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Research Article

Study on histopathological changes occurred in *Fasciola spp.* Infected liver in slaughtered buffaloes at Anand and Ahmedabad district, Gujarat, India

Pandya, S. S.^{1*}, Hasnani, J. J.², Patel, P. V.³, Ghodasara, D. J.⁴ and Shukla Ravi⁵

*¹Ph. D. Scholar, Department of Parasitology

²Professor and Head, Department of Parasitology

³Professor, Department of Parasitology

⁴Associate professor, Department of Pathology

⁵M. V. Sc., Department of Livestock Production and Management

Corresponding Author : pandyasuchit@gmail.com

Abstract

Keywords

Fasciola gigantica,
necrosis,
hepatic cell,
eosinophil,
hyperplasia,
haemorrhage.

In the present study a total of 701 and 743 liver samples were collected from Anand and Ahmedabad District, respectively for deriving the prevalence rate of Fasciolosis in slaughtered buffaloes. Out of these all collected samples, 30 *Fasciola gigantica* infected positive liver samples were collected from slaughter house for histopathological study. Liver were examined during post-mortem examination. Outer surface of the liver and bile duct opening in the liver were properly checked for the presence of the parasite. Surface of the liver was also incised for examining presence of parasite. Intestine of all 30 buffaloes infested with *Fasciola spp.* were also collected found normal and there was no pathological changes observed. Liver was found severely affected, enlarged, covered with rust colored patches, showing haemorrhagic tracts because of migration of flukes, perforation and presence of large number of immature flukes in the parenchyma and at the opening of the bile duct. Hundreds of immature and mature flukes were recovered after squeezing and tearing of liver. Microscopic section revealed focal necrosis with heavy infiltration of inflammatory cells. Portal triad area revealed proliferation of fibrous tissue. Hepatic cells showed degenerative changes and mild fatty changes. There were large numbers of multiple haemorrhagic tracts made up of erythrocytes and degenerating hepatic cells with polymorphs, eosinophils and mononuclear cells. Bile ducts showed hyperplasia, desquamation and degeneration of epithelium.

Introduction

Fasciolosis is regarded as one of the most important diseases of buffaloes in humid tropical regions of the world. Milk production is a livestock enterprise in which small-scale farmers can successfully engage in order to improve their livelihoods and this milk production is decreased by infection of *Fasciola spp.* parasite in buffaloes (Gupta *et al.*, 2012). Fasciolosis in buffaloes is asymptomatic, subclinical and/or chronic form of the disease, adversely affecting their reproductive cycle, weight gain, food conversion efficiency and productivity. The frequency of outbreaks increases between October and May, but sporadic outbreaks continue throughout the year (Agrawal, 1998). Acute form of Fasciolosis caused by migratory immature flukes in the liver

parenchyma is rare in cattle and buffaloes, and is difficult to diagnose as no eggs are detected in faeces. Chronic Fasciolosis mostly occurred in large ruminants viz. cattle and buffaloes (Chandra *et al.*, 2011). Cases of acute Fasciolosis in buffaloes are rarely reported from India except Banerjee *et al.*, 2001 who has reported one such outbreak in Nainital district of Uttarakhand. For the main purpose of this study is to understand the macroscopic and microscopic changes occurred in liver and bile duct of *Fasciola* infected buffaloes. The collected organs and tissue samples were kept in airtight container with the addition of 10% formaline solution for the prevention of the contamination.

Materials and Methods

1. Study area and sample collection

Samples were collected from local slaughter houses of Anand and Ahmedabad districts of Gujarat. Animal were examined during post-mortem examination for the presence of *Fasciola* infection in liver and bile duct of buffaloes. Infected liver and bile duct were collected in airtight container with proper label. For the prevention of contamination 10% formalin was added in the container. After collection samples were brought to the department laboratory for further processing.

2. Processing of collected samples

For microscopic examination tissue pieces of liver preserved in formalin transferred to 10% neutral buffered formal saline solution and were processed by paraffin embedding method. Sections were cut at 5-6 micron thickness with the help of microtome and stained with Ehrlich's Haematoxylin and Eosine (H&E) method for examinations as described by Luna (1960). Typical lesions were photographed at different magnifications. Liver and bile duct grossly examined by teasing surface of liver and bile duct with the help of scalpel.

