Anisakid nematodes in marine fishes

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Abstract: A total of 816 marine fishes belonging to four different fish species were examined for presence of anisakid larvae. Four species of anisakids were identified; *Anisakis typica*, *Phocanema decipiens*, *Porrocaecum decipient* and *Hysterothylacium aduncum*. The total prevalence was 65.81%. The prevalence in Mediterrean horse mackerel (*Trachurus mediterraneus*), Mediterrean silverside sand smelt (*Atherina species*), red porgy (*Pagrus species*) and Lizardfish (*Saurida undosquamis*) was 92.31%, 72.80%, 59.09% and 43.43%, respectively. The morphological characteristics of the detected anisakids were studied.
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1. Introduction:

Anisakidosis is a human disease caused by the ingestion of larval nematodes from the family Anisakidae, especially *Anisakis simplex*. The infection is acquired by eating raw seafood or undercooked fish and squid (Sakanari and McKerrow 1989). In humans the *Anisakis simplex* larvae can cause lesions at different points of the digestive tract, usually leading to severe pain. These parasites have also been implicated in allergies, from angioedema-urticaria to anaphylaxis (López Serrano *et al*., 2000; Audicana *et al.,* 2002 and Daschner *et al.*, 2002).

Larvae of Anisakid nematodes are a major problem for commercial fishing industries, and are potential human health hazards, both as causative agents of anisakiasis (Sakanari and Mckerrow, 1989), and as potential food-borne allergens (Moneo *et al*., 2000; Baeza *et al*., 2001; Arlian *et al.*, 2003 and Daschner and Pascual, 2005).

Human are accidental host in the life cycle, and the parasites almost never developed further within the human gastrointestinal tract (López Serrano *et al*., 2000 and Chai *et al.*, 2005). These nematodes produced clinical symptomatology characterized by serious gastrointestinal lesions (Eguia *et al*., 2003 and Montalto *et al*., 2005). Moreover, owing to the thermostability of *Anisakis simplex* allergens, the ingestion of safely cooked fish containing dead parasites can also be potentially dangerous and can cause severe allergic reactions such as contact dermatitis and asthma (Audicana *et al*., 2002; El-Daly *et al.*, 2004 and Audicana and Kennedy, 2008).

The aim of the present study was to Study prevalence and identification of anisakid nematodes infecting marine fishes at Sharkia province, Egypt.

2. Materials and Methods

A total of 816 marine fish belonging to four different fish species; 198 Lizardfish (*Saurida undosquamis*), 52 Mediterrean horse mackerel (*Trachurus mediterraneus*), 500 Mediterrean silverside sand smelt (*Atherina species*) and 66 red porgy (*Pagrus species*) were bought from fish markets at Sharkia Province, Egypt from August 2009 till the end of July 2010. The muscles, body cavity, internal organs and gonads were examined visually for the presence of larvae and by using Compression technique and digestion method according to Jackson *et al*. (1981) and Manfredi *et al*. (2000).

The collected larval stages of anisakid nematodes were washed in distilled water. They were cleared in lactophenol and permanently mounted in polyvol. Then left to dry in hot air oven at 40-50°C for 24 hours, and examined microscopically (Lucky, 1977, Kruse and Pritchard, 1982 and Moravec, 1994). The larvae were identified according to their morphological characteristic features using a light microscope (Olson *et al.*, 1983; Hurst, 1984; Chai *et al.*, 1995; Shih, 2004 and Shih *et al.,* 2010). Prevalence of anisakid larvae were reported as mentioned by Bush *et al.* (1997).

Results

I. Prevalence of larval anisakid parasites in the examined fish species

Table (1) showed the susceptibility of the examined fish species to larval anisakid nematodes infection. Mediterrean horse mackerel (*Trachurus mediterraneus*) was the most susceptible species as the infection rate was (92.31%), this was followed by Mediterrean silverside sand smelt (*Atherina species*) as the infection rate was (72.80%). While, red porgy (*Pagrus species*) and Lizardfish (*Saurida undosquamis*) were less susceptible to larval anisakid

infection as the infection rate was 59.09% and 43.43%, respectively.

Table (1): Prevalence of larval anisakid parasites in the examined fish species

Fish species	No. of examined fish	No. of infected fish	%
lizardfish	**198**	86	**43.43**
Mediterranean horse mackerel	**52**	48	**92.31**
Mediterranean silverside sand smelt	**500**	364	**72.80**
Red porgy	**66**	39	**59.09**
Total	**816**	**537**	**65.81**

II. Distribution of anisakid larvae in marine fishes:

The third-stage larvae of *Anisakis typica* were isolated from body cavity, mesenteries, liver surface, gonads and muscles of Lizardfish (*Saurida undosquamis*).

