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Research Article
Mode of Attachment and Pathogenicity of *Circumonchobothrium shindei* (Eucestoda: Ptychobothriidae) in *Mastacembelus armatus* of River Godavari, Andhra Pradesh, India

Anu Prasanna Vankara

Department of Animal Sciences, Yogi Vemana University, YSR Kadapa, 516003 Andhra Pradesh, India

Abstract

Background and Objective: *Circumonchobothrium shindei* is Ptychobothriid (Cestode) parasitizing the fresh water spiny eels of the family Mastacembelidae. The main objective of the study is to document the pathological changes induced by the pseudophyllidean cestode, *Circumonchobothrium shindei* in the intestine of *Mastacembelus armatus* (*M. armatus*). **Materials and Methods:** The Mastacembelid fishes were procured from the River Godavari during the year 2005-2009 and screened for the presence of ptychobothriid cestode and the normal and infected tissues of the fish were fixed, processed and permanent slides were prepared by the conventional techniques. **Results:** Histopathological changes include mechanical damage with desquamation of the epithelium, focal necrosis and increase in number of fibroblasts at the attachment point due to boring action of scolex. Vacuolation of sub-mucous cells and proliferative changes lead to the degeneration of the layers of the intestine. Muscularis layer does not show much damage. Parasites cause the dilation of blood vessels of the sub-mucosa resulting in degeneration of intestinal folds, shrinkage of villi and necrosis of epithelial cells. Villi get erupted at the region of attachment of the intestine. Inflammation and fibrosis associated with hyperplasia and metaplasia was observed. Increase in number of lymphocytes in the stratum granulosum and connective tissue layer was an indicative of inflammation. **Conclusion:** The main interpretation of the present study is that, the parasite is potentially pathogenic to the host and will certainly affect the fish productivity through mortalities, decreasing growth rate, declining the quality of the flesh and making the hosts more susceptible to secondary infections.

Key words: *Circumonchobothrium shindei*, *Mastacembelus armatus*, pathogenicity, vacuolation, proliferative changes, ptychobothriid cestode

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Corresponding Author: Anu Prasanna Vankara, Department of Animal Sciences, Yogi Vemana University, YSR Kadapa, 516003 Andhra Pradesh, India
Tel: 07032825689

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fishes are the ultimate sources of fine quality proteins but are affected by many parasites. Fish health is the primary requisite in the aquaculture but it is indefinitely affected by environmental factors, nutrition and pathogens causing fish diseases[1,2]. Parasitic infections in fishes are exceptionally common, particularly in wild populations from diverse aquatic environments where ecological necessities for intermediate hosts and parasite transmission are met[1]. Diseases caused by parasitic infections are restraining factors in aquaculture production and there is escalating proof that persistent infection in wild fishes[2-4]. Also, some parasites such as trematodes have a great zoonotic potential and can impact human beings[5,6]. The assessment of pathologies can be done with study of histology which in an accurate sense is a study of microanatomy of particular tissues which present a comprehensive outlook of the functioning of tissues and organs. A precise knowledge of histology provides an edge to the histologist to differentiate the normal tissue from an infected tissue. The methods employed for the preparation of material for such studies are known as histological or histopathological techniques. Fish diseases and histopathology are attaining a lot of significance for their wide range of uses as indicators of environmental stress since they provide a distinct biological end point of histological exposure[7]. Pathological outcomes of cestode might be due to the site preference of the adult parasite in the gastrointestinal tract and also to the encapsulation of larval stages in the tissues. Cestodes survive in a very perilous milieu with the help of a diverse kinds of hold fast organ (scolex) as there is an continuous movement of the gut lining, food gut surface and hold fast organ (scolex) may be acetabulate with 4 suckers, bothriate with 2 bothria (hold fast grooves) or bothridiate with 4 bothria (muscles hold fast organs). Sometimes, scolices may be equipped with hooks and spines or have a retractable rostellum or proboscis covered with fine hooks[7]. Histopathology is considered as an imperative tool to indicate the fish health and establish the effect of parasites on various tissues. The environmental factors are very significant in the recruitment, transmission, colonization, productiveness and continued existence of both the adult and larval parasites[8]. The host parasite relationship in cestodes is multifaceted one, involving relations between two and sometime more genetically system namely those of the parasites, its intermediate and its definitive host. Thus a cestode is suitably adapted to the morphology, physiology, biochemistry, immunology and ecology of its hosts. In low to moderate infections, pathological effects are confined to a small area around the attachment of the adult worm. The degree of damage is proportional to the deepness of scolex penetration into the tissue. It is negligible when parasites are clinging to the epithelial mucosa only but turn out to be brutal, with widespread granuloma and successive fibrosis, when the scolex anchors deeply in the muscle layer or entirely perforates into the intestinal wall[9-12]. The profundity of penetration of some species may be at variance in different host fishes[10]. Review of the literature supports the fact that majority of the pathological work was contributed on the clariid fishes[13-17] and only a meager work on the systematic, ecology and pathology of Mastacembelid fishes[11,12,18-24]. In the present study, the Mastacembelid fishes are found to be heavily infected with an adult cestode, *Circumonchobothrium shindei*. Hence, an attempt was made to study the pathological effects of the cestode on the intestine of the host.

