver flukes (Khaled, 2010). Two species most commonly concerned as the etiological agents of fasciolosis are *Fasciola hepatica* (*F. hepatica*) and *Fasciola gigantica* (*F. gigantica*). Both species can infect a wide variety of domesticated animals, wild life and humans. Fasciolosis is commonly distributed in countries where cattle and sheep are raised and there is a niche for Lymnaeid snail and the disease repeatedly reported in different continent such as America, Australia, Europe, Asia and Africa (El-Hoda *et al.* 2002).

Fascioliasis is an economically important parasitic disease as it causes huge economic losses in terms of reduction of milk and meat and high morbidity in all ages of animals (Saleha, 1991). The disease causes considerable economic impact due to mortality, liver condemnation, reduced weight gain (up to 20%) and reduced quality and quantity (3–15% loss) of milk production (Piedrafita *et al.* 2018).

Fasciolosis is associated with liver damage and hemorrhage due to migration of flukes through the liver parenchyma. There is also haematophagic activity of the adult flukes and damage to the bile duct mucosa by their cuticular spines due to fluke residence in the bile duct (Taylor 2007 and Urquhart 1996). Diagnosis of fasciolosis may be established based on the epidemiology of the disease, observations of clinical signs, and information on grazing history (Kassai 1999).

Bangladesh has a tropical monsoon climate characterized by rain-bearing winds, moderately warm temperatures, and high humidity. The geo-climatic condition of Bangladesh are highly favorable for the growth and multiplication of parasite (Shahid 2010). Due to the tropical climate the cousal agent of *Fasciola hepatica* and *Fasciola gigantica* is prevalent in this part of the world (Amin and Samad 1998). The prevalence of fascioliasis may differ in cattle. Earlier reports suggest around 19-53% prevalence of fascioliasis in cattle in various districts of Bangladesh (Rahman & Mondal, 1983), (Chowdhury *et al.*, 1994), (Affroze *et al.*, 2013).

In Dinajpur city there is much prevalence (65.78%) (Ducheyne *et al.* 2015) of facioliosis and in this study we tried to draw out the pathological condition associated with facsioliasis, which will be helpful for the proper diagnosis of this condition and also the key points for the comparison with other study on the basis of literature. So, the present study was conducted the following objectives:

- To evaluate the fascioliasis in the liver collected from slaughterhouses
- To assess the gross and histopathological features of infected liver

CHAPTER II

REVIEW OF LITERATURE

2.1 Bovine Fascioliasis

Fascioliasis, a serious infectious parasitic disease infecting domestic ruminants and humans, tops all the zoonotic helminthes worldwide (Haridy *et al.* 2002). The disease in predominantly caused by *F. hepatica* and / or *F. gigantica* (Soulsby, 1987).

Gupta & Kamra (1987) In country like Bangladesh and elsewhere too, providing animals' clean water to drink and prevent these enter into natural water reservoirs infested with snails shall greatly reduce the risk of acquiring fascioliasis. Ensiling of animal feed such as paddy straw likely to be infested with metacercariae kills not only the deposited metacercariae.

Gupta *et al.* (1988) But also increase the digestibility and nutritive quality of the feed. Elimination of snails from environment shall break the life cycle of the parasite. This can be achieved by application of molluscicides like copper sulphate, bayluside, trifenmorf with caution because this is not always possible due to environmental objections. Cleaning of ponds, ditches, canals etc., of unwanted aquatic vegetation can drastically reduce the mollusc population and almost eliminate all the molluscan eggs. Plant origin molluscicides, which degrade into simpler components within short period of application, may also be fruitful.

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

Spithill *et al.* (1997) Since then a number of parasite proteins identified and characterized from both *F. hepatica* and *F. gigantica* have been tested in vaccine trials with partial success in different laboratory and natural animal hosts. The current vaccine strategy implies the immunological mechanism by which the parasite metabolic products (enzymes) variously termed as 'defined, natural and conventional' antigens aiding in invasion, survival and evasion of host immune responses are neutralized rendering the parasite helpless. Such parasite proteolytic enzyme agents known as defined molecular candidate vaccine agents are being looked forward against *Fasciola* with bright prospects. Vaccination of cattle with same enzyme resulted in 50% reduction of worm burden but when the candidate vaccine agent was combined with fluke haemoglobin the protection was enhanced to 70%, and 100% failure of eggs to embryonate shed by such vaccinated animals. Contrarily, cathepsin L from *F. gigantica* failed to provide similar protection in cattle to homologous infection. The basic concept is that if such vaccination is successful it can reduce parasite transmission enormously. Lastly but not the least, prevention of human infection by avoiding consuming metacercariae is much discussed above. Measures are many but to follow these require patience and concern. Otherwise, human infection is like meeting a fatal accident as a result of careless driving. Treatment of animals with broad-spectrum fasciolicide would prevent contamination of environment with parasite eggs and subsequently infection of intermediate snail host and contamination of environment with metacercariae. On the basis of strategies used to identify these defined candidate vaccine molecules of *F. hepatica* and *F. gigantica*, three prototype antigens have been identified These are: (1) The fatty acid binding proteins (FABP), a cross protective antigen of *F. hepatica* recognized by cross reactive antibodies raised against the trematode Schistosoma mansoni, rendering cross protection against S. mansoni. (2) The glutathione stransferase (GST), a *Fasciola* molecule homologous to antigen previously shown to protect animals against S. mansoni and S. japonicum infection. (3) Cathepsin-L, the essential *Fasciola* molecules, constituting of cysteine proteinases and predicted to perform functions essential for parasite growth and survival. Under this category, *F. hepatica* cysteine proteinase recognized by the host post-infection inducing high levels of protection of up to 70% in sheep vaccinated with the purified cystiene proteinases.

Cobbold (1855) and Linnaeus (1966) Bovine fascioliasis (liver rot) is an economically important helminth disease caused by two trematodes viz. *Fasciola hepatica* (the common liver fluke) and *Fasciola gigantica*this disease belongs to the plant-borne zoonosis.

Hillyer (2005) Depending on the disease prevalence in a herd, these reductions can be significant. The direct economic impact of fascioliasis infection is increased condemnation of liver meat, but the far more damaging effects are decreased animal productivity, lower calf birth weight, and reduced growth in effected animals.

Marcos *et al.*(2008) Fascioliasis is generally a disease of ruminants such as sheep, cattle and goats and is also recognized as occasional zoonotic disease of man. Fascioliasis has the widest geographic spread of any emerging vector-borne zoonotic disease and affects an estimated 17 million people in more than 51 countries, worldwide.

Rana *et al.* (2014) Fascioliasis causes thickening and dilation of the bile ducts and toxic degeneration of the adjacent liver tissues leading to liver condemnation at slaughter. Heavy infections cause serious disease and high mortality in cattle and sheep. Recently, fascioliasis has only been recognized as a significant human disease; studies to determine the global morbidity caused by the disease are ongoing.

2.2 History of Fascioliasis

Malpighi, (1697) and Bidloo(1697) *Fasciola*, amongst the helminthic parasites, does not find its description until before Ebers' Papyrus (1550 B.C.) described Guinea worm in Old Testament; Hippocrates (460-377 B.C.) described the beef tapeworm (*Taenia saginata*) and hydatid cyst (*Echinococcus granulosus)* infections and Anstali (384- 322 B.C.), the large round worm (Ascaris). The generic name *Fasciola* is from L. dim. of fascia, meaning a band of a fillet. Jehan de Brie recognized the *Fasciola* hepatica in 1379. The first report of human infection dates back to 17th century.Then specimen of *Fasciola* was detected in gall bladder of man during post-mortem (Fortassin, 1804; Duval, 1842; Partridge, 1846) and during operation in an eight-year-old girl in 1872. Leukart and Thomas in 1883 unravelled its life cycle and implicated the role of snails as its intermediate host. Adolph Lutz in 1892 described its mode of transmission to definitive host in Hawaii.

Yilma and Malone (1998), Daniel (1995) and Dagne (1994) Tadelle and Worku (2007) Bovine fasciolosis is an economically important parasitic disease of cattle caused by Fasciolidae, which are trematodes of the genus *Fasciola*, the two most important species of this genus are *F. hepatica and F.gigantica.* The presence of fasciolosis due to

F. hepatica and F. gigantica in Ethiopia has long been known and its prevalence and economic significance have been reported by several workers.

According Alemu (2019) In Ethiopia, fasciolosis is a parasitic disease that affects most population of cattle. Fasciolosis exists in almost all parts of the country. It is caused by commonly known species of liver fluke that are *Fasciola hepatica and Fasciola gigantica* which mainly affects domestic ruminants. Fasciolosis is more apparent in young cattle and is usually chronic in nature, a dult flukes in the bile ducts cause inflammation, biliary obstruction, distraction of liver tissue and anemia. Snails of family Lymnaeidae are main intermediate hosts having great role on the transmission of the disease and the infection is acquired through grazing on swampy pasture. The epidemiology of fascioliasis is strictly linked to the geographical and environmental characteristics of the area where transmission occurs.

