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RESEARCH PAPER

Seasonal histopathological alterations caused by *Bothriocephalus acheilognathi* in *Schizothorax niger* of Kashmir valley

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Abstract : Liver and intestines affected by an cestode parasite *viz., Bothriocephalus acheilognathi* along with their host (*Schizothorax niger)* were collected randomly. *Bothriocephalus acheilognathi* causes damage to the intestinal tissues and induce complex host response; the affected fish were anemic and emaciated. The viscera were dark red on opening the abdomen. The intestinal contents showed lot of mucous and contained dark contents. The mucosal wall was red in colour and revealed necrotic surface. The histopathological changes were varied in severity with the season and parasitic burden. Intestinal infection of *Bothriocephalus* with *Adenoscolex* was usually seen together. Severe enteritis with heavy infilitration of inflammmtory cells and fibroblasts seen in lamina propia. Liver cells showed severe degenerative changes.

Key Words : *Bothriocephalus acheilognathi*, Schizothorax niger hyperplastic, Enteritis, Fibroblasts

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INTRODUCTION

B. acheilognathi have originated from China and Eastern Russia and is now found almost in every part of world commonly known as Asian fish tapeworm (Riggs, 1986). The pathogenic effects of *B.acheilognathi* include blockage and perforation of intestine, distended abdomen, inflammation, haemorrhaging, and loss of intestinal lining which can have severe impact on growth and condition of fish (Hansen *et al.*, 2005). The ability of endoparasites to detect even lowest metal concentration due to their enormous accumulation capacity has made them suitable water pollution

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biomonitors than their fish hosts (Elijah *et al*., 2010). Recently Sures (2007) indicated that parasites can also act as metal sinks for its fish host. The distribution of parasites varies not only in different species of fish but also seasonally and from one water body to other. The pathogenicity of parasitism has been reported to cause extensive damage to the host leading to the lower production of the fish (Rai, 1986). Pollution stress can influence the prevalence of parasites directly or indirectly, or the parasite infestation may decrease the host resistance to toxic pollutants (Khan and Thulin, 1991). Intestinal helminths of fish are of increasing interest as potential bioindicators for heavy metal contamination in aquatic habitats. Among these parasites cestodes and acanthocephalans in particular have an enormous heavy metal accumulation capacity exceeding that of established free living sentinels. Metal concentrations several thousand times higher in acanthocephalans than in host tissues has been described from field and laboratory studies.The objective of present study was to assess histopathological changes caused by *Bothriocephalus acheilognathi* in the in the liver and intestines of affected fishes.

MATERIAL AND METHODS

Gross pathology:

Fishes were systematically subjected to detailed macroscopic examination with special emphasis on liver, intestine and the lesions were recorded.

Histopathology:

Representative tissue samples from the liver, intestine affected by parasites were collected in 10% formalin. The tissue samples were processed for routine paraffin embedding technique and 5u thin section were stained with Haris haematoxylin and Eosin (Bernet *et al*., 1999).

Histochemistry:

Sister tissue section selected on the basis of histopathological examination was stained for following histochemical observations.

– Determination of acid and neutral mucin by combined alcian blue Periodic-acid Schiff (PAS) stain (Bancroft and Gamble, 2002).

/0 Determination of mast cells by toluidine blue staining protocol (Gandalfo *et al*., 2006).

RESULTS AND DISCUSSION

The affected fish were anemic and emaciated. The viscera were dark red on opening the abdomen. The intestinal contents showed lot of mucous and contained dark contents. The mucosal wall was red in colour and revealed necrotic surface (Fig. 1). The histopathological changes were varied in severity with the season and parasitic burden. During spring Intestinal infection of *Bothriocephalus* with *Adenoscolex* was usually seen together. Severe enteritis with heavy infilitration of inflammmtory cells and fibroblasts seen in lamina propia. Goblet hyperplasia was seen alongwith elucidation of acid mucopolysaccharide (Fig. 2). During summer intestinal infection compared to spring season, the enteritis changes with less severe. The lamina propria was infiltrated with inflammatory cells like granulocytes and lymphocytes. Mast cell infiltration was evident with scattered metachromatic granules evident about the lesion (Fig. 3). During autumn intestinal infection wirh severe enteritis was seen with infiltration of mononuclear cells and desquamated necrotic cells. Liver cells showed severe degenerative changes (Fig. 4). During winter enteritis was less severe with infiltration of cells in lamina propria and lamina epithelium (Fig. 5). Enterocysts showed degenerative changes. Goblet cells hyperplasia with presence of acid mucopolysaccharide was seen. The histopathological alterations observed in the intestine of fish (severe degenerative and necrotic changes in the intestinal mucosa as well as edema between submucosa and mucosa) might be the result of cestodes, and has previouslsly been attributed also to the uptake of toxic metals (Hanna *et al*., 2005). Uptake of metals occurs mainly through gills but may also occur via intestinal epithelium (Mohamed, 2008). Toxic lesions most common in the intestine of fishes exposed to cadmium chloride include atrophy in the muscularis, degenerative changes in the tips of villi and necrosis of submucosa (Kaoud *et al*., 2011). In the intestine of *Channa punctatus* exposed to HgCl, the degenerative changes in the tips of mucosal folds, 2 hypertrophy and necrosis were observed (Sastri and Gupta, 1978). Similarly, cellular debris, vacuolation in intestine of *Mugil auratus* exposed to inorganic and organic mercury were also observed in another study (Establier *et al*., 1978). The major damage induced by cestodes consisted of necrosis and sloughing of stomach and intestine epithelium. At the site of infection, numerous RCs and mucous cells were seen in the epithelium. Rodlet cells are exclusive to fish, and their ultrastructure is well

