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The macroscopic and associated histopathological abnormalities in liver and bile duct of buffalo (*Bubalus Bubalis*), naturally infected with amphistome, slaughtered in different areas of Kashmir valley

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ABSTRACT

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This study reports the infection of liver and bile ducts, carried out from July 2012 to June 2014 on 282 randomly selected buffaloes—*Bubalus bubalis*, infected with the amphistome trematode parasite. Liver samples of buffaloes infected with amphistome were collected from the different abattoirs of Kashmir valley. Histological studies of infected livers revealed severe damage resulting in disrupted hepatic cords, inflammation, atrophy and necrosis. Bile duct hyperplasia was prominent with proliferation of epithelial cells. Macroscopic examination revealed massive infection of adult fluke in bile ducts and intrahepatic ductules in 49 (17.37) cases. The predominant features were multifocal granulomatous nodules throughout the luminal surface of the bile ducts. Histopathological study of 4 µm thick tissue sections cut adjacent to and through the site of attachment of individual worm and stained with hematoxylin and eosin revealed intense infiltration of inflammatory cells such as lymphocytes, macrophages, plasma cells, eosinophils as well as fibrocytes. This was associated with fibrosis and thickening of the bile ducts. Due to high level of prevalence and intensity of natural infection, amphistomiasis appears to be endemic in this geographical region and probably represent one of the most important animal health problems. The purpose of this paper is to overview the gross pathology (Macroscopic) and histopathological

findings of amphistome infection of liver of buffalo. It is hoped that the study may draw attention to the need for educating farmers, regarding the economic importance of infection of these amphistome parasites and also for the development of control strategies to prevent the spread of infection to ruminants.

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1. Introduction

Livestock sector has the scope for generating more employment opportunities, especially for the marginal and small farmers and landless laborers who own around 70 per cent of the country's livestock (Baba et al., 2011). Livestock wealth is more equitably distributed than that of land (Kumar and Singh, 2008). Being an important source of income and employment for this section of society, the livestock helps in alleviating poverty and smoothing of income distribution (Birthal et al., 2002). Livestock is important both as savings and investments for the poor household and provides security or insurance through multiple ways in different production systems (Kitalyi et al., 2005). Buffaloes make a critical contribution to food self-sufficiency for households by providing milk, meat, skin, manure and traction. Internal parasites adversely affect the health and productivity of animals and also decrease the resistance of animals to various diseases, which may ultimately lead to higher mortality (Irfan, 1984). Parasitic infections are generally regarded as the most prevalent and important health problems of grazing ruminants. Trematode infection, although having a restricted distribution in temperate regions has been estimated to cost \$100 million (Boray, 1999). The parasite may also cause reduction in growth rate as well as reduction in the production of meat and milk. They usually graze in the open natural external environment; face regular natural problems such as they are very susceptible to many diseases including liver disease. Hyperplasia is a condition, which is characterized by increase in the number of cells in the tissue or organ. Proliferating cells are similar to their parent cells and of regular size and shape. These cells are capable of mitotic division and co-ordinate with the function of parent tissues. Hyperplasia occurs due to increased hormonal stimulus, physical or chemical function (Robbins and Kumar, 1994). One of the common causes of hyperplasia is chronic irritation. Induced hyperplasia of epithelium is caused by parasitic infestation in various organs (Cheville, 1989). Chronic inflammation, chronic injuries and regenerative hyperplasia of the bile duct epithelium may be related to malignant transformation (Shimonishi et al., 2000). In the present study we describe the macroscopic and histopathological findings of the natural infections in the liver of buffalo caused by amphistomes- a trematode parasite.

2. Materials and methods

Buffaloes were examined for the liver amphistome infection at the different abattoirs of Kashmir valley. Visual examination technique was used for random collection of the infected livers. Amphistomes from the infected livers were collected and preserved in 70% alcohol. These were then processed for staining in Carmine Alum and finally mounted permanently in Canada balsam. The amphistomes were then identified with the help of keys and relevant literature (Soulsby, 1982; Yamaguti, 1985). For histopathology, pieces from 10 infected livers were taken and immediately fixed in 10% formaline. Sections of 6-8 μ m thickness were prepared with paraffin embedded tissue by using standard histological techniques. Sections were stained with haematoxylin and eosin and mounted permanently in Canada balsam. Microphotographs of the tissue sections were prepared for histological observations.

3. Results

3.1. Gross pathology

The presence of amphistome in bile ducts caused remarkable fibrosis of sub mucosa and hyperplasia of the endothelial layer, such that villus-like structures and protuberances were formed in large bile ducts of the infected livers. Macroscopically a transverse cut across the infected liver, showed multiple bile ductules. These ductules as