The third-stage larvae of *Phocanema decipiens* were isolated from body cavity, mesenteries and liver surface of Lizardfish (*Saurida undosquamis*). Also from body cavity, mesenteries, liver surface, gonads and muscles of fishes of Mediterranean silverside sand smelt (*Atherina species*) and Mediterranean horse mackerel (*Trachurus mediterraneus*).

The third-stage larvae of *Porrocaecum decipient* were isolated from body cavity and mesenteries of Lizardfish (*Saurida undosquamis*) and red porgy (*Pagrus species*).

The third-stage larvae of *Hysterothylacium aduncum* were isolated from body cavity and mesenteries of Mediterranean horse mackerel (*Trachurus mediterraneus*).

III-Morphological description of the detected anisakid larvae:

1. Anisakis typica (Diesing, 1860):

The body measured 14.14-21.85 mm in length and 0.34-0.36 mm in width. The cuticle had transverse striations. Three lips were surrounding the mouth opening; two ventrolateral lips and one dorsal lip. Boring tooth was prominent and projected anterioventrally, ventral to the mouth. The excretory pore was present between the ventrolateral lips at which a single excretory duct opened. The oesophagus consisted of two parts, anterior muscular part was long and measured 1.25-2.55 mm and a posterior ventricular part was short and measured 0.49-0.79 mm. The tail was short, rounded and bears a distinct mucron at the posterior end, the anal opening was sub- terminal laying anterior the end of the body by 0.092-0.108 mm.

***2. Phocanema (Terranova or Pseudoterranova) decipiens* (Krabbe, 1878):**

The body measured 5.62 - 7.71 mm long and 0.13-0.16 mm width. The cuticle was transversely striated. A characteristic boring tooth was located near the ventral margin of the dorsal lip. The excretory pore appeared as a slit-like between the two ventrolateral lips at the base. The oesophagus was divided into anterior muscular part measured 0.64-0.83 mm long and posterior ventriculus glandular part measured 0.23-0.38 mm long. Intestinal caecum measured 0.49-0.72 mm long and extending anteriorly. Tail was short, bluntly rounded and had a small mucron at its tip. Anal opening was sub-terminal laying anterior the end of the body by 0.108-0.139 mm.

***2. Porrocaecum decipients* (Railliet et Henry, 1912)**

The body was relatively stout. It measured 15.24-19 mm long and 0.34-0.43 mm wide. A characteristic boring tooth was located near the ventral margin of the dorsal lip. The excretory pore situated 0.39-0.41 mm from the anterior end. The oesophagus was divided into anterior muscular part measured 0.99-1.14 mm long and posterior short ventriculus glandular part measured 0.085-0.09 mm long. Intestinal caecum was short extending anteriorly and it measured 0.15-0.19 mm long. Tail was short, bluntly rounded. Anal opening was sub-terminal laying anterior the end of the body by 0.15 - 0.21 mm.

***4. Hysterothylacium aduncum* (Ward et Magath, 1917)**

The body was elongate, tapering anteriorly and posteriorly. The body length was 10.45-11.4 mm and maximum width was 0.131-0.185 mm at the middle of the body. Three large lips were present at the anterior end; two large ventrolateral and one dorsal, the dorsal one was somewhat shorter. The lips were somewhat wider than the body. Interlabia were present. Excretory pore situated just behind nerve ring. Ventriculus was short and it measured 0.022-0.041 mm in length. Intestinal caecum run anteriorly at nearly half or more of the muscular oesophagus and it measured 0.277-0.308 mm in length. Ventricular appendix run posteriorly and it measured 0.678-0.77 mm in length. Tail was conical and armed with numerous spines situated at different levels and appeared as cactus-tail. The anal opening was sub-terminal lying anterior to the end of the body by 0.116-0.169 mm.

Table (2): Morphological characters of the third stage larvae of four anisakid nematodes obtained from marine fishes.

