Histopathological changes of the esophagus of the freshwater eels, *Macrognathus aculeatus* (Bl.) and *Mastacembelus pancalus* (Lac.) due to infection of metacercariae of *Clinostomum mastacembeli* Jaiswal, 1957

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Abstract *Macrognathus aculeatus* (Bl.) and *Mastacembelus pancalus* (Lac.) (Family: Mastacembelidae) are considered to be the most delicious fish in Andhra Pradesh due to the tenderness of their muscles. The metacercariae of *Clinostomum mastacembeli* Jaiswal, 1957 are found infesting the oesophagus of these fishes heavily. During present studies, histopathological changes caused due to the encystment of metacercariae of *C. mastacembeli* in the oesophagus of these fishes were observed. The four layers of the oesophagus namely; serosa, muscularis layer, submucosa and mucosa were found severely affected. Histopathological changes included decrease in the lumen diameter of the oesophagus, atrophy and necrosis of epithelial cells of serosa layer, shrinkage of villi due to thickening of muscularis layer and formation of small vacuoles in the sub-mucosal layers.

Keywords *Clinostomum mastacembeli*, Histopathology, *Macrognathus aculeatus*, *Mastacembelus pancalus*, Esophagus

Introduction

Fishes represent a major group of organisms which serve as hosts for many parasites including metacercariae (Hoffman

1967). Parasites are one of the chief causes of mass mortality in fish populations (Paperna and Vanas 1983; Orecka-Grabda 1991). With the increasing interests in aquaculture parasitic infestations are becoming threats for fish health management and aquatic crop production throughout the world. It is therefore an essential area for proper attention to be given by the scientists for sustainable aquaculture production.

Freshwater eels of the family Mastacembelidae inhabit a good number of metazoan parasites. Metacercarial infections particularly of *Clinostomum mastacembeli* are heavy in these fishes since they act as intermediate hosts for these digenetic trematodes. Family Clinostomatidae has a widespread distribution. These metacercarial stages are known to cause yellow grubs in the muscles and other organs of the fish and make them unsuitable for human consumption. If a human accidentally consumes the raw or semi cooked fish, the fluke attaches to human esophagus and causes injury. The invasion of metacercariae within a particular organ accounts for considerable pathological and histochemical changes in their hosts. Histopathology resulting from the presence of metacercariae in fish has been studied by Osborn (1911), Faust and Khaw (1925), Hunter and Dalton (1939), Hunter and Hunter (1940), Chandler (1951), Chapman and Hunter (1954), Hoffman and Dunbar (1963), Mawdesley-Thomas and Young (1967), Mahmoud et al. (1989), Mahdy (1991), Lo et al. (1992) and Mahdy et al. (1995). The histopathological changes caused by the metazoan parasitic fauna have not so far been studied in these fishes. *Macrognathus aculeatus* and *Mastacembelus*

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pancalus are found to be heavily infected with metacercariae of *Clinostomum mastacembei* Jaiswal, 1957. In the present study, these metacercariae were found in clumps around the esophagus. Histopathological changes have been well documented in the present study and it is the first of its kind from this fish.

Materials and methods

For histopathological studies, the infected esophagus of 18 *M. aculeatus* and 39 *M. pancalus* were fixed in standard

fixatives such as Bouin's fluid and Susa fluid for 24 hours. Esophagus of 15 uninfected fishes was also fixed separately to have a comparative study. After fixation, the material was thoroughly washed with water and dehydrated in graded series of alcohols (70%, 90%, 95% and 100%). The material was cleared in xylol and infiltrated in paraffin wax at 58°C and blocks were prepared. Transverse, longitudinal and saggittal sections of 3–8 μ thickness were cut with the aid of microtome. Iron hematoxyline, Heidenhain's Azan and Mallory's triple stains were applied for histological studies. Methods described by Pearse (1968) and Bancraft (1975) were followed.