MATERIALS AND METHODS

Study area: Godavari River is known for its lively environment, enriched by the nutrients proved to be a highly productive and prospective field to accomplish fishery research and fishing operations. It is the second longest river (900 miles) in India whose origin is at Trimbakeshwar near Nasik which is 380 km away from the Arabian Sea but floods to Southeast across South-Central India and finally unites in the Bay of Bengal. The river divides into two streams forming an extremely productive delta at Rajahmundry (80 km from the Bay of Bengal coast). It is a seasonal river which expands during monsoons and dried out during summer and the colour of water is generally brownish and muddy. Indravati River, Manjira, Bindusara and Sarbar are its tributaties[25-27]. Bhadrachalam, Rajahmundry and Narsapur are some of the important urban centres of Andhra Pradesh on the banks of River Godavari (Fig. 1a-c).

Fish collection and identification: A total of 494 *Mastacembelus armatus* were collected from the River Godavari and various fish markets in and around the river in different seasons by using different types of 'Nets and Gears' with the help of local fishermen during the study period 2005-2009. Fishes were thoroughly washed, photographed in fresh condition and preserved in 9-10% formalin solution. For larger fishes an incision on the abdomen was done and the gut contents were removed before preservation. The collections were made once in a month from 2005-2009. The fish was identified with help of standard books[28-30].

