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Priyanka Kumari
Department of Zoology, MLSM College, LNMU, Darbhanga, Bihar, India

Dr. MMR Nomani
Department of Zoology, MLSM College, LNMU, Darbhanga, Bihar, India

Corresponding Author:
Priyanka Kumari
Department of Zoology, MLSM College, LNMU, Darbhanga, Bihar, India

Study of intestinal histopathologies due to infestation of *Caryophyllaeus laticeps* (Pallas, 1781) in the common carp, *Cyprinus carpio*

Priyanka Kumari and Dr. MMR Nomani

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Abstract
The current investigation was undertaken to study the intestine histopathologies due to infestation of helminth parasite cestode, Caryophyllaeus laticeps (Pallas, 1781) in the Cyprinus carpio. Only symptomatic samples of diseased fishes comprised of juvenile and adults of common carp were collected and were patho-anatomical examined. The significant histopathological changes were observed in the intestine where the scolices attached, the vacuolization of the epithelial cells and rupture of the brush border and severe edema. Hyperplasia of the goblet cells and infiltration of inflammatory cells in lamina propia and its epithelium was desquamated in the infested silver carp. No changes were noticed in other organs of the affected fishes. In severe infestations the fishes showed growth retardation and mortality. Thus, it is clear that the endoparasites inhibiting intestine of C. carpio produced pronounced pathological changes in host tissue, all denoting impairment of intestinal physiology.

Keywords: *Caryophyllaeus laticeps*, *Cyprinus carpio*, helminth, histopathology, cestode, endoparasite

Introduction
Parasitic infections reportedly play a major role in disease occurrence (78%) in Indian freshwater aquaculture causing huge economic loss (Larka, 2006) [7]. Another study reports that Andhra Pradesh and West Bengal fish farmers put loss of 21 % and 26 % respectively in terms of production due to parasitic infestations and resultant diseases (Vineetha and Abraham, 2009; Ramudu and Dash, 2015) [16, 12].

The monozoic tapeworm *Caryophyllaens laticeps* (Pallas, 1781) is a common parasite of cyprinids. The population biology of C. *laticeps* has been investigated by several authors (Kennedy 1971; Anderson 1974; Pojmanska 1984) [6, 1, 11] and the morphology and fine structure has been described by Richards & Arme (1982) [13]. A light microscopical description exists of the host parasite interface of *C. laticeps* (Mackiewicz, *et al.* 1972) [9] and the ultra structure with electron microscope by Karanis and Taraschewsk (1993) [5].

The success and objectives of the fishery development programmes greatly depend on parasitological research and control of parasites by improving water quality since the improvement or increase in fish production/ fish yield can mainly be achieved from healthy fish stock (Thakur, 2021) [15].

So, the objectives of the current investigation was undertaken to study the intestine histopathologies due to infestation of helminth parasite cestode, *Caryophyllaeus laticeps* (Pallas, 1781) in the *Cyprinus carpio*.

Materials & methods
Frequent incidences of naturally occurring *Caryophyllaeus laticeps* infestation was recorded in the cultivated ponds/tanks/hatcheries in Darbhanga, Bihar during the period of observation (2020-2021). Collected samples were comprised of juveniles and adults of silver carp in most of the occasions. Only symptomatic and moribund samples of diseased fishes were collected and were brought to the laboratory for patho-morphological and patho-anatomical examinations. The specimens of *C. laticeps* were counted and measured with a binocular microscope as living worms under slight coverslip pressure. After that small bits of tissues (3-4 mm thick) from the intestine etc. of moribund or freshly killed diseased fish (silver carp) 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) [8]. 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) [8]. 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 juvenile in the fish culture ponds. The disease is caused by a cestode, *Caryophyllaeus laticeps* which attracted particularly the intestine of the fishes.

Fish Species affected: juveniles and adults of exotic carp, *Cyprinus carpio* has been found to be affected by this disease.

Clinical Symptoms and Gross pathology: Highly affected fishes showed growth retardation, weight loss, lethargic swimming behaviour, loss of appetite and tendency to take shelter near pond margin.

