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Histopathological study of parasitic effect of *Dactylogyrus vastator* (Nybelin, 1924) on the *Catla catla* in culture ponds in Darbhanga

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Abstract

The current investigation was undertaken to study the gill histopathologies due to infestation of helminth parasite, monogenetic trematode, *Dactylogyrus vastator* (Nybelin, 1924) in the Indian major carp, *Catla catla*. There were only symptomatic samples of diseased fishes comprised of fingerlings and adults of *Catla catla* collected and examined. Behaviour respond studied- partial suffocation, lethargic swimming and clinical sign observed as their gills were very pale in colour and had haemorrhagic and inflamed areas at the sites of parasite attachments. The significant histopathological lesions and changes were seen in the gills where the parasites were found attached to the lamellar tissues causing local erosions of the epithelium and hyperplasia of the secondary lamellar epithelium and fusion of adjacent secondary lamellae, vascular congestion leading to aneurysm besides, clumping of the tips in secondary lamellae, congested gill showed accumulation of blood leading to swelling hypertrophy in secondary lamellae, autolysis of epithelium and secondary lamellae and severe edema and necrosis were observed in gill of *C. catla*. No changes were noticed in other organs of the affected fishes. In severe infestations the fishes showed growth retardation. The histopathological analysis for parasites in fish will be useful tool for fish health management.

Keywords: *Dactylogyrus vastator*, histopathology, trematode, symptom, necrosis, *Catla catla*

Introduction

Monogenean species belongs to helminth parasites are well known for their high degree of host specificity (Jarkovsky *et al.*, 2004) [4]. The monogeneans are typically and often economically important ectoparasites of the skin or gills of fish. They are attached to the host surface by a characteristic opisthaptor which is species-specific and has hooks and hooklets (order Monopisthocotylea) or clamps (order Polyopisthocotylea). The monogenea are hermaphroditic flatworms and mainly ectoparasites of aquatic vertebrates. They are often found in the gills or on the external surface of the fish. The genus *Dactylogyrus* is the largest helminth genus, with more than 900 species and generally has high host specificity. Most *Dactylogyrus* species parasitize cyprinids although certain species are adapted to the more advanced fish families.

For the fish health management programme, it is essential therefore, to obtain timely and routinely precise diagnosis of any fish disease outbreak in fish culture pond. Throughout visual examination for external signs of the disease at the pond site should be followed by detailed Pathomorphological, Pathoanatomical and microscopical, examinations of squas and permanent stained preparations of the tissue samples of diseased fishes in the laboratory (Ramudu, & Dash, 2013) [8].

So, the objective of the current study was to isolate, identify and *Dactylogyrus sp.* infestation in Indian major carp, *Catla catla*.

Materials and Method

Frequent incidences of various naturally occurring diseases were recorded in the Indian major carp, *Catla catla* cultivated ponds/tanks in Darbhanga's various State Govt. ponds and also private fish culturists during the period (2020-2021) of observation.

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Only symptomatic and moribund samples of diseased fishes, catla were collected and were brought to the laboratory for examinations. Clinical examine start with Patho-morphological examination was comprised of identifying and locating any visible external lesions, erosions, haemorrhages etc. and formation of cysts/spores/patches on body surfaces, gills and fins and examined under the microscope for detecting aetiological agents. Patho-anatomical examination was then carried out for finding any visible lesions/inflammations in internal organs.

Small bits of tissues (3-4 mm thick) from the vital organs like skin, gills and intestine etc. of moribund or freshly killed diseased fish samples were collected and fixed in ten percent Neutral Buffered Formalin for 18-24 hours. It was prepared by dissolving 4 gm of sodium dihydrogen phosphate and 6.5 gm of Disodium hydrogen phosphate in 900 ml of distilled water. 100 ml of formaldehyde was then added and the fixative was ready for use. Fixed tissue samples were then processed and paraffin embedded blocks of all the tissues were prepared using the standard histological methods (Luna, 1968) [6]. Calcified tissues like skin and gills were decalcified in ten percent Nitric Acid which helped in getting perfect and unbroken serial sections of these tissues during microtomy. Tissue blocks were cut into serial sections (5-7 thick) by a rotary microtome. For routine staining of the histological sections, Ehrlich's Haematoxylin (H.) and alcoholic Eosin (E.) stains were prepared and used according to Luna (1968) [6]. Photomicrographs of the most characteristic regions of histopathological lesions in the stained tissues of diseased fish samples were taken.

Result

This disease has been found to occur predominantly in the nursery and rearing ponds and also in the large fish culture ponds. Frequent mass mortalities of fish in the rearing and stocking ponds due to this disease have been observed. The disease is caused by a monogenetic Trematode, *Dactylogyrus vastator* which attracted particularly the gills of the fishes.