2.3 Taxonomy of Fascioliasis

Stiles and Hassall, (1898) and Odhner (1911) The genus *Fasciola* Linnaeus, 1758 belongs to the family Fasciolidae established by Ralliet in 1895 followed by erection of subfamily Fasciolinae.

Odhner (1905) while describing the trematodes of North Africa divided the family Fasciolidae into three subfamilies, viz., 1) *Fasciolinae* Stiles et Hassall with the genuses *Fasciola* and a new genus for Dist. magnum Bassi later replaced as Fascioloides (Ward, 1917); 2) *Fasciolopsinae* (Odhner, 1911); and 3) *Brachycladiinae* (Odhner, 1905). Travassos, (1929) while analysing the family Fasciolidae conclusively divided it into four subfamilies with addition of subfamily *Omphalometrinae* to the above description.

Faust (1929) Stunkard and alvey, (1930) raised subfamily Brachycladiinae Odhner, 1905 to a new family, *Brachycladiidae* (Faust, 1929) and along with family Fasciolidae (Ralliet, 1895) affiliated these with the superfamily Fascioloidea (Stiles, 1910). In 1930, Stunkard and Alvey briefly analysed the prevalent opinions on the taxonomy of Fasciolidae and from biological point of view reunited the families *Fasciolopsidae* and *Campulidae* as a single family Fasciolidae (Ralliet, 1895) with three subfamilies earlier suggested by (Odhner, 1911) but replacing *Brachycladiinae* (Odhner, 1905) by subfamily *Campulinae.*

In (1935), Skrjabin and Shul'ts initially recognized the classification of (Stunkard and Alvey, 1930) but in the same year Ozaki, a Japanese researcher, recognized only the unified family Fasciolidae comprising four subfamilies, viz., *Fasciolinae, Fasciolopsinae, Campulinae* and *Nasitrematinae*. Later they were convinced that these classifications could not withstand criticism at this particular juncture in thedevelopment of the taxonomy of fasciolid and reconciled with (Odhner, 1926) in elevating the subfamily *Campulinae* to the status of Family and suggested that these two families could be placed under single superfamily, *Fascioloidea* Stiles et Goldberger, 1910 affiliated with the suborder *Fasciola*ta (Skrjabi, 1935). Further, they supported the concept of Fascioloidea formulated by (Faust, 1929) except proposal of a new family, *Brachydadiidae* (Faust, 1929), in place of considering the latter a synonym for the same *Campulidae.*

<u>Taxonomic Classification</u>

Kingdom Animalia
Phylum Platyhelminthes
Class Trematoda
Subclass Digenea
Order Echinostomiformes
Family Fasciolidae
Genus *Fasciola*
Species *F. hepatica/ F. gigantic*

(Soulsby, 1965)

Figure 1: Taxonomic Classification

2.4 Epidemiology of Fascioliasis

2.4.1 Etiology

Walker *et al.,* (2008) Fasciolosis is caused by two digenetic trematodes *F. hepatica* and *F. gigantica*. Adult flukes of both species are localized in the bile ducts of the liver or gallbladder. Bovine fasciolosis is a parasitic disease of cattle caused by trematodes usually *Fasciola gigantica* and rarely *Fasciola hepatica* in the tropics. The life cycle of these trematodes involves snail as an intermediate host.

Rana *et al.*, (2014) Fascioliasisis a common disease of cattle and other ruminants caused by *F. hepatica* and *F. gigantica*.

2.4.2 Geographical Distribution

Rana *et al.*, (2014) *F. hepatica* measures 2 to 3 cm and has a cosmopolitan distribution. *F. gigantica* measures 4 to 10 cm in length and the distribution of the species is limited to the tropics and has been recorded in Africa, the Middle East, Eastern Europe and south and eastern Asia (Torgerson & Claxton, 1999). The disease is worldwide in distribution and is liable for causing extensive economic losses to the livestock industry encompassing reductions in weight gain, milk yield and fertility.

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

2.4.3 Host Range of Fascioliasis

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

2.4.4 Source and Mode of Transmission

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

2.4.5 Prevalence of fascioliasis

Howlader *et al.* (2016) A study was conducted during the period from October, 2016 to January, 2017 to determine the prevalence of bovine fascioliasis at Mirzaganj upazilla under the Patuakhali district in Bangladesh. A total of 92 bovine cases was recorded as study population for the present study. Tentative diagnosis of fascioliasis was made on the basis of history, clinical signs, physical examination findings; whereas confirmatory diagnosis was made on the basis of coprological examination. The overall prevalence of fascioliasis was 44.57%, the age of the study population (cattle) were divided into three groups i.e. 1-2 years; 2-4 years; 4-6 years and their prevalence (%) of fascioliasis were found 47.83%, 41.37% and 41.17% respectively. The higher prevalence of fascioliasis was recorded in female 52.83% followed by male 33.33%. Among the study population, the highest prevalence was recorded in cross breed (60%) cattle than local or indigenous (42.68%) cattle.

Elshraway and Mahmoud, (2016) The main objectives of a study were to determine the prevalence of fascioliasis infections in cattle and buffaloes, slaughtered in El-Kharga city slaughterhouse at New Valley Governorate. The slaughtered animals were daily inspected for liver fascioliasis all over 2016. Macroscopic fascioliasis was detected from a total of 2251 basing on animals specie, sex, season, and *Fasciola* spp. in addition to microscopic examination of blood, fecal samples which collected from female cattle and buffalo (50 each). The total prevalence rate of *Fasciola* sp. infection occurs in the study area were about 695/2251 (30.88%) from the total cattle and bovine slaughtered carcasses. The incidence of fascioliasis was 4/12 (33.33%) and 678/2200 (30.82%) for females and males cattle carcasses, respectively, while the infection rate in buffalo carcasses was 1/4 (25.00%) and 12/35 (34.29%) for females and males buffalo carcasses, respectively. The moderate fasciolosis infection in cattle and buffaloes slaughtered at the municipal abattoir of El-Kharga, Egypt. The highest fascioliasis infection was recorded during winter and autumn. It constitutes a major cause of economic losses at El-Kharga abattoir and threat public health.

In adult cattle, the infection usually takes a chronic course, with no obvious clinical signs. Significant production losses occur in the herds having a prevalence of *F. hepatica* infection of 25 % or above (Vercruysse and Claerebout, 2001). The cercariae of liver flukes were observed from a pond first time by Otto Muller in 1773 (Andrews, 1999). A large variety of animals, such as sheep, goats, cattle, buffalo, horses, donkeys, camels and, rabbits, show infection rates that may reach 90% in some areas (Farag, 1998).The snail usually habitat along the edges of stagnant ponds, marshy lands and ditches which may be a reason of increased prevalence of Fascioliasis in the animals bathing in stagnant water (Ulmer, 1971; Saladin, 1979). Both *F. hepatica* and *F. gigantic* are prevalent in Pakistan (Maqbool *et al.*, 1994, 2002; Siddiqui and Shah, 1984; Chaudhry and Niaz, 1984; Masud and Majid, 1984; Sahar, 1996).

M. A. *et al.* (2014). The prevalence of fascioliasis in cattle slaughtered in the Sokoto metropolitan abattoir was investigated. Faeces and bile samples were collected and processed using formal ether concentration technique. Gross lesions from 224 out of 1,313 slaughtered cattle were randomly selected and examined. Out of the 224 cattle examined, 95 (42.41%) were males and 129 (57.59%) were females. Out of 95Male cattle examined, 27 (28.42%) were infected and out of 129 females 35 (27.13%) were infected. Based on breed, infection rates were 31 (31.0%), and 31 (25.2%) for breeds of Sokoto Gudali and Red Bororo respectively. No infection was recorded in White Fulani breed. Lesions observed were more in males than in females and more in Red Bororo than in Sokoto Gudali. Overall, prevalence of infection with *Fasciola* was 27.68%. There was no statistically significant association between infection and breed and between infection and sex of the animals sampled ($P > 0.05$). Regular treatment of all animals with an effective flukicide, as well as snail habitat control, tracing source of animals, public enlightenment about the disease, proper abattoir inspection, adequate and clean water supply to animals, and payment of compensation of condemned tissues and organs infested with the parasite by government were suggested.

