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Original Article

Occurrence and Intensity of Anisakid Nematode Larvae in Some Commercially Important Fish Species in Persian Gulf

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Abstract

Background: Anisakid nematodes are common parasites of fish, mammals, fish-eating birds, and reptiles with a worldwide distribution, causing diseases in human, fish and important economic losses.

Methods: A preliminary epidemiological study was carried out on Anisakid nematodes larvae in some commercially important fish species to evaluate the anisakid nematode larvae from greater lizardfish, (*Saurida tumbil)*, Japanese thread fin bream (*Nemipterus japonicus*), crocodile longtom (*Tylosurus crocodilus crocodiles*) and longfin trevally (*Carangoides armatus*) from the Persian Gulf of Iran.

Results: The collected larvae were identified mainly as the third larval stage (L3) of *Hysterothylacium* larval type A, B and C, *Anisakis* sp., *Raphidascaris* sp., *Pseudoterranova* sp. and *Philometra* sp. (Nematoda: Philometridae). The prevalence of Anisakid larvae infection of examined fishes was 97.2% in *N. japonicus*, 90.3% in *S. tumbil,* 20.5% in crocodile longtom and 5.5% in longfin trevally. *Anisakis* type III for the first time was different from *Anisakis* type I and *Anisakis* type II.

Discussion: Zoonotic anisakids by high prevalence in edible fish could be a health hazard for people. So health practices should be considered in these areas.

Introduction

Zoonotic diseases are important threats in economics value and produce animal protein. These diseases can impair public health and wellbeing (1). Humans suffer from multiple parasitic foodborne zoonoses that many of which are caused by nematodes (2). The behavior of homo sapiens has important role in the parasitic zoonosis. Human anisakidosis is a recognized zoonosis that is directly connected to the reactions that could lead to inflammatory lesions, allergic response and eosinophilic granulomas (3, 4). Most wild and farmed fish species can be infected by parasites at same stage of their development (5), although literature indicates that only some of them cause disease in human (6). Like pathogenic bacteria, chemical residues, pesticides and heavy metals, parasites are considered as an important risk factor for evaluation fish quality (7).

Nematodes are one of the most common parasites found in aquatic organisms, particularly those belonging to the anisakidae family that includes parasites of considerable economic and medical importance (5, 8). These nematodes from the superfamily Ascaridoidea (family Anisakidae with three *subfamilies:* Anisakinae; Raphidascaridinae and Geoziinae) are extensive distributed parasites in the Asia (Iran, Korea, Japan), Europe (The Netherlands, France, the United Kingdom, Spain, Germany, Italy), Africa (Egypt), and the Americas (Alaska, Hawaii, Canada). Their live cycles comprise of invertebrates and fish as intermediate hosts, and fish, sea mammals, mammals as well as fish- eating birds being definitive hosts (9). Although these parasites can infect humans after consumption of raw or insufficient cooking fish, there is some evidence suggesting that risk related to not only on the presence of living organisms (10, 11) but it is also connected to the dead parasites that capable of producing proteins and could induce hypersensitivity and allergic reactions (12-14). During infection, other clinical manifestations are important. Some of these signs consist of epigastric pain, as well as systematic or gastrointestinal symptoms, fever, diarrhea, vomiting, gastric and duodenal ulcers (15-17) and intestinal obstruction (18, 19).

While the parasites and their impact on human health have been investigated in many fish species worldwide, there has been a little study of parasites in commercial fish in Persian Gulf. The purpose of this study was to determine how the prevalence and mean intensity of nematode infections varied according to commercially important fish species in Persian Gulf.

Material and Methods

From October 2010 through April 2012 the nematode larvae were examined from four species of fish, including *Saurida tumbil* (n=31), *Nemipterus japonicus* (n=37), *Tylosurus crocodilus crocodiles* (n=34) and *Carangoides armatus* (n=30). For this purpose fish were examined for the nematode larvae from Choebded, Boushehr and Dayyer ports from Iran's shores of the Persian Gulf (26.0000° N, 52.0000° E) following the method of Cannon and Deardorff (1, 20). Fish were transported to the central laboratory of Aquatic Animal Health in Chamran University of Ahvaz, Iran and each specimen was individually measured for the total length (cm) and weight (g) (Table 1).