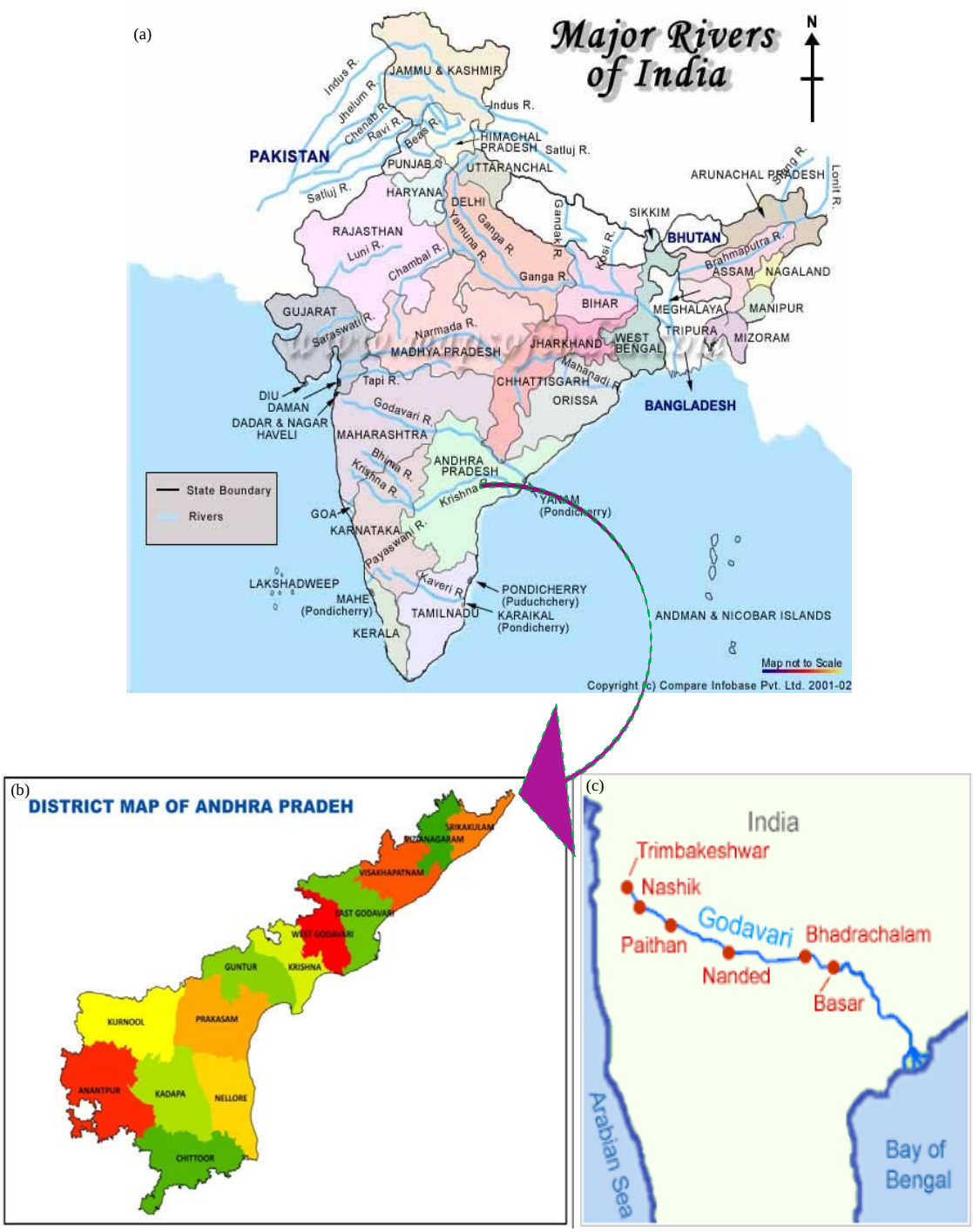

Fig. 1(a-c): (a) Geographical map of India showing Godavari river flowing Andhra Pradesh state, (b) Andhra Pradesh map and (c) River Godavari

The fish were sectioned and the alimentary canal especially intestine was examined for cestodes. The excised intestine was then cut open, washed in petri dish with 0.1% sodium chloride solution and examined thoroughly for the cestodes. The cestodes were collected and preserved in A.F.A (Alcohol-85 mL, formalin-10 mL and acetic acid-5 mL) which acts as an idyllic fixative for the whole mount preparations and processed for further studies.

The normal and infected fish tissues were fixed in Bouin's fluid for 6-7 h before making a permanent preparation. The

fixative helps to avoid dissolving of cells by their own enzymatic action, to prevent post-mortem decomposition or putrefaction caused due to bacterial invasion, to harden the tissue so that they do not disintegrate on subsequent treatment, to enable the tissue cells resist the varying osmotic pressures of the different reagents to be subsequently applied and to render the cell resistant to shrinkage during subsequent processing. Post fixation is followed by series of washing and dehydration in graded alcohols (50, 70 and 95% and then twice in absolute alcohol for 20-30 min in each grade), clearing in xylene and embedding in paraffin wax (58-62°C). The blocked tissues were sectioned at 4-5 microns in the rotary microtome (RM2235) and stained in Eosin Haematoxylin double staining method and finally mounted using Canada balsam or DPX mountant[31,32]. Sections of the tissues on the slides were examined under the Nikon Alphashot microscope and photomicrographs were taken.

RESULTS AND DISCUSSION

The parasite recurrently obtained from the intestine of *M. armatus* was taken into a petri dish (Fig. 2a). *Circumonchobothrium shindei* is a pseudophyllid cestode parasite commonly occurring in the intestine of freshwater fishes (Fig. 2b, c). It is a common cestode parasite of *M. armatus* and *Ophiocephalus punctatus*. These parasites are attached to the intestine by an aspiration affect with pseudosuckers located in the scolex.

Alimentary canal of the host consists of oral cavity, oesophagus, stomach, intestine, rectum and anus. Intestine consists of four layers namely, serosa, muscularis layer, submucosa and mucosa. The outermost serosa layer of the digestive tract is thicker at the region of intestine. Serosa consists of connective tissue fibers, cells, blood vessels and nerves. Next to serosa is the muscularis mucosa consisting of inner circular and outer longitudinal muscles. A thin layer of loose connective tissue consisting blood vessels and nerves acts as a basement membrane between the muscle layers. Third layer of intestine is submucosa, often referred to as 'sub-epithelial layer' is highly vascularized and extends into villi and lamnia propria. This layer forms the central core of the villi and a thin core layer between muscle bundles. Mucosa being the innermost layer gives off finger like projections into the lumen, consisting of columnar epithelium. The columnar epithelial cells consist of absorptive cells and mucus secreting cells (Fig. 2d, e).