2.4.6 Risk Factors

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

2.4.7 Incidence of Fascioliasis

Ollerenshaw and Rowlands, (1959) However, the increasing number of dams and irrigation canals built to boost energy and food production has also increased the number of potential snail habitats and with them the danger of liver fluke infestation. Hammond

(1973) and Graber (1976) reported that sheep are very susceptible to acute fascioliasis and that its periodic outbreaks cause high economic losses. Once infected with flukes, goats are also very susceptible to the disease. In cattle, acute fascioliasis often remains undetected and develops into chronic fascioliasis.

Daynes, (19690, Ogunrinade and Ogunrinade, (1980) Fascioliasis is a parasitic disease in ruminants that can cause major economic losses. In Africa, fascioliasis is a serious problem in the humid and subhumid zones.In the arid and semi-arid zones, where climatic conditions are less favourable for liver flukes, fascioliasis incidence has until recently been comparatively low.

Maqbool *et al.* (2002) The probable reasons of increased infection rate of Fascioliasis may include, (i) development of resistance due to improper use of *Fasciolicides* including frequent use of same drug for a longer time with inappropriate doses (Boray, 1990; Fair-weather and Boray, 1999) (ii) Lack of regular evaluation of local available drugs against any parasitic disease or no use of specific drug against any parasitic disease (Jabbar *et al.*, 2006). (iii) Socio-economic status of the farmers to treat the nuisance (Jabbar *et al.*, 2006). The probable reason for highest prevalence in winter might be the availability of optimal conditions of environment for the transmission, growth and development of parasitic life cycle stages (Rowcliffe and Ollerenshaw, 1960). This includes temperature ranging from 23-26oC for development of eggs (Rowcliffe and Ollerenshaw, 1960; Thomas, 1883) and maximal growth of snails (Kendall, 1953) and humidity level upto 90 % caused by plenty of water available facilitates embryonation (Andrew, 1999), emergence of miracidium from eggs due to increased activity of cilia (Thomas, 1883 a,b) and liberation of cercariae from snails (Alicata, 1938; Dixon, 1966). However, *Fasciolosis* was recorded throughout the year.

2.5 Pathogenesis

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

2.6 Pathology

Freeland, (1976) The disease fascioliasis is by most authors clinically described to occur in three phases, viz., acute, sub-acute and the chronic. The acute phase commences between 2-6 weeks after ingestion of substantial number of metacercariae and the species involved. In *F. hepatica* it may be 2000 metacercariae for sheep while for *F. gigantica* 300 metacercariae can produce similar condition. In large ruminants, buffalo and cattle, ingestion of 1000 metacercariae of *F. gigantica* produces acute disease clinically. The acute phase is characterized by severe haemorrhage caused by the migrating juvenile flukes in the hepatic tissue rupturing the blood vessels. The liver parenchyma, particularly the ventral lobe associated with gall bladder, is severely damaged assuming an uneven surface covered with blood clots. In late stage between 11-25 weeks of infection depending on the species of parasite and host involved, the animal squats in a specific posture on its right abdomen resting the ground and head turned to opposite direction. The animal disinclines to stand or move and feed. Between 3-7 days the condition worsens and the animal lays flat with extended body, blood froth may ooze out through mouth and nostrils before death. At necropsy, the liver is enlarged, haemorrhagic covered with fibrous clots and necrotic tunnels with migrating flukes. The ventral lobe is covered with fibrous exudates and from sub capsular haemorrhage the blood stained fluid pass into abdominal cavity with ascitis. In small ruminants multiple adhesions of adjacent organs with liver are present. Sheep may die suddenly without exhibiting any clinical manifestations, or following weakness, anorexia and pain.

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

2.7 Immunology

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

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

Mulcahy and Dalton, (2001) Other *Fasciola* isoenzymes viz., cysteine proteases (cathepsin L and B) are considered to play main role in tissue invasion and immune evasion.

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

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

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

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

2.8 Diagnosis

Dargie, (1987), Vercruysse and Claerebout, (2001) The diagnosis of Fasciolosis is based on the detection of eggs in feces or *F. hepatica* specific antibodies in serum. Recently, a method based on detection of *F. hepatica* specific copro-antigen has been developed and commercialized (Mezo *et al.*, 2004). The sensitivity (Se) and specificity (Sp) of these tests have been determined after experimental infection (Cornelissen *et al.*, 2001) or by using two distinct populations, a positive population selected from an enzootic area and a negative population from a fluke free area. (Ibarra *et al.*, 1998; Mezo *et al.*, 2004; Salimi- Bejestani *et al.*, 2005). The current diagnostic tests for Fasciolosisin cattle are qualitative only, yet the level of infection is considered an important factor in determining production losses.

Arriaga *et al.* (1989) The current trend in the diagnosis of more common bovine diseases, including Fascioliasis is to use the same samples of milk that are collected on farm for routine monitoring of animals productivity and quality of milk (Hill *et al.*, 2010; Mars *et al.*, 2010), reducing the associated costs and disturbance to animals as a result of handling sampling. The MM3-SERO ELISA is a sensitive and highly specific test for the sero-diagnosis of cattle Fasciolosis and can be reliable to use with milk samples. It is an excellent method of estimating withinherd prevalence of infection when used with bulk samples (Mezo *et al.*, 2009, 2010). The immune enzymatic techniques as indirect ELISA have been found very suitable for the diagnosis of Fasciolosisdue to their high sensitivity and the possibility of many sera samples.

Ibarra *et al.* (1998) these techniques based on detection of antibodies have been successfully utilized to detect early infection long persistence of high levels of immunoglobulin, even though animals have been successfully treated, makes interpretation more difficult.

Langley and Hillyer, (1989) in detected antigenemia as early as 2 weeks after infection in cows. It is concluded that it is very important and useful to combine two enzymatic assays, indirect and direct ELISA, to achieve a more reliable knowledge of the real infection status of the host. Results of iELISA using different antigens of *F. gegantica* for detecting antibodies against *Fasciola* in sera may be used in cattle. The diagnostic sensitivity, specificity and accuracy of the assay can be calculated according to Timmreck, 1994 and Smith, 1995**.** During migratory phase of infection, *F. hepatica*antigens are available to the immune system, and it is possible to detect them by serologic probes as sandwich-enzymelinked immunosorbent assay SEA.

Marin (1992) When the parasite is established in the bile ducts less antigen is there available to the immune system, and its detection must be directed to fecal or bile samples. It has been demonstrated that most of pathological damage takes place when flukes are migrating through peritoneal cavity and liver parenchyma before their establishment in the bile ducts. It is very important to use early diagnostic techniques to reduce the great losses in cattle. An indirect-enzyme immune-linked immunosorbent assay IEA allows an early diagnosis of *Fasciolosis*. *F. hepatica* antibodies can first be detected by indirect-ELISA between 3 and 6 weeks after infection during the liver migratory phase of immature worms.

2.9 Treatment

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

2.10 Prevention and Control

PanAmerican Health Organization, (2003) Hygienic feeding habits are the best preventive measures for keeping parasitic infections. So is true for fascioliasis. Besides promoting public health awareness through education in endemic areas are proving to be increasingly effective in prevention of fascioliasis diseases these days. So far fascioliasis is concerned, avoiding of watercress plants, water chestnuts and vegetation grown in aquatic environment, consuming raw has far reaching consequences in endemic areas.

Thorough washing of edible aquatic vegetation with running water can get rid of metacercariae to great extent and treating vegetation with 1% citric acid, 1% acetic acid (12% commercial vinegar), liquid soap (1.2%) or potassium permanganate solution (24 ppm) kills and detaches all metacercariae.

CHAPTER III

MATERIALS AND METHODS

3.1 Study Area

This study was design to focus on the Pathological status of facioliosis in bovine liver collected from slaughter house of Dinajpur city of Bangladesh, The experiment plan was prepared in the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Sciences, Hajee Mohammed Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh.

Figure 2: Map of the Area study

3.2 Duration of the study

This study was conducted during the period of January to June, 2020 in Pathology Laboratory Hajee Mohammad Danesh Science and Technology University.

3.3. Selection of slaughter house area

A total 75 samples were collected randomly from different slaughter houses of Dinajpur city of Bangladesh. The samples were collected from slaughter houses transported to laboratotry. The samples were subjected to be collection for their gross alteration on palpation, incision and recorded any changes which observed in the normal tissue texture of liver parenchyma and bile duct. Possible protective measures like protective clothing, sterile instruments and appliances were used for the collection and transportation of the sample in the laboratory for further processing.

3.4. Materials required for necropsy examination and sample collection

- Sample (bovine liver)
- Scissors
- Forceps
- Gloves
- Musk
- Scalpel
- 10% formalin.

3.5. Trimming of tissue is cutting a fixed tissue or organ to create a flat surface with correct orientation.

Figure 3: Trimming of tissue

3.6. Study Method

During necropsy the collected samples having gross lesions were fixed in 10% buffered neutral formalin for histopathological studies. Formalin fixed tissue samples were processed, embedded with paraffin, sectioned and stained with Haematoxylin and Eosin stain as per standard method (Luna, 1968).