To determine the presence of nematode larvae, each fish were dissected and intestine, stomach, muscles, ovary and body cavity were observed carefully. The collected nematode larvae were washed several times using saline solution and stored in 70% ethanol. They were cleared in glycerine for examination and were identified using stereo- and light microscopy. After clarification in glycerine, larvae were identified at the genus level by a light microscope equipped with a camera lucida, according to the morphological criteria (21-23). The prevalence (%), mean intensity (MI) and mean abundance (MA) were calculated (24). Also, relative frequencies of caught fish according to weight (gr), length (cm) and sex of fish was calculated.

Results

The overall prevalence of anisakids in fish from Persian Gulf was 90.3% in *S. tumbil*, 97.2% in *N. japonicus*, 20.5% in *T. crocodilus croc-*

odile and 5.55 in *C. armatus*. Of 31 *S. tumbil*, 37 *N. japonicus,* examined, 34 *T. crocodilus crocodile* and 36 *C. armatus* 28, 36, 7 and 2 were infected by at least one parasite, respectively. Parasite species were identified *Anisakis* sp.*, Pseudoterranova, Hysterothylacium* type A*, Hysterothylacium* type B*, Hysterothylacium* type C*, Raphidascaris* type PG *and Philometra* sp. Data on infection parameter are presented in Table 1-2. A total of 646, 206, 18 and 11 nematode were recovered from *S. tumbil*, *N. japonicus*, *T. crocodilus crocodile* and *C. armatus*, respectively.

The intensity of infection in each species of fish is shown in Table 1. Larvae were found mainly within abdominal cavity excepted of *Philometra* sp. that was in ovary. One of our specimens was considered as *Anisakis* type III, because the larvae were different from *Anisakis* type I and *Anisakis* typeII by lacking a mucron on the posterior extremity, possessing an elongated ventriculus, a nerve ring located from the 0.19 mm (0.1-0.29) from the anterior end. The excretory pore was located below the tooth and muscular esophagus was 0.67mm (0.59- 0.75) long. The anus was located 0.13 mm (0.09–0.18) from the posterior end. Third-stage larvae were usually found encysted in a coiled, spring-like state on the wall of gonads, intestines and stomach. Total body length and width of the larvae were 5mm-8mm and 0.19mm-0.2mm, respectively.

The 8 larvae collected from 2 species of fish (*S. tumbil* and *T. crocodilus crocodile*) were similar to *Pseudoterranova* sp. The description of *Pseudoterranova* sp. was based on measurements of 8 larvae from these fish. Color dark red; length 7 mm (6 – 8) maximum width 0. 2 mm (0.1-0.3); 3lips; possessing boring tooth; excretory pore ventral between subventrallips; nerve ring located from the 0.31mm (0.36-0.45) from anterior end; muscular oesophagus was 1.07 mm (0.9 - 1.3) long followed by a glandular ventriculus 0.38 mm long (0.27-0.49) and 0.16 mm (0.08–0.2) width; intestinal caecum 0.4 mm long (0.31 - 0.43) extending anteriorly slightly further than the anterior margin of the ventriculus; anus 0.2 mm (0.15-0.24) from the posterior end; tail bluntly rounded with small mucron.

Hysterothylacium type A was described according 445 larvae collected from *S. tumbil*, *N. japonicus*, *T. crocodilus crocodilus* and *C. armatus*. Body was 11mm (8-14) long by 0.2 mm (0.15-0.3) wide at greatest width. Boring tooth projecting anterioventrad. A nerve ring located from the 0.35 mm (0.27-0.46) from the anterior end. The excretory pore was located below the nerve ring and muscular esophagus was 0.9mm (0.59- 1.3) long followed by a glandular ventriculus 0.13mm long (0.06-0.2) and 0.09 mm (0.08 – 0.1) width. Rectal gland was spherical shaped, numbering 4. Tail was conically shaped and 0.15 mm (0.19- 0.21) long with a single spine.