Criteria	*Anisakis typica*	*Phocanema decipiens*	*Porrocaecum decipient*	*Hysterothylacium aduncum*
1) Anterior end				
a- Boring tooth	Present	Present	Present	Absent
b- Excretory pore	Present between the ventrolateral lips	Present between the ventrolateral lips	situated 0.39-0.41 mm from the anterior end	situated at the level of nerve ring
2) Ventricular region				
a- Ventriculus	Long (0.49 – 0.79 mm long)	Relatively short (0.23-0.38 mm long)	Short (0.085-0.09 mm long)	Short (0.022-0.041 mm long)
b- Intestinal caecum	Absent	Long (0.49-0.72 mm long)	Short (0.15 – 0.19 mm long)	Short (0.277-0.308 mm long)
c- Ventricular appendix	Absent	Absent	Absent	Long (0.678-0.77 mm long)
3) Posterior end				
a- Anal opening	Sub-terminal laying anterior the end of the body by 0.092-0.108 mm.	Sub-terminal laying laying anterior the end of the body by 0.108-0.139 mm	Sub-terminal laying anterior the end of the body by 0.15 - 0.21 mm	Sub-terminal laying anterior the end of the body by 0.116-0.169 mm
b- Mucron	Present and prominent	Present and small	Absent and posterior end was blunt	Absent and posterior end has a cactus-shaped tail

4. Discussion

Anisakid nematodes are parasites of commercially important marine fish and its prevalence is of great concern for both human health and economic reasons.

The presence of some anisakid larvae in marine fishes had been investigated, and the number of fish species have been found to be receptive to anisakid infection (Smith 1984; Manfredi *et al.*, 2000; Luque and Poulin, 2004; Cruz *et al.*, 2007; Abd-Al-Aal *et al.*, 2008 and Ahmed *et al.*, 2010).

In the present study the morphological description of anisakid third stage larvae obtained from marine fishes was in accordance with that described by Shih (2004).

In the present investigation, the total prevalence of examined fish species reached to be 65.81%.

The previous studies which have been carried out to investigate the infection of Lizardfish (*Saurida undosquamis*) and red porgy (*Pagrus species*) with anisakid larvae were few. From the obtained result, the prevalence of anisakid larvae in Lizardfish (*Saurida undosquamis*) and red porgy (*Pagrus species*) was 43.43% and 59.09%, respectively. Similarly, Abd Al-Aal *et al.* (2008) and Ahmed *et al.* (2010) reported that 41.86% and 48.76% of Lizardfish (*Saurida undosquamis*) were infected with anisakid larvae, respectively. While, Abd El-Maksoud (1992) isolated anisakid larvae from red porgy (*Pagrus species*) with a prevalence of 63.1%.

In the present study, anisakid larvae were detected at a higher prevalence to be (92.31%) in Mediterranean horse mackerel (*Trachurus mediterraneus*). Such finding substantiated what has been reported by Abd Al-Aal *et al.* (2008). They revealed that 100% of Mediterranean horse mackerel (*Trachurus mediterraneus*) were infected with anisakid larvae. On the other hand, the lower prevalence was recorded by Abd El-Maksoud (1992), Adroher *et al.* (1996), Abd El-Ghany (2007) and Labib *et al.* (2009) d 30%, 39.4%, 48.33% and 18.8%, respectively.

Dealing with infection of Mediterranean silverside sand smelt (*Atherina species*) with anisakid larvae; the prevalence rate of infection reached to be 72.80%. However, different authors reported lower prevalence rate as Amer *et al.*(2007), Abd Al-Aal *et al.* (2008), Ibrahim *et al.*, (2009), Labib *et al.* (2009) and Mohamed (2010) detected 20%, 47%, 20,3%, 33.8% and 23.12%, respectively.

