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GENERAL INFORMATION

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CONTRIBUTION ON LARVAL ANISAKID NEMATODES IN SOME IMPORTED FISH

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Abstract

A total of 235 frozen imported fish, 115 mackerel (*Scomber scombrus*) and 120 Atlantic horse mackerel (*Trachurus trachurus*) were collected from fish markets at Sharkia Province, Egypt, and examined for the presence of anisakid larvae. The obtained results revealed that the overall infection rate with nematodes larvae was 62.97%. The infection rate in mackerel (*Scomber scombrus*) and Atlantic horse mackerel (*Trachurus trachurus*) was 53.91 and 71.66% respectively. Four species of anisakid larvae were identified; *Anisakis simplex*, *Phocanema decipiens*, *Porrocaecum decipient* and *Hysterothylacium aduncum* with prevalence 52.76, 26.81, 9.36 and 4.25% respectively. The morphological characteristics of the recorded anisakid larvae and the best methods for prevention and control their potential risk were discussed.

INTRODUCTION

Imported fish considered as one of the cheapest and promising source of protein that overcome the problem of limited abilities of animal production (Norman, 1951). Fish like all living organisms are suffering from parasitism, either as final or intermediate host (Shih, 2004). Fish and fish products have long been considered a vehicle of food borne parasitic infections leading to human illnesses (Brown and Dorn, 1977).

Nematodes from superfamily Ascaridioidea, (Family: Anisakidae) commonly named Anisakid, are parasites of many water organisms. Low specificity in the choice of hosts, both intermediate and definitive, causes their geographical distribution is wide (Mhaisen *et al*., 1988). The most widespread genera, *Anisakis* Dujardin, 1845; *Contracaecum* Railliet and Henry, 1912; *Pseudoterranova* Mozgovoy,1951; and *Hysterothylacium* Ward et Magath, 1917. In their intermediate host fishes anisakid larvae occur encapsulated in and on the viscera or free in the body cavity beneath the liver serosa or within the parenchyma of the liver and also in muscles (Szostakowksa *et al*., 2002). Humans act as accidental hosts by ingesting raw marine fish and invertebrates infested with larvae (Szostakowksa *et al*., 2002 and Doupé *et al*., 2003) causing significant clinical disease known as Anisakiasis or Anisakidosis in a number of countries (Zhou *et al.*, 2008).

The nematodes that cause anisakiasis are larvae of *Anisakis simplex* in most cases, followed by larvae of *Pseudoterranova decipiens.* Other larval anisakid nematodes, such as *Contracaecum spp*., *Contracaecum osculatum* and *Heterothylacium aduncum*, are very rarely found in humans (Ishikura, 2003). *Anisakis*, *Pseudoterranova* and *Contracaecum* have similar life cycles (Lymbery *et al.*, 2002). The definitive hosts of these larvae are either marine mammals (*Anisakis simplex*) or piscivorous birds and mammals (*Contracaecum*) or elasmobranches (*Terranova*) and teleost fishes (*Hysterothylacium*), while aquatic invertebrates and fish are intermediate hosts. In the intermediate host, the larvae penetrate the intestine and invade the celomatic cavity or the muscle, where they molt into the third stage and become encapsulated (Bernardi, 2009). Based on the definitive hosts, in which adult worms occur, *A. simplex* and *P. decipiens* are called whaleworm and sealworm, respectively (Nagasawa, 2005).

Infection by Anisakids can affect the commercial value of fish, particularly when larvae are located in the musculature and thus represent some economical loss for the fisheries industry (Angot and Brasseur, 1995).

During the last decade there has been renewed interest in the importance of the anisakid nematodes. Surveys have been conducted throughout the world to determine the occurrence and distribution of the anisakids in fish, especially those hosts of commercial value. So, the objective of the present study was to demonstrate and identify anisakid nematodes infecting some imported fishes.

MATERIALS AND METHODS

Collection of fish samples:

A total number of 235 frozen imported fish including 115 mackerel (*Scomber scombrus*) and 120 Atlantic horse mackerel (*Trachurus trachurus*) were collected from fish markets at Sharkia Province, Egypt, during the period extended from April 2010 till March 2011. Identification of fish was carried out according to Hisck (1987). Each sample was carried in a plastic bag and kept in the deep freeze (-18˚C) until examination.