Morphology of parasite: The body shape of *Dactylogyrus vastator* is elongated and cylindrical, length varied from 0.4 to 1.2 mm with a breadth of 0.2 to 0.27mm around testis and forward from here it narrow down towards the neck region. Two pairs eyes present at the anterior end a haptor measures about 120µm in diameter. It contains two large hooks or humuli or anchors and 7 pairs of marginal hooks of 29-33µm length. The body contains a cirrus and a vagina. The gut caeca unite together behind testis. The genital pore is situated slightly posterior to the bifurcating point of intestine.

Fish Species affected: Fingerlings, juveniles and adults of *Labeo rohita*, *Catla Catla*, and *Cirrhinus mrigala* have been found to be affected by this disease. Epizootic conditions due to this parasitic infestation in the rearing and large fish culture ponds have been recorded particularly in Indian major carps causing mass mortalities of these fishes.

Clinical Symptoms and Gross pathology: Highly affected fishes showed growth retardation, weight loss. partial suffocation, lethargic swimming behaviour, loss of appetite and tendency to take shelter near pond margin. Gills of fishes became very pale in colour and showed haemorrhagic and inflamed areas where the parasites got attached and had excess secretion of mucus. Squas preparation of a portion of

the affected gills showed the parasites attached to the gill tissue which confirmed like kidney, liver, spleen and intestine did not show significant gross pathology.

Histopathology: In normal gill of catla revealed typical teleostean plan. The primary and secondary lamellae were easily differentiable. The cartilaginous skeletal structure, several layered epithelium and blood vessels all appeared well ordered (Figure-1). The leaf like primary gill lamellae were arranged in two rows and from their lateral rides project the secondary lamellae. The mucus cells were mostly observed in inters lamellar epithelium between secondary lamellae (Figure-1).

Congested gill showed accumulation of blood thereby inducing swelling. Significant histopathological changes in the gill tissue was the excessive proliferation of secondary lamellar epithelial cells due parasites. There was local tissue erosion at the site of parasite attached. Extreme hyperplastic condition was observed in the secondary lamellar epithelium in the adjacent regions (Figure-2). Multi-focal haemorrhagic areas were seen in the primary lamellae in certain region of the gill section (Figure-3). Excessive proliferation of the secondary lamellar epithelium due to hyperplasia led to the fusion of adjacent lamellae blocking or reducing the flow of water between the lamellae. In certain site where the parasites were found in abundant numbers in the gills, the extent of hyperplasia of secondary lamellar epithelium and the degree of fusion of adjacent lamellae were such that the secondary gills lamellae lost their identity totally and the whole gills appeared like slender road with only the showed degenerative changes. Other vital organs of the affected samples did not show any histopathological changes (Figure-3).

Diagnostic histopathological lesions identified: Detection of the parasites in the squas preparation of the gill of the affected fishes helped in the diagnosis of the disease. The main histopathological lesion were seen in the gills where the parasites were found attached to the secondary lamellar epithelium and fusion of adjacent gill lamellae were the tissue level reactions caused by the parasites.



Fig 1: Section of normal gills of *Catla catla*.H.E x100



Fig 2: Gill of *Catla catla* (Ham) infected with *Dactylogyrus vastator* showing the parasites (P) attached to the secondary lamellae causing lamellar hyperplasia (LH) and haemorrhages (H) in the primary lamellae. H. E., X 200.

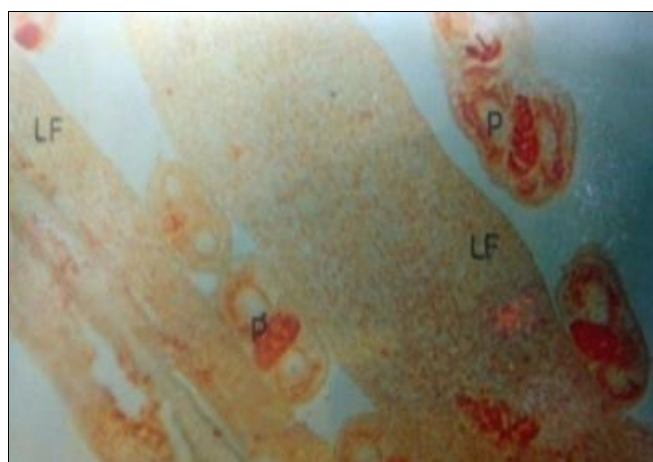


Fig 3: Gill of *Catla catla* (Ham) with *Dactylogyrus vastator*. infection showing extremely hyperplastic secondary lamellae leading to lamellar fusion (LF) due to parasites. H.E., X 200.