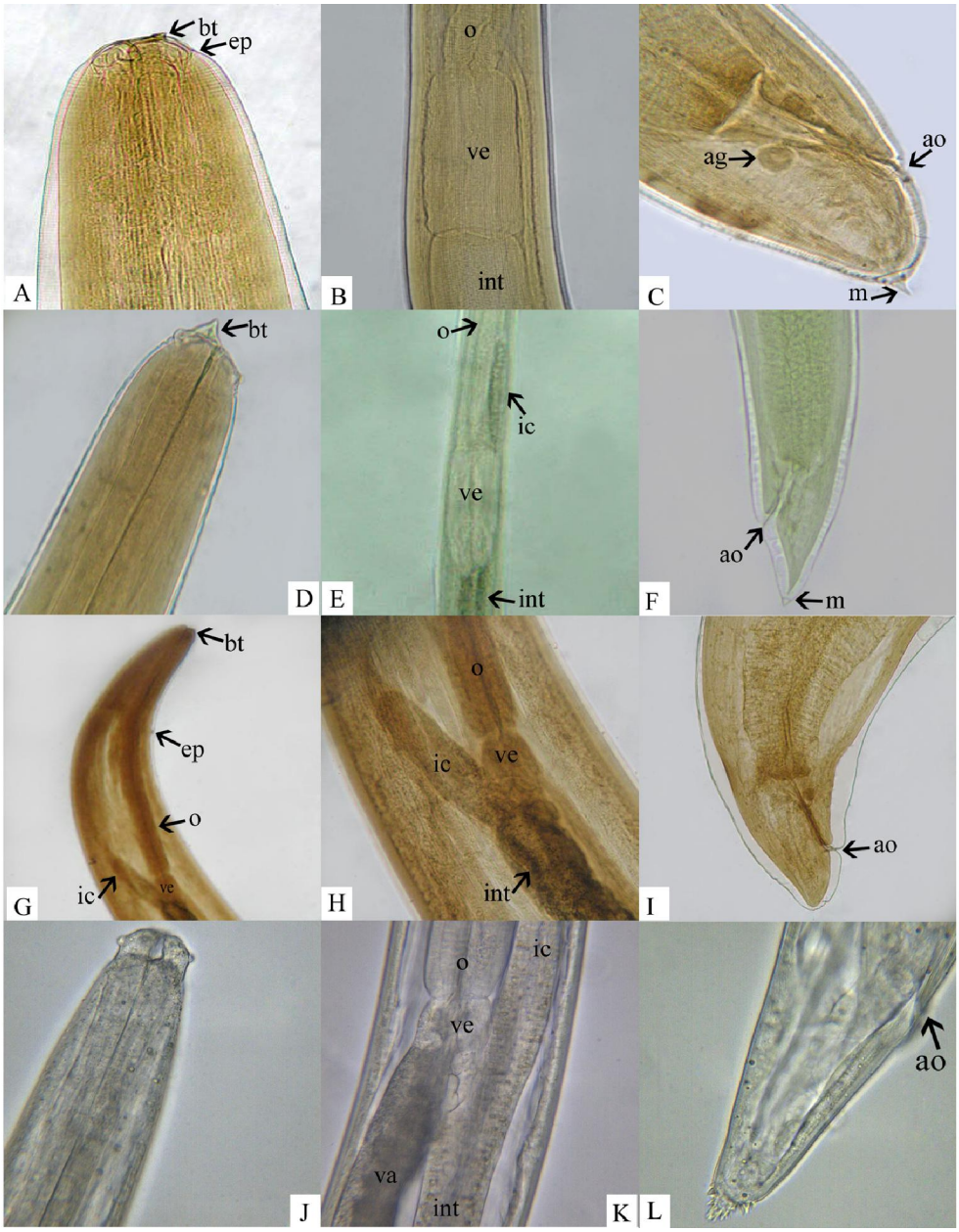

Plate (1): A, D, G, J Cephalic end of *Anisakis typica, Phocanema decipiens, Porrocaecum decipient* and *Hysterothylacium aduncum* showing boring tooth (bt), excretory pore (ep) esophagus (o), ventriculus (ve) and intestinal caecum (ic). B, E, H, K Ventricular region of *Anisakis typica, Phocanema decipiens, Porrocaecum decipient* and *Hysterothylacium aduncum* showing esophagus (o), ventriculus (ve), intestinal caecum (ic), ventricular appendage (va) and intestine (int). C, F, I, L posterior end of *Anisakis typica, Phocanema decipiens, Porrocaecum decipient* and *Hysterothylacium aduncum* showing mucron (m) and anal opening (ao) (Camera digital).

In our study, anisakid larvae were found in muscles, body cavity, mesenteries, liver surface, gonads of the infected marine fish. Smith (1984) suggested that the distribution of anisakid larvae are mainly governed by the conditions encountered within host tissues and are possibly related to the availability of nutrients. Although most larvae were found attached to the viscera or free in the body cavity of infected fish, their importance as a potential source of human infection cannot be excluded (Smith and Wootten, 1975).

It is worth mentioning that the results are proving that attention must be paid for cooking fish well where the larvae of anisakids invade the human

body through the digestive tract and settle in the liver, intestine, spleen, heart, muscle and causing pathological illness as well as the type of *Anisakis simplex*. In addition of the previous mention, it occurs severe urticaria through contact with human skin when cleaning fish or by eating them through digestive tract.

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