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Article

Comparative histomorphological study of non affected and affected liver by fascioliasis in Black Bengal goat

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Abstract: The purpose of this study was to comparative histomorphological investigation of the non affected and affected liver by fascioliasis in Black Bengal goat. The average weight of affected livers was 511.20±14.10 gm which was significantly ($p<0.001$) higher than non affected livers (371.70±13.25 gm). The average length and girth of affected liver were 27.50 ±0.63 cm and 34.00±0.71 cm, respectively which were also significantly ($p<0.001$) higher than the length (21.80±0.61 cm) and girth (26.60±0.80 cm) of non affected liver of Black Bengal goat. The gross changes in acute form, affected livers were enlarged than normal. The livers were slightly swollen with rounded edges and the color became paler than normal. The capsule was more or less thick, opaque and rough than normal smooth thin capsule. The gross changes of the liver in chronic fascioliasis characterized by increase in the size of the organ due to inflammatory changes in the parenchyma and fibrosis of the bile ducts containing adult flukes. In acute cases microscopic changes were found mainly in the portal area. The grossly visible spots or tracks was represented by the presence of numerous eosinophils admixed with few lymphocytes and monocytes in the parenchyma of liver and accompanied by hemorrhage and edema. In chronic fascioliasis the hemorrhagic tracks or tunnels were represented by the areas infiltrated with fibroblasts admixed with neutrophils, lymphocytes and few mononuclear cells in the area previously migrated by young flukes. So it was concluded that liver of Black Bengal goats are mostly affected with fascioliasis which severely change the structure of that organ.

Keywords: histomorphological; liver; fascioliasis; Black Bengal goat

1. Introduction

Goats as far as known, were probably the first domestic ruminant after dogs around 9000-7000 BC (Herre and Rohrs, 1973). There is very little information about the origin of dwarf goats mostly found in this region. It is believed that Asiatic breeds of domestic goats are generally considered to derive from a single wild species capra hircus aegagrus. The dwarf goat might be descended from a different origin and perhaps from African ancestors (Epsterin, 1971). Black Bengal goats are dwarf breed and are known to be famous for its adaptability, higher disease resistance, fertility, fecundity, early sexual maturity, larger litter size, delicacy of meat and superior skin quality (Devendra and Burns, 1983; Husain *et al.*, 1998). However, some authors reported that their growth rate is slow, milk yield is as low that sometimes it is not sufficient for their kids and higher kid mortality (Amin *et al.*, 2000, 2001; Chowdhury *et al.*, 2002). Though majorities of the Black Bengal bear black coat color, black and white, brown, brown and white and white coat colors are also common in the Black Bengal population. Black Bengal goat constitute nearly 90% of the total goat population of the country and the remaining are Jamunapari and their crosses (Husain *et al.*, 1998). Parasitism is one of the main causes limiting livestock productions in most of the tropical and sub-tropical countries of the world. In Bangladesh,

parasitism has been considered as one of the major constraints of livestock production (Jabbar & Hag, 1983). The incidence of parasitic diseases in the domestic ruminants of Bangladesh is also high and as a result hardly any livestock industry could develop here. Besides a large number of helminth parasites these are constantly deteriorating the health and productivity of the ruminants, *Fasciola gigantica* is by far the pre-dominant species of outstanding importance. The pathogenic effect of this parasite is extended over a large number of domestic ruminants; cattle, sheep, goats and buffaloes are mostly affected and drain a substantial economic loss to the country annually. Infection with *Fasciola gigantica* is regarded as one of the most common single helminth infection of ruminants in Asia and Africa (Hammond & Sewell, 1990). This disease causes enormous economic losses all over the world and these losses are due to reduction in milk and meat production, condemnation of liver, loss of draught power, reproductive failure and mortality (Diaw *et al.*, 1998). The World Health Organization (WHO, 2006) has estimated that 2.4 million people are infected with *Fasciola hepatica* and a further 180 million are at risk of infection. So, for controlling the disease in this area, appropriate preventive control strategies have to be designed to reduce the impact of the disease on goat production in Bangladesh. 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; Menkir *et al.*, 2007), Swai and Unlicky (2009) 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$.