Results and Discussion

In the present study, the histopathological changes in Fasciolosis in buffaloes were observed during study period. A total of 30 positive *Fasciola gigantica* infected liver and intestines were collected from slaughter house. For that we used to regularly go to the slaughter houses of Anand and Ahmedabad districts. Liver were examined during post-mortem examination. Outer surface of the liver and bile

duct opening in the liver were properly checked for the presence of the parasite. Surface of the liver was also incised for examining presence of parasite. Intestine of all 30 buffaloes infested with *Fasciola* were found normal and there was no pathological changes observed.

Grossly the body cavities of slaughtered buffaloes were found to contain large amount of straw colored fluid with fibrin flakes. There was oedema in brisket and dewlap region and petechial haemorrhage in muscles in some of the buffaloes. Liver was found severely affected, enlarged, covered with rust colored patches, showing haemorrhagic tracts, perforation and presence of large number of immature flukes in the parenchyma and at the opening of the bile duct. At places blood filled cavities were also seen in the parenchyma. Hundreds of immature and mature flukes were recovered after squeezing and tearing of liver (Figure I). Gross lesions were confined to the liver which was enlarged, haemorrhagic, highly congested and had wide-spread greyish creamy deposits on its surface. Migrating flukes caused extensive destruction of liver parenchyma marked with haemorrhages (Figure II). Microscopically the sections of liver showed haemorrhagic tracts, wide-spread area of necrosis and haemorrhages. It also revealed focal necrosis with heavy infiltration of inflammatory cells. Portal triad area revealed proliferation of fibrous tissue. Hepatic cells showed degenerative changes and mild fatty changes. There were large numbers of multiple haemorrhagic tracts made up of erythrocytes and degenerating hepatic cells with polymorphs, eosinophils and mononuclear cells (Figure III & IV). Bile ducts showed hyperplasia, desquamation and degeneration of epithelium. The epithelium of bile duct both close to and distal to the sites of fluke penetration was highly hyperplastic and thickened with numerous eosinophils and mononuclear cells infiltration into the lamina propria (Figure V).



Figure I. Gross specimen of *Fasciola gigantica*



Figure II. Gross specimen of liver showing enlarged, haemorrhagic, highly congested liver and had wide-spread greyish creamy deposits on its surface

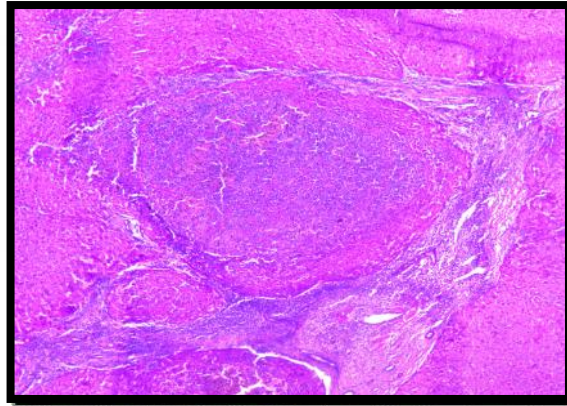


Figure III. Microscopic section of liver showing focal necrosis and infiltration of inflammatory cells (H & E stain x 50)

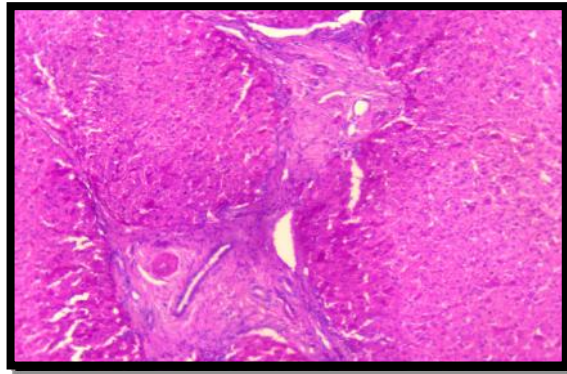


Figure IV. Microscopic section of liver showing proliferation of fibrous connective tissue in portal triad area. (H & E x 120)

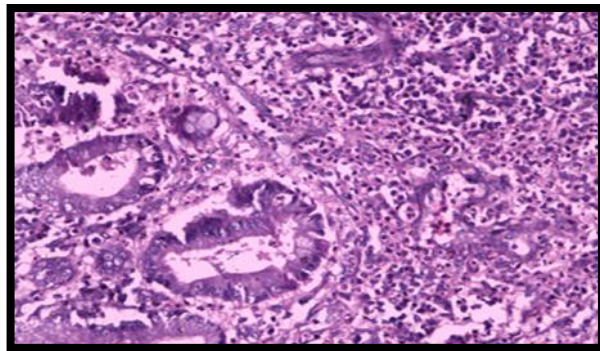


Figure V. Microscopic section of liver showing proliferation of the bile ducts and infiltration of mononuclear cells