Histopathology

The normal intestine sections of the host fish *Cyprinus carpio* showed ordered architecture and cellular organization of the cells constituting atypical teleostean intestine (Figure-1.). Exhibited well ordered arrangement of the usual four layers i.e., serosa, muscularis, submucosa and mucosa. The mucosa is formed of columnar epitheliul cells thrown into a number of folds or villi. The nuclei occupied basal position. The submucosa, constituted of loose connective tissue, firms the central core of the villi known as lamina propia. The submucosal cells are gramular, richly vascular and eosinophilic in nature. The muscularies is composed of outer thin longitudinal muscules and inner thick circular muscles. The outer most thin vascular covering is the serosa shows distinct mucus secreting and brush border cells (Figure-1.).

The intestinal epithelium of the infected host fish was found to be locally compressed by the pressure of scolices. Besides, at the site where scolices attached, the vacuolization of the epithelial cells and rupture of the brush border were quite prominent and severe edema was also apparent (Figure-2.). Besides, infiltration of eosinophilic granulocytes formed an important pathological changes due to infestation of *Caryophyllaeus laticeps*. Macroscopically, excess mucus secretion and shedding of host tissues were clearly apparent. Again cellular infiltration, particularly of fibroblasts, was also noted at the site of attachment (Figure-3). Further, there was infiltration of inflammatory cells in lamina propria and its epithelium was desquamated. Besides, hyperplasia of goblet cells was also obvious (Figure-4). Other vital organs of the affected samples did not show any histopathological changes.

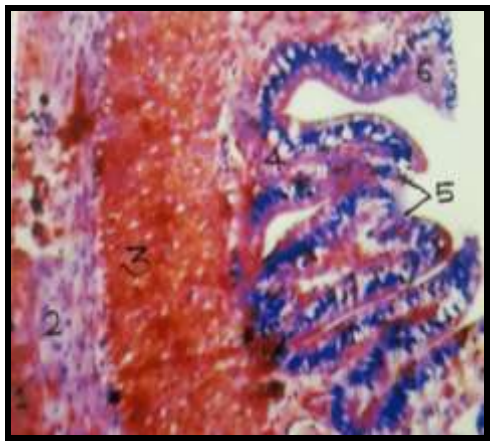

Fig 1: T. Section of normal intestine of *Cyprinus carpio* showing normal cellular organization H. & E., X 400.

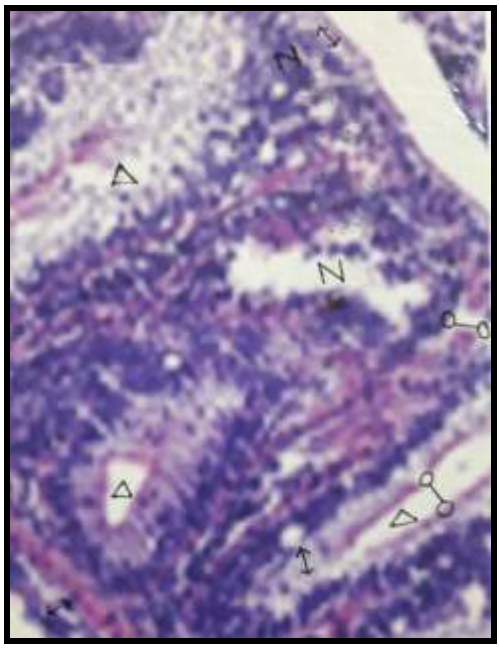

Fig 2: T.S. of intestine of the *Cyprinus carpio* reflecting vacuolization of epithelial cells ( ), rupture of brush border cells (o-o), necrosis (N) and severe edema ( ) due to infestation of *Caryophyllaeus laticeps*. H. & E., X 400.