Hysterothylacium type B was described according 229 larvae collected from *S. tumbil*, *N. japonicus*, *T. crocodilus crocodilus* and *C. armatus*. Length of body 7mm (6–8) maximum width 0. 08 mm (0.05-0.14); lacking boring tooth; excretory pore opening just below nerve ring; nerve ring located from the 0.24mm (0.19-0.26) from anterior end; muscular oesophagus was 0.5 mm (0.45 – 0.59) long followed by a glandular ventriculus 0.05mm long (0.04-0.06) and 0.03 mm (0.02 – 0.04) width ; intestinal caecum 0.1 mm long (0.09 - 0.14); anus 0.13 mm (0.12-0.16) from the posterior end and tail conically shaped with a single spine.

The 114 larvae collected from 2 species of fish (*S.tumbil* and *N. japonicus*) were similar to *Hysterothylacium* type C. The following description is based on measurements of larvae from these fish. Body was 7.3 mm (5.5-9.2) long by 0.13 mm (0.05-0.2) wide at greatest width. Boring tooth projecting anterioventrad. A nerve ring located from the 0.28 mm (0.26-0.31) from the anterior end. The excretory pore was located below the nerve ring and muscular esophagus was 0.61 mm (0.42- 0.81) long followed by a ventriculus 0.04 mm long (0.03-0.6) and 0.04 mm (0.03 – 0.05) width. Rectal gland was spherical shaped, numbering 4. Tail was 0.15 mm (0.19- 0.21) long. Type C was very different from other *Hysterothylacium* in this study by possessing a tuft of 8-10 spinelike projections distally on the tail.

The 3 larvae collected from *S. tumbil* were similar to *Raphidascaris* type A. By possessing a ventricular appendage and no intestinal cecum, this nematode differs from all other larvae collected. Body was 4.5 mm (3.1- 5.2) long by 0.16 mm (0.08-0.2) wide at greatest width. Esophagos was 0.5 mm (0.41-0.61) long. Nerve ring located in anterior. Four of rectal glands were spherical and tail was conically shaped.

Philometra sp. with the intensity of 1-5 parasites per fish was isolated from the ovaries of *S. tumbil* and described as *Philometra* sp. The body filiform of the gravid females nematode was yellowish brown to reddish body coloration, cuticle smooth, length about 49.63 mm, maximum width 2.31 mm, with rounded ends; the posterior part of the body is distinctly narrower than the anterior part. The esophagus is narrow, swollen near the mouth to form a distinct bulb 0.13 to 0.17 (0.14) mm long and 0.13 to 0.22 (0.19) mm wide, which is followed by the posterior cylindrical part of the esophagus. The overall length of the esophagus is 1.31 to 1.64 (1.54) mm, the maximum width of its cylindrical part was 0.16 to 0.18 (0.17) mm (Fig. 1).

