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Pathological manifestation of the *Acanthocephalus dirus* in *Thunnus albacares*

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ABSTRACT

Objective: To describe the pathological manifestation of the *Acanthocephalus dirus* (*A. dirus*) infestation in *Thunnus albacares* (*T. albacares*) from southeast coast of India.
Methods: The parasite-infested fishes were collected from Nagapattinam landing centre of Tamil Nadu from southeast coast of India. The Acanthocephala morphology, gross pathology, histopathology and histochemistry were investigated.
Results: *T. albacares* were severely infested with *A. dirus*, the group of Acanthocephala attached to the posterior region of the intestine. The adult worm proboscis was cylindrical and the length and width ranging between 2.7–6.4 mm and 0.8–1.3 mm, respectively. Histopathologically, the infested intestinal mucosal epithelium, stratum granulosum, lamina propria, muscular and serosa layers were highly degraded. The lesions were infiltrating with basophil-like inflammatory cells. The parasite-affected lesions were histochemically positive for alcian blue, azo dye, toluidine blue and oil red O.
Conclusions: This is the new host for the parasite *A. dirus* in *T. albacares*. On the basis of histological and histochemical findings, the lesions were highly damaged due to the parasitic infestation. The high density of the parasite and severe penetration of the proboscis into the intestinal tissues are the main reason for the pathogenicity in the host.

1. Introduction

The yellowfin tunas [*Thunnus albacares* (*T. albacares*)] belonging to the family of Scombridae occur in worldwide tropical and temperate seas and contribute to many national fisheries food[1]. It is widely distributed in the eastern and western coast of Indian waters and lives strictly in marine habitats. Acanthocephalans are thorny or spiny-headed worms with aquatic life cycles. Fishes are their final hosts. They are endoparasitic worms of slender cylindroids or slightly flattened forms[2]. Acanthocephala is frequently reported in intestines of wild marine and freshwater fishes[3-6]. The *Acanthocephalus dirus* (*A. dirus*) is rarely observed in fishes and other vertebrates throughout the world. Adult acanthocephalans survive in the fish intestine where they absorb nutrients through the integument while anchoring to the intestinal wall by an armed praesoma[7].

The infected fish intestines suffer from irreversible mechanical damage due to the attachment of the acanthocephala armed proboscis which also affects the nature of the intestinal structure and leads to pathological changes. Several reports on the harmful effects of many acanthocephala on the digestive tract of the different fish species are available[8]. The pathological effect is localized around individual acanthocephala in low infection, but in the high parasitic load, the total destruction of the tissue nature extends due to the adherence of the collective pathological changes[9].

Several reports are available on *A. dirus* infestations in fresh water fishes such as *Lota lota*, *Culaea inconstans* and *Catostomus commersoni* in Great Lakes[10]. However, to our knowledge, this is the first report of marine fish, *T. albacares*, infested with

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acanthocephalan (*A. dirus*) and we provide the detailed description regarding the pathological lesions in the infested fish intestinal tissue.

2. Material and methods

The infested *T. albacares* were collected from Nagapattinam landing centre (10°45'36.25" N and 79°50'59.54" E) of Tamil Nadu, southeast coast of India. Fish were dissected and examined for parasites via a longitudinal incision of the intestine. The infected intestinal lesions were excised then preserved in 10% neutral buffer phosphate formalin. The biopsies were dehydrated with a graded series of ethanol, processed and embedded with paraffin wax. The thin section of 4 μm was incised with a rotary microtome and stained with Harris haematoxylin and eosin[11]. Alcian blue staining method was conducted by Bancroft and Stevens[12]. The mast cells analysis was used for toluidine blue staining method by Migliaccio *et al.*[13]. Lipid accumulation of the infected intestinal was studied by oil red O stain used by Young and Heath[14]. The sections were treated with azodye stain method used by Malaty[15] for detecting the activity of acid and alkaline phosphatases. The stained sections were observed under a phase contrast microscope (20× magnification). The collected parasites were identified according to the method of Amin[4] and Bhattacharya[16]. Parasite taxonomy was carried out as per World Register of Marine Species and the Integrated Taxonomic Information System[17]. The host fish, yellowfin tuna, was identified based on the method of De Bruin *et al.*[18].

