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Original Article

Anatomic pathology of cattle and goat liver collected from slaughterhouses in Mymensingh city of Bangladesh

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A B S T R A C T

The morphologic abnormality of cattle and goat livers (collected from slaughterhouses in Mymensingh city of Bangladesh) were investigated in the present study. The livers of slaughtered cattle (n = 14) and goat (n = 20) were examined by gross visualization, palpation and incision as well as histopathology. The total liver lesion in cattle was 71.4% and in goat was 75%. Grossly, various pathological changes, mainly thickened, rough capsule which was whitish in color, necrotic foci, thickened bile ducts with matured flukes, fibrosis were seen. Histopathologically, necrosis, degenerative changes of hepatocytes, dilated sinusoids, inflammatory infiltrates, granuloma, bile duct hyperplasia, periportal and periductal fibrosis were found in cattle and goat livers. Extensive fibrosis was seen portal areas of liver by Goldner's trichrome staining. Migratory tracts and immature flukes were seen in intrahepatic bile ducts. Most common cause of liver lesions found in the present study was Fascioliasis in both cattle and goat. It could be concluded that the major gross and microscopic alterations in the livers of cattle and goat due to *Fasciola gigantica* infection reflected tissue damage, which can lead to substantial financial losses in animals and great health problems in human. Therefore, special care and attention are required to ensure that seriously damaged livers of food animals are not passed on for human consumption, and extensive monitoring and epidemiological surveys are necessary to cope this adverse situation.

Introduction

Animal protein mainly comes from the livestock, which is very essential for life. The main sources of animal protein are cattle and goats in Bangladesh. There are about 25.7 million cattle and 14.8 million goats in Bangladesh (Banglapedia, 2021) which play an important role in economy and fulfill the requirements of animal protein for human consumption. In Bangladesh, the livestock sector serves around 20% of the population directly and 50% indirectly (Bhowmik *et al*., 2020). Food animals are beneficial because they provide high-quality protein to humans, but they also function as disease transmission vectors. Obtaining safe animal food is very challenging. Cattle and goat diseases cause economic losses and may pose an epidemiologic and zoonotic concern (Mellau *et al*., 2010). Parasitic infection is the most serious impediments to livestock production in Bangladesh (Kabir *et al*., 2010).

Liver is a nutritious and delicious food due to enrichment with iron, copper, the B vitamins and preformed vitamin A. Livers of cattle and goat are widely available from butchers and supermarkets and commonly used as human food in Bangladesh. On the other hand, the liver is the largest gland in the body that have a variety of important biological functions, including detoxification and the creation of proteins and biochemicals required for digestion and growth (Abdel-Misih & Bloomston, 2010). The liver is susceptible to a variety of diseases due to its strategic position and multifaceted functions. Liver is damaged by parasites and diseases, rendering them unfit for human consumption and a huge amount of liver is condemned from abattoirs every year causing loss of animal protein and economic loss. Besides,

diseased liver has an effect on animal's total output by deteriorating their health condition. Many infectious and non-infectious factors can produce pathological alterations in the liver. The most common diseases or disease conditions of liver found in slaughtered food animals are fascioliasis, hepatitis, tuberculosis, hydatidosis, cysticercosis, abcesses, necrosis and cirrhosis in Brazil (Mendes *et al*., 2007), Tanzania (Mellau *et al*., 2010), Sudan (Mohamed, 2021). But the presence of such liver diseases or disorders of food animals was poorly studied in Bangladesh. The affected parts of liver are trimmed, or the entire liver is condemned in slaughterhouses after inspection (Kara *et al*., 2009; Cadmus and Adesokan, 2009). Thus important source of protein is getting wasted every year from slaughterhouses.

Condemnation of liver because of many disorders in slaughterhouses or meat inspection practices needs to find out. Studies of slaughter house liver in several geographic regions showed that the reasons for liver condemnation differ depending on the regional ecology. Chemical poisoning now a day is a common condition that causes hepatic degeneration in the liver, death of hepatocytes and proliferation of fibrous tissue. Unrestricted uses of pesticides in Bangladesh agriculture (Kobir *et al*., 2020) and due to industrialization, many pastures have already been contaminated with industrial effluents but their effect on liver is also not clear. Gross alterations due to such an unexpected poisoning need to address. Therefore, this study demonstrated the gross and histomorphological changes of slaughterhouse liver of food animals (cattle and goat) and that will be helpful to determine the reasons for condemnation of liver from slaughterhouses.

