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Histopathology of Heart of Freshwater Spiny Eel, *Mastacembelus armatus* Naturally Infected with *Tetracotyle* Metacercaria (Trematoda: Strigeidae)

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ABSTRACT

Freshwater spiny eel, *Mastacembelus armatus* Lacpéde, 1800 hosts a number of adult parasitic groups and metacercarial stages of avian digenetic trematodes and acts as an intermediate hosts in the completion of life-cycle of these parasites in birds. The fish shows heavy infestations with the larval stages, *Tetracotyle* metacercaria of *Cotylurus* species (96%) of the parasitic fauna of *M. armatus*. These metacercariae were distributed among various body organs like heart, liver, kidney and gonads. Heart tissue was heavily infected with this metacercariae and severe pathological changes were made with light microscopic as well as transmission electron microscopic studies. Pathologic changes like atrophy due to pressure, inflammatory cells in the vicinity of capsule and loss of striation of cardiac muscle were noted which might have reduced the cardiac efficiency of the host.

Key words: Trematoda, Strigeidae, *Tetracotyle*, *Mastacembelus armatus*, heart, TEM

INTRODUCTION

Helminth parasites are a diversified group of parasites inhabiting almost every organ of the vertebrate host which cause pathological, physiological and biochemical changes in the infected tissues and affects host physiology and thus induce stress in the host animal (Cheng, 1974, Chubb, 1977, 1982; Pardeshi *et al*., 2012). Host-parasite interactions are noticeably deleterious to the host (Holmes and Bethel, 1972; Holmes, 1979; Minchella and Scott, 1991). Parasites may cause mechanical damage in the host due to its attachment to host tissue or may be toxic damage due to release of harmful substances by the parasite. Parasites reduce the absorption rate and interrupt the metabolic processes of the host. Several ecological factors play a vital role to maintain the complicated relationship between the host and the parasite. Various physiological conditions such as feeding, resistance, immunology and their environment almost always conjointly play a crucial role in connection with course and manifestations of fish diseases due to parasitic infection. However, the extent of damage occurred to the host is dependent on the parasitic burden, type of tissue and also the health status of the host.

Fishes represent a major group of animals which serve as hosts for many metacercariae (Hoffman, 1967). Histopathology due to metacercarial infections in fish is 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) and Mawdesley-Thomas and Young (1967). The pathological consequences of parasitic disease of fish have proven that the parasites are one of the chief causes of mass mortality in fish populations (Paperna and Van As, 1983; Orecka-Grabda, 1991). Freshwater eel, *Mastacembelus armatus* of the family Mastacembelidae inhabits a good number of metazoan parasites. Metacercarial infections are heavy in this host. They act as intermediate hosts for avian digenetic trematode *Tetracotyle* sp. *Tetracotyle* metacercaria of the family strigeidae parasitizes almost every organ of *M. armatus*, mostly heart, liver, body cavity and gonads. Studies have been made on strigeids infesting fishes by Orecka-Grabda (1991), Dolezol and Crompton (2000), Coleman (1993), Paperna (1991), Tort *et al.* (1987) and Dezfuli *et al.* (2005). Although they are greatly detrimental, the histopathological and histochemical changes caused by the metazoan parasites of these fishes have not been carried out so far from this region. Pathological changes caused by the parasites are dominant species in the host fishes and the histopathology caused by such parasites is always a challenging field for a parasitologist to work with. The advanced techniques of electron microscopy aid to determine the extent of sub-cellular damage in the infected heart of *Mastacembelus armatus*. Transmission Electron Microscope (TEM) studies provide a detailed account of the pathological changes in the tissues of the host fish. Fairly, an excellent amount of work is contributed by Orecka-Grabda (1991) and Dezfuli *et al.* (2002, 2003, 2005).

MATERIAL AND METHODS

Material: Hearts of 494 *Mastacembelus armatus* measuring 18-52 cm (Mean±SE = 36.42±6.69 cm) in total length were analyzed during the study period 2005-2007. The fish were then dissected, sexed and the hearts were observed *in situ*.

Methodology: Normal and infected heart tissues were fixed in standard fixatives such as Bouin's fluid and Susa fluid for 24 h. After post treatment process, the tissues were 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 taken with the aid of microtome. Iron haematoxylene, Heidenhain's Azan and Mallory's triple stains were applied for histological studies. The methods were followed from Pearse (1968) and Bancraft (1975).