There is no available information on histomorphological study of non affected and affected liver by fascioliasis in Black Bengal goat. So the present study has been undertaken to evaluate the comparative histomorphological study of non affected and affected liver by fascioliasis in Black Bengal goat.

2. Materials and Methods

2.1. Experimental area and duration

The investigation was carried out in the Department of Anatomy and Histology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur. Livers of goats were collected from different slaughter houses of Dinajpur town for detailed histomorphological examinations during the period from 1st July, 2014 to 30 June, 2015. Non infected goat was reared in the farmer house.

2.2. Experimental animals

The experimental animals of this study were goats those submitted for slaughtering in the slaughter house. The experimental animals were two types such as-

a) Black Bengal goats affected with fascioliasis
b) Black Bengal goats not affected with fascioliasis

2.2.1. Selection of Black Bengal goats affected with fascioliasis

Black Bengal goats affected with fascioliasis were primarily selected from Butchers house by observing clinical signs and symptoms such as-

Pallor of the mucous membranes and development of anaemia, emaciation and progressive loss of body condition, submandibular oedema and ascites (bottle jaw), loss of appetite, diarrhoea, ruffled hair coat, distended and painful abdomen, disinclination to move, significant effect on production etc which are tentative diagnosis of fasciolosis, confirmative diagnosis of fascioliasis was done by observing live parasites in the liver, bile duct and gall bladder of slaughtered goat. Faeces examination was done in the laboratory of Department of Anatomy and Histology to identify the eggs of *Fasciola gigantica.* Age of the goats were determined by dentition.

2.2.2. Selection of Black Bengal goats not affected with fascioliasis

Selection of Fasciola non affected goat was confirmed by faeces examination under McMASTER method which is as follows-

This method is based on the principle that the eggs are floated up in a counting chamber. A special type of slide devised by McMaster is required in this technique. Two glass slides joined together and the space between them divided to form two counting chambers of 0.15ml capacity each is the McMaster slide. In this method a known volume of faeces (5 to 10g) is thoroughly suspended in a known volume (50 to 100ml) of saturated salt solution (sp.gr. 1.2). The suspension may be strained through a 150 mm mesh sieve to remove the coarse

particles. A portion of the suspension is withdrawn with the help of a pasteur pipette or with an ordinary plastic transfer pipette, and allowed to run into the chambers of the McMaster slide. The slide is stand for3 to 5 minutes to allow the eggs to float. The eggs in the two chambers are counted using low powers objectives (X10) and eyepiece (X10). The number of eggs per faeces may be calculated by using the following formula:

$$\text{Number in one gram} = \frac{\text{Number in two chamber}}{0.3} \times \text{dilution factor}^{*}$$

$$^{*}\text{Dilution factor} = \frac{\text{Total volume of supensin in ml}}{\text{Total volume of faeces}}$$

Negative result i.e absence of *Fasciola gigantica* eggs was confirmed that goat was not infected with fascioliasis. Age of the goat was determined by dentition.

2.2.3. General managment of goats not affected with fascioliasis

After confirmation regular anthelmintic was used to prevent the further infection upto slaughter**.** The goat was reared in conventional farming system. There was semi-concrete floor system for experimental goat. Surface of the floor was even bedding material. There is both natural and artificial air flow in the house. Both roughage and green grass were offered to him. Some times they were supplied Jackfruit leaves, Banana leaves, Mango leaves etc. Concentrates supplied by the farmers were rice polish, wheat bran ,anchor bran, boiled rice, broken maize, broken rice, tiloil cake, mustard oil cake, pulse husk, salt etc. Regular vaccination was done for the goat to prevent the contagious diseases.

