Histopathological effects of larval trematodes on the digestive gland of freshwater snail species, *Vivipara bengalensis* and *Lymnaea acuminata*

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Abstract Comparative histopathological effects were observed on the digestive glands (hepatopancreas) of freshwater snail species, *Vivipara bengalensis* and *Lymnaea acuminata* infected with single (furcocercous cercariae) and double infection of digenean trematode larvae (gymnocephalous cercariae + metacercariae), respectively. Digestive glands of both the snail species revealed degenerative changes in their digestive gland tubules. The latter became irregular in shape, reduced in size with enlarged lumen and inter-tubular space besides rupturing of digestive tubules. These changes were correlated with the size and types of trematode larvae, single or double infection and degree of parasitemia. Autolytic necrosis of numerous tubules was found in digestive glands infected with double and very severe single infection. None of the tubules was found to be invaded by sporocyst, redia and cercaria. However, metacercariae were seen to invade digestive tubules of *Lymnaea* species.

Keywords Digestive gland · Histopathological effects · *Lymnaea acuminata* · Larval trematodes · *Vivipara bengalensis*

Abbreviations

ADGT	Atrophic digestive gland tubule
AN	Autolytic necrosis
C	Cercaria
CEC	Columnar epithelial cells
CT	Connective tissue

DG	Digestive gland
DGT	Digestive gland tubule
L	Lumen
MC	Metacercaria
R	Redia
SP	Sporocyst
TP	Tunica propria

Introduction

Gastropod snails have been reported to be vector of certain larval digeneans (Choubisa and Sharma 1986; Choubisa 2008a). These snails harbour various developmental stages such as sporocysts, rediae and cercariae of adult trematodes. During their multiplication and growth they obtain nutrients from infected tissues like digestive gland, gonads/ovotestis and other visceral organs of snail host. This leads not only to the diverse histopathological changes in the snails, but physiological disturbances too (Huffman and Fried 1985; Soomro et al. 2005; Huffman et al. 2009). Besides the snail species, various species of bivalves (Pelecypoda) also serve as vectors for many digeneans. Like snails these bivalves also harbour sporocysts, rediae, cercariae and metacercariae of trematodes in their digestive gland, reproductive system and other parts of body where these larvae cause various damages (Jonsson and Andre 1992; Valderrama et al. 2004). However, there is scant information on comparative histopathological account of the digestive gland infected with single and double infection of different types of larval digeneans (Choubisa and Sharma 1986). Similarly, cases of developmental stages as cercariae and metacercariae of different trematode species occurring in the same snail species are rarely reported. The

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present investigation attempts to report the histopathogenesis of large sized active furcocercous cercaria, *Cercaria gurayai* (Choubisa 1990) and gymnocephalous cercaria, *Cercaria johrii* (Choubisa 1985) and metacercaria (unknown species) in the digestive gland of *Vivipara bengalensis* and *Lymnaea acuminata*, respectively.

Materials and methods

A total of 85 mature specimens of *V. bengalensis* and 40 of *L. acuminata* were collected from the perennial river Som Kamla of Udaipur district of Rajasthan (India) in the summer season (2011). They were brought to the laboratory and maintained in separate aquaria containing fresh water and aquatic plants. The next day, larval digeneans were recovered from infected snails and identified as earlier reported (Choubisa 2008a). The infected and uninfected digestive gland (hepatopancreas) of these snail species were processed for histology as described in previous reports (Choubisa 1988). The stained sections were observed using Olympus Ch20i TR microscope equipped with Olympus E 450 digital camera.

Results and discussion

Out of 85 specimens of *V. bengalensis*, 22 (25.4 %) were found to be infected with furcocercous cercariae, *Cercaria gurayai* and their large sized sporocysts (single infection). Two specimens (5 %) of *Lymnaea* species were found to be infected with gymnocephalous cercariae and their rediae and unidentified metacercariae (double infection). In aforesaid snails a group of 7–10 uncysted metacercariae were found. Digestive glands of both the snail species infected with single or double species of trematode larvae appeared as loose mass with brownish coloration.