Materials and methods

Ethical approval

The ethical standards committee of the Bangladesh Agricultural University Research System (BAURES) approved the research work with the reference number BAURES//ESRC/VET/14 dated 11.12.2020.

Sample collection and gross observation

From various slaughterhouses in Mymensingh City Corporation, Bangladesh, a total of 14 cattle (male) and 20 goats (both male and female) livers of adult age were collected during the study period from January, 2021 to August, 2021. The detailed history of the slaughtered cattle and goat were not possible to collect as there was lack of proper ante-mortem examination. However, postmortem examination was carried out in details at slaughter, and the gross alterations were noticed and recorded carefully.

The samples of liver were examined grossly for the identification of any pathological changes immediately after slaughtering both externally and internally by visualization, palpation and incision of liver. The samples of liver were incised at the ventral sides, cutting bile ducts open to check thoroughly for the existence of the parasite. After gross examination, the samples were packed into small containers of 10% formalin. Then the sample containers were carried carefully to the Anatomy and Histology Department, Bangladesh Agricultural University, Mymensingh, Bangladesh and proceed for microscopic examination.

Hematoxylin and eosin (H & E) staining

The liver samples (lesion area with normal tissue) were fixed into 10% neutral buffered formalin (NBF) immediately after collection by standard method. Then, dehydration of NBF fixed liver tissues was carried out with ascending graded alcohols and after embedding in paraffin sectioning was done using a sliding microtome at 6 µm thickness. The deparaffinized sections were stained with hematoxylin and eosin (H and E). The histopathological alterations were studied using a light microscope (LABOMED, Labo America Inc., CA 94538). Morphological abnormalities, taken into account: necrosis, hepatic degeneration and atrophy, inflammatory variations in portal area, granuloma, varying levels of fibrotic lesions (periportal fibrosis, periductal fibrosis and diffuse fibrosis) and proliferation of bile ducts.

Goldner's trichrome staining of liver tissue sections

Goldner's trichrome staining was performed to visualize connective tissues as blue color, particularly collagen, in tissue sections. The basic composition of the Masson-Goldner trichrome is 3 dyes: Weigert's hematoxylin for nuclei, a mixture of acid dyes (Fuschine Biebrich acid scarlet) for cytoplasm and brilliant green for collagen. Procedure in brief, tissue sectiones were deparaffinized and rehydrated. The sectioned tissues were then stained for 3 minutes in Weigert's iron hematoxylin and rinsed in running tap water for 15 minutes. Tissue sections were dipped in ponceau acid fuchsin for 5 minutes and were quickly rinsed 3 times in 1% acetic acid solution. They were then dipped into phosphomolibdic acid orange-G for 15 minutes and rinsed in 1% acetic acid and then dipped in light green stain for 5 minutes. They were carried out 3 quick rinses in 1% acetic acid solution and dipped in 96% ethanol for 1 minute and two changes in 100% ethanol (2 minutes each). The slides were dipped 2 times in xylene, 5 minutes in each and were mounted with cover slip using DPX. The connective tissue collagen fibers appeared greenish blue under light microscope.

Image processing and Statistical analysis

Photomicrographs at high and low-power magnification were taken with a digital camera (DS-Fi1, Nikon). Adjustment of photographs for contrast, brightness and sharpness, layout and lettering were performed in Adobe Photoshop 7.0J and Adobe Illustrator 10.0J.

Results

Gross and histomorphology of normal liver of cattle and goat

Unaffected liver was reddish-brown in color with four lobes, namely left lobe, right lobe, caudate lobe and quadrate lobe. The lobation of liver was more distinct in goat than in cattle (Figure 1.A-B). A typical serosa (visceral peritoneum) covered the liver, which was overlain by a thin connective-tissue capsule. The consistency of liver was firm-elastic. The unaffected cattle and goat livers showed the typical histo-architecture of liver (Figure 1.C-D). In brief, connective tissue from the capsule extended into the liver lobes as interlobular connective tissue to surround individual hepatic lobules and supported the vascular and bile duct systems. The expanded areas of interlobular connective tissue contained a lymph vessel, branches of the hepatic artery and portal vein, and a bile duct which together with supportive connective tissues formed the portal canals or portal areas (Figure 1.C-D). Liver parenchyma consisted of hepatocytes which were arranged in rows in the hepatic laminae which radially placed around the central vein (Figure 1.C-D).