Electron microscopic studies: Biological tissue samples of heart were cut into a rectangular pieces with the aid of BP handle blades and were fixed in 2.5% Glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 24 h at 4°C. These tissues were then post fixed in 0.5% aqueous Osmium tetroxide in the same buffer for 2 h. After the post fixation samples were dehydrated in a series of graded alcohols, infiltrated and embedded in Spurr's resin (Spurr, 1969) which gets hardened upon heating. Once hardened, both semi thin and ultra thin sections were cut with a glass knife oma Leica Ultra cut UCT- GA- D/E- 1/100 ultra microtome. Semi thin section of 200-300 nm thick was stained with toludine blue and Ultra thin sections (50-70 nm thickness) were mounted on a thin metal grid. Then the ultra thin sections were stained with saturated aqueous Uranyl acetate and counter stained with 4% lead citrate and these grids are transferred into a special chamber in the TEM. Sections were observed at various magnifications under transmission electron microscope (Model: Hitachi, H- 7500 from JAPAN) at RUSKA Lab, College of Veterinary Sciences, SVVU, Rajendranagar, Hyderabad, India. The methods were followed from the Electron

Microscopy principles and techniques by Bozzola and Russell (1999). This part of methodology is purely assisted by the technical people at RUSKA laboratory, College of Veterinary Sciences, SVVU, Rajendranagar, Hyderabad, India.

RESULTS

Though Metacercarial stages have a low metabolic demand when compared to adult digeneans but heavy burdens of Metacercariae of trematode, *Cotylurus* sp. (Digenea: Strigeidae) is always detrimental because of the mechanical damage exerted by the weight or pressure of the cysts in the pericardium and thus impairs the normal functioning of the heart tissue of the host. Macroscopically, visible capsules were found encysting the heart and other organs (Fig. 1). These metacercariae are not only restricted to the pericardial cavity of the heart, but also distributed among the various organs of the body like liver, gonads, kidneys and coelomic cavity etc (Fig. 2).

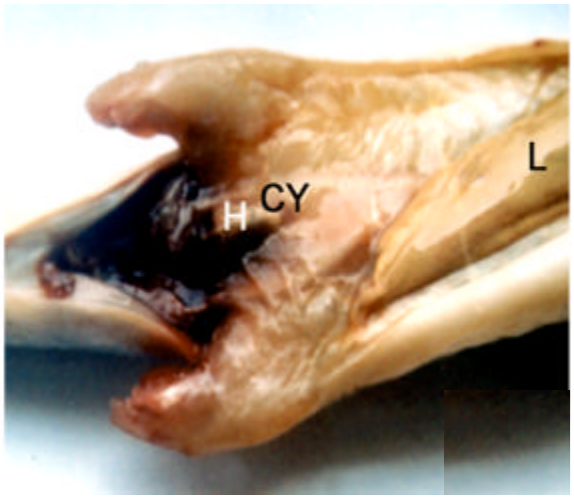

Fig. 1: Heart surrounded by the strigeid metacercaria, H: Heart, CY: Cysts of strigeid

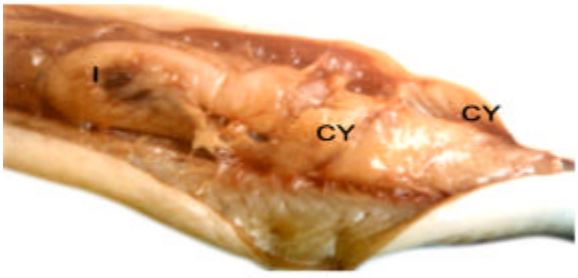

Fig. 2: Strigeid metacercaria in the body cavity and other organs, CY: Cysts of strigeid, I: Intestine

Vast majority of the parasitic cysts were observed either singly or as aggregative masses on the surface of bulbous arteriosus. Each capsule normally contains only one tetracotyle, occasionally 2-3 larvae were found encysted in a single capsule (Fig. 3) and the larva after excystation from the cyst is shown in Fig. 4. Hearts of 90% of the total fish examined were found infected with the metacercarial cysts of *Cotylurus* sp. Majority of parasites appear like white grape-like clusters or aggregations (Fig. 2) on the surface of bulbous arteriosus. Some were found embedded in a granulomatous proliferation of heart epicardium surrounding ventricle, space between ventricle and bulbous arteriosus (Fig. 4). Occasionally, in chronic infections, cysts are found in the heart myocardium by piercing pericardium and causing severe pathologic effects (Fig. 5). The