2.3. Gross morphological examination

A total of 10 affected and 10 non affected livers along with gall bladders showing grossly visible abnormalities were collected for detailed morphological studies. The length and girth of the livers and gall bladders were measured by using measuring tape. The weight of the livers and gall bladders was measured by using digital weight machine. All the changes were noted. Incisions were given to the affected area to study the nature or the macroscopic cessions, whenever needed. 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 Anatomy and Histology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University.

2.4. Microscopic examination

During slaughter, affected and non affected livers 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).

2.5. Statistical analysis

The data recording the study of the affected and non affected livers were tabulated and subjected to analysis. Statistical analysis of the data was done by paired t- test using SPSS 10.0 programme. The weight, length and girth between the affected and non affected livers were compaired by t-test.

3. Results

On gross and microscopic examination of 10 non affected and 10 affected livers by fascioliasis in Black Bengal goat that were collected freshly from different slaughter houses were subjected for different examinations. The comparative biometry (weight, length, girth) of the non affected and affected livers by fascioliasis in Black Bengal goat was shown in Table 1. The average weight of affected liver was 511.20±14.10 gm which was significantly ($p<0.001$) higher than non affected liver (371.70±13.25 gm). The average length

and girth of affected liver was 27.50 ±0.63 cm and 34.00±0.71 cm, respectively which were also significantly ($p<0.001$) higher than the length (21.80±0.61 cm) and girth (26.60±0.80 cm) of the non affected liver.
Two types of changes were recorded during the course of study. These were gross changes and microscopic changes. Two forms of fascioliasis were found during these investigation namely acute and chronic infections.

3.1. Gross changes of liver

In acute form, affected livers were enlarged than normal. The livers were slightly swollen with rounded edges and the color became paler than normal. The capsule was more or less thick, opaque and rough than normal smooth thin capsule (Figures 1 & 2). Numerous small and large pin point hemorrhagic patches scattered over the parietal surface which are completely absence in normal liver (Figures 3 & 4). Light hemorrhagic spots or elongated tracks occurring on the surfaces of the affected liver. Some cases numerous swelling and round shape of the organ were visible mainly at the parietal surface of the right lobe which were absent on normal liver surface. The surfaces of the affected livers in some instances exhibited few depressed areas which were absent in normal livers (Figures 5 & 6) and when cutting these depressed areas appeared as tracks or tunnels filled with bloody exudates (Figures 7 & 8). There also recorded pipe stem appearance of the liver caused by the migration of the parasites which were also absent in the normal liver. Sometimes immature flukes were found in the tunnels of affected liver where as both tunnels and immature flukes were absent in normal liver (Figures 9 & 10).
The gross changes of the liver in chronic fascioliasis characterized by increase in the size of the organ due to inflammatory changes in the parenchyma and fibrosis of the bile ducts containing adult flukes which were completely absent in normal livers (Figures 11 &12).The affected livers were greatly enlarged, hard in consistency and their edges become round because the lobes were not distinct which represented 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 on the ventral surface of the liver. The liver as a whole become flabby and easy to cut, hemorrhagic patches and white necrotic foci of abcessiation was found which are totally absent in non affected liver (Figures 13 & 14). Sometimes due to migration in the liver or death of the fluke, there causes necrosis and due to necrosis the part of the liver become harden but there was no calcification and during cutting there produce no gritty sound. The left lobe was uneven on the both parietal and visceral surfaces and displayed much more thickening than the right. On cut section numerous yellowish-white migratory tracks and the markedly dilated, thickened wall bile ducts were noticed as well as different sizes of liver flukes can be seen. In some cases, hepatic nodule of different sizes and shapes separated by invading tracks of fibrous connective tissue were distinctly seen. Such livers were lethery in consistency and did not cut easily. The capsule was thick and opaque. The periphery of the caudate and the left lobe appeared to be thin and whitish due to fibrosis. The hapatic nodules are also enlarged and hard in consistency.