3.6.1. Materials required for histopathology

Equipment and appliances:

- Samples (bovine liver)
- 10% formalin
- Chloroform
- Paraffin
- Alcohol
- Tape water
- Xylene

- Hematoxylin and Eosin stain
- Distilled water
- Clean slides
- Cover slips
- Mounting media (DPX).
- Microscope
- Microtome

3.6.2. 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 abnormal diseased tissues were collected side by side.
- The thickness of the tissues were as less as possible (5mm approximately).

2. Fixation: 10% formalin was added in the plastic container (10 folds of the tissue size and weight) for fixation of samples.

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 change.).

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 5-61am thickness. The sections were allowed to spread on luke warm water bath (40-42 °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.

3.6.3. Routine Hematoxylin and Eosin staining procedure

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

3.6.5. 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.

3.6.6. 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.

3.6.7. Staining protocol

The sectioned tissues were stained as described below:

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.3.11.

3.7. Processing of tissue

Fig. 4: Hydration of tissue

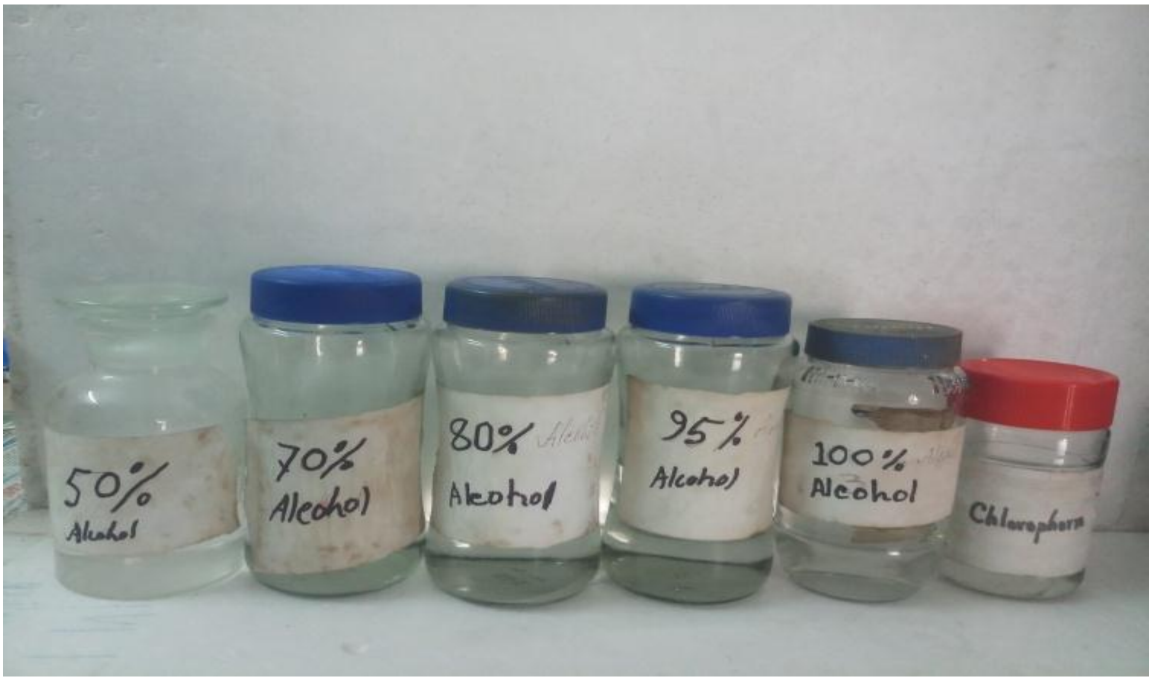


Fig. 5: Rehydration of tissue

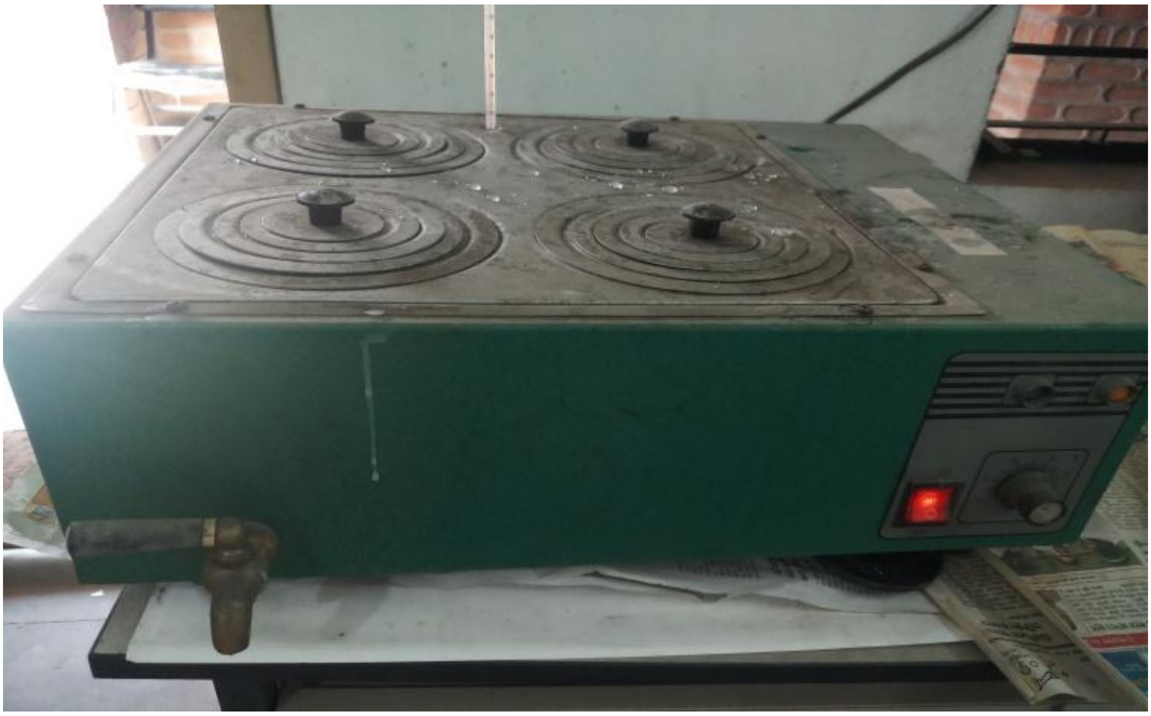


Fig. 6: Cocking of tissue

Fig. 7: Blocking of tissue

Fig. 8: Sectioning of tissue

Fig. 9: Distilled water and gelatin agar by using Placement of slides

3.8. Staining of tissue

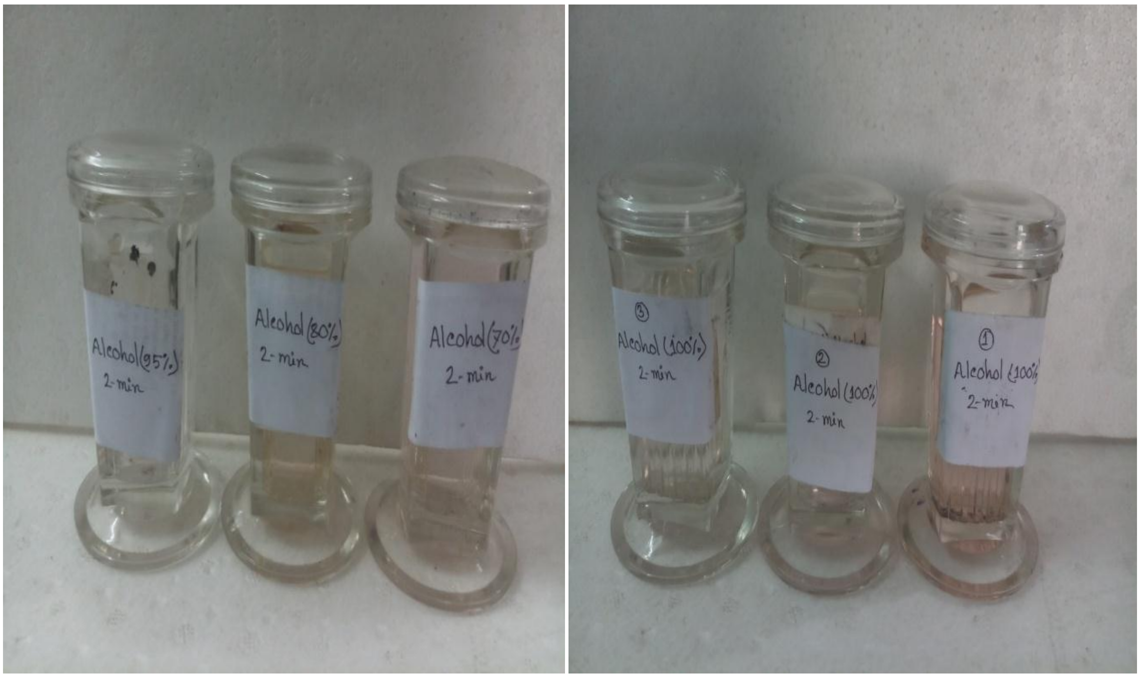


Fig. 10: Alcohol 2 minute each one

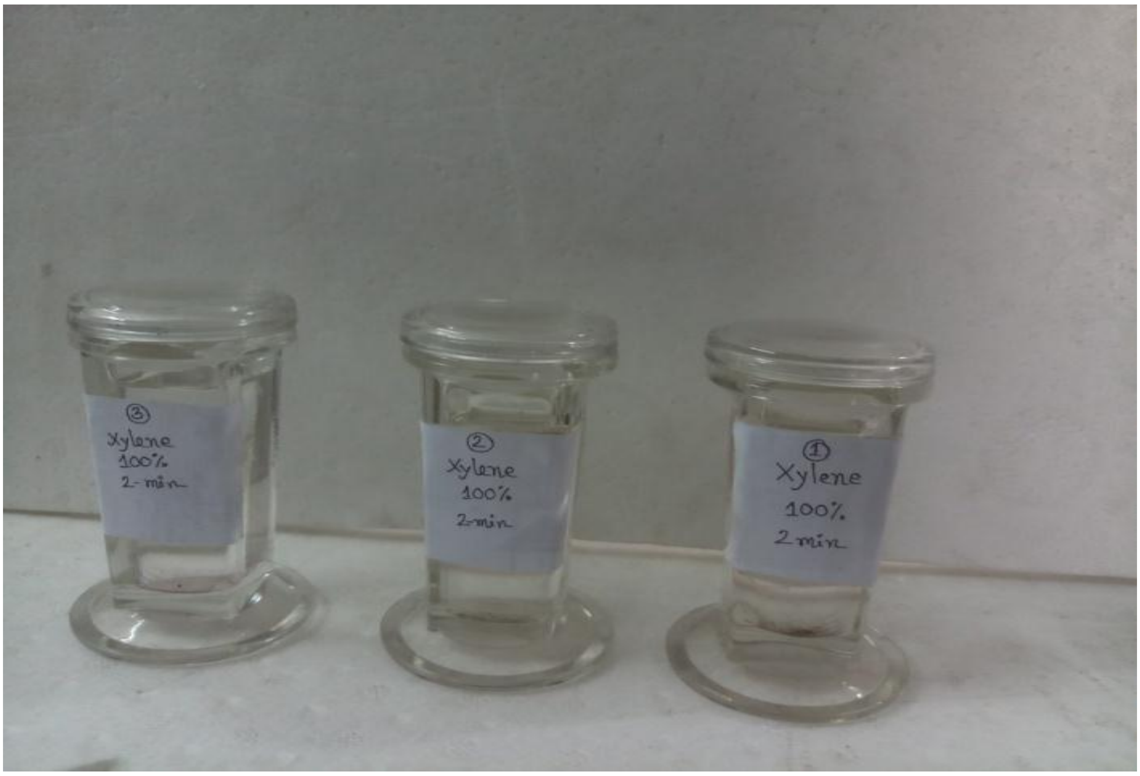


Fig. 11: Xylene 2 minute each one

Fig. 12: Distilled water 10 minutes

Hemotoxylene 10 minutes

Lithium carbonate few drop

Eosin treatment 30 or 60 second

Fig. 13: Xylene 2 minutes

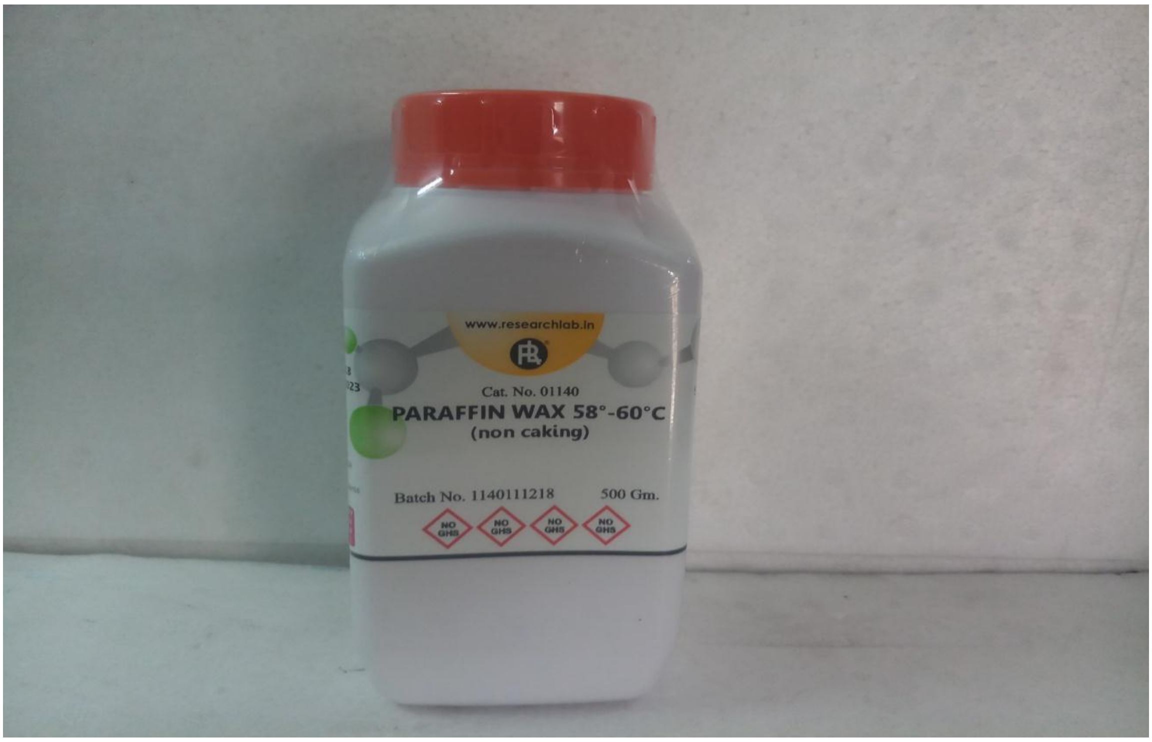
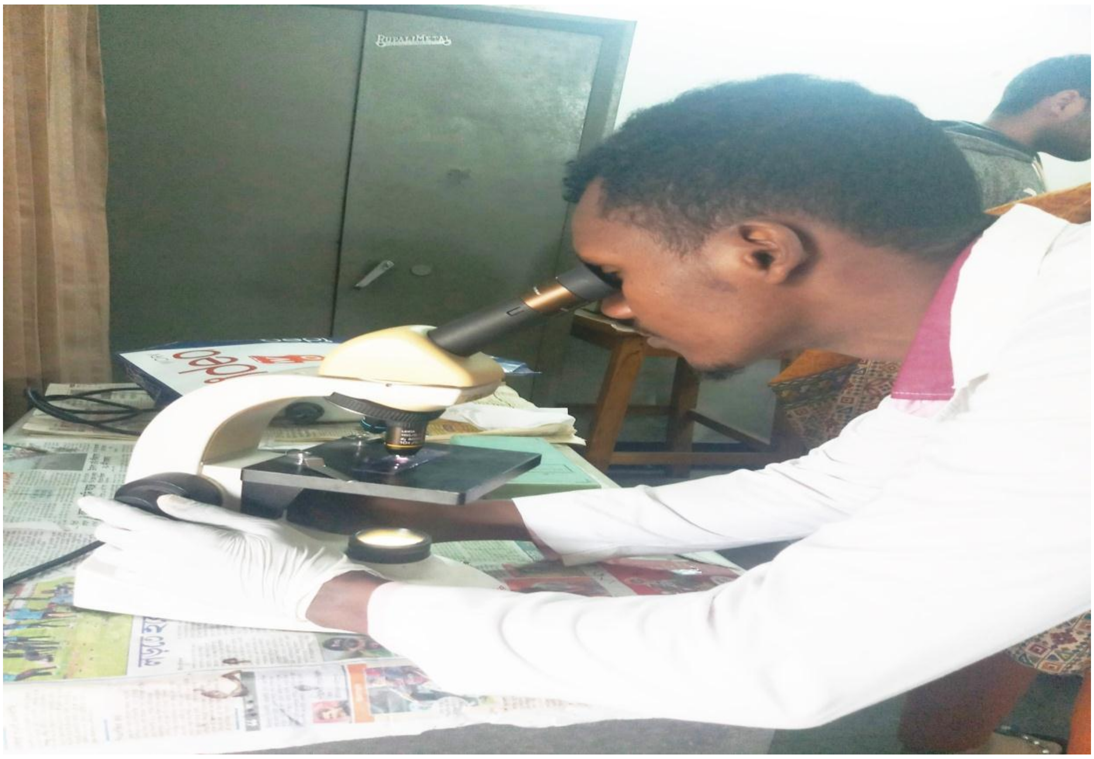