Hepatocytes contained a centrally located spherical nucleus with one or more prominent nucleoli and cytoplasm.

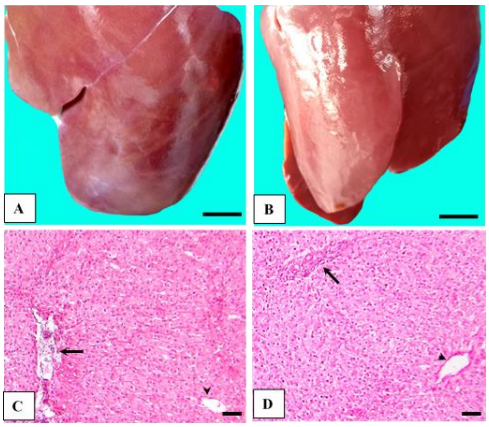

Figure 1. Gross (A-B) and histo-architecture (C-D) of normal liver of cattle and goat. A-B: Normal reddish-brown colored liver of cattle (A) and goat (B) showing the parietal surface. The lobation of liver was more distinct in goat than in cattle. C-D: Histology shows regular arrangement of liver tissues around central vein (arrow head) and portal area (arrow). C-D: H & E stain. Bars: A-B = 5 cm and C-D = 100 µm.

Gross pathological changes in liver of cattle and goat

Cattle and goat livers were obtained randomly to study the anatomic pathology of liver. Grossly, in affected/diseased livers of cattle and goat, various pathological changes, like thickened capsule, necrotic foci, proliferation of bile ducts, thickened bile ducts, the presence of adult flukes, hemorrhagic tracts and fibrosis were found by visualization, palpation, and incision (Table 1).

Table 1. Gross pathological observations of affected liver of cattle and goat.

Species	Total no. of liver	Presence of lesions (%)	Absence of lesions	Major lesions
Cattle	14	10 (71.4%)	04	1. Enlarged liver 2. Proliferation of bile ducts 3. Necrosis 4.Thickened bile duct (pipe-stem liver) 5. Presence of adult fluke 6. Hemorrhagic tracts 7. Fibrosis 8. Calcification within biliary ducts 9. Hard consistency 10. Thickened capsule
Goat	20	15 (75%)	05	1. Necrosis 2. Fibrosis 3. Thickening of bile duct 4. Presence of adult flukes in bile ducts 5. Hemorrhagic tracts 6. Multifocal nodules

Cattle liver showed a variety of pathological changes. Necrotic lesions were found on livers which were edematous, vast patches of necrosis showing as pale, yellowish, punctate lesions and capsule was wrinkling (Figure 2.A). Hyperplasia of bile ducts and hemorrhagic tracts were seen at cut surface of liver (Figure 2.B). Liver flukes (*F. gigantica*) came out following sectioning of bile ducts (Figure 2.C). The hemorrhagic or migratory tracts and necrosis were produced by *F. gigantica* which was found on bile ducts sectioning. The migratory tracts varied in size (small to large) and filled with blackish brown exudates were distributed throughout the liver (Figure 2.D). Biliary ducts that have thickened excessively (Figure 2.E) because of formation of fibrous tissue around it resemble the pipe-stem liver found in 3 livers of cattle (42.9%). In addition to Fascioliasis, other findings were related to other diseases or disease conditions of liver, like hepatitis, perihepatitis and cirrhosis. Calcification of the bile ducts was found in 2 livers of cattle (28.6%) assured by the gritty sound felt during bile duct sectioning.

