

Histopathology and other aspects of echinostome infection in freshwater fishes of the South Western Ghats, India

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Abstract. While investigating the trematode parasite infestation in the freshwater fishes of South Western Ghats, India we came across echinostome infection in three species of fishes *Pseudosphromenus cupanus*, *Lepidocephalichthys thermalis* and *Aplochelus lineatus*. Detailed histopathological studies on the infected kidney of the freshwater fish *L. thermalis* revealed severe damage and degeneration of their cells. The prevalence, intensity and mean abundance of infection of *Echinostoma* sp. in the three fishes were also recorded. The prevalences of *Echinostoma* sp. infection in the freshwater fishes *P. cupanus*, *A. lineatus* and *L. thermalis* were 5.55%, 66.66% and 25%, the intensities of infection were 4.5, 36 and 1, and the mean abundances were 0.25, 24 and 0.25, respectively.

Keywords: *Echinostoma* sp.; freshwater fishes; histopathology; Western Ghats.

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Introduction

Fishes serve as hosts for many parasites including metacercariae and adult trematodes and that may result in various pathological effects (Seppänen et al., 2009; Jyrwa et al., 2016).

Echinostomes are a group of digenetic trematode parasites characterized by the presence of well developed collar and collar spines. They are predominantly parasites of birds, and also of mammals including humans (Yamaguti, 1958; Esteban and Muñoz-Antoli, 2009) and

have a range of second intermediate hosts including gastropods, bivalved molluscs, fishes and amphibians. The nature and structure of the echinostome cysts, and their localization vary with hosts (Huffman and Fried, 1990; Fried, 2001).

Echinostoma spp. have been recognized as important zoonotic agents and cases of human echinostomiasis have been reported from various geographical areas (Chai et al., 1994; Haseeb and Eveland, 2002; Sripa et al., 2010; Chai et al., 2012; Sah et al., 2018). Eating raw or improperly cooked or processed fish are the main sources of infection to humans. According to Toledo and Esteban (2016) human echinostomiasis is attributed to a minimum of 24 species that are endemic to Southeast Asia and the Far East, including the mainland China, Taiwan, India, Korea, Malaysia, Philippines and Indonesia. Various studies on human echinostomiasis have been reported from India (Maji et al., 1993; Tandon et al., 2014).

The paper describes the pathological effects of *Echinostoma* sp. on the kidney of the freshwater fish *L. thermalis*. The paper also describes the prevalence, intensity and mean abundance of *Echinostoma* sp. infection in the freshwater fishes *P. cupanus*, *A. lineatus* and *L. thermalis*.

Materials and methods

Histopathology

Infected tissues were washed thoroughly with water and preserved in buffered formalin for about 24 h. After thorough washing the specimens were dehydrated in alcohol series & acetone and were cleared in methyl benzoate. Paraffin blocks were prepared with molten wax maintained at 58 °C. Serial sections having 7 µm thick taken using a Microtec rotatory microtome (Germany) were stained with haematoxylin and eosin to study the histopathology and cellular infiltration at the site of parasite

attachment. The DPX mounted sections were observed under Nikon ECLIPSE Ni-U phase contrast research microscope and photographed with the Nikon Y-TV55 camera attached to the microscope.

Specimen preparation and studies on prevalence, intensity and mean abundance of infection

Host specimens were collected from small rivulets in Wayanad using sweep net. The collected fishes were sacrificed by cervical rupture and all organs were examined under a Labomed (Luxeo 4Z) stereozoom microscope for larval digeneans. Metacercariae were transferred to 0.75% saline and studied under Nikon ECLIPSE Ni-U phase contrast research microscope (Nikon, Japan). Permanent slides of excysted metacercariae were prepared after fixing the flukes in 5% formalin and staining them in acetocarmine. The procedure outlined by Cantwell (1981) was followed for staining. Prevalence, intensity and mean abundance of infection were measured following Bush et al. (1997).