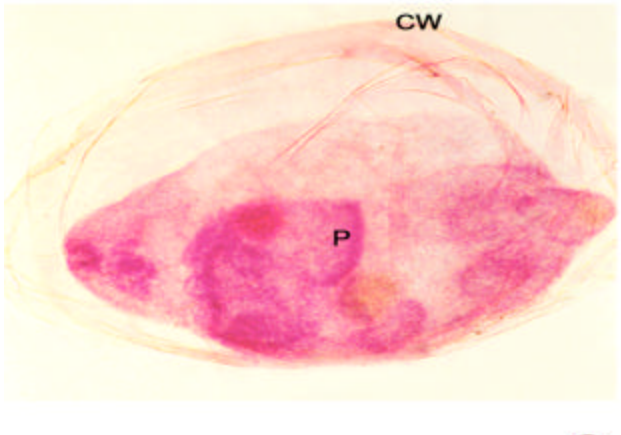

Fig. 3: *Tetracotyle* metacercaria in the cyst 20X, CW: Cysts wall, P: Parasite

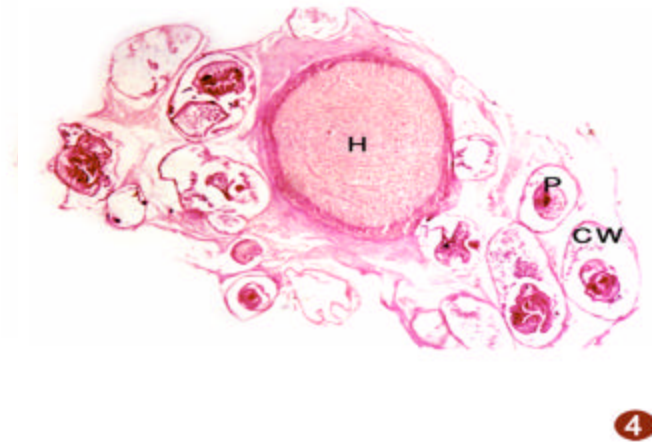

Fig. 4: T.S of infected heart and metacercariae surrounded around the heart 20 X, H: Heart, P: Parasite, CW: Cysts wall

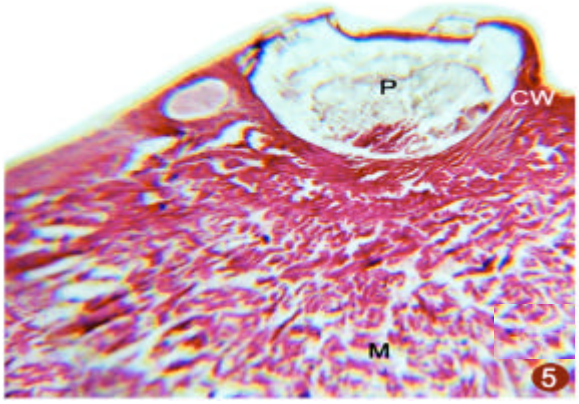

Fig. 5: L.S of heart showing the penetration of strigeid metacercaria towards the heart pericardium P: Parasite, CW: Cyts wall, M: Myocardium

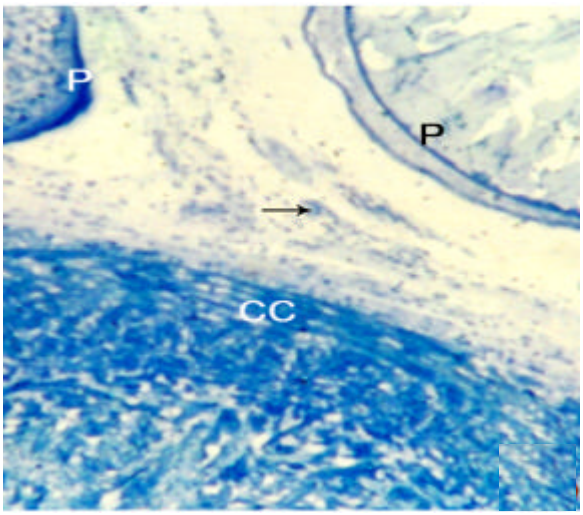

Fig. 6: T.S. of heart showing pericardium and myocardium affected by the cysts surrounding the heart. 50X, P: Parasite, CC: Cuboid cells. Arrows indicating various inflammatory cells in and around the cyst and the excysted parasite

surrounding pericardial tissue is atrophied by the pressure of the capsules, the cells of pericardium into an flattened fibrous cells) (Fig. 6). Metacercarial cysts are surrounded by capsules formed by in the vicinity of capsule, shows severe metaplasia (a reversible change of hexagonal shaped cells host's inflammatory response (Fig. 6). Capsule is formed by a series of concentric whorls of fibroconnective elements and measures 0.25-0.4×0.35-0.58 mm in thickness. A number of elongated