Sample Preparation:

The collected fish was allowed to thaw within 3-4 hours under the running tap water; the fishes were autopsied separately in Petri dish according to Kabata (1985).

Examination of fish:

The muscles, body cavity and internal organs were examined visually for the presence of larvae and by using Compression technique and digestion method according to Manfredi *et al*. (2000).

Identification of nematode larvae:

A) Preparation of permanent specimens

The collected larvae 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 (Kruse and Pritchard, 1982 and Moravec, 1994).

B) Identification of mounted larvae:

The mounted larval nematodes were identified according to their morphological characteristic features using a light microscope (Chai *et al.,* 1995; Shih, 2004 and Shih *et al.,* 2010).

RESULTS AND DISCUSSION

From the zoonotic point of view, it is well known that the anisakid nematode resemble a real hazard to the public health Abd El-Maksoud (1992). Out of 235 examined fish 148 (62.97%) have been found to be infected with anisakid larvae (Table 1). The infection rate was 53.91% and 71.66% in mackerel (*Scomber scombrus*) and Atlantic horse mackerel (*Trachurus trachurus*) respectively.

Lower prevalence rate in different hosts of fish was recorded by Abd El-Maksoud (1992) in marine fish 34.09%, Raef (1994) the Mediterranean Sea fishes 3.96%, Amer *et al*., (2007) Mediterranean sand smelt fish 20%, Shagar and El-Ashram (2007) Red Sea fishes 45.9% and Abd Al-Aal *et al*. (2008) marine fish 37.64%. A higher percentage was reported by El-Daly *et al.* (2004) smoked fish 90%, El-Gazzar *et al*. (2004) in smoked and frozen herring fish as (78% and 81%), Aref (2006) smoked herring fish 66.7% and Nada and Abd El-Ghany (2011) marine fish 65.81%. Dealing with the infection of *S. scombrus* with anisakide larvae, the prevalence rate was 53.91%. These result nearly similar to Mahmoud (1986) and Abd El-Ghany (2011) who reported that the prevalence rate of infection in *S. scombrus* was 51.98% and 57.97% respectively. The higher prevalence was observed by Abd El-Maksoud (1992) 80.9%.While the lower prevalence was reported by Alves and Luque (2006) 25.6% and Abd El-Ghany (2007) 48.15%. On the other hand, the prevalence rate of nematodes larvae among *T. trachurus* was

71.66%. Lower prevalence was reported by Abd El-Maksoud (1992) 30%, Adroher *et al.* (1996) 39.4% and Abd El-Ghany (2007) 48.33%. These variations in the prevalence of infection may be attributed to the number of examined fish, locality, weight and length of fish, condition encountered within the host tissue and to the availability of nutrients (Woo, 1995 and Paperna, 1996).

The prevalence of *Anisakis simplex, Phocanema decipiens, Porrocaecum decipient* and *Hysterothylacium aduncum* was 52.76%, 26.81%, 9.36% and 4.25% respectively (Table 2). However, Adroher *et al*. (1996) recorded that the prevalence of *Anisakis simplex*, *Hysterothylacium aduncum* and *Hysterothylacium sp*. was 26.1, 31.1 and 1.7% respectively. Essia *et al.* (1998) found that the percentage of *Anisakis simplex* and *Pseudoterranova decipiens* was 22.5% and 3.75% respectively. On the other hand higher percentages were recorded by Wharton *et al.* (1999) who found Anisakis larvae in 82% of 100 *Chelidonichthys kumu*, in 84% of 100 *Nemadactylus macropterus* and 100% of 36 *Thyristes atun*. El-Gazzar *et al*. (2004) detected that the prevalence of *Anisakis simplex* and *Pseudoterranova decipiens* in smoked and frozen herring fish was 41, 7, 28 and 7% respectively. Shagar and El-Ashram (2007) found that the percentage of *Anisakis simplex* and *Hysterothylacium fabri* was 35.5 and 1.6%. Ahmed *et al*. (2010) recorded that the prevalence rate of *Anisakis simplex*, *Pseudoterranova decipiens* and *Porrocaecum decipient* was 24.79, 28.92 and 4.13% respectively. Abd El-Ghany (2011) detected that the prevalence rate of *Anisakis simplex*, *Phocanema decipiens*, *Porrocaecum decipient* and *Hysterothylacium sp.* was 25.65%, 42.50%, 0.45% and 4.89% respectively.