Attachment of the scolex to the intestine resulted in some mechanical damage, due to boring action on the tissue,

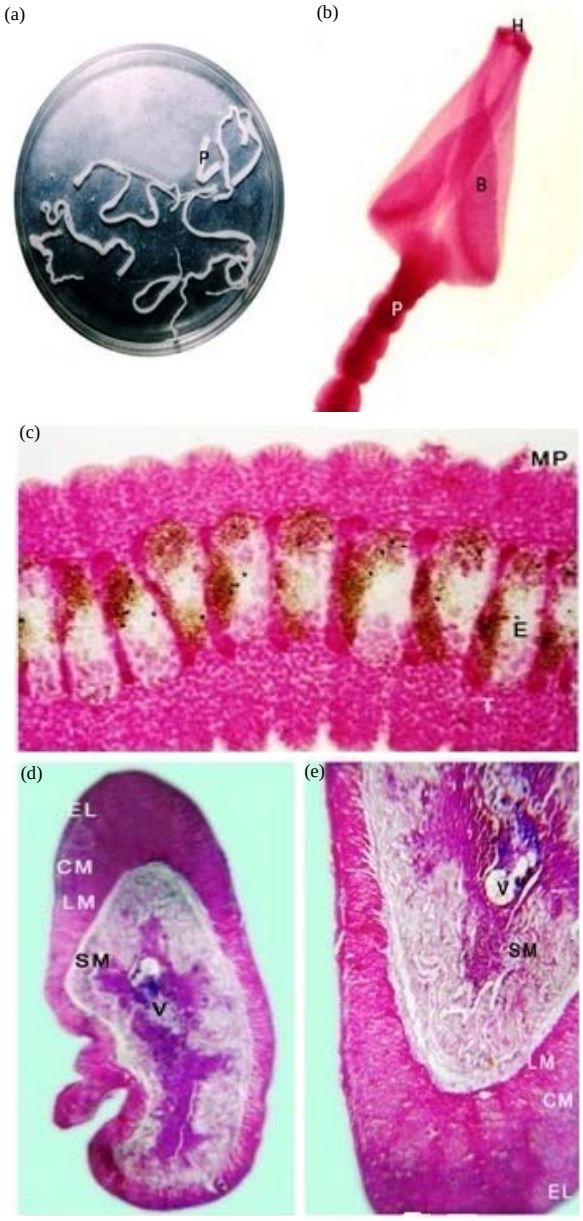

Fig. 2(a-e): (a) *Circumonchobothrium shindei*-Worm entire worm in petri dish, (b) Scolex of worm enlarged view 40X, (c) Mature proglottid of worm 40 X, (d) Transverse section (T.S) of intestine of *Mastacembelus armatus* (Entire view) 20X and (e) T.S. of intestine enlarged view 50 X

EL: Epithelial layer, CM: Circular muscle layer, LM: Longitudinal muscle layer, SM: Sub mucosa, V: Villus, P: Parasite, H: Hooks, B: Bothria, MP: Mature proglottid, E: Eggs

causing desquamation of the epithelium, focal necrosis and increase in number of fibroblasts at the attachment point. Vacuolation of sub-mucous cells and proliferative changes which lead degeneration of the layers of the intestine was