3.2. Microscopic changes of liver

In acute cases microscopic changes were found mainly in the portal area. The grossly visible spots or tracks was represented by the presence of numerous eosinophils admixed with few lymphocytes and monocytes in the parenchyma of affected liver and accompanied by hemorrhage and edema which features were not found in non affected liver (Figures 15 & 16). The presence of thrombi in the branches of portal and hepatic veins of affected liver was also observed in this study. On the other hand, the migrating flukes produced little reaction in the host tissue and in the later stages they became surrounded by macrophages, eosinophils and granular tissue. In the zone of reaction the blood vessels were highly congested or ruptured. In some sections, the center of the tract contained a mass of degenerating neutrophils which were thought to be the part of a migrating immature flukes. Due to migration there destruction of hepatic cords, extra vascular erythrocytes were also seen. Dissolution of many hapatocytes, hepatic necrosis along with reactive cells and found total architechtural destruction of the hepatic parenchyma where as no extra vascular hepatocytes were observed in non affected liver. Hepatic cords were disorganized, swollen hepatocytes with acidophilic cytoplasm and pyknotic nuclei while other hepatocytes showed coagulative necrosis, as well as dilated hepatic sinusoids that engorged with blood. Also haemorrhagic migratory tracts were noticed within the necrotic hepatic cords. Portal veins were surrounded with cellular infiltration mainly eosinophils, macrophages and lymphocytes with kuffer cells activation, inaddition to hepatocytic ballooning degeneration. 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 advanced stages of *Fasciola gigantica* infection livers 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.

In chronic fascioliasis the hemorrhagic tracks or tunnels were represented by the areas infiltrated with fibroblasts admixed with neutrophils, lymphocytes and few mononuclear cells in the area previously migrated by young flukes. In many sections, the lobular architectures were found to be distorted 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 destructions of the normal lobuler architechture where as normal portal triad was observed in case of non affected liver (Figures 17 & 18). Liver section showed atrophy, necrosis and fatty changes due to chronic fascioliasis in goat. 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.

4. Discussion

The average weight of affected and non affected liver by fascioliasis in Black Bengal goat was 511.20±14.18 gm and 371.70±13.25 gm, respectively. The result is closely agreed with Getty (1975) who stated that average weight of liver of sheep and goat was 550 gm to 700 gm. The variation in weight of liver may be due to variation in breeds of goat.

4.1. Gross changes of liver

The gross changes of the liver in acute form, the livers were slightly swollen or enlarged with rounded edges and the color became paler than normal with numerous small and large hemorrhagic patches scattered over the parietal surface. In chronic Fascioliasis characterized by increase in the size of the organ due to inflammatory changes in the parenchyma and fibrosis of the bile ducts containing adult flukes. Gross changes produced by *Fasciola gigantica* infection were almost similiar with the earliar findings (Dow *et al.* 1967 and Soulsby 1982). 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 fedding habit of these premature parasites (Sengupta and Iyer, 1968). Ansari-Lari and Moazzeni (2006) reported more or less same result on the prevalence of liver condemnations due to Fascioliasis. 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 changes in acute Fascioliasis described by these authors may be summarized as light hemorrhagic spots or elongated tracks occurring on the surfaces of the liver.