3. Results

The yellow to orange in colour group of *A. dirus* were found attached to the posterior end of the *T. albacares* intestine and the lumen was blocked. The infested sites were injured, reddish, swollen and thickened (Figure 1). The infested intestine was entirely damaged to the hook adherent tissue with distended appearance of parasite caused by extensive tissue damage.

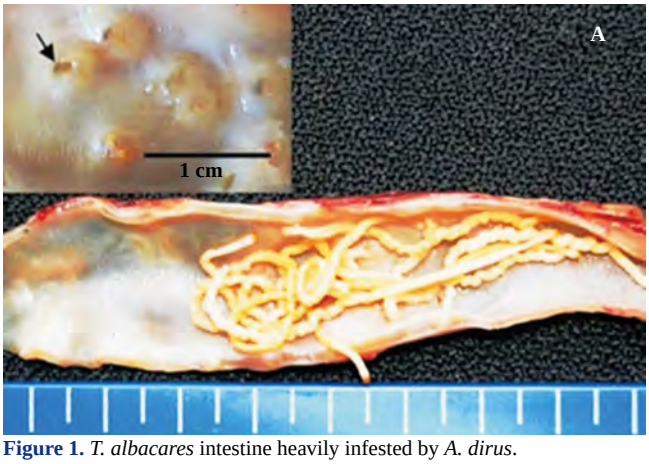

Figure 1. *T. albacares* intestine heavily infested by *A. dirus*. Insets show a close-up view of the parasite-adherent site with the distended appearance (A). Ruler: 1 cm/bar.

The total length and width of the parasites ranged between 2.7–6.4 mm and 0.8–1.3 mm, respectively (Figure 2A, B). Proboscis was cylindrical with 14–17 alternate longitudinal rows of evenly curved hooks, each rows consist of 11–13 hooks. The first 7 rows of hooks increased in size. The length and width of the proboscis ranged between 875–930 μm and 340–410 μm, respectively. Male and female acanthocephalans were identified based on the sexual organs bursa and genital opening, respectively (Figure 2C, D).

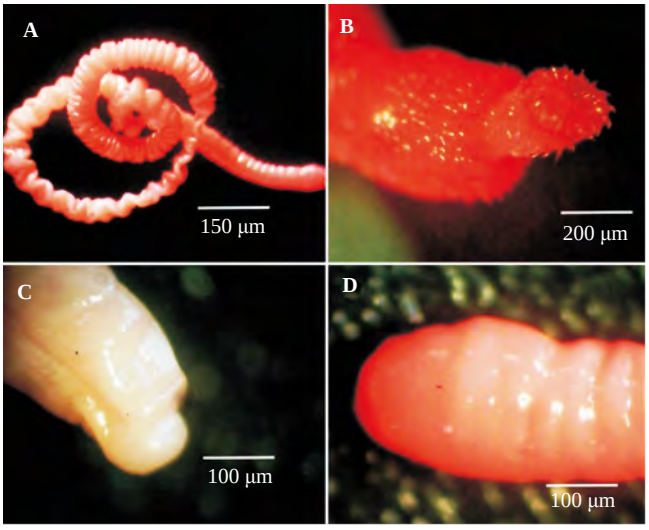

Figure 2. Lateral view of a complete male specimen of *A. dirus* (A), anterior end of proboscis (B), dorsoventral view of bursa (C), and dorsoventral view of genital opening (D).

Histologically, the *A. dirus*-infested intestinal lesions showed degraded mucosal epithelium, stratum granulosum, lamina propria, muscular and serosa (Figure 3A). The parasite attached nearby lesions and showed granulocytes inflammatory infiltrations were intermingled with fibroblast (Figure 3B, C). The lipid droplets were also observed within the cells in deeper parts of the infected host intestine (Figure 3D). The large, last row of spines on the proboscis was anchored firmly in the surrounding connective tissue capsule (Figure 3E). The actual trunk of the worms is also visible nestled between microvilli. Again "gaps" were visible in each of these figures but these were thought to be an artifact. The higher magnification view of the proboscis in Figure 4E illustrates a proliferation of erythrocytes and leukocytes surrounding the area of proboscis attachment (Figure 3F).