Histology of healthy digestive gland

Healthy digestive glands of both the snail species, *V. bengalensis* (Fig. 1a) and *L. acuminata* (Fig. 2a) can be easily identified due to their dark coloration. The gland is composed of numerous oval to elongated branched or unbranched tubules having lumen surrounded by single layer of epithelial cells, digestive/absorptive, secretory and undifferentiated cells. Digestive gland tubules are generally connected to each other by fine connective tissues (Fig. 1a) but in *Lymnaea* species these are compactly arranged (Fig. 2a). The whole mass of digestive gland was found enclosed in the outermost membrane, the tunica propria.

Fig. 1 **a** Histology of healthy digestive gland of *V. bengalensis* stained with haematoxylin and eosin (x 100). **b** Digestive gland infected with single infection of sporocysts and their furcocercous cercariae *C. gurayai* showing various degenerative changes associated with moderate (x 100) and **c** Severe (x 100) parasitemia

Histopathological changes in parasitized digestive gland

In both the snail species infected digestive glands appeared brown or grey in colour, swollen and friable. In heavily

Fig. 2 **a** Histology of healthy digestive gland of *L. acuminata* stained with haematoxylin and eosin (x 100), **b** Digestive gland severely infected with double infection of cercariae (*Cercaria johrii*) and metacercariae showing disorganisation (x 400) and **c** Autolytic necrosis of digestive gland tubules (x100)

infected digestive glands, tunica propria was either irregular or ruptured due to parasitic burden.

In general, areas of inter-tubular connective tissues were occupied by parthenitae. None of the digestive gland tubules was found to be infected or invaded by sporocysts, rediae and cercariae (Fig. 1b, c and Fig. 2b, c). It was

interesting to note that some tubules had massive infection involving a number of metacercariae and other had only one larva (Fig. 2b, c). The infected tissue discerned two types of damage; (i) mechanical and (ii) physiological. Mechanical damage was observed in the form of degenerating changes such as rupture of tunica propria, reduced diameter, irregular shape of digestive tubules and consequently enlargement of inter-tubular areas, decrease in height of columnar epithelial cells as well as increase in volume of intra-tubular space or lumen and complete or partial destruction and blocking of individual tubules, few neighbouring tubules however remained unaffected (Fig. 1b and Fig. 2b). Physiological damage was observed in the form of loss of tissue integrity and necrosis or lysis of epithelial cells in heavily infected digestive gland (Fig. 1c and Fig. 2c). These histopathological alterations were not present in all cases and depended on the degree of parasitemia and were more often found to be related with rediae of gymnocephalous cercariae and metacercariae rather than with sporocysts of furcocercous cercariae.

Whether snail species infected with single or double infection, the variety of histopathological changes in digestive glands and ovotestis induced by larval digenae trematode parasites was found to be dependent upon the severity of infection, size and types of larvae. The mechanical damages of the digestive tubules appear to be the cumulative effect of the larval migration/movement, feeding, growth and their multiplication. The physiological changes as autolysis and/or necrosis are possibly the result of the release of proteolytic enzymes from the ruptured digestive cells/or enzymatic secretion and metabolites from trematode larvae. This lends support from previous reports of certain snail species (Choubisa 1988, 2008b). Starvation autolysis was due to squeezing of digestive tubules at different loci and consequently no food was able to pass into the tubules. Therefore, there is a possibility of intra-cellular digestion. Hence, atrophy of digestive tubules was generally found in heavy infection, especially in double infection as has been observed in earlier studies (Mohandas 1974; Choubisa 1988). Another possible reason for reduction in size of epithelial cells and increasing of inter and intra-tubular spaces of glands is reduction in the amount of stored nutrients and energy due to increasing demands of the developing host gonads as well as developing or multiplying parthenitae (Bertman 1980). It has been discussed in earlier studies (Mohandas 1977; Choubisa 1988) that redial stages cause more mechanical and physiological damage as compared to sporocysts because of their mouth and locomotory organs while their pharyngeal glands and gut contribute for the physiological damages. It has also been observed that rediae engulf the host's digestive cells and utilize the hydrolases (Choubisa 1988, 2008b) for their extra cellular digestion. Thus it can

be conjectured that redial stages are more destructive for the host tissues in the present cases. Besides this, other contributory factors can be parasitic secretions and excretory products that produce toxic effects (Erasmus 1972). In the present study, intensity of destruction was greater in case of dual infection, as the uncysted metacercariae and rediae of gymnocephalous cercariae possess well developed digestive system and locomotory organs.

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