Results

Histopathology

The histological study of infected kidney of *L. thermalis* showed severe damage (Figure 1) on its structure. The heavy burden of encysted metacercariae in the kidney resulted in the degeneration of cells. The tissues surrounding the cyst were necrotic. Degeneration in the epithelial cells of renal tubule with hypertrophied cells and narrowing of tubular lumen was evident (Figure 2). The cells surrounding the metacercarial cyst was necrotic and the pathogenic effects included the degeneration of glomerulus and hematopoietic tissue (Figure 3). Acute granuloma was evident. The histopathology revealed severe tissue damage in the infected fish.

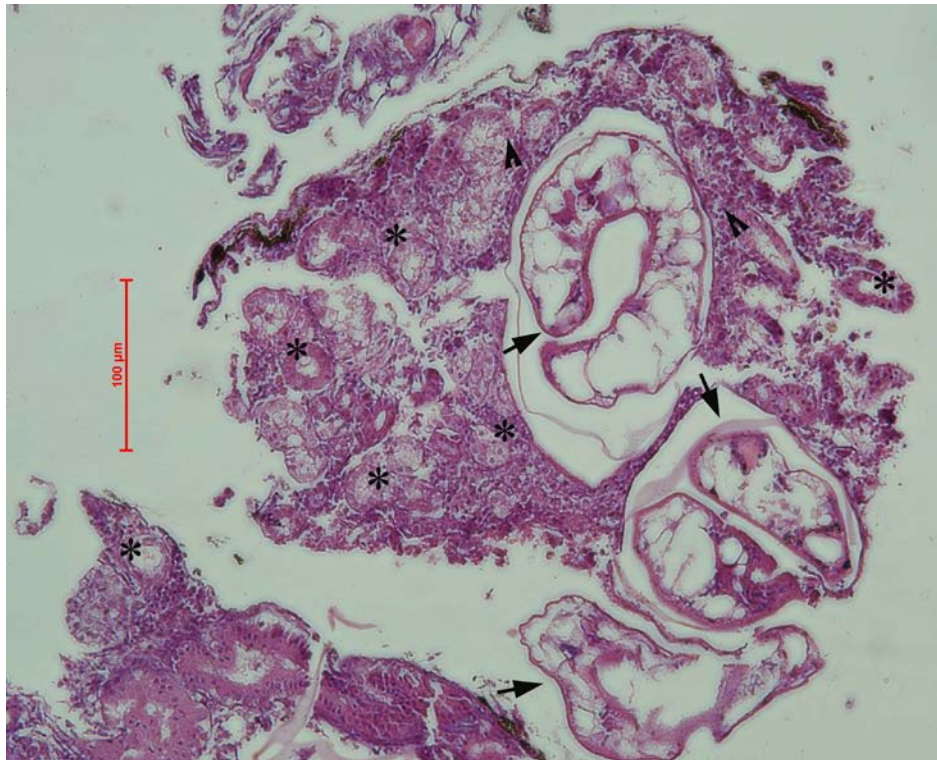


Figure 1. Photomicrograph of the transverse section of infected kidney showing severe tissue damage. Metacercaria (arrow), tissue damage (arrow head) and degeneration of cells in the renal tubule (asterisk).