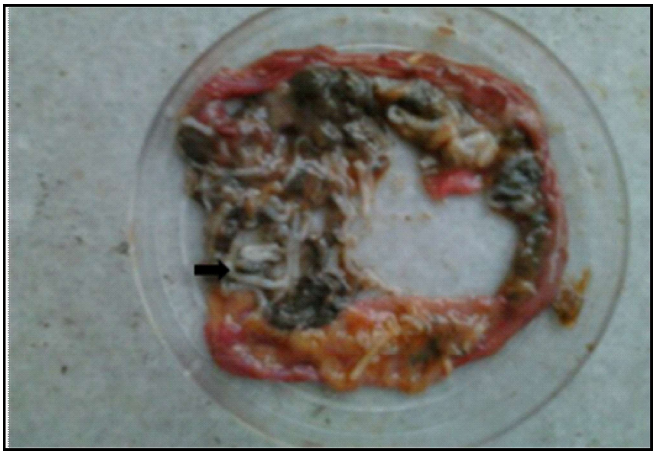

Fig. 1: The mucosal wall was red in colour and revealed necrotic surface. *Bothriocephalus* seen in contents (arrow)

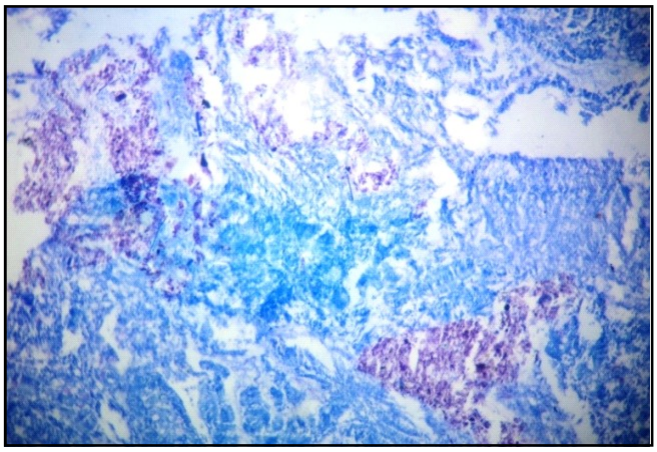

Fig. 3: Mast cell infiltration was evident with scattered metachromatic granules during summer season. Toluidine blue stain X 25

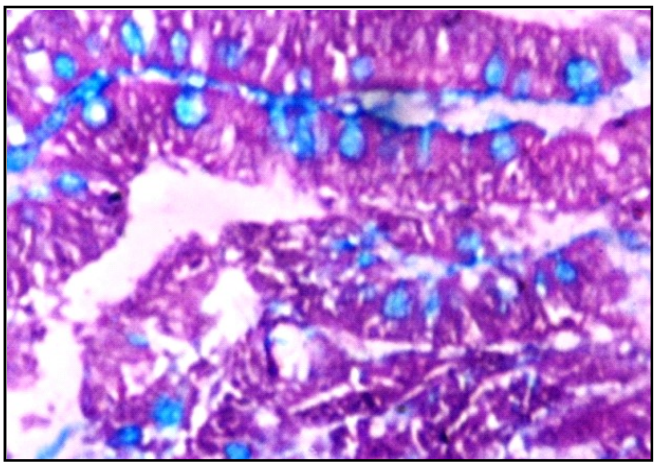

Fig. 2: Goblet hyperplasia was seen alongwith Elucidation of acid mucopolysaccharide during spring season. Alcian blue PAS staining X 80

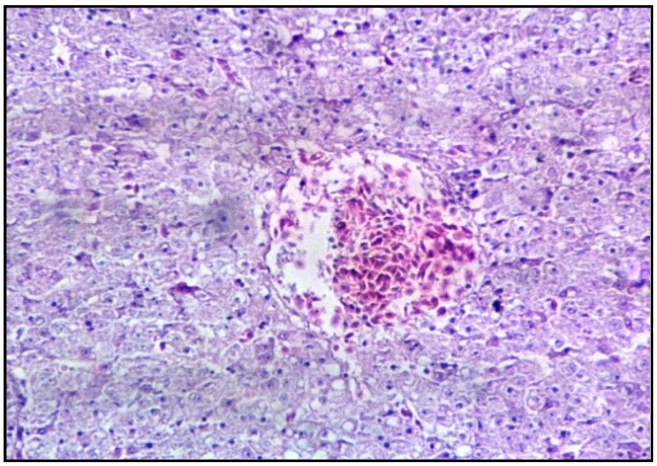

Fig. 4: Liver: Cells showed severe degenerative changes during autumn season. H and E X 69