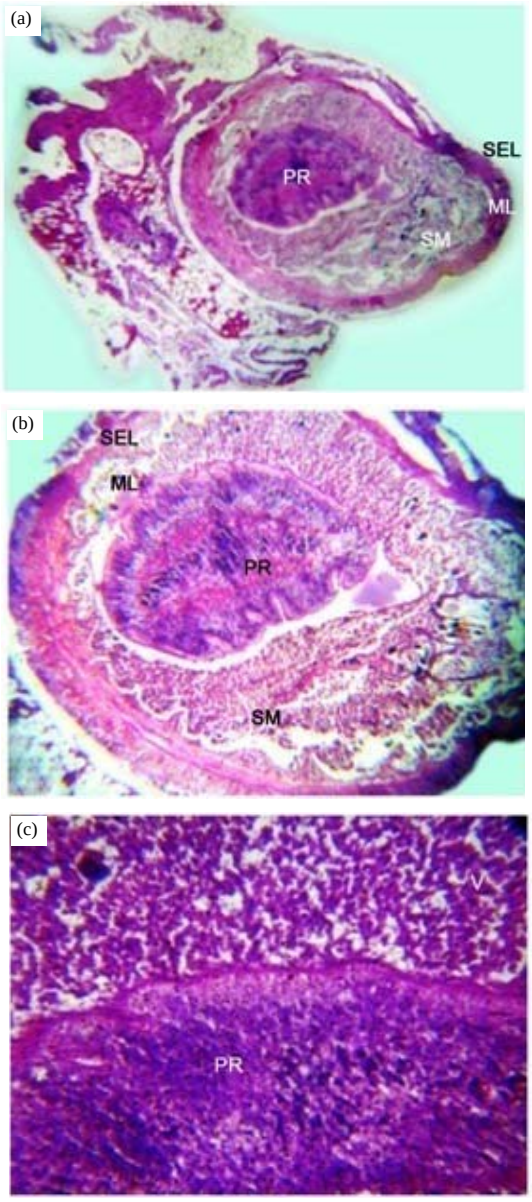

Fig. 3(a-c): (a) T.S of intestine showing proglottid of the cestode, 20X, (b) Section of infected intestine enlarged 50X and (c) Transverse section of intestine showing the disintegration of villi and infiltration of various inflammatory cells in the close vicinity of parasite 200X

SM: Sub mucosa, PR: Proglottid, SEL: Sub-epithelial layer, ML: Muscle layer, V: Villus

observed (Fig. 3a). Muscularis layer does not show much damage. Parasites cause the dilation of blood vessels of the sub-mucosa to some extent which results in degeneration of intestinal folds. Shrinkage of villi and necrosis of epithelial cells was also noticed (Fig. 3b). The parasites invaded the mucous membrane of the intestine where the extensive damage to villi is seen. Villi get erupted at the region of attachment of the intestine. Inflammation and fibrosis associated with hyperplasia and metaplasia was observed. Increase in number of lymphocytes in the stratum granulosum and connective tissue layer was an indicative of inflammation (Fig. 3c). In the present study, inflammation of the tissue at the site of infection is worth mentioning.

Helminth parasites affect the host's physiology by invading and inhabiting almost each and every organ and thus not only altering the morphology of the organ but also severely interrupting the metabolism and nutritive capacity of the host. A survey of the literature revealed that studies pertaining to helminth infection of fish are meager when compared to other vertebrates[33-37]. 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.

The infection of cestodes and their attachment modes in the intestine of fishes are well-documented in marine fishes especially sharks and rays[38]. But such type of studies on the freshwater fishes is very rare[39-44]. Cestodes are triumphant in surviving and growing well until reproduction within the host. The worms are found hanging in the intestinal lumen with its scolex attached to the intestinal villi. The present study shows similar pathological observations made by many earlier workers on the intestinal pathologies of cestodes[45,46]. Cestode infections cause mechanical damage by desquamifying the epithelium, focal necrosis, increasing the number of fibroblasts at the site of attachment point and harshly damaging the villi. Cestodes also cause inflammation and fibrosis related with hyperplasia and metaplasia, increase in number of lymphocytes in the stratum granulosum and connective tissue layer[47,48]. Thus all these effects cause physiological imbalance resulting in reduced immunity of the hosts against secondary infections. Also, the heavy infections of cestodes normally block the intestinal passage completely resulting in reduced growth of the fish[49]. This type of histopathological studies can give a better idea to identify the stress induced by the parasite on the damaged tissue and thus can be helpful to future scientists to suggest some preventive measures to ease the stress in these tissues.

CONCLUSION

The present study concludes that the infection by the adult cestode, *Circumonchobothrium shindei* in the intestine

of *Mastacembelus armatus* will affect the fish health, growth and productivity of the fish. The host is in loss, not able to drive away the parasite or to kill it by secreting toxins in cavity formed by surrounding villi.

SIGNIFICANCE STATEMENT

This type of histopathological studies will uncover the damage and stress imposed by the parasite on the host tissue and such type of pathological studies will definitely benefit the future aqua culturists to analyze the possible consequences of the various cestode infections in the hosts and thus enable them to provide certain deterrent measures to improve the quality of the fish and their health.

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