Alcian blue stain was positive for sulfated and carboxylated acid mucopolysaccharides and sialomucins, and the mucus secreting goblet cells contained abundant acid mucopolysaccharides (Figure 4A). The proboscis vicinity cells were stained positively for toluidine blue, and metachromatic mast cells accumulations were observed (Figure 4B). Oil red O stain was positive for lipid droplets in infested intestine. This lipid accumulation was associated with increased oxidative stress (Figure 4C). The red granule of the azodye deposits indicated the acid phosphatase sites and bluish granules indicated the alkaline phosphatase sites (Figure 4D).

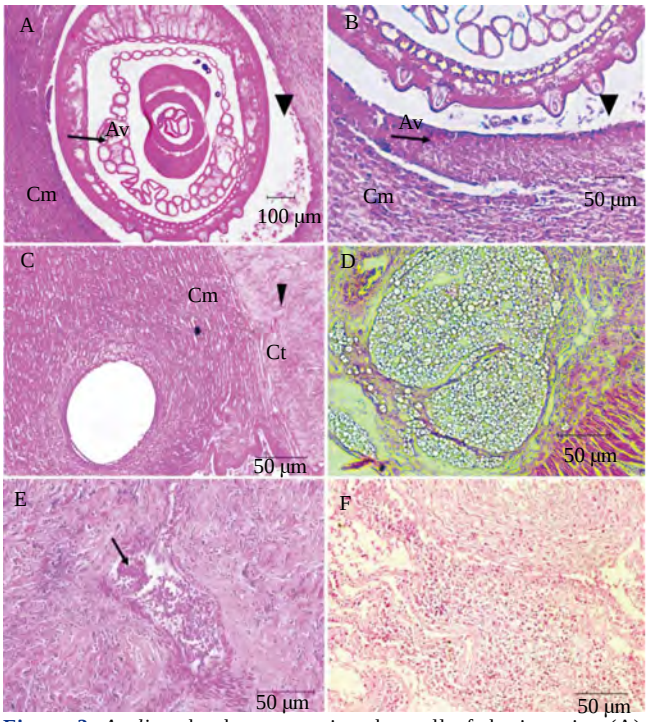

Figure 3. *A. dirus* hooks penetrating the wall of the intestine (A); Enlargement of parasite hooks (arrow) surrounded by a connective tissue capsule (B); Spines on the proboscis anchored firmly in the surrounding connective tissue capsule (C); Lipid droplets seen within cells in the deeper parts of the infected intestine (D); Detached epithelium along with clumps of red blood cells lying free in the lumen (E); Proboscis of the parasite surrounded by a connective tissue capsule (F).
Av: Alveolar lobe; Cm: Circular muscles; Ct: Connective tissue capsule.

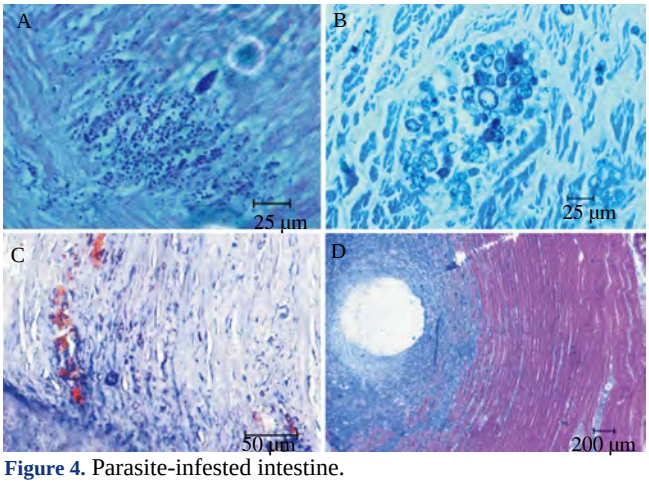

Figure 4. Parasite-infested intestine.
A: Stain alcian blue positive for acid mucopolysaccharide; B: Stain toludine blue positive for matachromatic mast celles; C: Stain oil red O positive for oil droplet; D: Stain azodye positive for acid and alkaline phosphatase.