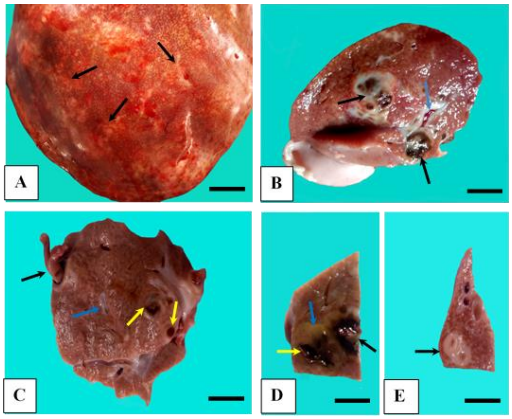

Figure 2. Gross pathology of the affected liver of cattle. A: Necrotic lesion (black arrows) was found on livers which were edematous, vast patches of necrosis showing as pale, yellowish, punctate lesions and capsule was wrinkling. B: Bile ducts hyperplasia (black arrows) and hemorrhagic tracts (blue arrow) were seen at cut surface of liver. C: Hyperplasia of bile ducts (yellow arrows) and migratory tracts (blue arrow) were seen and flukes (black arrow) came out following sectioning of bile ducts. D: The migratory tracts packed with exudates that are blackish brown in color (yellow arrow) produced by *F. gigantica* (black arrow) and necrosis around it were found in the liver. E: Liver with significantly thickened bile ducts was seen on the cut surface. Bars: A-E = 5 cm.

In goat liver, the affected area of liver became red-brown to pale/grayish in color, hard in consistency and enlarged. Necrotic and fibrotic lesions were observed on the parietal surface of liver (Figure 5.A). After sectioning, the inner liver showed necrosis, fibrosis, hemorrhagic tracts, thickened bile ducts and presence of matured flukes in bile ducts (Figure 5.B,C) and multifocal nodules (Figure 5.D). Hemorrhagic tracts were found in 09 (45%) infected goats which were associated with Fascioliasis and distributed throughout the liver with small to larger in size.

Finally, 10 (71.4%) of the 14 cattle livers were revealed to have various lesions, whereas 15 (75%) of the 20 goat livers were affected.

Histopathological changes in liver of cattle and goat

Histopathology of affected/diseased cattle and goat livers showed a variety of pathological changes (Table 2). Particularly, in cattle, bile duct hyperplasia and focal necrosis were seen (Figure 3.A) and the lobular architecture was lost. Degenerated hepatocytes infiltrated with

inflammatory cells and migratory tracts composed of eosinophilic debris were observed (Figure 3.C). Section of immature *F. gigantica* was discovered in intrahepatic biliary duct (Figure 3.D). The dilatation of the biliary ducts was associated with necrotic alterations in the columnar cells surrounding the biliary ducts with huge proliferations (42.85%) were found (Figure 3.A,C).

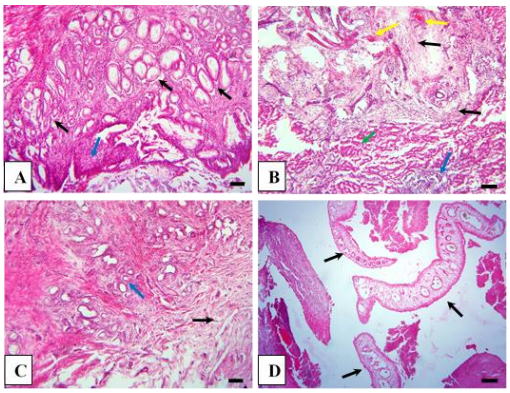

Figure 3. Histopathology of the affected liver of cattle using routine (H&E) stain. A: Hyperplasia of bile ducts (black arrows) and focal necrosis with more darkly stained hepatocytes (blue arrows) were seen. B: Liver fibrosis (black arrows) with replacement of hepatic parenchyma, granuloma (blue arrow), congestion (yellow arrow) and necrotic hepatocytes (green arrow) were found. C: Migratory tracts (black arrows) composed of eosinophilic debris and degenerating hepatocytes infiltrated with inflammatory cells and hyperplasia of bile ducts (blue arrows) were seen. D: Immature *F. gigantica* (black arrows) was observed in an intrahepatic biliary duct. Bars: A-C = 100 µm, D = 50 µm.

In the hepatic parenchyma, there were focal infiltrations of mononuclear cells as well as in the portal areas along with severe necrosis and widespread portal cirrhosis (Figure 3.B). Hydropic changes characterized by swelling of hepatocytes and vacuolization of the cytoplasms of hepatocytes were found (Figure 4.A). Periductal fibrosis along with periportal fibrosis was seen (Figure 4.B). Blue color fibrous connective tissue proliferation around bile ducts and portal areas were found in Goldner's trichrome stained sections (Figure 4.A,C,D).