4.2. Microscopic changes of liver

The histopathological examination also revealed the presence of numerous eosinophils admixed with few lymphocytes and accompanied by hemorrhage and edema in acute fascioliasis. This study partially correlated the findings of Dow *et al.* (1967) who recorded the changes of liver in experimentally produced Fascioliasis in calves. However, the occurrence of thrombi in the branches of portal and hepatic veins as observed by this author were not seen in this study. On the other hand, the migrating flukes produced little reaction in the host tissue and in the later stages they became surrounded by macrophages, eosinophils and granular tissue. Early of the flukes into the bile ducts resulted in proliferation of the epithelium producing a granular mucosa which was found in the present study. Ross *et al.* (1967) described similar pathological changes in acute fascioliasis in calves and pig livers, respectively. According to Ross (1966) acute pathological lesions could only be produced by developing flukes prior to their entry to the bile ducts. The histopathological changes in chronic fascioliasis were characterized by infiltration of fibroblasts admixed with lymphocytes and few mononuclear cells in the area previously migrated by young flukes. Similar statement was made by Dow *et al.* (1967) and Smith *et al.* (1972). Huge proliferations of fibrous connective tissue associated with infiltration of lymphocytes and plasma cells in the portal area were commonly seen. Liver section showed atrophy, necrosis and fatty changes due to chronic fascioliasis in goat. 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 space were seen. Thickening of the bile ducts and fibrosis in a portal area due to chronic fascioliasis also found. Migratory tract with lymphocytic infiltration was more common case. Sections of immature flukes surrounded by clear spaces were found in the dilated and thickened bile ducts. The adult *Fasciola gigantica* was noticed in cross section in the lumen of the thickened bile ducts. These findings substantiated the observations of Acosta-Fereira *et al.*(1980), Ross (1966), Ross *et al.* (1967), Dow *et al.* (1967), Sengupta and Iyer 1968, Uzoukwn and Ikeme1978, Smith *et al.* (1972). They added that the cytoplasmic blebs at the free margin of the epithelial lining cells were the first indication of intense hyperplasia. In this investigation, no brilliant cell was seen in the areas of adenomatous proliferation of the biliary epithelium in the goat liver as described by Shirai (1976) in cattle liver. No calcification in the wall of the bile ducts in chronic fascioliasis in goat could be seen in this study. Similarly, according to Simesen (1968) and Pullan *et al.* (1970), the bile ducts of the liver in sheep never calcified, contrary to findings in cattle Ross, 1966 and Dow *et al.* (1967). Ross *et al.* (1967) reported that there was no calcification in the pig liver in chronic fascioliasis. The difference in calcification might be due to species variation.

Table 1. Comparative biometry of non affected and affected liver by fascioliasis in Black Balgal goat.

Points	Type of Liver (n=10)	Mean ± SE	Significance
Weight (gm)	Affected Liver (n=10)	511.20±14.18	***
	Non Affected Liver (n=10)	371.71±13.25	
Length (cm)	Affected Liver(n=10)	27.50±0.63	***
	Non Affected Liver (n=10)	21.80±0.61	
Girth (cm)	Affected Liver (n=10)	34.00±0.71	***
	Non Affected Liver (n=10)	26.60±0.80	

SE= Standard Error, (***) = Highly significant (p<0.001), n= number of sample

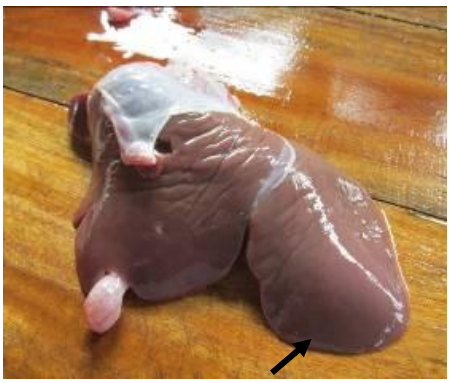

Figure 1. Liver not affected with fascioliasis show normal elongated edge.

Figure 2. Liver affected with fascioliasis show swollen, rounded edge.

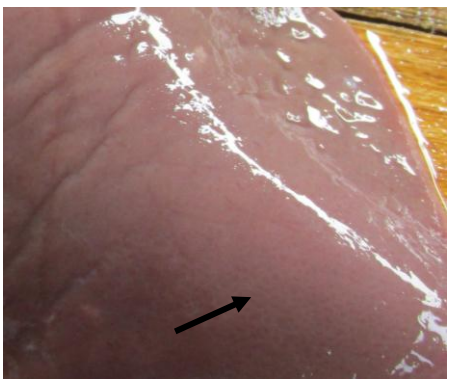

Figure 3. Absence of pin point hemorrhagic patches over the parietal surface of non affected liver.

Figure 4. Small and large pin point hemorrhagic patches scattered over the parietal surface of affected liver.