4. Discussion

The pathological consequences of parasites in fishes are well-documented and serve as an evidence to support the view that parasitization is one of the main causes of mortality in fish population. This study recorded the occurrence and pathology of *A. dirus* from Nagapattinam coastal waters, especially as an internal parasite of the marine sciaenid fish, *T. albacares*. In case of heavy infestations, they can cause occlusion of the gut and invasion or migration of parasites into the uncommon sites to the infested fish.

Histopathological studies revealed the structural abnormalities such as disruption of infected intestine cells, degeneration, necrosis and also damage of blood vessels in the intestinal tissues. The deposition of hemosiderin pigments of infected fish of Lekki Lagon may probably have some relevance with the poor growth, reproduction, lethargy of the infected fishes[19]. In the present study, the number of goblet cells and their sizes were found to be increased in the affected region, and the increased mucus secretions were observed from the surface of the intestine. Accumulation of lymphocytes and the presence of a large number of granulocytes as well as fibroblasts suggest inflammatory responses.

The differences in the cellular components in the inflammatory tissue of the stone loach appeared to be connected with the amount of collagens presenting in the infested region[20]. The manifestation of the granulocytes, a few macrophages and a small amount of collagens is used as an indicator for acute inflammations. A chronic inflammatory state is associated with the presence of a large number of collagens, giant cells and fibroblasts[21]. Similarly, in the present study we also observed same fibroblasts, granulocytes, lymphocytes and macrophages of the inflammatory tissues. These changes could be due to the acanthocephala proboscis penetration into the host and the length of the parasite. The absence of giant cells in inflammated tissues indicated the acute inflammation. In some of the earlier reports, inflammatory reactions involving cellular responses, either accompanied by the formation of granulomas, have not been reported for numerous piscine parasites belonging to different groups[22].

The chronic intestinal damage leads to the inflammatory responses and serious illnesses or a high mortality induced by acanthocephalan-infected fishes. Pathogenic effects and the ultrastructure of acanthocephalans are due to the attachment of the adult acanthocephala in the digestive tract and also in the encapsulation of larval stages in the intestinal tissues. The extent of damage is proportional to the depth and width of the proboscis adherence into the host intestine[4]. There were more significant pathological changes observed in yellowfin tuna as a result of *A. dirus* infection. Acanthocephalan infections were negligible when parasites were attached to the epithelial mucosa only. The intestines of acanthocephala-infested fishes suffer from physical disturbance in the form of peristalsis and food movement can exert powerful drag on attached parasites. The most of the intestinal acanthocephala severely damaged the fish's intestine, mainly due to their superficial relationship with the tissue. The high intensity of the parasitic infestation and severely penetration of the proboscis into the host intestinal tissues are the main reasons for the pathogenicity in the host. There was evidence that irritation of the tissue due to constant mechanical damage or parasitic encystrnent may lead to the formation of extra cellular infiltration[23].

Acid phosphatase is a lysosomal enzyme that plays a vital role in the physiology of the intestine. The increase of the activity of this enzyme may be related to autolysis of any foreign substances and microbial agents. Similar observations have been reported in the intestine of some birds and rodents with a trematode infestation[24].

The role of alkaline phosphatase in transport of glucose 6-phosphate from the intestinal lumen, the moderate decrease in the level of this enzyme in the infected ileum, especially in the villi, may be explained by the destruction of the intestinal epithelium and altering of its absorption power[25,26]. Here it is first well documented the marine fish infestation and its pathological effect with special staining of the lesions.

The severe pathological changes caused by the acanthocephalan have totally destroyed the architecture of the intestinal tissues. The damage was based on the parasitic proboscis' length, hooks' length and parasitizing durations. Therefore, in recent years there has been recognition that sub-lethal physiological effects on hosts which may lead to alterations in the behavior of infected fishes may also play an important role in regulating populations through demographic effects of marine fishes in Tamil Nadu coast.

Conflict of interest statement

We declare that we have no conflict of interest.

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