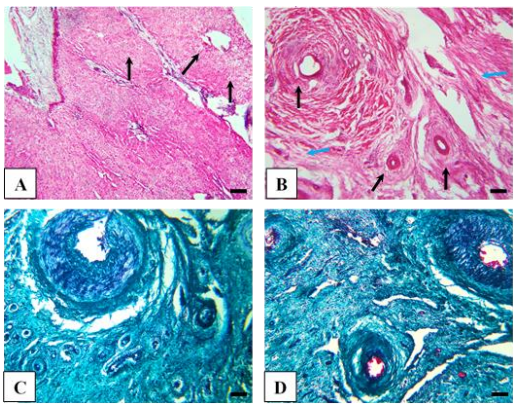

Figure 4. Histopathology of the affected liver of cattle using routine (H&E; A-B) and special Goldner's trichrome stain (C-D). A: Hydropic changes of hepatocytes characterized by swelling of hepatocytes and vacuolization of the hepatocyte cytoplasms. B: Periductal fibrosis (black arrows) and periportal fibrosis (blue arrows) were found. C &D: Blue color fibrous connective tissue proliferation around bile ducts and portal areas was seen. Bars: A-D = 100 µm.

Table 2. Histopathological findings of affected cattle and goat livers.

Lesions	Cattle (%)	Goat (%)
Hyperplasia of bile ducts	42.85%	35%
Necrosis	42.85%	45%
Periportal fibrosis	57.14%	45%
Periductal fibrosis	42.85%	35%
Inflammatory infiltrates	71.4%	65%
Hepato-lobular atrophy	28.57%	20%
Granuloma	14.28%	15%
Hemorrhage & Congestion	42.85%	30%
Presence of flukes	57.14%	45%
Degenerative changes of hepatocytes	71.4%	65%
Dilated sinusoids	28.57%	25%
Hydropic changes	14.28%	25%

Lesions of Fascioliasis were found in 7 goat livers (45%) out of 20 liver samples, the lesion of which was similar to cattle liver affected with Fascioliasis. Immature flukes were present in the intrahepatic biliary ducts (Figure 5.E) and the hemorrhagic tracts were produced by migration of immature flukes which were then infiltrated with inflammatory cells and in the bile ducts as well as in the hepatic portal triads mild fibrosis was also found (Figure 5.F,G). In chronic Fascioliasis, cirrhosis in liver was produced by severe fibrosis with replacement of hepatic parenchyma and nodularity of parenchyma was showing atrophy of hepatocytes and dilated sinusoids (Figure 5.H).

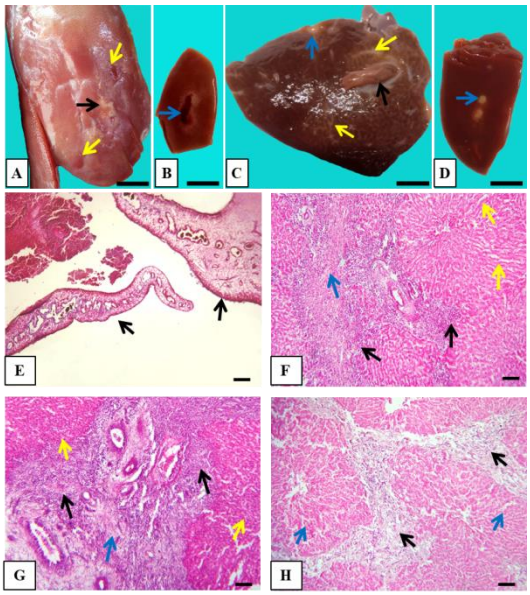

Figure 5. Gross (A-D) and histopathological (E-H) examination of affected liver of goat. A: Necrotic (yellow arrows) and fibrotic lesions (black arrow) were found on parietal surface of liver. B: Hemorrhagic tract (blue arrow) was seen on the surface of liver. C: Adult fluke (black arrow) came out following sectioning of bile ducts as well as presence of fibrotic (yellow arrows) and necrotic lesions (blue arrow). D: Multifocal nodule (blue arrow) was seen. E: Immature *F. gigantica* (black arrows) were discovered in the intrahepatic biliary ducts. F: Multifocal portal granuloma (black arrows) and

migratory tracts (blue arrow) were seen with dilated sinusoids (yellow arrows). G: Granulomatous inflammation (black arrows) in portal areas, along with migratory tracts (blue arrow) and necrosis of hepatocytes (yellow arrows) were seen. H: Liver fibrosis (black arrows) with replacement of hepatic parenchyma was seen. Nodularity of parenchyma was showing atrophy and dilated sinusoids (blue arrows). Bars: A-D = 5 cm, E-H = 100 µm. E-H: H & E stain.