epitheloid cells, vacuolated cells, Eosinophilic Granular Cells (EGCs), granulocytes and melanocytes were observed within the highly compact fibrous capsule wall near the interface between host and parasite. The space between the parasitic tissue and capsule border is filled with slightly homogenous eosinophilic material, some vacuolated cells and some melanocytes (Fig. 6). Quite often, the space within the cyst is filled with tissue debris. Parasite within the oval to round compact fibroconnective capsule is optically very clear, oval shaped with two prominent pseudosuckers (Fig. 6). In some cysts, there is an optically empty space between the parasite and outer wall in which the live parasite moves freely. The free movement of the parasite within the cysts wall also induces several pathological and degenerative changes within the host myocardial tissue. Some cysts are enclosed by a thick fibroconnective tissue capsule composed of fibroblasts and are profusely surrounded by inflammatory cells, macrophages and lymphocytes. Some larvae appeared without cyst wall in the tissues. It may be assumed that the larvae might have break open the cyst wall and entered the tissues (Fig. 6). In severe cases, where metacercarial burden per heart exceeds more than 300-400, the adjacent myocardial muscle bundles show coagulative type necrosis where the nuclei of cells are lost (Fig. 7). Also it shows pressure atrophy and reflected various degrees of degeneration which includes granulations and vacoulation of sarcoplasm. Electron microscopic studies reveal the degenerative changes at sub-cellular stages. Cardiac muscle bundles showed hyaline degeneration which includes loss of striation and edema of myofibres (eosinophilic with pyknosis of their nucleus). Some cardiac muscle bundles showed myolysis (complete degeneration of muscle fibres) and are replaced by macrophage aggregates and lymphocytes together with melanomacrophages while some other cells show profuse aggregation of a large number of inflammatory cells, mainly macrophages and lymphocytes forming a tunnel of cellular elements between muscle bundles (Fig. 8). Various cell organelles like mitochondria, nucleus, ribosomes and endoplasmic reticulum show degenerative changes which includes distortion in the shape and size, loss of orientation etc. (Fig. 9). Also, the fusion of granules of Eosinophilic Granular Cells (ECGs) was observed in close vicinity of metacercaria. Some cysts showed macrophage aggregates and melanomacrophages within the parasite capsule (Fig. 10).

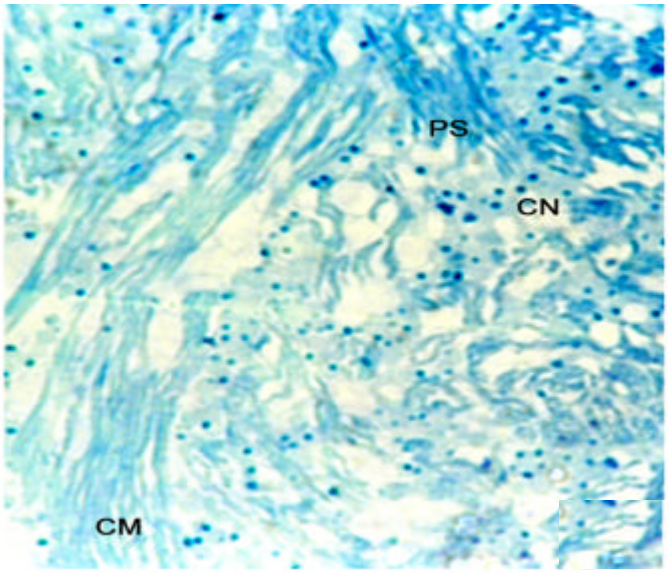

Fig. 7: T.S of heart showing the pericardial cells exhibiting metastasis, CM: Cardiac muscle, PS: Pseudosuckers, CN: Coagulative necrosis

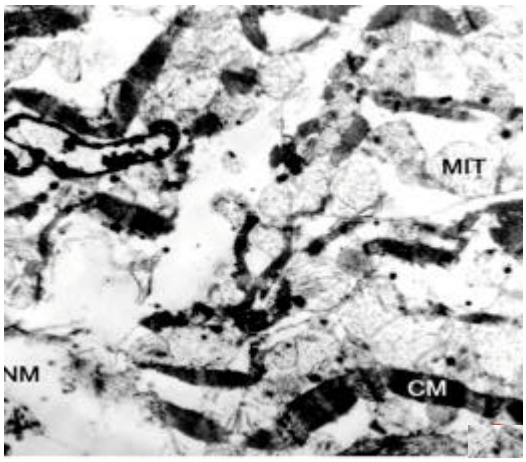

Fig. 8: Completely distorted mitochondria disrupted nuclear membrane and disintegrated cardiac muscle fibres with loss of orientation. 4K, 7160 X (TEM), MIT: Mitochondria, NM: Nuclear membrane