Fig. 1 Dissected fish showing clumps of metacercariae enclosed in a silvery layer and some encysted metacercariae visible from the rupture of silvery layer

Fig. 2 Metacercarial clump and Clinostomum excysted from the cyst

Fig. 3 Clinostomum metacercariae excysted from the cysts

Fig. 4 Metacercaria anterior portion showing suckers

Fig. 5 Metacercaria posterior portion showing rudimentary testes

Fig. 6 TS showing esophagus surrounded by metacercariae at 20 $\times$

Fig. 7 T.S of normal esophagus at 50×

Fig. 8 T.S of outer stratified epithelial layer and muscle layer of normal esophagus at 200×

Fig. 9 Section of villus of normal esophagus showing mucosal cells, lamina propria at 200×

Fig. 10 Infected esophagus enlarged view at 50×

Fig. 11 Disintegration of stratified epithelial layer and muscle layer of esophagus at 200×

Fig. 12 TS of infected esophagus showing shrunken villi and lumen at 200×

Abbreviations: C- Clinostomum metacercaria, OS- Oral sucker, VS- Ventral sucker, AT- Anterior testis, PT- Posterior testis, CW- Cyst wall, ML- Muscle layer, LP- Lamina propria, MC- Mucosal folding, SEL- Stratified epithelial layer, V- Villi, O- Esophagus, SV- Shrunk villi, L- Lumen, SL- Silvery layer, M- Clinostomum Metacercaria, TS - Transverse sections

Results

In the present study, it was found that the metacercariae of trematode parasite, *C. mastacembeli* adhere to the esophagus of host fishes. These metacercariae were found attached in clusters to esophagus as a white spongy ball in *M. pancalus*. In *M. aculeatus*, the metacercariae were very rarely found

attached to the esophagus. They were, however, found distributed individually in the body cavity. The pathological changes were noted in the infected esophagus when compared to the uninfected one.

All these metacercarial cysts were found enclosed in a silvery layer (which might be of host origin) giving a shiny ball like appearance to the cysts (Fig. 1). The heavily

infected fish with these metacercariae around the esophagus appeared like a patient suffering from goitre due to the bulging of esophageal region of the fish. Macroscopically, the esophagus of each fish was found surrounded by 30–40 cysts (Fig. 2). Each cyst normally consisted of only one compactly packed metacercaria. Each cyst measured $1.025\text{--}1.18 \times 1.52\text{--}1.81$ mm in thickness. The cyst composed of concentric whorls may be formed as a result of host immune response. When teased the live metacercariae, escapes from the cysts (Fig. 3). These were actively mobile and measured $4.95\text{--}10.86 \times 1.26\text{--}2.60$ mm in dimensions (Figs. 4 and 5). Heavy infestation of these parasites in the form of cysts exerted pressure on the esophagus resulting into the atrophy of tissue walls (Fig. 6) thus was impairing the normal functioning of the esophagus. Grossly, there seemed to be no change, but at microscopic level, disintegration of different layers was observed as compared to the normal. In uninfected esophagus (Figs. 7, 8 and 9). The stratified epithelial cells of the outer most serosa layer were found disintegrated and ruptured at the site of the attachment (Fig. 10). Shrinkage of longitudinal muscles was clearly evident. Muscularis mucosa layer was found thickened and shrunk thereby reducing the lumen diameter (Fig. 11). Vacuolization was found in the submucosa layer (Fig. 11). Mucosal folding was found shrunk and disintegrated (Fig. 12). Esophagus had become completely atrophied due to the heavy infection of the parasite (Fig. 6). The columnar epithelial cells of mucosa were also disintegrated (Fig. 11). Metaplasia or change of cell shape from columnar to stratified condition was seen at the site of cyst attachment.

Discussion

Helminth parasites affect the host's physiology by invading and inhabiting almost each and every organ and thus altering not only the morphology of the organ, but also severely interrupting the metabolism and nutritive capacity of the host. Pathogenicity of host involves an interaction between the parasite and the host. The effect of parasitism on the fish host is of considerable importance because these fish are one of the important table items for man. These parasites are of zoonotic importance as there are reports of *Clinostomum* sp. causing laryngopharyngitis in humans (Hiral et al. 1987; Isobe et al. 1994; Maejima et al. 1996). Pathophysiological studies on clinostomatid metacercarial infection in the liver of fishes have been extensively studied by Osborn (1911), Hunter and Hunter (1940), Gupta and Agarwal

(1983a, 1983b, 1984), Kalantan et al. (1986, 1987), Malek and Mobedi (2001), Laxma Reddy et al. (2006). Present study on the histopathology of esophagus of fish infected by clinostomatid metacercaria is first of its kind. From the present study it is evident that the Mastacembelid fishes, especially *M. aculeatus* and *M. pancalus* serve as perfect hosts for metacercaria of *C. mastacembeli*.

The histopathology of the esophagus of the infected fish is noteworthy showing the extensive damage to the epithelial cells of the outer most serosa layer. Shrinkage of muscle layers, mucosal folding, vacuolation and atrophy of the esophagus is clearly evident.

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