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Fish innate immunity against intestinal helminths

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ABSTRACT

Most individual fish in farmed and wild populations are infected with parasites. Upon dissection of fish, helminths from gut are often easily visible. Enteric helminths include several species of digeneans, cestodes, acanthocephalans and nematodes. Some insights into biology, morphology and histopathological effects of the main fish enteric helminths taxa will be described here. The immune system of fish, as that of other vertebrates, can be subdivided into specific and aspecific types, which *in vivo* act in concert with each other and indeed are interdependent in many ways. Beyond the small number of well-described models that exist, research focusing on innate immunity in fish against parasitic infections is lacking. Enteric helminths frequently cause inflammation of the digestive tract, resulting in a series of chemical and morphological changes in the affected tissues and inducing leukocyte migration to the site of infection. This review provides an overview on the aspecific defence mechanisms of fish intestine against helminths. Emphasis will be placed on the immune cellular response involving mast cells, neutrophils, macrophages, rodlet cells and mucous cells against enteric helminths. Given the relative importance of innate immunity in fish, and the magnitude of economic loss in aquaculture as a consequence of disease, this area deserves considerable attention and support.

1. Helminth intestinal parasites of fish

In fish, as in other vertebrates, the digestive tract is one of the primary routes of microbial and parasitic infections [1], and serves as a primary barrier limiting or preventing the entry of harmful organisms [2]. The intestinal canal affords a remarkably benign and rich environment for otherwise vulnerable enteric parasites, offering them protection and nutrients [3].

Helminths, also commonly known as parasitic worms, are multicellular organisms. There is no clear consensus on the taxonomy of helminths. Helminths include some turbellarians, ectoparasitic flukes (Monogenea), endoparasitic flukes (Aspidogastrea and Digenea), and Cestoda, all belonging to the phylum Plathyhelminthes; acanthocephalans, nemathelminths (nematodes), and hirudineas or leeches belonging to Anellida [4]. Helminth parasites are of considerable medical [5] and veterinary importance [6]. Parasitic helminths in natural habitats are able to reduce drastically their host fitness, which therefore have evolved powerful countermeasures to control infection [7]. Recently, Shinn et al. [8] provided estimates of economic loss associated with notable parasite infections in some of the world's leading finfish production industries. The successful infection of helminth largely depends on their capacity to evade and/or manipulate the generally efficient immune system of hosts [5,9,10]. Nevertheless, interaction between helminth and the piscine immune system are under-investigated [10].

There are numerous studies of the effects parasitic helminths have on the alimentary canal and associated organs of fish [11–18]. As part of the infection process, certain intestinal worms induce structural modification to their host's tissues (Table 1), and most likely are responsible for alterations to normal intestinal physiology [48]. Certain types of enteric helminths of fish (*e.g.*, digenetic trematodes, cestodes) usually do not cause severe, visible damage to the intestine, mainly due to their relatively superficial relationship with the host tissues [49] (Table 1a). In contrast other helminths such as acanthocephalans typically cause much more severe damage due to deep penetration of many species into the gut tissues [50] (Table 1b).

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Parasitic helminths excrete or secrete (ES) a variety of molecules into their hosts. The ES products of trematodes, cestodes and nematodes contribute to immune evasion strategies of the parasites through different mechanisms [51]. Research into the ES substances produced by helminths infecting fish is still very much in its infancy with only a few scattered observations on nematode-fish models (see Refs. [6,52]) and a tapeworm-fish system [10,53]. From the earlier studies of fish-helminth conducted by the authors, no tegumental secretions packaged into extracellular vesicles were observed, however that does not exclude the possibility that a fraction of ES proteins, not packaged in vesicles, may be produced by parasite. A description of each enteric helminth taxon is provided below.

1.1. Digenean (endoparasitic flukes) (Table 1a, Fig. 1a)

The Digenean Flukes or digeneans (formerly digenetic trematodes) form a class of flatworms or platyhelminths. Flukes reproduce as adults and again as larvae, hence the name "di-genetic" or two births. Unlike generalized flatworms, most digeneans have two sucker-like holdfast organs. About 70 families with over 5000 species are known from all fishes. The digeneans that produce significant damage to their hosts as sexual adults are mainly those that occur in non-gut sites. With reference to parasitized intestine, the site of infection by most digeneans is restricted almost entirely to the paramucosal lumen, mucosa or epithelial tissues (Fig. 1a) [54]. Most intestinal digeneans probably feed by browsing on the mucosa or epithelial tissues, mucus, blood, products of host's digestion, and products of their own histolytic secretion [55]. Many digeneans with at least one sucker attach to the mucosal surface of the fish digestive tract (Fig. 1a) [22,56]. Therefore, the main damage is the destruction of the mucosal epithelium covering the villi, with subsequent necrosis and degeneration [54,56].