Periportal fibrosis in goat liver was 45% and periductal fibrosis was 35%. Congestion along with proliferation of fibrous tissues and bile ducts were also found (Figure 6.A,B). Granuloma (15%) and bile ducts hyperplasia (35%) were also seen in goat liver. The 45% goat liver was found with necrotic lesions in which the hepatocytes became damaged and dead and produced an inflammatory response. Necrotic mass (focal necrosis) and inflammatory infiltrates were found in goat liver (Figure 6.C). Centrilobular necrosis of hepatocytes with inflammatory cell infiltration and congestion were seen (Figure 6.D). Extensive fibrosis in liver tissues especially in portal areas was revealed by Goldner's trichrome staining, a staining that is used to visualize connective tissues as blue color, particularly collagen (Figure 4.C-D & Figure 6.E-F).

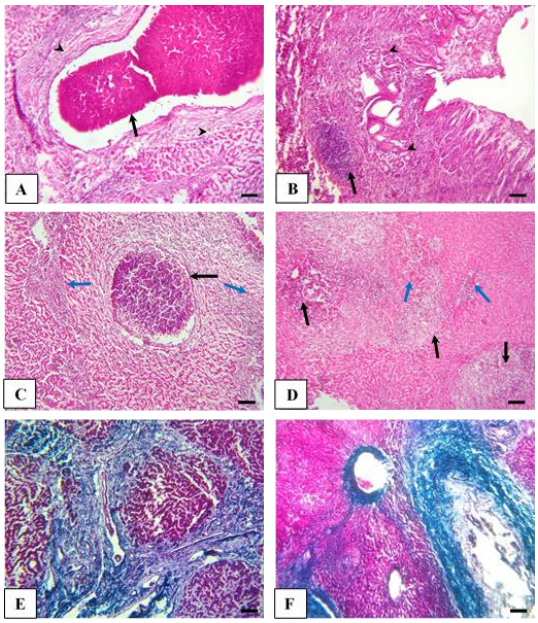

Figure 6. Histopathological examination of the affected liver of goat. A: Fibrosis (arrowheads) and congestion (black arrow) were found. B: Granuloma (black arrow) along with hyperplasia of bile ducts (arrow heads) was seen. C: Necrotic mass (black arrow) and inflammatory infiltrates (blue arrow) were seen. D: Centrilobular necrosis of hepatocytes with infiltration of mononuclear cells (black arrows) and congestion (blue arrows) were found. E-F: Blue color fibrous connective tissue proliferation was seen. A-D: H & E stain; E-F: Goldner's trichrome stain. Bars: A-F = 100 µm.

Discussion

In the present study, the collected cattle and goat livers from slaughterhouses showed various types of pathological changes along with some normal livers. 71.4% cattle livers (n = 14) and 75% goat livers (n = 20) were found affected in different conditions during this investigation. Grossly, the affected liver showed thickened capsule, enlarged liver, hard consistency, discoloration, necrotic foci, hemorrhagic tracts, fibrosis which are associated with different disease conditions of livers. Similar results have been reported by Talukdar *et al*., 2011 in goat, Marcos *et al*., 2007 in cattle and Jamil *et al*., 2017 in buffalo. At the stage of slicing of liver yellowish and brownish-black exudates and adult liver flukes (*F. gigantica*) were observed in the bile ducts in present study which was also observed by Jamil *et al*., 2017 in buffalo liver affected with *F. gigantica.* The hemorrhagic or migratory tracts and necrosis throughout the liver tissues were produced by *F. gigantica* that was observed in the current investigation. Talukdar *et al*., 2011 and Khan *et al*., 2015 also showed that hemorrhagic patches or elongated tracks on the liver surfaces and necrosis throughout the liver tissues were produced by *F. gigantica*. Formation of fibrous tissue in liver parenchyma and around the bile ducts resembled the pipe-stem liver found in cattle and goat in this study. Marcos *et al*., 2007 found that approximately two thirds of hepatic tissue from *F. hepatica* infected cattle showed cirrhosis (62.5%). Calcification of the bile ducts was present in 2 (28.6%) livers of cattle assured by the gritty sound felt during bile duct sectioning and no calcification in goat. This result is similar to the findings reported by Ross, 1966 and Marcos *et al*., 2007 in cattle.