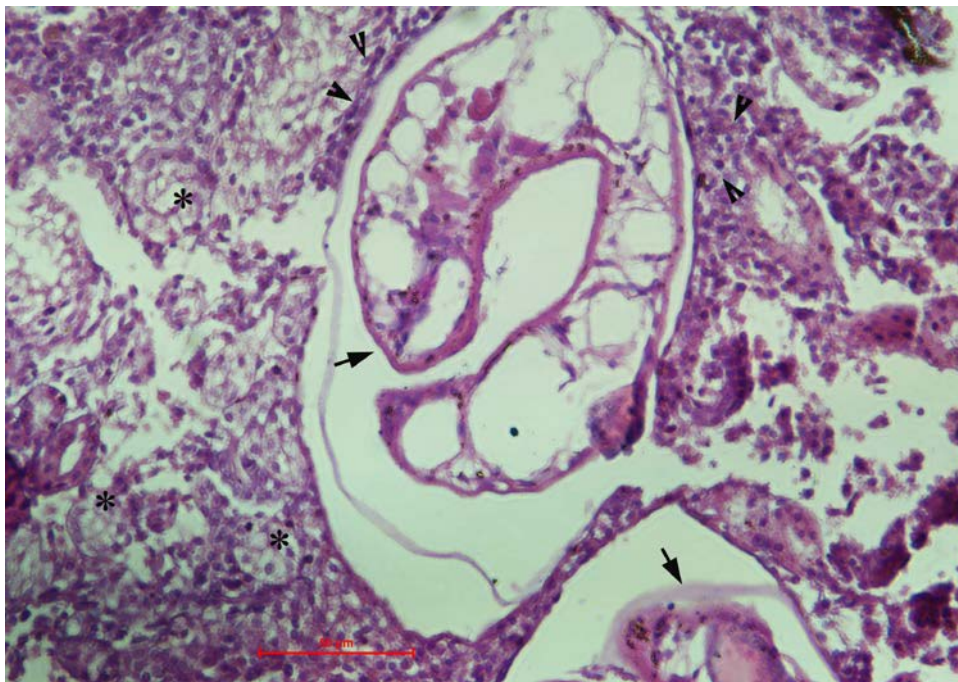


Figure 2. Photomicrograph of the transverse section of infected kidney showing degeneration of epithelial cells in the renal tubules (asterisk), necrotic cells surrounding the metacercariae (arrow head) and metacercariae (arrow).

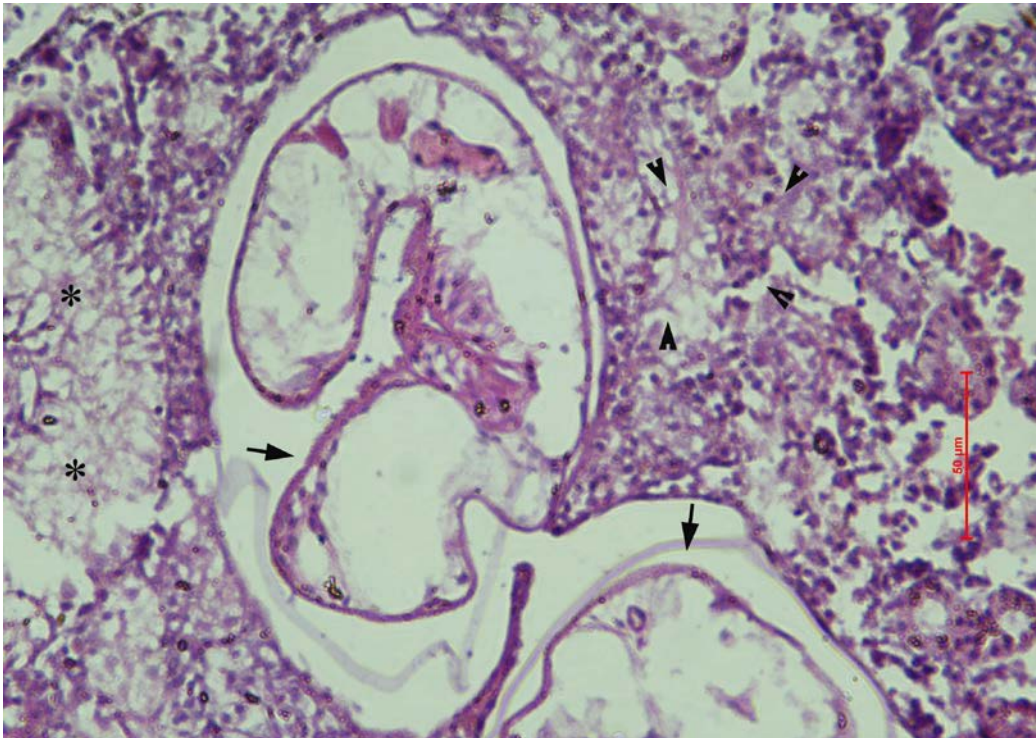


Figure 3. Photomicrograph of the transverse section of infected kidney showing degeneration of glomerulus (asterisk) and hematopoietic tissue (arrow head); metacercariae (arrow).

