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Research Article

Histopathology of the liver naturally infected with *Gigantocotyle explanatum* in Buffaloes

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Abstract

Keywords

Hepatic fluke,
Hemorrhage,
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abattoir

Aim: This study was undertaken to study the histopathology of the liver suffering with the natural infection of Hepatic fluke (*Gigantocotyle explanatum*) in buffaloes. **Material & Method:** Total of 30 positive *Gigantocotyle explanatum* infected liver were collected from local abattoirs after examining grossly the outer surface of the liver and bile duct opening for the presence of the parasite during post-mortem. Tissue pieces were preserved in 10% neutral buffered formal saline solution and were processed and stained with H&E stain for histopathological examination. **Result:** The predominant features were pale, enlarged firm liver with thickened distended bile ducts and multifocal granulomatous nodules throughout the surface. Histopathological study of tissue sections which were cut adjacent to nodule revealed intense infiltration of inflammatory cells such as lymphocytes, macrophages, plasmacells, eosinophils as well as fibrocytes along with extensive proliferation of fibrous connective tissue in the portal areas with mononuclear cell infiltration. **Conclusion:** Amphistomosis appeared to be most neglected parasitic disease which is widely prevalent in near by areas affecting animal health and production. This study describes the pathology of the disease and its impact which helps in taking initiatives for making strategies regarding proper control and prevention of the disease to lower its incidence rate.

Introduction

India has largest livestock population in the world. It has about 98 million buffaloes, which is 57% of total population in the world. They contribute to 1.48 million metric tons of meat, amounting 24.54% of the total meat produced in the country (FAO, 2008). They make a critical contribution to food sufficiency for households by providing milk, meat, skin, manure and traction. Liver is one of the most important vital organs of the animal body which helps in maintaining the health status of the animal. Parasites like amphistomosis and fasciolosis causes severe damage to the liver affecting its availability for human consumption and causing ill effects on animal health status which poses severe direct and indirect economic impact on the livestock production. As a result the economic benefits remain marginal due to prevailing diseases, poor nutrition, poor animal health and general lack of veterinary care.

Gigantocotyle explanatum is a very common amphistome found in the liver of buffalo and has a wide geographical distribution in India. Parasite requires an intermediate host; species of snail, genus *Lymnaea*. The adult worms reside in the main bile ducts and intrahepatic ductules. The parasite may cause severe hepatic damage leading to mortality, reduction in growth rate as well as reduction in the production of meat and milk. Keeping in view the prevalence and importance of this parasite, it was felt necessary to study the detailed pathology of the liver infected with this hepatic fluke.

Materials and Methods

A total of 30 positive *Gigantocotyle explanatum* infected liver were collected from slaughter house by regularly visiting 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. Tissue pieces were preserved in 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 Eosin(H&E) for histopathological examination as described by Luna(1968). Typical lesions were photographed at different magnifications.

Results

In the present study, the histopathological changes in Amphistomosis in buffaloes were observed during study period. Liver depicted variation in comparison to the normal which was appreciable both grossly and microscopically.

Gross lesion:

Grossly, Liver was found to be slightly hard inconsistency and common bile ducts were highly distended. The affected part of liver and bile duct appeared pale, enlarged and firm inconsistency. The capsule of liver was somewhat thickened and studded with few haemorrhagic spots on the surface. The lumen of the bile duct was blocked by large number of parasites. Some flukes were also lying free in the lumen of bile ducts. The mucous surface formed pedunculated structure sat the points of attachments of parasites. A transverse cut across the infected liver, showed multiple bile ductules. These ductules as well main bile ducts showed thickened wall with parietal chronic inflammation involving mucosa and submucosa. The lumen of bile duct contained mature flukes and yellowish green necrotic materials, which even covered the parasites. The affected bileducts were markedly thickened, distended and enlarged. Some of the flukes were attached to the papillae of the rumen.

Microscopic lesions:

Microscopic examination of 5µm thick section of liver cut through the granulomatous nodule revealed intense infiltration of inflammatory cells in the nodular part of the host tissue. These inflammatory cells include lymphocytes, macrophages, plasmacells, eosinophils as well as fibrocytes. Liver tissue from the vicinity of the bile duct showed extensive proliferation of fibrous connective tissue in the portal areas with mononuclear cell infiltration. Sections also showed degenerated cells and necrotic debris. Sections of bile ducts showed marked proliferation of mucosal glands with hyperplastic changes in the mucosal epithelium.