The histopathological alterations observed in liver of cattle and goat in present study were necrosis, inflammatory cells infiltration, migratory tracts, bile duct hyperplasia, portal cirrhosis and the loss of lobular architecture. Talukdar *et al*., 2011 reported that Black Bengal goat infected with liver flukes showed necrosis, the migratory tracts with infiltration of lymphocytic cells, atrophy, as well as fatty changes. Interestingly, immature flukes were discovered in the intrahepatic biliary ducts in the present study and the hemorrhagic tracts were produced by the migration of immature *F. gigantica* which were then infiltrated inflammatory cells and mild fibrosis was also observed. These findings are similar to Ahmedullah *et al*., 2007 in buffalo liver affected with *Fasciola gigantica*. They also reported hyperplastic alterations in epithelial cells of the bile duct, and periductal connective tissue growth. These findings are consistent to the observations of Ross, 1966, Sengupta and Iyer, 1968, Trivilin *et al*., 2014 and Khan *et al*., 2015.

Chronic liver diseases frequently lead to cirrhosis which is often accompanied by progressive loss of liver function. The chronic Fascioliasis gradually progress to continuous fibrous connective tissue proliferation along with mononuclear cells infiltration and ultimately results in cirrhosis as we found in present study. Periductal fibrosis along with periportal fibrosis was seen microscopically as blue color fibrous tissue proliferation around the bile ducts as well as portal areas in Goldner's trichrome staining. The density of *Fasciola* spp is highly associated to the severity of hepatic fibrosis and hyperplasia of bile ducts in naturally infected cattle (Marcos *et al*., 2007). Bile duct hyperplasia, periportal fibrosis as well as lymphocytic infiltration were also found in cattle livers by Faccin *et al*., 2016 which is in agreement with present findings. Granulomatous inflammation was characterized by the focal infiltration of mononuclear cells in the cattle and goat liver with a nodular appearance. In the buffalo liver, there was a nodular appearance and infiltration of mononuclear cells, indicating nodular hepatit (Ahmedullah *et al*., 2007).

Condemnation of liver often results in economic losses as well as losses of animal proteins. Liver rejection was caused

by a variety of diseases and afflictions, including necrosis, Fascioliasis, hydatidosis cysticercosis, calcification, abscess, hemorrhages, and liver cirrhosis in cattle in Sudan (Mohamed, 2021). Most common reason of liver condemnation in cattle and goat found in the present study was Fascioliasis. Ahmedullah *et al*., 2007 detected infection with *Fasciola gigantica* was in 22.5%, hydatidosis (2.5%), abscesses (3.75%), and haemorrhages (2.5%) in livers of buffalo obtained from different slaughterhouses in Barishal, Bangladesh. Mellau *et al*., 2010 in their survey report showed that Fascioliasis was the main cause of condemnation of liver at slaughterhouses in Tanzania. Fascioliasis is a re-emerging and widespread zoonosis that affects a wide range of human populations, in addition to its veterinary as well as economic significance around the world (Mas-Coma *et al*., 2005, Esteban *et al*., 2003).

Conclusion

The total liver lesion in cattle is 71.4% and in goat is 75% in slaughterhouses of Mymensingh, Bangladesh. Most common cause of liver lesions in cattle and goat found in the present study was Fascioliasis and granulomatous lesion with unknown cause. More emphasis should be given to these findings because it could result in economic losses and the possibility of zoonotic transmission of these pathogens to humans. Therefore, both veterinary personnel as well as planners of public health in the Bangladesh must use extreme caution to ensure that critically injured livers are not passed to be eaten by humans, despite their poor nutrition and health hazards.

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Conflicts of interest

There is no conflict of interest among the authors.

Author Contributions

The experiment was designed by MRK and MP. LA, NS, DCP and MP undertook the experiment; LA, MP, NS and MRK interpreted the results; MRK and LA wrote up the draft. MRK, NS, MP and MNHS checked the manuscript critically. All authors read and agreed on the final version of the manuscript.

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