Fig. 14: Paraffin wax



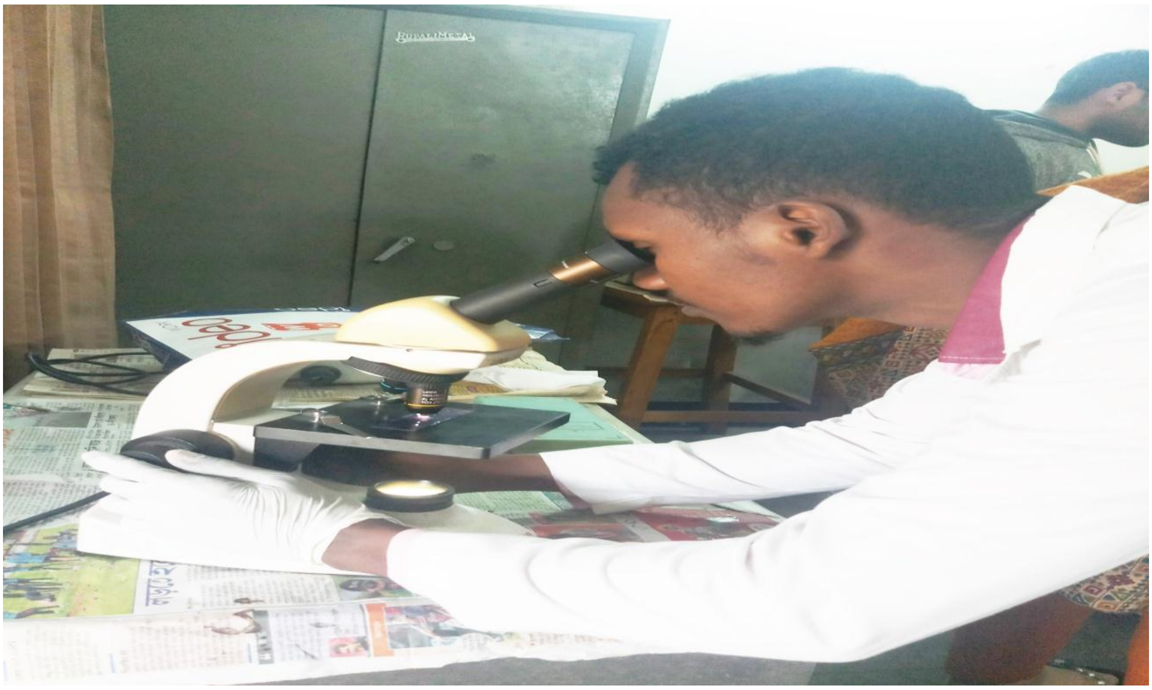


Fig. 15: Observation of slides

3.9.Experimental layout

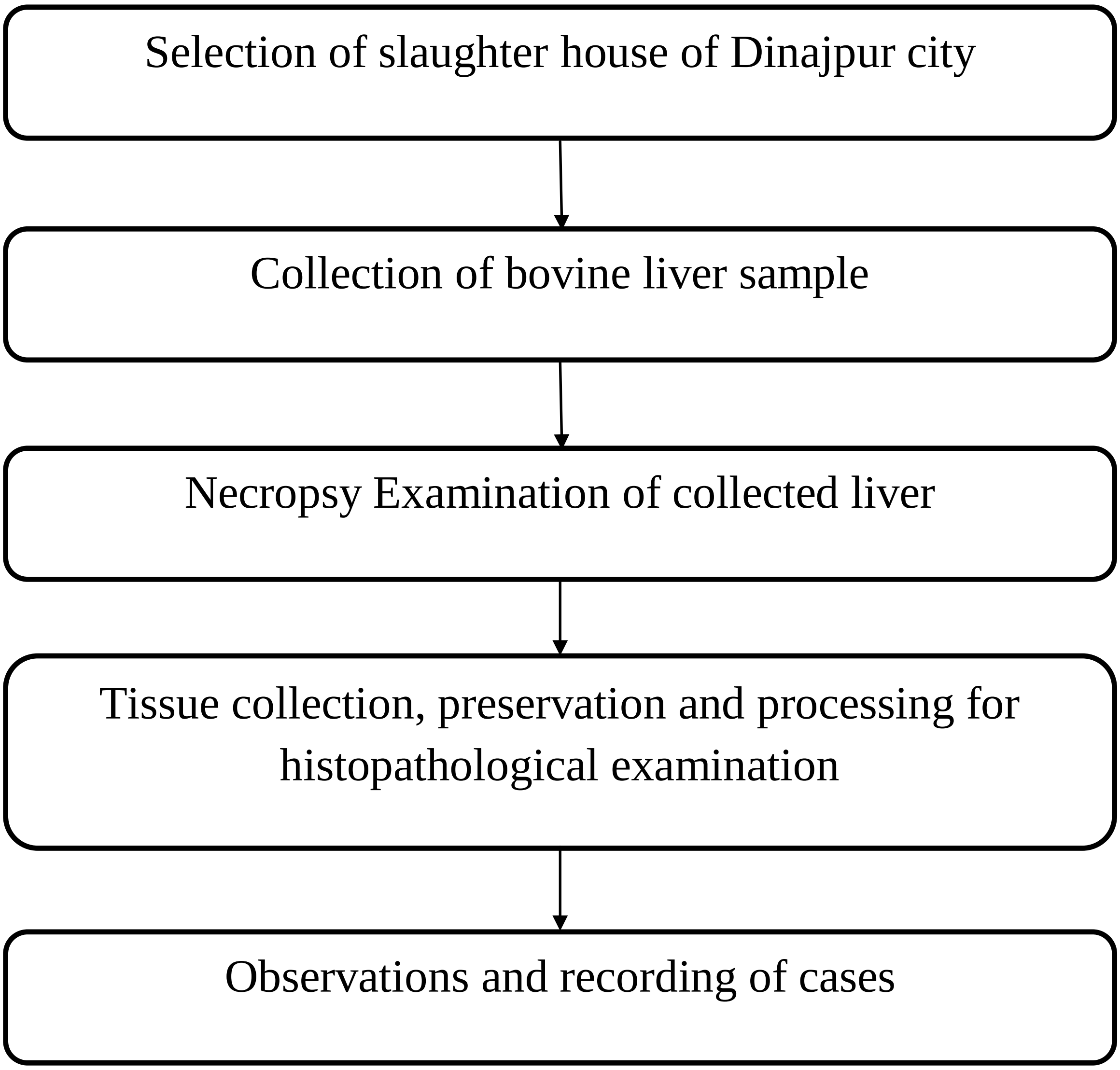


Figure 16: Experimental layout

CHAPTER IV

RESULTS

4.1 Overall prevalence of fascioliasis

Out of 75 samples, 8 samples were positive. The result of the present study revealed that the overall percentage of fascioliasis in slaughter houses samples was 17.3%.