well as the main bile ducts showed thickened wall with chronic inflammation involving mucosa and sub mucosa. The lumen of the bile ducts contained mature flukes and yellowish green necrotic materials, the parasites were found thickly covered with these necrotic materials. No calcium deposits were observed in any of the examined case. A longitudinal cut across the main bile ducts showed numerous trematode parasites, measuring on an average 5.8×3.9 mm, being attached throughout the luminal wall (Fig.1). Similar situation were observed when other intrahepatic ductules of the biliary passage were dissected out. When the parasites were pulled out with forceps, there appeared granulomatous nodules, 0.9–3.0 mm in size, on the luminal surface (Fig. 2). The blackish nodules were actually the sites of attachments of the amphistome trematode parasites and were created due to the powerful sucking action of the acetabulum of the trematode. This is clearly evident in the longitudinal microscopic sections cut across the nodule with the trematode attached in situ (Fig. 3). The degenerated cells and necrotic debris were evident in the studied section (Fig. 4). Migrational tracks were distributed at random throughout all areas of the livers. Colour progressively changed from brown-red in the smallest tracks to gray surrounded by a red zone of hyperemia. In the Microscopic examination of 6-4 μ m thick section cut through the granulomatous nodule revealed intense infiltration of inflammatory cells in the nodular part of the host tissue (Fig.5). Chronic infections were indicated by moderately dilated main bile ducts protruding somewhat on the visceral surface of the central and left lobes and progressively narrowing from the hilus towards the periphery. In the lumen of opened ducts yellowish green necrotic materials exudate contained numerous mature amphistomes. The walls were moderately thickened and fibrotic. Some areas of the lobe were commonly somewhat atrophied and cirrhotic with irregular scars distributed at random on the relatively uneven surfaces. The hepatic lymph nodes were slightly enlarged and greenish-brown on the cut surface. During the Parenchymal Phase Migrating immature flukes in the liver inflicted extensive mechanical and toxic damage to hepatocytes and other tissue components in tracks and closely surrounding areas. (Fig. 6). In smaller tracks tendency to haemorrhages was slight. In the larger tracks the macrophages produces pronounced haemorrhages. Often tissue elements surrounding the tracks were affected by a pronounced coagulative necrosis. The fine-grained brown material in the peripheral areas of some tracks was similar to the residue of ingested substance in the caeca of immature flukes and apparently had been defaecated by them. An incomplete rim of degenerated hepatocytes surrounding such tracks appeared more dark-stained compared to other areas. Healing migrational tracks were demonstrable in different stages in the large migratory areas.

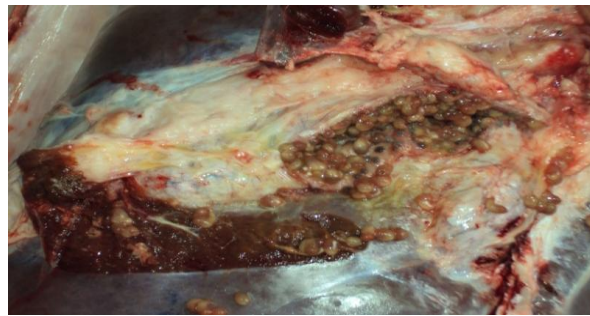


Fig. 1. Massive infection of adult amphistomes in bile duct and intrahepatic ductules.

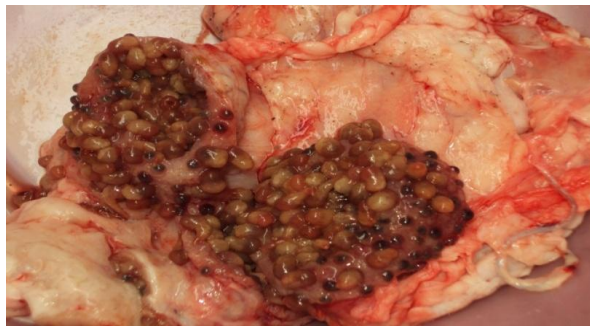


Fig. 2. The blackish granulomatous nodules on the luminal surface (0.9–3.0 mm in size).

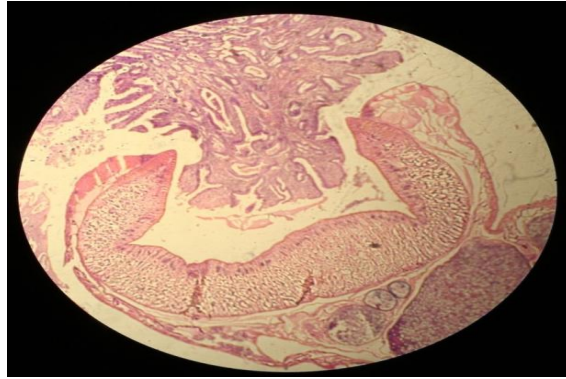


Fig. 3. The longitudinal microscopic section cut across the nodule with the trematode attached.

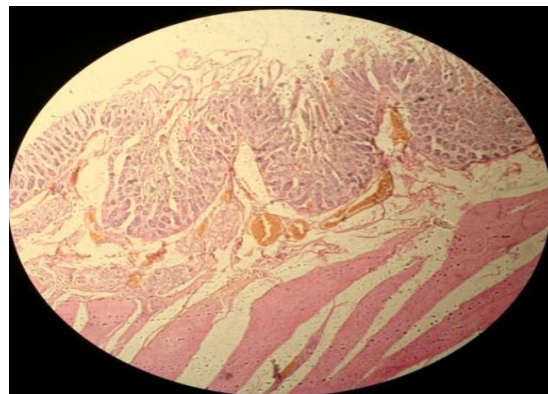


Fig. 4. The degenerated cells and necrotic debris were evident in the studied section.