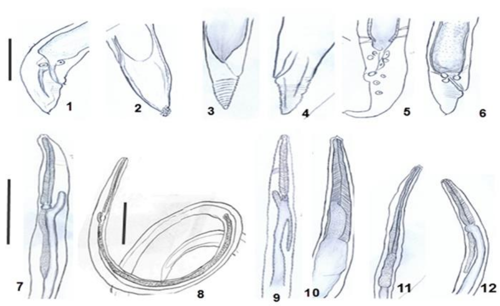

Fig. 1: Various anisakid morphotypes found in the present study. 1, posterior end showing anal glands and tail conically shaped with a single spine of *Hysterothylacium* type B;2, posterior end showing 8-10 spinelike projections distally on the tail of *Hysterothylacium* type C; 3, Tail bluntly rounded with small mucron of *Pseudoterranova* sp.;4, lacking a mucron on the posterior extremity of *Anisakis* sp;5, Four spherical rectal glands and conically shaped tail of *Raphidascaris* type A; 6, Tail with conically shaped and a single spine of *Hysterothylacium* type A;7,The relationship between various appendage of the digestive tract of *Hysterothylacium* type A; 8, *Hysterothylacium* type B;9, *Hysterothylacium* type C; 10, *Pseudoterranova* sp; 11, *Anisakis* sp.; 12, *Raphidascaris* type A. Scale bars 100 µm in (1–6) and 250 µm in (8), and 200 µm in (7) and (9-12)

Discussion

The purpose of this study was to determine how the prevalence and mean intensity of nematode infections varied according to commercially important fish species in Persian Gulf. Due to the great potential of Persian Gulf gurnard to be increasingly exploited as a food resource, information on the occurrence and spatial distribution of Anisakid larvae are important (6, 25, 26). In order to illuminate further this aspect, we separately analyzed the Anisakid larvae infection data for *S. tumbil*, *N. japonicus*, *T. crocodilus crocodile* and *C. armatus*. Little is known about the prevalence of zoonotic parasites including anisakids in fish or of anisakiasis in humans in Persian Gulf. Low intensity of *Anisakis* sp. and *Raphidascaris* were collected from long tail tuna fish (*Thannus tonggol*) caught from north parts of Persian Gulf (27).

Anisakis sp. was identified with the frequency of 4% from the intestine and stomach of *Scomberomorus commerson* and the body cavity of *N. japonicus* from Bandar Abbas port, southern of Persian Gulf (28). In similar study, *Anisakis* sp. and *Contracaecum* sp. were reported by Adel et al. on *S. commerson* (29).

Based on obtained results it is obvious that *S. tumbil* has a more diverse fauna of parasites with more intensity than other fish species that this can be attributed to the host body conditions and to the different life patterns. Also, throughout the study, three types of *Hystratylacium* sp. were determinant species with a high prevalence between another nematode. Third-stage larvae from this genus were found in four examined species of fish. Considering the three larval types of *Hystratylacium* collected, type B was most prevalent, followed by type A and type C. *Hystratylacium* type B had the highest intensity of infection followed by *Anisakis* sp, *Philometra* sp, *Pseudoterranova* and *Raphidascaris* type PG. This pattern of intensity was similar to intensity of *Hystratylacium* larvae in Hawaiian Island (30).

Anisakis type III for the first time was found in this study and it was different from *Anisakis* type I (15, 21, 31, 32) and *Anisakis* type II (21, 33-35) by lacking a mucron on the posterior extremity, possessing an elongated ventriculus. By increasing the weight and length of survived fish relative frequencies of anisakid nematode larvae were decreased. This observation was similar to report of Bagherpour et al. that studied correlation between intestine helminthes with sex, season and size in *Brachirus orientalis* from north of Persian Gulf (36).

In present study, relative frequencies of anisakid nematode larvae were not affected by sex, although this frequency in female fish was higher than male fish. Knudsen suggested that this difference might be related to different feeding habits of both sex of fish (37).

Many of fish species may harbor nematode larva of food hygienic important (38-40). Among of them, nematode larvae of the genus *Anisakis* and *Pseudoterranova* (family *Anisakidae*) found in the muscle tissue and perioral cavity of a wild range of fish species are known to cause anisakiasis and psedoterranoviasis, respectively (38). *Contracaecum* and *Hysterothylacium*, are also considered able to infect man, but rarely (41, 42). Hence, it is vital to recognize epidemiology and ecology of parasites present in infected fish (10, 39).