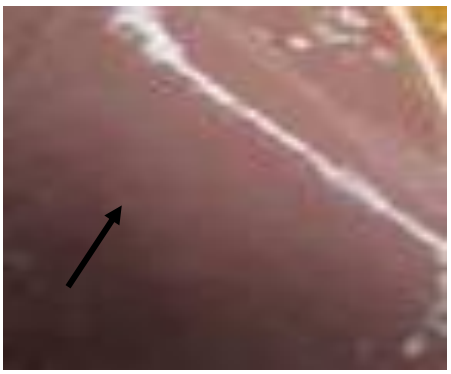

Figure 5. Liver not affected with fascioliasis did not show any depressed areas on it's parietal surfaces.

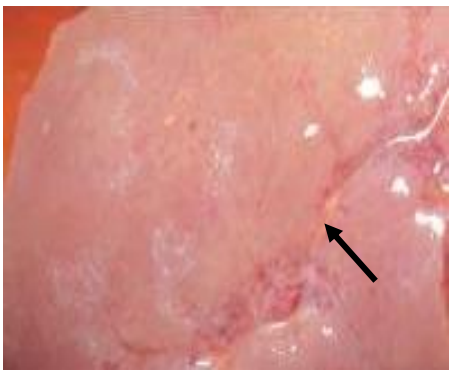

Figure 6. Liver affected with fascioliasis show few depressed areas on it's parietal surfaces.

Figure 7. Absence of bloody exudates on cutting of non affected liver.

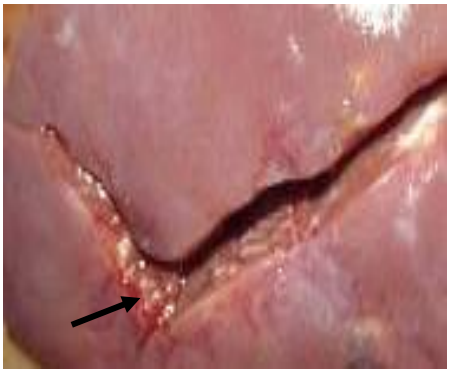

Figure 8. On cutting affected liver show tracks or tunnels filled with bloody exudates.

Figure 9. No immature fluke in the tunnels of non affected liver.

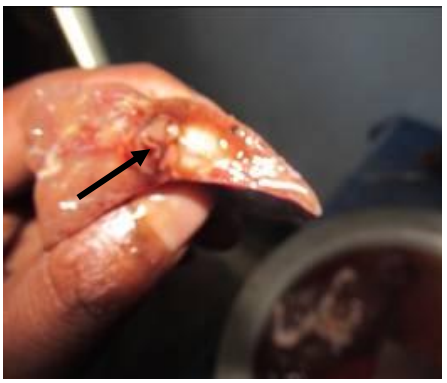

Figure 10. Immature fluke in the tunnels of affected liver.

Figure 11. No fibrosis of bile duct and no adult flukes found in the non affected liver.

Figure 12. Fibrosis of bile duct containing adult flukes in the affected liver.

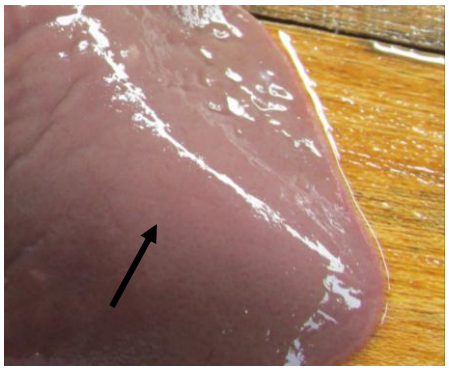

Figure 13. Absence of white necrotic foci on the parietal surface of non affected liver.

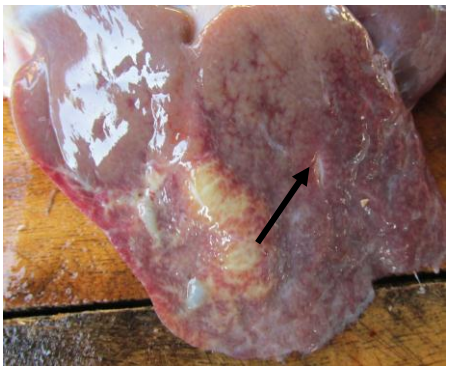

Figure 14. White necrotic foci on the parietal surface of affected liver.