Discussion

The present study correlates with the Alwar (1949) who studied the pathogenesis and post-mortem lesions of

amphistomes including *Gigantocotyle explanatum*. Kulasiri and Seneviratne (1956) studied both gross and histopathological lesions and reported pale, enlarged liver with distended bile ducts. Histopathological sections showed striking changes in the bile duct and portal triad with marked distortion of lobular architecture of liver. Kulkarni et al. (1974) described incidence of hepatic amphistomiasis in cattle, sheep and goats in Andhra Pradesh. He showed pinkish fibrin like material in areas where erosion of bile duct epithelium had taken place with hypertrophy and hyperplasia of bile duct epithelium and pronounced inflammatory changes with round cell infiltration near portal triad in histopathological sections. Jha et al.(1977) reported inflammation of the mucosa with associated mucoid diarrhoea in ruminal amphistomosis while haemorrhage, pronounced periductal fibrosis and other hyperplastic changes in liver in hepatic amphistomosis in bovine. Upadhyay et al. (1987) reported histopathological changes in bile duct of buffaloes infected with *Gigantocotyle explanatum*. Hasnani et al. (1988) noticed striking changes in bileducts with severely destructed lobular structures of liver with appearance of cut sections of *Gigantocotyle explanatum* in the lumen of bileducts along with hypertrophy and hyperplasia of bileduct epithelium with a marked proliferation of mucosal glands and severe infiltration of macrophages and lymphocytes. Hasnani(1992) observed gross finding of *Gigantocotyle explanatum* which were pertaining to bile ducts and nearby liver tissue including portal triad. He reported that liver was slightly hard inconsistency and common bile ducts were highly distended. The affected part of the liver and bile duct appear pale, enlarged and firm inconsistency. Microscopically lesions were mostly confined to the vicinity of the bile ducts, showed extensive proliferation of fibrous connective tissue in the portal triad area with mononuclear cell infiltration, atrophy of liver lobes, individualization of hepatic cells with degeneration and proliferation of Von-kupffer's cells. Ghosh and Chauhan(1994) studied occurrence and pathology of naturally occurring biliary amphistomiasis in buffaloes and cattle. Grossly, the liver was enlarged and the cut surface of the liver revealed numerous flukes in the lumen of the bileducts. Histopathologically, there was marked proliferation of bileducts forming irregular acinous or tubular structures, arteriole sclerososis and lymphocytic infiltration in the interlobular connective tissue. Khatoon et al. (2003) described histological changes in the liver of buffaloes infected with *Paramphistomum cervi* and observed severe hepatic damage, bile duct hyperplasia, varying degrees of fibrosis of portal tracts and infiltration of lymphocytes and macrophages. Liver infected with amphistomes of buffaloes was examined for histopathological study by Prasad et al. (2007) who noticed thickened liver capsule along with biliary hyperplasia, periportal fibrosis and dilatation of portal along with central veins and sinusoids. Puttalakshamma (2010) examined gross lesions of liver infected by *Gigantocotyle explanatum* and reported hepatomegaly, change in consistency of liver along with obstruction of bile duct due to presence of large number of flukes. Varma and Swamy (2006) studied histopathology

of liver and rumen of buffaloes infected with amphistomes and found haemorrhagic tracks in the liver parenchyma which contained erythrocytes, neutrophils and a few macrophages in the early stage. At the point of attachment, they observed necrosis, marked hypertrophy and hyperplasia of the bile duct epithelium.

In the present study gross lesions were confined to the liver which was enlarged, haemorrhagic, highly congested and had wide-spread greyish creamy deposits on its surface. Microscopically, sections of liver showed haemorrhagic tracts, wide-spread area of necrosis haemorrhages and focal necrosis with heavy infiltration of inflammatory cells. There were large numbers of multiple haemorrhagic tracts made up of erythrocytes and degenerating hepatic cells with polymorphs, eosinophils and mononuclear cells. The above findings were in accordance with the findings of Haque et al. (2011) who reported haemorrhagic tracts, widespread area of necrosis and haemorrhages in the section of positive liver samples. Migratory tracts 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.

Conclusion

Due to high level of prevalence and intensity of natural infection, amphistomosis appears to represent one of the most important animal health problems which poses severe economic impact and serious health risks to the consumers. To mitigate such impacts proper deworming programs coupled with good husbandry practices would likely to be initiated to decrease the incidence rate.

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