In the present study, the anisakid larvae were found free in body cavity, intestine and liver surface or encapsulated attached to external intestinal wall of infected fish Fig. (1) A, B. All obtained larvae were found dead, this may be due to the prolonged period of freezing the imported fish. Thus we can emphasizes the role of freezing in destroying the anisakid larvae according to (Moller, 1991) who recorded that the fish inhabiting nematode stages show a remarkable resistance to low temperature, but are destroyed readily by freezing at -20°C for more than 24 hours.

Table (1): The prevalence of larval anisakid nematodes in the examined fish species.

Fish species	No. of examined fish	No. of infected fish	Percentage of infected fish
Mackerel (*Scomber scombrus*)	115	62	53.91
Atlantic horse mackerel (*Trachurus trachurus*)	120	86	71.66
Total	235	148	62.97

Table (2): The prevalence of larval anisakid nematodes in examined fish species.

Fish species	Total No. of examined fish	***Anisakis simplex***		***Phocanema decipiens***		***Porrocaecum decipient***		***Hysterothylacium aduncum***	
		No. of infected fish	**%**	**No. of infected fish**	**%**	**No. of infected fish**	**%**	**No. of infected fish**	**%**
Mackerel	115	53	46.08	36	31.30	17	14.78	10	8.69
Atlantic horse mackerel	120	71	59.16	27	22.5	5	4.16	0	0
Total	235	124	52.76	63	26.81	22	9.36	10	4.25

Anisakid larvae have been detected worldwide in a large variety of fish species, approximately 200 fish species (Chen *et al.,* 2008). Anisakids often have major pathological effects in the alimentary tract and associated organs of their natural host species; they are reported to erode the gastric lining, resulting in autodigestion of the stomach wall by the host, causing ulceration with connective tissue proliferation and granulation, they also can perforate the gastrointestinal tract and cause peritonitis Smith (1999). Although most of the fish-born parasitic zoonoses of man have an apparently limited geographical distribution, yet people's food habit represents an important factor in influencing the incidence of these diseases (Abd El-Maksoud, 1992). Besides the problems related to consumer's health when the live larvae are ingested such as anisakidosis or allergic problems, the products excreted or released by the larvae are recognized as proteases, protease inhibitors or antioxidants, which can cause changes in fish muscle during storage Vedacik (2009). In general, proper identification of anisakis species infecting host fishes is very important to both human health and fish disease diagnosis.

Morphological description of the detected anisakid larvae

Anisakis simplex (Rudolphi, 1809) Fig. (2) A, B.

Host: *Scomber scombrus* and *Trachurus trachurus*

Habitat: body cavity, intestine, liver surface and encapsulated on intestine wall.

This third stage larva was slender in shape and whitish in colour. The body measured was 20 – 24 mm in length and 0.37 - 0.51 mm in width. The cuticle had fine transverse striations. The mouth opening surrounding with three lips which were two ventro-lateral and one dorsal and having a characteristic anterio-laterally boring tooth at its anterior end. The excretory pore was situated between the ventro-lateral lips. The mouth leaded to oesophagus which measured 1.9 – 2.6 mm long and provided with an elongated ventriculus. The intestinal caecum was absent. The tail was broadly rounded and measured 0.19- 0.24 mm long and provided with mucron.

***Phocanema* (*Terranova* or *Pseudoterranova*) *decipiens* (**Krabbe, 1878) Fig. (2) C, D.

Host: *Scomber scombrus* and *Trachurus trachurus*.

Habitat: body cavity, intestine.