4.2. Gross lesions

Gross lesions of fasciolosis found in the liver were presented in Table 1 and Figure 17. About 14.6% liver were increased in the size (hepatomegaly) due to inflammation of parenchymal layer with pinpoint hemorrhages on the parietal surface. There was 16% sample with paleness in some areas which was due to the necrosis. Fibrosis was indicated by congestion and firm whitish areas within parenchyma which was about 10.6% and was the indication of chronic Fascioliasis. In 17.3% sample there was roughened and thick capsule with whitish or reddish discoloration throughout the capsule

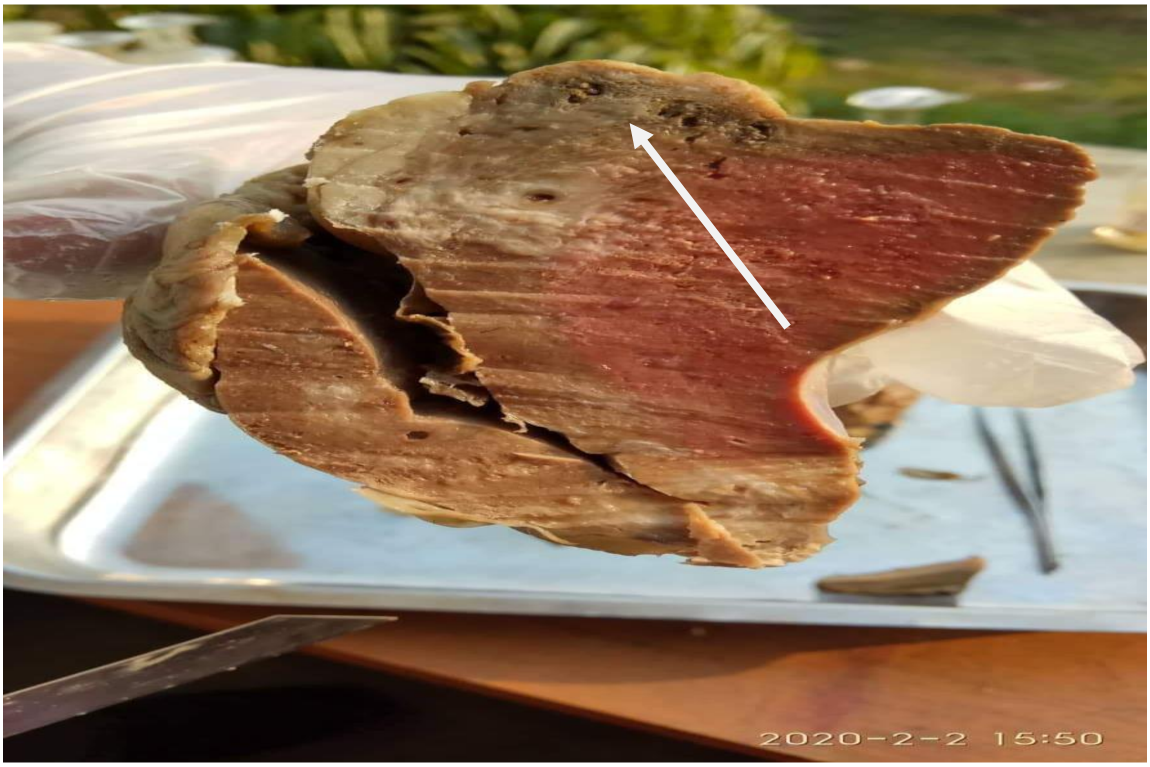


Fig. 17: Gross lesions of Fascioliasis infected liver

Grossly there was engorgement of bile the duct with fibrosis in about 10.6% of liver sample. Several twisted flukes including both mature and immature were clearly visible and blocked within the swollen and the fibrotic bile ducts. The infected bile duct were also filled with blackish brown exudates in about 13.3% sample and giving the pipe stem appearance of the liver were noticed.

Table 1: The gross lesions of *Fasciola* in male and female of bovine liver

Gross lesions found in liver	No. of infected liver	Percentage
Fibrosis	8	10.6%
Flukes inside bile duct	12	16%
blackish brown exudates	10	13.3%
Roughened and thick Parenchyma	9	12%
Necrosis	12	16%
Discoloration	13	17.3%
Increase size of liver	11	14.6%

4.3. Histopathological Lesions

Microscopically, depending on the duration and intensity of the infection, various changes were observed in the structures of *Fasciola* infected cattle liver. In histopathological study of acute Fascioliasis, there was lot of hepatocytic alterations like swelling of individual hepatocytes by increase in size and characterized by opaque cytoplasm (Fig. 18) and sometime there was fatty change in which clear vacuoles appeared in the cytoplasm with peripherally located nuclei (Fig. 19). Several abscesses including necrotic debris surrounded by a large numbers of inflammatory cells mainly neutrophils, histiocytes, eosinophils and lymphocytes and bounded by fibrous connective tissue capsule (Fig. 20). Congestion was commonly found was due to dilation of central vein and sinusoids, engorged with a large number of RBCs (Fig.21). Migratory tract with lymphocytic infiltration was observed in few sections and some slides showed the cross section of mature liver fluke with heavy infiltration of inflammatory cells (Fig.22).

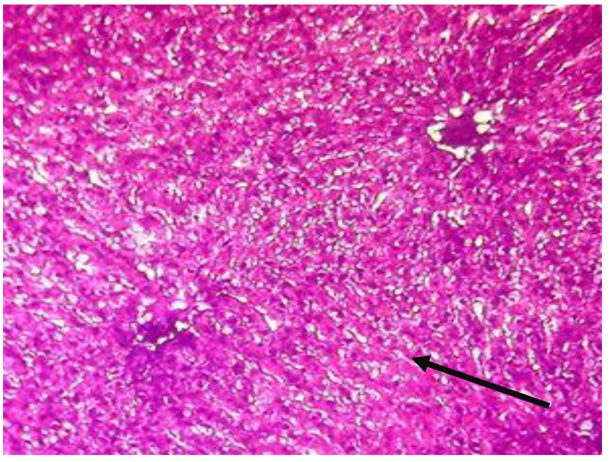


Fig. 18: Swelling of individual hepatocytes by increase in size and characterized by opaque cytoplasm (Black arrow)

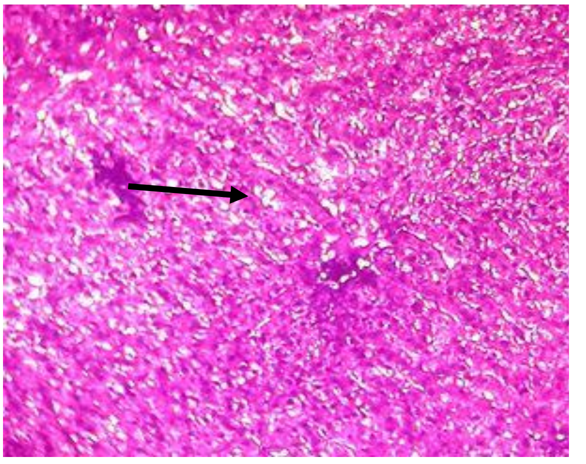


Fig. 19: Fatty change with clear vacuoles appeared in the cytoplasm with peripherally located nuclei (Black arrow)

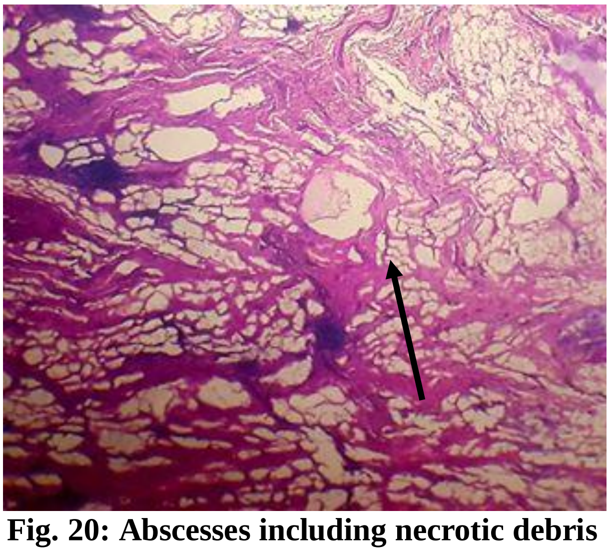


Fig. 20: Abscesses including necrotic debris surrounded by a large numbers of inflammatory cells (Black arrow)

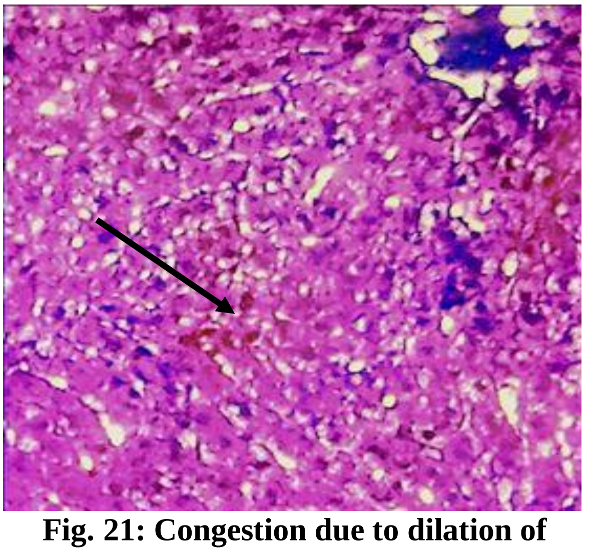


Fig. 21: Congestion due to dilation of central vein and sinusoids and engorged with a large number of RBCs (Black arrow)