According to old belief of southern parts of Iran, consuming of raw fish in order to cure the jaundice was useful; therefore, it is necessary that doctors consider Anisakis larvae in patients with signs of abdominal pain and Phlegmonous enteritis (29). The life cycle of Anisakidae consist of small crustaceans as the first intermediate host, fishes and cephalopods as the second host, and marine mammals as the definitive host (42). Klimple and college followed the general nematode life cycle pattern and the adults in the final host (43). The stage L1-L3 are within the eggs and subsequently in intermediate or paratenic host (L3) and as preadult stage (L4) and adults in cetacean final host (whales and seals)(43, 44). Humans are considered as accidental hosts of the parasite following consumption of the secondary hosts. A large number of cephalopod and marine fish species are connected with the transmission of the disease (44, 45). It is important to know that fish of different species can play a vital role in the distribution of anisakids in the environment.

This study investigated some popular fish in the Persian Gulf of Iran and demonstrated considerable infections with potentially zoonotic parasites, including *Anisakis* type III, *Raphidascaris* type PG, *Philometra* sp. and *Pseudoterranova* sp. and *Hysterothylacium* types A, B and C. These parasitic nematodes are recognized as important pathogens that cause vital problems for human health and foodborne zoonosis (43). Since the 1988, a standardized nomenclature was used for three different terms: 1) anisakidosis caused by any members of the family Anisakidae, 2) anisakiasis caused by members of the genus *Anisakis*, and 3) pseudoterranovosis caused by members of the genus *Pseudoterranova* (10, 46). According to this assortment we can recognize anisakidosis, anisakiasis and pseudoterranovosis in this study in Persian Gulf. In addition, we indicate to the zoonotic potential of anisakids to infest humans in costal population in Persian Gulf. Most potential transmission routs are raw, undercooked and lightly marinated seafood according the habit and culture of people in the region (43, 47). Most of these infections induce chronic rather than acute disease with asymptomatic which being diagnosed after the expulsion of the nematodes by coughing, vomiting or defaecation (11). Interestingly, more than 90% of causes are caused by a single larva with peculiar signs (16, 35, 46, 48).

Parasites have also been considered favorable biological tags for discriminating different stocks or populations of marine fishes, and Anisakis larvae have been reported as a good tool for stock recognition of fishes in Europe and Asia (49-51).

Conclusion

With these studies, we can be optimistic regarding the control of zoonotic nematode infections in the future and use these parasites as biological tags to estimate various aspects of the marine fisheries in Persian Gulf.

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Table 1: Fish species from which anisakid larvae were collected in the present study

Fish species	N	Mean length (cm)	Mean weight (gr)	Prevalence (%)	MI±SD (Range)	MA±SD
Saurida tumbil **(Lizardfish)**	31	38.7±6.8	477.1±260.8	90.3	23.07±21.3 (3-74)	20.83±15.6
Nemipterus japonicas **(Japanese threadfin-bream)**	37	25.4±7.2	236.4±353.2	97.2	4.72±3.4 (1-45)	5.56±4.7
Tylosurus crocodilus crocodile **(Crocodile needlefish)**	34	74.4±5.3	357.1±320.3	20.5	1.57±2.02 (1-6)	0.32±5.9
Carangoides armatus **(Longfin trevally)**	36	20.7±6.2	90.5±20.7	5.55	4.5±1.09 (2-4)	0.25±0.6

N is the number of fish sampled, prevalence is the % of infected fish and (MI) is the mean number of larvae in the infected fish host ±SD, (MA) is the mean abundance

Table 2: L3 larvae of the Anisakidae family detected in fish of Persian Gulf

Host	N	Infected organ/s	*Anisakis* sp.	*Pseudoterranova* sp.	*Hysterothylacium* type A	*Hysterothylacium* type B	*Hysterothylacium* type C	*Raphidascaris* type PG	*Philometra* sp.
Saurida tumbil	28	Body cavity/ ovary	17	7	161	332	113	3	13
Nemipterus japonicus	36	Body cavity	2	0	64	103	1	0	0
Tylosurus crocodilus crocodile	7	Body cavity	3	1	1	6	0	0	0
Carangoides armatus	2	Body cavity	2	0	3	4	0	0	0

N is number of fish with Anisakid larvae