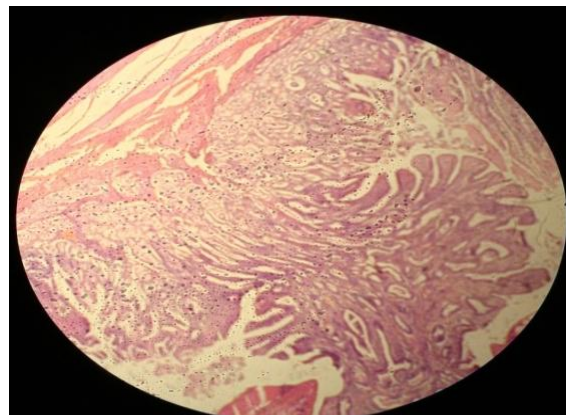


Fig. 5. The Microscopic section cut through the granulomatous nodule revealed intense infiltration of inflammatory cells in the nodular part of the host tissue.

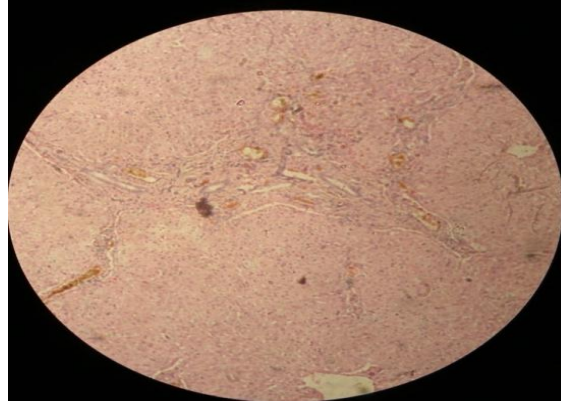


Fig. 6. Migrational tracks were distributed at random throughout all areas of the livers.

Macrophages were abundant, mainly in the areas of larger tracks. The reabsorbed areas of tracks were progressively replaced by proliferating fibroblasts. Proliferation of bile ductuli was extensive in healing tracks and in altered portal triads. The epithelial cells in bile ductuli were hyperplastic and not of uniform appearance. The most superficial areas of mucosa were commonly necrotic.

4. Discussion

The most of the available literature on the infection of bile ducts by trematode parasites is on cattle, it appears evident that most of the studies reported are related to the infection by the *Fasciola hepatica* in cattle (Serra-Freire, 1995) as well as in human beings (Pile et al. 2000). Human fascioliasis has also been reported to be endemic in some part of Far and Middle East (Bassily et al., 1989). Wild herbivorous mammals have also been found to be susceptible to *F. hepatica* infection (Menard et al. 2001). Fascioliasis is a zoonotic disease that infects wide varieties of mammalian hosts all around the world (Mas-Coma et al., 2005). There are also reports of fascioliasis in site other than liver, such as subcutaneous tissue and epididymis, brain, lungs, stomach and caecum (Makay et al., 2007). There is a common occurrence of *E. explanatum* and *F. gigantica* in the bile ducts of buffalo. The former is much more prevalent than the latter and are recorded in 17.37% of the cases examined. The reason could be the availability of the snail intermediate host in this geographical region. The description of natural infection by *E. explanatum* is sporadic (Upadhyay et al., 1987; Hafeez and Rao, 1989; Wiedosari et al., 1991; Ahmedullah et al., 2007).

In chronically infected livers the mast cells are apparently increased, as is the case in cirrhotic livers (Micijels 1939). It is noteworthy that majorities of the slaughtered animals investigated in the present study are not healthy and suffer from loss of weight. They are not usually considered for any further use and are sold to be slaughtered. They are brought here from different states of India but also from adjoining districts and far off districts of Kashmir. The predominant necrotic, histopathological and inflammatory findings in livers are consistent with those reported for *Fasciolopsis buski* infection in intestine of pigs (Haque and Siddiqi, 1978). The marked hyperplasia and hypertrophy of the mucosa of the bile ducts, associated with fibrosis is similar to that reported for *F. hepatica* infection in Black pigs (Maria Teresa et al. 2009), but they never observed any nodular lesion which are widespread, whereas in this study nodular lesions are widespread in all the cases examined. The reason may be the hold fast organ of the trematode, which in our case is an amphistome, remains firmly attached to the host tissue with their hold fast organ, causing nodule to form. In an experimentally infected pig, an intense fibrosis tissue encapsulating *F. hepatica* has been reported (Nansen et al. 1972). In the present study, such encapsulated flukes are never observed even in massive infection. My study concerning the histopathology of naturally infected amphistome livers confirm previous reports (Keck et al., 1967, Morrill et al., 1942, Nieberle, et al., 1967, Ross et al., 1966). In the present material considerable alterations during the parenchymal phase occurred in the migratory areas. Frequent thrombosis, significant haemorrhages, and extensive damage were associated mainly with the largest tracks. (Sandritter, et al., 1967, Schlichiit, 1963).

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