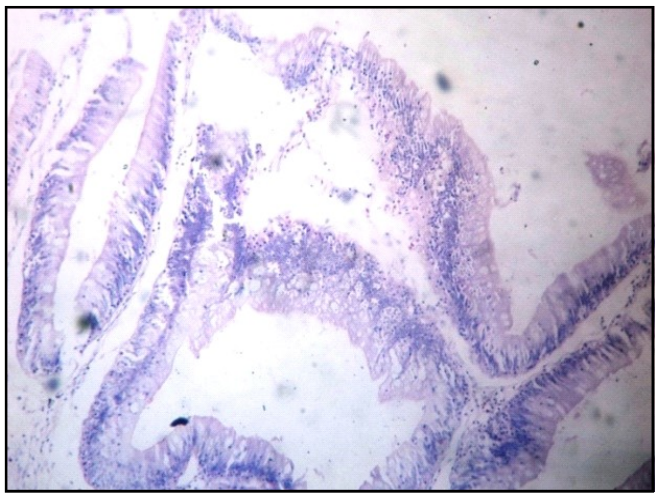

Fig. 5 : Intestine: Enteritis was less severe with infiltration of cells in lamina propria and lamina epithelium during winter season. H and E X 28

known. Data from several recent surveys of wild and farmed fish support the suggestion that RCs are an immune cell type closely related to other piscine inflammatory cells such as mast cells (Dezfuli *et al*., 2008; Jordanova *et al*., 2007; Reite, 2005; Reite and Evensen, 2006 and Vigliano *et al*., 2009). The attachment organ of endoparasitic helminths often provokes inflammation of the host gastrointestinal tract. Inflammation is a protective reaction of the host in response to injury, resulting in specific chemical and morphological alterations in cells and tissues (Suzuki and Iida, 1992). Severe enteritis with heavy infilitration of inflammmtory cells and fibroblasts wre seen in lamina propia. Lamina epithelialis showed severe desquamation. Necrotic intestinal villi were evident. Goblet hyperplasia was seen alongwith elucidation of acid mucopolysaccharide. It appears that several peptides involved in the regulation of intestinal mucus secretion are released during inflammation (Fairweather, 1997; Lamont, 1992 and Plaisancie *et al*., 1998). Fish mucus is involved in a wide range of functions, including respiration, reproduction, excretion, feeding, ionic and osmotic regulation, and protection against and resistance to, disease (Schroers *et al*., 2009; Shephard, 1994; Smirnova *et al*., 2003 and Yan *et al*., 2007). It has been reported that, in some fish species, mucous cells produce and release defensive materials (Cho *et al*., 2002 and Nakamura *et al*., 2001). In the present study, in parasitized *Schizothorax niger*, hyperplasia of intestinal mucous cells and enhanced mucus secretion were documented. Cestode bodies were often covered with an adherent mucus blanket. Our data are in agreement with the suggestion that the mucus gel layer protects the underlying epithelium as a physical barrier against pathogens and their toxins (Lamont, 1992 and Schroers *et al*., 2009). *C. rudolphii* larvae induced severe damage within the tunica propria and on the external surface of the stomach and intestine, with conspicuous granulomas. Data on fish granulomas provoked by helminths have been reported by Taraschewski (1988 and 1989) and Karanis and Taraschewski (1993). It has been reported that mast cells are major effector cells in the immune response to helminth infection (Dezfuli *et al*., 2008 and Sharp *et al*., 1989) and suggested that mast cells or their products are pivotal in mediating leukocyte recruitment to inflammatory sites (Mekori, 2004). Mast cells have been associated with defense against bacteria (Wedemeyer *et al*., 2000) and metazoan parasites (Dezfuli *et al*., 2000 and 2008; Dezfuli and Giari, 2008 and Reite, 2005). Their primary function is considered to be stimulating the activation of cells such as neutrophils to kill pathogens (Reite and Evensen, 2006), but some evidence suggests that they may also participate directly in killing microbes (Murray *et al*., 2007; Silphaduang *et al*., 2006 and Silphaduang and Noga, 2001). Liver of fish is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver and exposing it to a much higher levels than in the environment, or in other organs (Oliveira-Ribeiro *et al*., 2002). The liver of fish infected with cestodes showed marked histopathological changes. Degeneration and necrosis of the hepatocytes might be due to the cumulative effect of metals and the increase in their concentrations in the liver. These results agreed with Authman and Abbas (2007) who stated that the liver has an important detoxical role of endogenous waste products as well as externally derived toxins as heavy metals. The cellular degeneration in the liver might also be due to oxygen deficiency as a result of gill degeneration and/or to the vascular dilation and intravascular haemolysis observed in the blood vessels with subsequent stasis of blood (Mohamed, 2001). Many authors have reported similar histopathological alterations in the liver of fish exposed to metals (Athikesavan *et al*., 2006; Triebskorn *et al*., 2007 and Van *et al*., 2007).

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