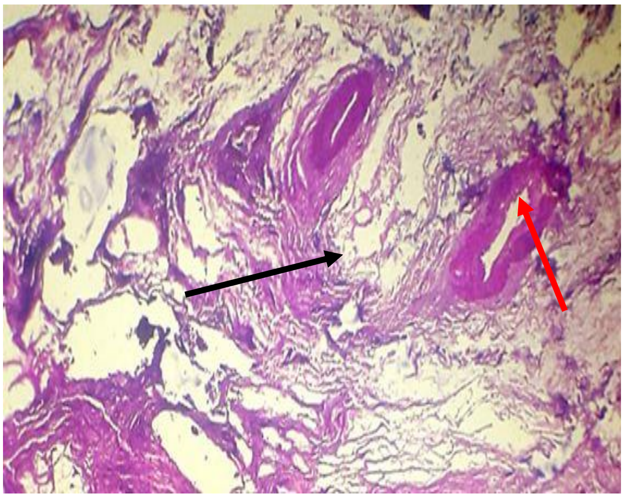


Fig. 22: Migratory tract with lymphocytic infiltration (Black arrow) and cross section of mature liver fluke with heavy infiltration of inflammatory cells (Red arrow)

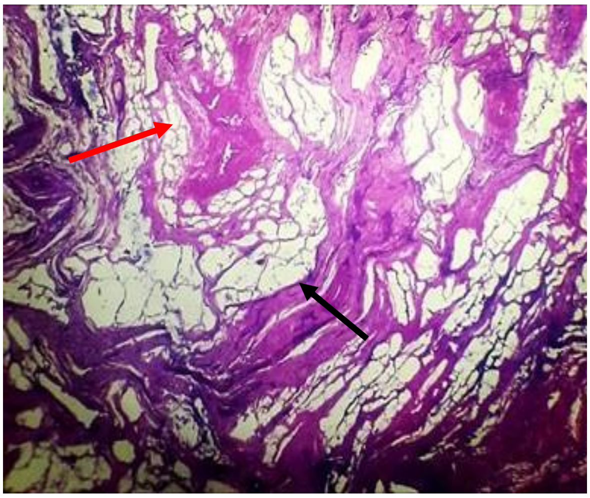


Fig. 23: Fibroblastic proliferation in the previous migratory tract (Black arrow)and biliary cirrhosis with extensive proliferation of fibrous connective tissue around the intra-hepatic bile ductules (Red arrow)

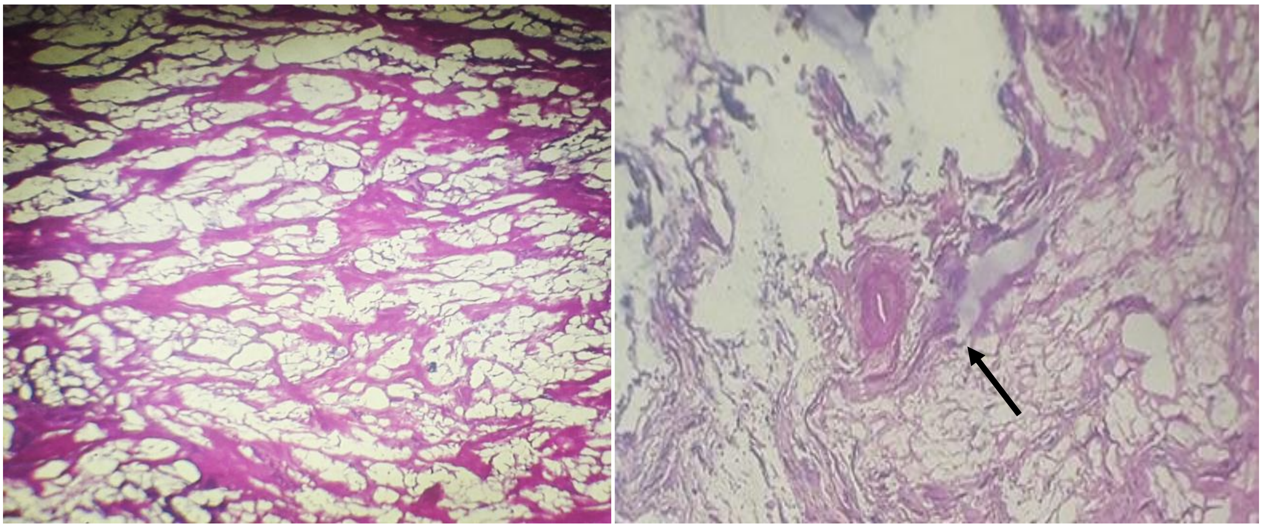


Fig. 24: Liver cirrhosis, indicated by different dark and light spots and proliferated fibrous connective tissue around the regenerative hepatic lobules (Black arrow) was infiltrated with inflammatory cells

The histopathological lesions of chronic Fascioliasis were characterized by infiltration of fibroblasts admixed with lymphocytes and few mononuclear cells in the area previously migrated by young flukes. Huge proliferations of fibrous connective tissue associated with infiltration of lymphocytes and plasma cells in the portal area were commonly seen and microscopically bile duct lesions revealed primary biliary cirrhosis with extensive proliferation of fibrous connective tissue around the intra-hepatic bile ductules with infiltration of mononuclear inflammatory cells of the bile ducts (Fig.23).

Liver cirrhosis was indicated by different dark and light spots and proliferated fibrous connective tissue around the regenerative hepatic lobules was infiltrated with inflammatory cells (Fig. 24).

CHAPTER V

DISCUSSION

5.1. The gross pathological changes observed on the liver

The liver was enlargement and pin-point hemorrhages on the parietal surface of the liver which is partly due to the inflammatory changes and fibrosis that took place in the liver parenchyma. The affected bile ducts were markedly thickened and there was fibrosis in the duct wall. These findings are congruent with the earlier findings of Velusamy *et al*. (2002) proved that thickening of bile duct is due to chronic nature of *Fasciola* infection and later, this finding is in agreement with previous studies of Okaiyeto *et al.* (2008). While the same results were achieved most of the above lesions in chronic Fasciolosis in Black Bengal goats Talukder (2010).

5.2. The histopathological features of liver

Histopathologically, the chronic hepatic cells showed variable degrees of cell swelling, degeneration and deposition of bile pigment. Similar results were also recorded by Mac Gavin *et al.* (2001). Correspondence with study of Talukder *et al.* (2010) reported atrophy, necrosis and fatty changes in chronic Fascioliasis in goat. In our study liver abscess cases is in agreement with study of Sayed *et al.* (2008) proved that invasion of the liver by migrating immature liver fluke damages the tissue and provide anaerobic condition, that allowed the germination and proliferation of bacteria that induce hepatocellular necrosis and abscess formation. In present study liver paranchyma showed infiltration of poly morphonuclear inflammatory cells; neutrophil and eosinophil with mononuclear inflammatory cells which were observed in the proliferated fibrous tissue among hyperplastic newly formed bile ductules. Our results are in agreement with two previous studies of Doy et. al. and Wiedosari *et. al* (1984 and 1991) proved that the migration of immature liver flukes through the tissue causing hemorrhage and irritation, and brought the cellular inflammatory reactions and this study is partially correlated with the finding reported by Dow *et al.* (1967) mentioned the infiltrations of inflammatory cells in experimentally produced Fascioliasis in calves. Reported liver cirrhosis and biliary cirrhosis in our study are in agreement with previous studies who described cirrhosis as portal, multilobular and biliary according to the fibrous connective tissue distribution Swarup *et al.* (2001) and Eguale *et al.* (1987 and 2003). In the present observation, glandular hyperplasia of bile duct walls, which produced a thick and adenomatous picture was marked. It was mostly seen in the main ducts containing many adult flukes. Several studies detected that the presence of mature flukes within the lumen of intra-hepatic bile ducts which brought a continuous irritation and led to hyperplastic proliferations which were emphasized by papillomatous projections and formation of newly formed bile ductules. This damage was caused by the spiny surface of the flukes and their feeding habits on the hyperplastic bile ducts Modavi *et al.* and Shaikh *et al.* (1984 and 2004).

CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

In conclusion fascioliasis in cattle is one of the major constrains for cattle development in Bangladesh. The hot and humid climates in fact make this country a paradise for parasitic animals. Epidemiological investigation of fascioliasis is considered as tools for controlling bovine parasitic infection. Although the present study results have some limitation because low sample size, large study area and low duration of the study may lead improper diagnosis. The present study revealed that the histopathological study of acute Fascioliasis, there was lot of hepatocytic alterations like swelling of individual hepatocytes by increase in size and characterized by opaque cytoplasm and sometime there was fatty change in which clear vacuoles appeared in the cytoplasm with peripherally located nuclei. All the findings could be helpful for proper diagnosis as well as could be the key point of further research work.Finally some recommendations were suggested as like as,

- To carry out further studies on isolation, morphology and characterization of *Fasciola* sp.
- To develop good management practices like hygienic condition, vaccination program to minimize the risk of the disease.

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