The body measured 7.83 - 11.41 mm long and 0.29-0.33 mm width. The cuticle was transversely striated and wrinkled. There was a characteristic boring tooth 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 0.93-1.08mm long and posterior ventriculus glandular part measured 0.35-0.74 mm long. Intestinal caecum short and extending anteriorly. Tail was short, bluntly rounded measured 0.1-0.16 and had a small mucron at its tip.

Porrocaecum decipients (Railliet and Henry, 1912) Fig. (2) E, F.

Host: *Scomber scombrus* and *Trachurus trachurus*.

Habitat: body cavity.

The body was relatively stout. It measured 13.12-18.9 mm long and 0.31-0.39 mm wide. A characteristic boring tooth was located near the ventral margin of the dorsal lip. The excretory pore situated 0.28-0.34 mm from the anterior end. The esophagus was divided into anterior muscular part measured 0.82-1.11 mm long and posterior short ventriculus glandular part measured 0.06-0.08 mm long. Intestinal caecum was short extending anteriorly. Tail was short, bluntly rounded.

Hysterothylacium aduncum (Ward and Magath, 1917) Fig. (2) G, H.

Host: *Scomber scombrus*.

Habitat: body cavity.

The body was elongate, tapering anteriorly and posteriorly. The body length was 9.33-10.7 mm and maximum width was 0.11-0.17mm 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 intestinal caecum run anteriorly at nearly half or more of the muscular oesophagus and it measured 0.19-0.27 mm in length. Ventricular appendix run posteriorly and it measured 0.53-0.62 mm in length. Tail was conical and armed with numerous spines situated at different levels. These morphological characters of larval nematodes were similar to that obtained by (Moravec, 1994; Abdel-Rahman, 1995; El-Gazzar, *et al*., 2004; Shagar and El-Ashram, 2007; Shih *et al.*, 2010 and Nada and Abd El-Ghany, 2011).

Anisakiasis is a serious zoonotic disease occurs when humans who act as incidental host in the life cycle eat raw or lightly cooked marine fish that are infested with anisakis larvae. So a number of control measures are used to prevent anisakiasis as recommended by the Food and Drug Administration (FDA, 2001); cleaning (evisceration) of fish as soon as possible after they are caught reduces the number of larvae penetrating into the muscles from the mesenteries, the consumer should avoid the consumption of the whole small fish or the abdominal regions of the fish, eating well-cooked fish by subjecting the fish to temperatures more than 60°C at least 1 min when cooking fish conventionally or heating to over 74°C for at least 15 seconds when microwave cooking, finally eaten well-frozen fish at -35°C or below for 15 hours or at -23°C for at least 7 days kills the larvae of nematodes.

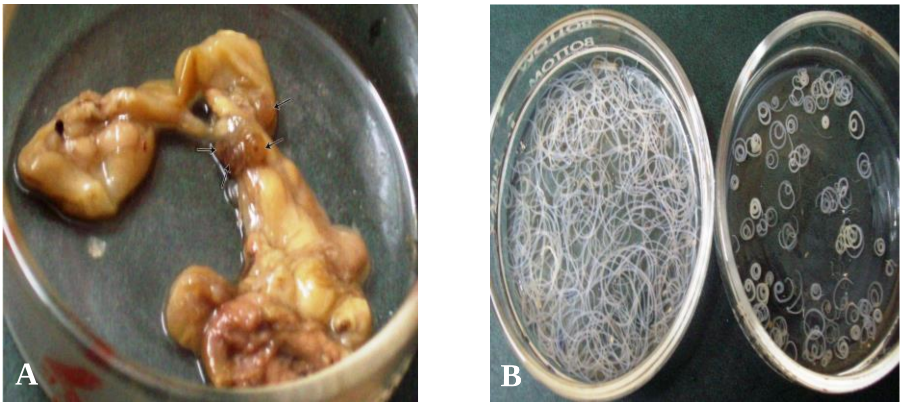

Figure (1): A- Encapsulated larvae attached to external intestinal wall of *Trachurus trachurus* (arrows). B- Anisakid larvae recovered from infected fishes.

