Natural trematode infection in liver of water buffalo (*Bubalus bubalis*): histopathological investigation

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Abstract This study reports the infection of liver and bile ducts, carried out from November 2008 to April 2010, on 730 randomly selected water buffaloes—*Bubalus bubalis*, infected with the amphistome trematode parasite *Explanatum explanatum* (Creplin, 1847) Fukui, 1929. Macroscopic examination revealed massive infection of adult fluke in bile ducts and intrahepatic ductules in 131 (18%) 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. 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.

Keywords Trematode · Histopathology · Liver · Water buffalo · *Expalnatum explanatum*

Introduction

Amongst the livestock in India, buffaloes are of great economic importance as they are closely associated with the life activities of resource-poor rural farmer. They make a critical contribution to food self sufficiency for households by providing milk, meat, skin, manure and traction. They usually graze in the open natural external environment, face regular natural calamities such as draught or flood and as such are very susceptible to many diseases. As a result the economic benefits remain marginal due to prevailing diseases, poor nutrition, poor animal health and general lack of veterinary care. *Expalnatum explanatum* (= *Gigantocotyle explanatum*) is a very common trematode parasite found in the liver of water buffalo and has a wide geographical distribution in India and neighboring countries. It is localized in the main bile ducts and intrahepatic ductules. The parasite may cause mortality, reduction in growth rate as well as reduction in the production of meat and milk.

The digenetic life cycle of *E. explanatum* requires an intermediate host; species of snails, genus *Lymnaea*. The adult worms reside in the bile ducts of buffalo liver. The eggs are discharged along with the feces of the host. Free swimming larva called miracidium is hatched out of the egg and penetrates the snails of the specific genus and undergoes a series of larval development, changing to sporocyst, redia, daughter redia and finally cercaria. The cercariae emerge out of the snail into the external environment, swim free and finally get encysted on vegetation to become metacercaria, where they remain until ingested by the final host. In the intestine of buffalo the cyst wall dissolves and the young trematode become free. The trematode penetrates the intestinal wall to reach the peritoneum, migrate to the surface of liver, penetrates and finally reaches the bile ducts.

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In the present study we describe the macroscopic and histopathological findings of the natural infections in the liver of water buffalo caused by this trematode parasite.

Materials and methods

The study was conducted over a period of 18 months from November 2008 to April 2010 on buffaloes which are regularly slaughtered at the local abattoirs in Patna district. Livers were collected from the abattoirs soon after the slaughter and brought to the laboratory. The livers were examined for histopathological changes and dissected to expose the bile ducts. The common bile ducts and intrahepatic ductules were cut open and detailed investigation was carried out. Samples having gross appearance of lesions with or without parasites in situ were fixed in Bouins fixative, processed and embedded in paraffin. Tissue section of 4 μ m thickness were cut adjacent to and through the site of attachment of individual worm and stained with hematoxylin and eosin for routine histopathology.

Results

On the average 30 animals are slaughtered daily. During the period of investigation it became possible to examine 730 randomly selected livers, out of which 131 (18%) were found to be infected. The intensity of *E. explanatum* infection varied across the study period. In majority of cases the number of flukes per infected liver cross over 200, and such cases were considered as having a massive infection. In a particular case, a chosen piece of 225 g of infected liver contains over 357 fluke. Out of 131 infected livers 107 (82%) were having massive infection. In 14 (2%) of the infected livers examined, co-infection with *Fasciola gigantica* was also detected. In such cases *E. explanatum* were found to be restricted to main bile ducts whilst *F. gigantica* were localized deep in the intrahepatic ductules.

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 parietal chronic inflammation involving mucosa and submucosa. The lumen of the bile ducts contained mature flukes and yellowish green necrotic materials, so much so that the parasites were found thickly covered with these necrotic materials (Fig. 1). 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 6 $\times$ 4 mm, being attached throughout the luminal wall (Fig. 1, arrow 2). Similar

Fig. 1 A cut through the buffalo liver infected by the trematode *Expalnatum explanatum*, showing lesions along with dilated and thickened bile duct. Arrow 1 points to the granulomatous nodules, the point of attachment of parasites and arrow 2 indicates numerous adult flukes attached throughout the luminal surface of the bile duct which has been cut open

situation were observed when other intrahepatic ductules of the biliary passage were dissected out. When the parasites were pulled apart with forceps, there appeared granulomatous nodules, 2–3 mm in size, on the luminal surface (Fig. 1, arrow 1). Those bluish black nodules were actually the sites of attachments of the amphistome trematode parasites and were created due to the powerful sucking action of the holdfast organ or 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. 2).

Microscopic examination of 4 μ m thick section cut through the granulomatous nodule revealed intense infiltration of inflammatory cells in the nodular part of the host tissue. These inflammatory cells included lymphocytes,

Fig. 2 Micrograph of a longitudinal section through a granulomatous nodule with the trematode *Expalnatum explanatum* attached in situ, showing how the acetabulum of the fluke holds fast on the host tissue, thereby creating the nodule (H&E $\times$ 100)

macrophages, plasma cells, eosinophils as well as fibrocytes, which were indicative of the chronic nature of infection (Fig. 3). Lymphocytes were predominant among the inflammatory cell that might be a manifestation of strong immune response of the host. A fibroblastic reaction was also indicative due to the presence of fibroblasts and fibrocytes in the tissue section. Also seen in the section were degenerated cells and necrotic debris (Fig. 4).

Discussion

Considering the available literature on the infection of bile ducts of cattle by trematode parasites, it appears evident that most of the studies reported are related to the infection by the *Fasciola hepatica* in cattle (Serra-Freire 1995) as

Fig. 3 Buffalo liver. Histological study of granulomatous nodules shows chronic inflammation with infiltration of macrophages, eosinophils, plasma cells and lymphocytes (H&E $\times 400$)

Fig. 4 Buffalo liver. Histological study of the bile duct shows fibrosis. Fibroblast and fibrocytes are visible in the tissue section (H&E $\times 400$)

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 (see Makay et al. 2007).

Interestingly in the present study, infection of liver by *F. hepatica* is not detected in any of the cases examined; instead there is a common occurrence of *E. explanatum* and *F. gigantica* in the bile ducts of water buffalo. The former is much more prevalent than the latter and are recorded in 18% 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).

It is noteworthy that majority 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 not from the Patna district alone but also from adjoining districts and far off districts and are said to include even those which are brought from Bangladesh. 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), however they never observed any nodular lesion, 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.

If parasitism is considered a biological indicator of an animal's welfare and state of health than judging from the high level of prevalence, intensity and abundance of natural infection, amphistomiasis appears to be endemic in and around this wide spread region and probably represents one of the most important animal health problems. Furthermore, considering the lack of veterinary care and facilities it also appears to be one of the most neglected diseases of cattle and related livestock. It is hoped that this

study, besides dealing with the histopathological aspect, will also provide a starting point for educating the small-holder farmer regarding the economic importance of infection of these amphistome parasites and also for the development of control strategies.

Since the transmission of infection is linked to intermediate host and is food-borne, epidemiologically based control strategies is suggested. People should be educated to avoid livestock from feeding on watercress and other aquatic plants and drinking from road side ditches, stagnant water bodies and flooding zones.

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