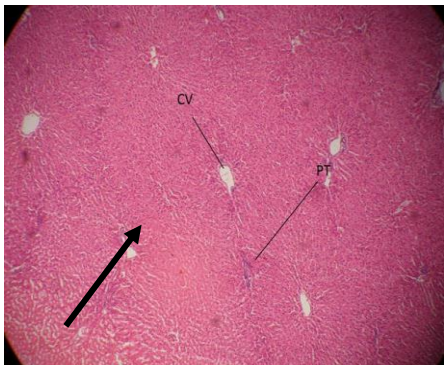

Figure 15. Eosinophil, lymphocytes and monocyte were not found in the parenchyma of non affected liver (H & E X 10).

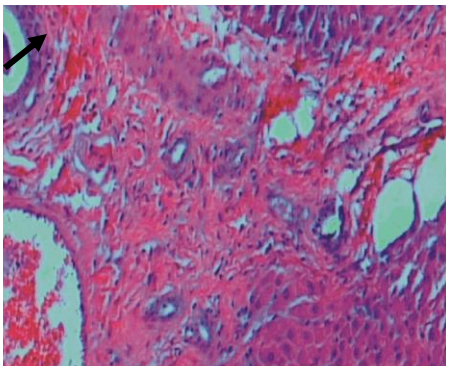

Figure 16. Numerous eosinophils admixed with few lymphocytes and monocyte in the parenchyma of affected liver (H & E X 10).

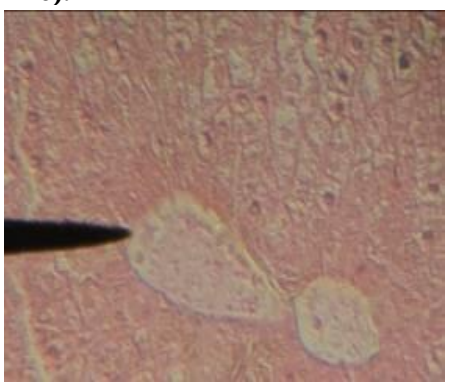

Figure 17. Normal portal triad were observed in the non affected liver. (H & E X 10).

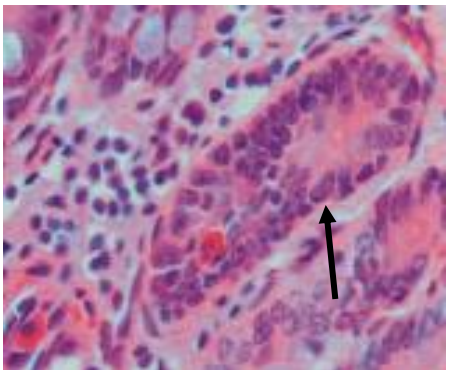

Figure 18. Hyperplasia, fibrous connective tissue and closure portal triad were observed in the affected liver. (H & E X 40).

5. Conclusions

Liver of Black Bengal goats are mostly affected with parasitic disease like fascioliasis. Fascioliasis (*Fasciola gigantica*) is greatly responsible for hepatic damage of Black Bengal goat and demands immediate attention for taking control and eradication measures. Livers of male and female goats both sexes were found to be affected with fascioliasis but severity is higher in Female goats than those of male goats. Probably fascioliasis (*Fasciola gigantica*) is the major cause of reduction of meat production of Black Bengal goats. The prevalence of fascioliasis (*Fasciola gigantica*) is higher in older animals. Regular anthelmintic should be used in case of Black Bengal goat from very early age to avoid infection by fascioliasis. Infected goat should be administered proper anthelmintic at proper dose without any delay. Balanced diet and proper housing system should be maintained to avoid fascioliasis.

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Conflict of interest
None to declare.

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