Prevalence, intensity and mean abundance of infection

The freshwater fishes, *P. cupanus*, *A. lineatus* and *L. thermalis*, collected from Moolithodu of Wayanad were infected with *Echinostoma* sp. The metacercariae (Figure 4) were found encysted in the kidney of *P. cupanus* & *L. thermalis* and mesenteries of *A. lineatus*. The prevalence, intensity and mean abundance of infection varies with hosts (Table 1).



Figure 4. The excysted metacercaria of *Echinostoma* sp.

Table 1. Prevalence, intensity and mean abundance of infection in fish hosts

Host	Prevalence of Infection (%)	Intensity of Infection	Mean abundance
<i>Pseudosphromenus cupanus</i>	2/36 (5.55)	4.5	0.25
<i>Lepidocephalichthys thermalis</i>	2/3 (66.66)	36	24
<i>Aplocheilus lineatus</i>	2/8 (25)	1	0.25

Discussion

The genus *Echinostoma* Rudolphi, 1809 belongs to the family Echinostomatidae Looss, 1899 with *Echinostoma revolutum* (Froelich, 1802) Dietz, 1909 as its type species. More than 130 species of *Echinostoma* have been reported from various parts of the globe (Nasir, 1960; Joet and Umathevy, 1966; Lie and Basch, 1967; Fried et al., 1990; Huffman and Fried, 1990; Toledo et al., 2009). The *Echinostoma* reported from India includes, *Echinostoma revolutum* (Froelich, 1802) Dietz, 1909; *E. govindum* Moghe, 1932; *E. bhattacharyai* Verma, 1936; *E. chasma* Lal, 1939; *E. ivaniosi* Mohandas, 1973; *E. eduardoi* Ghosh and Chauhan, 1977; *E. minimum* (Verma, 1936) Srivastava, 1982.

In the present study the histopathology revealed severe granuloma in the infected kidney. The development of granulomas of fibrotic tissue and leukocytes around Echinostome cysts in tadpole kidneys were observed by Holland et al. (2006) and similar responses were observed in adult frog's kidneys (Martin and Conn, 1990). According to Evans (1974) histopathology of kidney of cutthroat trout infected with *Sanguinicola kiamathensis* showed necrosis or hypertrophy of renal epithelial cells and the degeneration of epithelial cells of the renal tubule, and this may lead to the renal failure. According to Fried et al. (1997) echinostomes can induce oedema and inhibit tadpole growth. Holland et al. (2006) observed oedema and lack of visible glomeruli in the *Echinostome* infected tadpoles. The present study showed disruption of the glomeruli by metacercarial cysts and this may lead to the degeneration of the glomerulus. The study suggests that the disruption of the glomeruli could have a grave impact on renal function.

According to Poulin and Mouillot (2005) the prevalence, intensity, or abundance of infection by a particular

parasite differs among its host species and the present study agrees with the findings of Poulin and Mouillot (2005). An earlier study of Chai et al. (1985) revealed a high infection rate (41.6%) and density (8.1) of *E. hortense* in the loach, *Misgurnus anguillicaudatus* from Korea whereas in the present study the prevalence, intensity and mean abundance of *Echinostoma* sp. infection in the loach, *L. thermalis* were relatively higher: 66.66%, 36 and 24, respectively.

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All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted.

Conflict of interest

The authors declare that there is no conflict of interest between them.

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