The present study correlates with the Kumar *et al.* (1982) who reported severe pathology in the liver and bile-ducts, Massoud and Vedadi (1983) noticed thickening of the main bile ducts and glandular hyperplasia of the main bile duct mucosa. They have reported little calcium deposition on the surface mucosa of the bile ducts. Hypertrophy of blood vessels was more pronounced and some showed thrombosis. Monolobular fibrosis in those parts which were not disrupted by the massive migratory tracks was pronounced and constricted the liver lobules and produced pseudo-lobulation.

Doy and Hughes (1984) documented early migration of immature *F. hepatica* and associated liver pathology in cattle and noticed liver pathology which showed atypical progressive tissue disruption with a neutrophil and lymphocyte infiltration and a marked eosinophilia, mesothelium of the liver capsule had undergone metaplasia becoming ultimately highly active and columnar, Swarup and Pachauri (1987) recorded typical lesion of early chronic stage of infection, most important being the disruption of hepatic cells, loss of normal architecture of liver parenchyma, cellular infiltration and fibrotic and hyperplastic changes in the bile-duct, these all findings are in line with the present study.

In other study, Sadjjadp *et al.* (1997) noted lobular hepatic chord fibrosis, liver cell atrophy and regeneration, congestion and edema of sinuses, increase and activation of kupffer cells and fibrous tissue, cellular infiltration and proliferation of bile ducts in portal triads. They have also noticed trauma, hyperplasia and regeneration of epithelia, globule leukocyte in the epithelium, glandular hyperplasia, calcium deposition, plasma cell infiltration and fibroplasia of bile ducts which is completely correlates with the present study.

Sheikh *et al.* (2004) revealed severe damage resulting in disrupted hepatic cords, inflammation, atrophy and necrosis, bile duct hyperplasia was prominent with proliferation of epithelial cells, Nishant *et al.* (2005) reported large number of RBCs, degenerated hepatic cells, neutrophils and occasional eosinophils within the migratory tracts, they have also found large scale destruction of hepatocytes and nuclei of this hepatic cells appeared lysed, leaving behind only reticulin network and they have noticed that most important changes were seen in the bile duct which became thickened due to hyperplasia and hypertrophy of epithelium, the above authors findings are incorporate with the present study.

In the present study Liver was found severely affected, enlarged, covered with rust colored patches, showing haemorrhagic tracts, perforation and presence of large number of immature flukes in the parenchyma and at the opening of the bile duct, gross lesions were confined to the liver which was enlarged, haemorrhagic, highly congested

and had wide-spread greyish creamy deposits on its surface (Plate 2). Microscopically sections of liver showed haemorrhagic tracts, wide-spread area of necrosis and haemorrhages. It also revealed focal necrosis with heavy infiltration of inflammatory cells. Portal triad area revealed proliferation of fibrous tissue. Hepatic cells showed degenerative changes and mild fatty changes. There were large numbers of multiple haemorrhagic tracts made up of erythrocytes and degenerating hepatic cells with polymorphs, eosinophils and mononuclear cells (Plate 3 & 4).

The above similar findings were recorded by Chandra *et al.* (2011) who reported haemorrhagic tracts, wide spread area of necrosis and haemorrhages in the section of positive liver samples, migratory tracts were full of necrotic debris, cellular and haemorrhagic mass, mononuclear cell reaction was observed along the side of necrotic haemorrhagic tracts and these were encircled by proliferative connective tissue infiltrated by mononuclear cells, Affroze *et al.* (2013) who documented fasciola infected liver appeared larger with tensed capsule and bile ducts were dilated, thickened with fibrous tissue masses forming the characteristic pipe-stem liver.

Conclusion

In general edematous condition found in Fasciola infected buffaloes. Fasciola causing remarkably damage to the both liver and bile duct. Migratory tracts are formed on the surface of the liver and wall of bile duct become thickened. Haemorrhagic lesions were found on the migratory tract of the fluke. Microscopically infiltration of erythrocytes and degenerating hepatic cells with polymorphs, eosinophils, plasma cells and mononuclear cells. Bile ducts showed hyperplasia, desquamation and degeneration of epithelium.

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