Observations on the histopathological changes caused by myxosporidian infections in minor carps

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Abstract Myxozoan infections result in large scale histopathological features depending on various factors. Earlier studies on pathological changes caused by myxozoan infections in piscine hosts have been reported from different localities. This study is based on the histopathological features caused by the members of genera *Myxobolus* Bütschli, 1882 and *Thelohanellus* Kudo, 1933 in the gill and fin tissues of commercially significant minor carps, *Labeo calbasu* and *Labeo gonius* of the genus *Labeo* collected from the four different districts of the north-eastern Indian state of Tripura (latitude 22°51'–24°32'N and longitude 90°10'–92°21'E) spanning over a total geographical area of 10,491 km².

Keywords Histopathology · Myxozoan · Minor carps · Tripura

Introduction

The Myxozoans are capable of causing major deleterious effects on its hosts (Kent et al. 2001). Myxozoan infections

result in large scale histopathological features according to the infection stage and host susceptibility; histozoic parasite species elicit greater pathological response compared to coelozoic species (Feist and Longshaw 2008). There are several earlier reports of histopathological studies of myxosporidian gill infections from different localities (Shulman 1957; Dykova and Lom 1978; Molnar 1979; Sanaullah and Ahmed 1980; Ventura and Paperna 1984). Recently an appraisal of histopathological studies of the myxozoan infections was done by Dykova and Lom (2007). Sanaullah and Ahmed (1980) reported that myxosporidian infection was a primary cause of disease condition in carp ponds of Bangladesh which along with secondary infections leads to mortality.

In the present work, one thousand two hundred and thirty-nine host fishes comprising four minor carp species *Labeo calbasu*, *L. bata*, *L. gonius* and *Cirrhinus reba* within range of 5–35 cm of total body length were examined in a period of two years. Histopathological studies were done on gill epithelium and fin tissues that were grossly infected by myxozoan group of parasites comprising the members of genera *Myxobolus* Bütschli, 1882 and *Thelohanellus* Kudo, 1933.

Materials and methods

The host tissues along with the parasites were fixed and preserved in 5 % NBF. Hard tissues were decalcified using 10 % aqueous formic acid and 25 % aqueous sodium citrate before dehydration in alcohol series. The tissues cleared in xylene or benzene were transferred into hot air oven containing paraffin wax maintained at a temperature of 600–650 °C for infiltration process. For attaining

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maximum purification four changes was done in the paraffin wax. After the infiltration process, paraffin wax moulds or blocks were made for embedding the tissues containing the parasites. Tissues were kept in different orientations as per L.S. or T.S. sections. Utmost care was taken to prevent formation of white areas, water bubbles and dry spaces in the paraffin blocks.

The sectioning was done using Manual Rotary Microtome (Leica RM 2235). Neatly trimmed blocks were attached to the holder of the microtome and the thickness of the microsections was fixed at $7\ \mu$. Haematoxylin was the nuclear stain and eosin was the cytoplasmic stain used. The stained histopathological slides were studied under transmission light microscope and microphotographs of the tissues were taken using a dedicated digital camera.

The pathological changes caused by *Myxobolus* spp. (Infection prevalence-50/1239; 4.04 %) on the gills of the host fishes, and by *Thelohanellus* spp. (Infection prevalence-83/1239; 6.70 %) on the fins of the hosts were investigated in the present study.

Results and discussion

Pathological damages caused by protozoan infections

In gills

The gills carrying a heavy load of parasite cysts was highly mucus laden, had a very thick coat of slime on the surface and showed a pale appearance. Large number of spherical white parasite cysts (plasmodia) of about 1 mm in size were attached to the tips of the gill filaments. The sections of gills revealed that the functional respiratory components of the gills namely the gill lamellae were affected by the parasitic infection (Plate 1).

The tips of the gill lamellae were enveloped by large mass of plasmodia enclosed by the flattened gill epithelial cells. A large amount of mucus secretion was visible as a result of host tissue response. Swelling of the host tissues was observed in the attachment site of the large sized cysts or plasmodia. There was no cellular changes in the adjacent uninfected tissues. Large-scale gill myxoboliasis hampers

Plate 1 Photomicrographs of gill sections of *Labeo calbasu* infected with *Myxobolus* spp. **a** Sections of normal uninfected gills. **b** A section of the gill showing developing cyst(plasmodia) attached on the gill lamellae. **c** Photo micrograph comparing uninfected gills and

a cyst attached gill filament. **d** Enlarged view of cyst attached on the gill filament, here the cyst wall has ruptured and the gill epithelium has been disintegrated, a large amount of mucus has been secreted as a result of host tissue reaction. Scalebars **a**, **c**, **d**—50 μ m, **b**—500 μ m

Plate 2 Photomicrographs of fin sections of *Labeo gonius* infected with *Thelohanellus* spp. **a, b** Comparing sections of uninfected fins and fins infected with cysts of *Thelohanellus* spp. **c, d** Sections showing erosion and dissolution of cartilaginous tissue at the site of

attachment of large sized cyst. **e** Section showing the younger stages of spores adhering to the peripheral layer of the host tissue. *Scalebars* **a, d**—200 μm, **b, c**—500 μm, **e**—50 μm

the normal respiratory function of the gills by replacing the lamellar spaces by large-sized plasmodia, thereby reducing the surface of air exchange and leading to stress and anoxic conditions. Molnar (1979) observed that the damage caused by myxozoan infection paves way for secondary microbial infections.

The present observation correlates with the results of histopathological studies done by Dykova and Lom (1978) on the gills of fishes infected by *Henneguya* spp. where

hyperplasia of the gill epithelium and disintegration of the gill lamellae was reported. Inflammatory response of host tissue after the maturity of spores was also reported, the same pathological response was observed in the present case also. Kalavati and Narasimhamurti (1985) also observed hypertrophy and vacuolisation of cytoplasm in the host cells surrounding the mature cyst which envelope the whole gill lamellae in the histopathological studies on gills of *Channa punctatus* infected by *Henneguya*

waltirensis and further stated that when the gills were very densely infected the host fishes showed extreme lethargy and motionless, there was very slow movement of the operculum during respiration.

In fins

Elongated and oval shaped cysts of about 3 mm in size was found attached to the inner portions of the fin rays and on the cartilaginous portions of the dorsal and caudal fins of the host tissues. The disintegration and erosion of the hoisting cartilaginous tissues was very evident. The integrity of the fins had been lost due to the parasitic infestation (Plate 2).

The histopathological changes observed in the fin sections in the present study correlates with the observations of Molnar (1982) which described the pathological changes seen in the caudal and pectoral fins of *Cyprinus carpio* infected with *Thelohanellus nikolskii*. Very remarkable deformation of fin characterised by distortion, breakdown and degeneration of the fin rays into powder like fragments and an external coat made of thick hyaline material around the cyst ectoplasm was observed and this was were reported by Molnar (1982) also. The gross damages caused in fins of host tissues by the protozoan infection result in overall weakness and slow swimming movements, finally making them susceptible to secondary bacterial and viral infections. From the present study, it is evident that a myxosporidian infection causes grave pathological changes in the gill and fin tissues of the minor carps.

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