1.2. Cestoda (tapeworms) (Table 1a, Fig. 1b, c)

Tapeworms or cestodes form a large class of the flatworms with more than 5000 species identified. The common name comes from the long series of body segments which resemble a tape measure. All tapeworms are permanent parasites. Adults cestodes inhabit the digestive tract or, occasionally, associated organs of vertebrates definitive hosts [57,58]. Tapeworms usually consist of a chain of segments (proglottids) each with a set of reproductive organs and lack a digestive system, absorbing nutrients through a specialized outer layer of the body. Eucestoda possess a distinct anterior holdfast organ called the scolex (Fig. 1b, c), which varies remarkably in shape among the 11 orders [57]. The extent of damage caused by cestodes as in other helminths is generally related to the intensity of infection and depth of parasite penetration within the host tissue (Table 1a). Most tapeworms generally do not induce severe damage to the fish digestive tract, provoking only destruction of the superficial layer of the intestinal wall at the point of scolex attachment (e.g., *Cyathocephalus truncatus* see Table 1a, Fig. 1b, c). More rarely tapeworms penetrate more deeply, closely approaching the muscular layer and inducing both a complete loss of the intestinal architecture and an enhanced inflammatory response (e.g., *Monobothrium waganeri* see Table 1a).

1.3. Acanthocephala (thorny headed worms) (Table 1b, Fig. 1d, e)

These worms form a small phylum in the Animal Kingdom. The name "acanthocephala" means thorny headed. Acanthocephalans are all permanent parasites in the intestine of most vertebrates. More than 1500 species are known, the vast majority of which are parasites of fish and use crustaceans as intermediate hosts. Acanthocephalans attach in the gut of their host with a globular or cylindrical and retractable, thorny proboscis. In addition to the intensity of infection, the extent of damage caused by acanthocephalans is related to the depth of parasite penetration within the host tissue (Table 1b). Some acanthocephalan genera parasite of fish, including *Acanthocephalus* [50] and *Pomphorhynchus* (Table 1b) penetrate deeply through the intestinal wall and provoke extensive damage to the alimentary canal (Fig. 1d). At the site of attachment the acanthocephalan parasite destroys the mucosal folds (Table 1b, Fig. 1d, e) and the proboscis hooks penetrate into the epithelium of adjacent villi for secure anchoring. Folds more distant from the worms remained intact.

1.4. Nematoda (roundworms) (Table 1c, Fig. 1f)

The phylum Nematoda are comprised of 256 families and more than 40,000 species [59]. Most free-living forms are small to microscopic, but parasitic forms are large. It is believed that there are 125 families of zooparasitic nematodes, including species that exploit both freshwater, marine and brackish water fishes. Roundworms, as the name implies, are circular in cross-section, and often take the form of an elongate cylinder, tapered at each extremity. The body of nematode is covered by a thick, elastic and tough cuticle, which could be smooth, or more generally bears fine transverse striations at regular intervals [60]. Most species are dioecious, sexually dimorphic and oviparous.

Due to the rapid development of marine aquaculture, the importance of nematodes as fish pathogens is increasing [60]. Most of our knowledge on nematodes as fish parasites is founded in the numerous papers and monographs of Moravec [see 60]. Nematode parasites harm their host in different ways, such as causing mechanical injury, atrophy of tissues, castration, and occlusions of the alimentary canal and blood vessels [61,62]. While most references on nematode parasites are concerned with the prevalence and intensity of infection, there are some studies on the pathogenecity of fish intestinal nematodes (Table 1c).

2. Fish immune system

The Latin word "*immunis*" means "exempt from", and the term immunology refers to the field of research on defence mechanisms against infectious diseases [63]. The immune system has evolved to discriminate between self (e.g., host tissue) and non-self (e.g., pathogens, foreign bodies). Defence mechanisms include two broad categories which differ between them mainly for the receptor types used to recognize pathogens [64]:

I) Specific (adaptive or acquired) immunity, which responds to an invading pathogen and then reacts in an appropriate manner to eliminate it. The adaptive immune recognition is mediated by antigen receptors with narrow specificities. Upon repeated exposure to the pathogen, the specific immune system produces a faster and more adequate response. Lymphocytes are the primary cell types involved in specific immune responses [63,65].

II) Aspecific (non-specific or innate) immunity, which provides an immediate response to an invading organism and is composed of physical barriers, cellular and humoral components [66]. The innate immune recognition is mediated by germline encoded receptors with a broad specificity [67]. The innate defence is the only mechanism available to invertebrates and is of primary importance in vertebrates, especially in fish where the acquired immune response is sluggish compared to the instant and relatively temperature-independent innate immune response [66,68]. Innate