Discussion

Dactylogyrosis disease caused by the monogenetic trematode, *Dactylogyrus vastator* was found to cause severe damage in the gills of the highly affected fishes. Adults of these parasites having a row of hooks were found attached to the host's gill tissues and sometimes sucker as well. These hooks caused much damage to the gill tissue by penetrating through it. When large numbers of parasites were found attached to the gill, they caused extensive damage and trauma which made portals of entry for opportunistic invaders which in turn might caused further damage to the host.

The spread of *D. vastator* in Indian freshwater system including the current study area, in all likelihood took place through introduction of the exotic carps in aquaculture practices (Jalali and Barzegar, 2005) [3]. The identifying feature of *D. vastator*, which separate it from other closely related species such as *D. intermedius*, considered during the current case was the total length of dorsal anchor, shaft and root which are comparatively larger in *D. vastator*. Besides, the haptor armature and male copulatory organs also formed a basis of identification of the species was the total length of dorsal anchor, shaft and root which are comparatively larger in *D. vastator*. The species has special pathogenic effects on the gills of fish (Jalali and Barzegar, 2005) [3].

Histopathological changes and the extent of tissue damage on the gills of the highly affected fishes examined for the studies

were quite characteristic. Large numbers of the *Dactylogyrus sp.* parasites were found lodged and attached to the secondary lamellar tissue and induced excessive proliferation of the secondary lamellar epithelium due to hyperplasia around the sites of parasite attachments. At the sites of parasite attachment, the lamellar epithelium and the capillaries were found eroded leading to the destruction of the gill tissue. Also, at the places where the parasite concentration was more, multi-focal haemorrhagic areas were noticed in the primary lamellar tissues in certain zones of the gill sections. Fusion of adjacent secondary lamellae resulting from hyperplasia of the secondary lamellar epithelium due to the attachment of the parasites in the gills led to blocking or reducing the flow of water between the gill lamellae, reducing the flow of water between the gill lamellae. This resulted in asphyxia conditions in the affected fishes leading to mortality. Another significant histopathological change observed in certain regions of the gill section of the highly affected samples was the extreme hyperplasia of the secondary lamellar epithelium which rendered the whole gill look like slender rods with the cartilage cells placed centrally. The cartilage cells showed necrotic changes and had multi-focal haemorrhagic areas in the cartilaginous tissue of the affected gills.

The above histopathological changes observed in gills of Indian major carps, catla the host fish resemble to those reported earlier (Jalali and Barzegar, 2005; Baker *et al.*, 2007; Ramudu and Das, 2015; Saptiani *et al.* 2017) [3, 1, 9, 11].

Similarly, quite in conformity with the present findings Ramudu and Das (2015) [9] observed swelling of gill arch, loss of primary and secondary lamellae, and high secretion of mucous and epithelial hyperplasia in Indian major carps. Khalil, *et al.* (2018) [5] reported that gills of infected sea bass and sea bream infected by *Dactylogyrus sp.* showing parasitic infestation at the gill's tip, granulomatous inflammation also showing parasitic infestation, embedded trophonts, at the gill's tip also, and showing parasitic infestation, embedded trophonts. The results of the present histopathological studies on Dactylogyrosis of Indian major carps are in perfect agreement with those of Molnar (1972) [7] and Khalil (2018) [5].

Blood congestion and aneurysm observed in this study may be attributed to pillar cells injury resulting into sudden increased blood flow inside lamellae as suggested by Rosety-Rodriguez *et al.* (2002) [10]. Here the explanation behind congestion occurring due to parasitic infestation in fish gills given by Hadi and Alwan (2012) [2] also needs to be mentioned. They have suggested that congestion has inverse relationship with oxygenation. Hence, observed congestion is indicative of low oxygen supply to the gills as a result of gill lesions particularly fluid deposition leading to edema caused by the higher prevalence of *Dactylogyrus vastator*.

Conclusion

The main histopathological lesions and changes were seen in the gills where the parasites were found attached to the lamellar tissues causing local erosions of the epithelium. Hyperplasia of the secondary lamellar epithelium and fusion of adjacent secondary lamellae, increased secretion of mucus, edema, vascular congestion and clubbing of lamellar tips were the significant tissue level reactions caused by the parasites.

The findings accomplished that disease, Dactylogyrosis caused by the monogenetic trematode, *Dactylogyrus sp.* was observed predominantly in the nursery and rearing ponds. Fingerlings and adults of Catla were found highly susceptible

to this disease than other IMC. The infection may visualize with necked eyes and need to manage promptly to secure the health of Indian major carps.

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