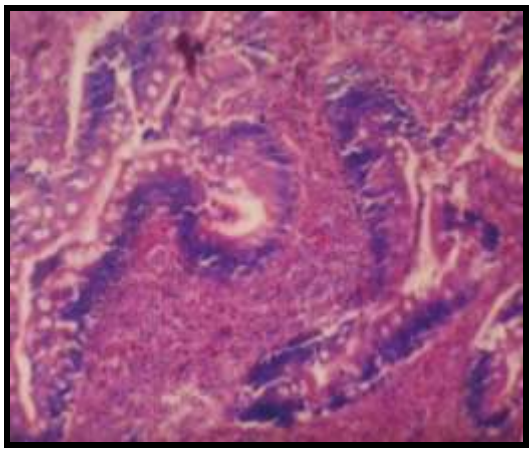

Fig 3: Infiltration of inflammatory cells in lamina propia in intestine of *Cyprinus carpio* due to infestation of *Caryophyllaeus laticeps*. H. & E., X 400.

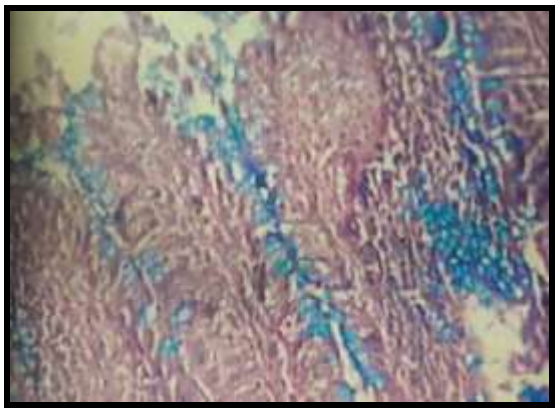

Fig 4: *Caryophyllaeus laticeps* induced hyperplasia of goblet cell and desquamated epithelial cells in intestine of *Cyprinus carpio*. H. & E., X 400.

Discussion

The present study describes the histopathologies due to the infestation of *Caryophyllaeus laticeps* (Pallas, 1781) in the silver carp, *Cyprinus carpio*. *C. laticeps* is a monozoic tapeworm of class cestoda under the family caryphallidae. It was found to parasitize large numbers of cyprinid fishes (Gibson, 2013) [2] of European countries, North Africa and West Asia (Hanzelova *et al.*, 2015) [3] but now has almost worldwide distribution.

The host-parasite interface shows much of the tissue alteration caused by *C. laticeps* seems to be related to its mode of attachment. Like other members of family this caryophyllid has no specific organs of attachment such as loculi, acetabula or bothria. Thus, in absent of such organs, it apparently effects attachment by gripping numerous small portions of the intestinal epithelium of host ensuring a very tight host-parasite interface. Resulted, the epithelial cells undergo heavily compressed, and eventually degenerate. The intermediate layer between the scolex and the epithelial brush border called the 'mucoid layer' might have an adhesive function in addition to mechanical attachment (Mackiewicz *et al.* 1972; Hayunga 1979) [9, 4]. In the present investigation similarity found, it is shown that the parasite as well as the host contributes to this layer.

The intestine of the *Cyprinus carpio* infected with *C. laticeps* revealed vacuolization in the epithelial cells, ruptured brush borders, infiltration of eosinophilic granulocytes and fibroblasts at the site of attachment, hyperplasia of goblet cells and hyper secretion mucus observed in present study resemble those of Wali *et al.* (2016) [17].

In the present investigation, the hosts' cellular defense reaction against the worms, such as the accumulation of eosinophilic granulocytes, is a normal reaction of fish against a parasite (Rowley and Mainwaring 1988; Karanis and Taraschewsk 1993) [14, 5].

Conclusion

The findings accomplished that infestation by cestode, *Caryophyllaeus laticeps* was observed predominantly in the rearing/ stocking ponds. Juveniles and adults silver carp was found susceptible to this disease. The intestinal histopathologies of the *Cyprinus carpio* infected with *C. laticeps* revealed vacuolization in the epithelial cells, ruptured brush borders, infiltration of eosinophilic granulocytes and fibroblasts at the site of attachment, hyperplasia of goblet cells and hyper secretion mucus observed.

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