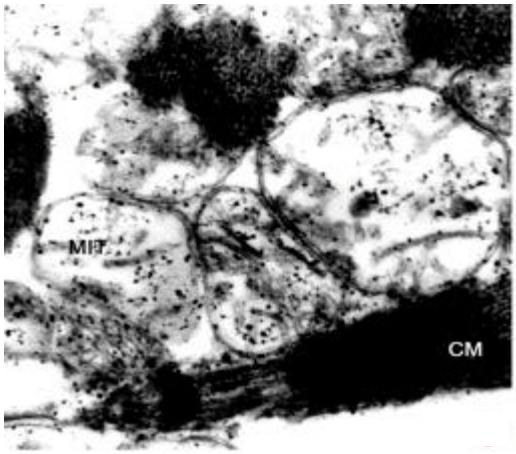

Fig. 9: Ruptured heart muscle cells showing enlarged mitochondria. 20 K, 35800X (TEM)

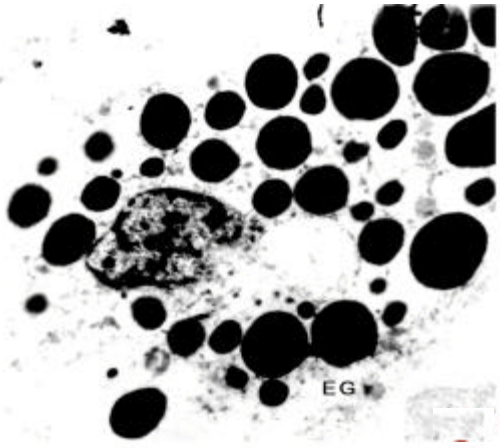

Fig. 10: Electron microphotograph of fused granules within the inflammatory eosinophilic granular cells, 5K, 8950 X (TEM), EG: Eosinophilic granular cells

DISCUSSION

Natural infections of strigeid metacercariae occur in amphibia, reptiles and mammals but are perhaps most frequent in fish where they cause a number of pathologies. Heavy infections of strigeids cause a serious problem in fish stocks reared commercially. Of these strigeids, some metacercariae induces host mortality (Orecka-Grabda, 1991) while some were not the direct cause of the fish mortalities (Blair, 1976). There is very less work done on the cardiac pathology of fish infected by the strigeid metacercarial forms. Some metacercariae are location restricted such as *Diplostomum* which infect the eye lens, *Neascus* sp. infecting the skin of the host fish but some metacercariae of the related *Cotylurus* sp. are not restricted to a particular location Odening *et al.* (1970), Olson (1970), Swennen *et al.* (1979) and Orecka-Grabda (1991) but have been found distributed throughout the body organs including the swim bladder (Swennen *et al.*, 1979), the eyes and body cavity (Blair, 1976; Smith and Noga, 1993) and the kidney and liver Orecka-Grabda (1991). The frequency of metacercaria range from medium-to-high number and the presence of this number of metacercaria are almost always mild and can compromise organ functions by slightly displacing fish tissue. However, very large numbers sometimes impair the regular functioning of the organs.

There are several instances which reflect the indirect effect of these metacercariae on their host fishes. Paperna (1991) analyzed that the infection of heterophyid metacercariae may result in the mortality of warm water fish species due the severe gill damage and reduction in respiratory tolerance. Hoole *et al.* (2001) reported that the metacercariae of *Apharyngostrigea cornu* are the cause of haemorrhaging and mortality of cyprinid fishes. Orecka-Grabda (1991) noticed the high intensities of *Ichthyocotylurus erraticus* metacercariae on the hearts of salmonids are the chief cause of mass mortality of these fishes. However, there are instances which account for the impact of these metacercariae on their heart performance. Tort *et al.* (1987) renowned the changes in the *in vitro* pumping performance of the hearts of rainbow trouts. Watson *et al.* (1992) observed that the fishes infected with a strigeid, *Apatemon gracilis* exhibited cardiac dysfunction, as well as poorness of condition and stunted growth due to the reduction in the cardiac output to 20 to 40%.

CONCLUSION

In the present study the pathologic effects- atrophy due to pressure, inflammatory cells in the vicinity of capsule and loss of striation of cardiac muscle at the microscopic level are the tremendous variations found on the heart of the fishes. Heavy infections may bring about death to the host slowly but the pathologic effects at microscopic level might result in reduced performance of the heart. However it is wonderful to note that the fish are surviving with this infection but with reduced cardiac efficiency.

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