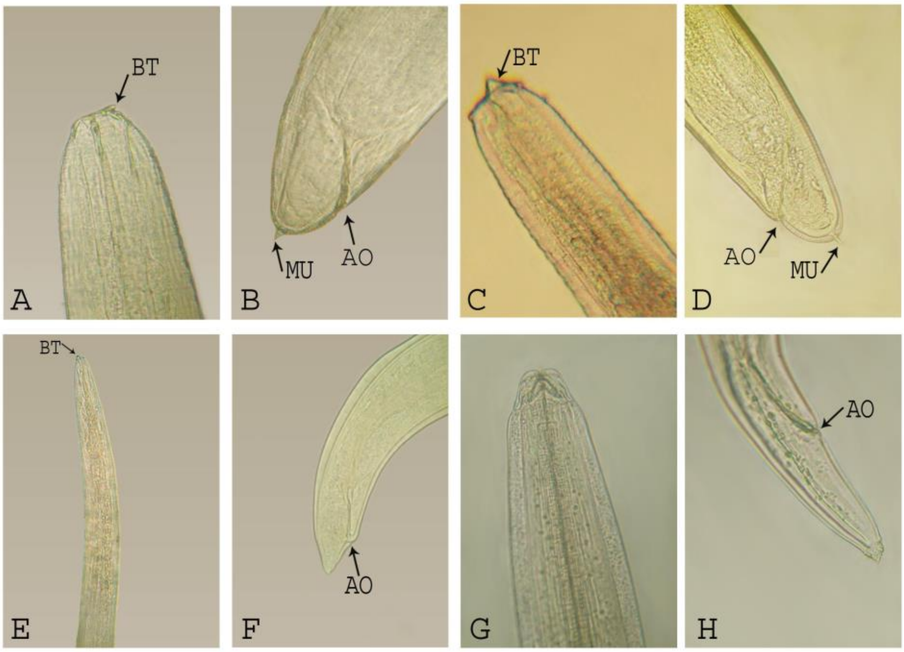

Figure (2): A- *Anisakis simplex* anterior end (X200), B- *Anisakis simplex* posterior end (X400), C- *Phocanema decipiens* anterior end (X200), D- *Phocanema decipiens* posterior end (X200), E- *Porrocaecum decipients* anterior end (X100), F- *Porrocaecum decipients* posterior end (X100), G- *Hysterothylacium aduncum* anterior (X100) and H- *Hysterothylacium aduncum* posterior end(X100).

Abbreviations: *Boring tooth (BT) *Mucron (MU) *Anal opening (AO)

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اسهامة عن يرقات ديدان الانيساكيد فى بعض الأسماك المستوردة

ولاء طلعت الأكيابى

قسم بحوث صحة الاسماك ورعايتها– المعمل المركزى لبحوث الثروة السمكية– مركز البحوث الزراعية – وزارة الزراعة – مصر

الملخص العربى

اجريت هذة الدراسة على عدد ٢٣٥ من الاسماك المستوردة جمعت من الاسواق فى محافظة الشرقية وتنتمى الى نوعين من الاسماك (١١٥ سيوار ، ١٢٠ باغة) وذلك للتعرف على انواع يرقات ديدان الانيساكيد ومدى الاصابة بها حيث أن هذه الديدان تؤثر على صحة الانسان.

أظهرت نتائج الفحص أن معدل الاصابة الكلى بيرقات ديدان الانيساكيد ٦٢.٩٧ % حيث كان معدل الاصابة٥٣.٩١ ، ٧١.٦٦% فى السيوار والباغة على التوالى. تم عزل اربعة انواع من يرقات ديدان الانيساكيد من الاسماك المصابة وهى يرقات ديدان الانيساكس سيمبلكس والفوكانيما ديسيبنس والبروسيكم والهستيروسيلكم ادنكم وقد تم تسجيل نسبة الاصابة بيرقات ديدان الانيساكس سيمبلكس والفوكانيما ديسيبنس والبروسيكم والهستيروسيلكم ادنكم كالاتى٥٢.٧٦ ، ٢٦.٨١ ، ٩.٣٦ ، ٤.٢٥% على التوالى. وقد تم وصف ومناقشة الخصائص المورفولوجية وافضل الطرق للوقاية والسيطرة على المخاطر المحتملة ليرقات ديدان الانيساكيد.