SOME STUDIES ON LARVAL NEMATODES (ASCARIDIDAE) INFECTION IN SARDINA PILCHARDUS WITH REFERENCE TO ITS PUBLIC HEALTH IMPORTANCE

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Abstract

A total of 50 fresh Sardine fish (*Sardina pilchardus*) of Red Sea was examined for the prevalence of *Contracaecum* larvae. The higher incidence of distribution and intensity of infection were in the liver tissues (100% and 8.7 larvae/fish), respectively. The lower values were detected in abdominal mucles (22% and 0.53 larvae/g of fish muscles). Tow species of *Contracaecum* larvae were recorded and described morphometrically. These larvae are considered as a first record from Red Sea *Sardina pilchardus* in Egypt. As a causative agent of human anisakiasis, their importance from public health point of view was discussed.

Application of physical control for killing the Contracaecum larvae in fish with thermal procedures as frying for 5 minutes on each side, grilling for 15 minutes at 60-80^{o}C on each side respectively, as well as freezing at-18oC for 48 hours proved to be quite sufficient to kill all larvae in the fish.

INTRODUCTION

Larval anisakids are among the most common nematodes of marine fishes. They reduce the commercial value of infested fish as they are transmissible to man. These larvae are of great significance as invadors of tissues, especially liver, gonads, mesentery and body muscles. Also, if consumed alive in raw or inadequately cooked fish, they can cause a dangerous parasitic syndrome in man (Cheng 1982).

Contracaecum species belongs to Anisakis larvae, which occur as adults in marine mammals, and as third stage larvae in fish hosts. It has been recorded in a large number from U.S.S.R. fish (Shulman and Shulman-Albova, 1953). Moreover, Kulochkova (1980) stated that 48.8% of the herring harbourded larval *Anisakis* sp., and the most abundant ones were *Contracaecum* larvae (60%). Woo (1995) pointed out that, in the U.S.A., a recent study has shown that all of the 171 examined

mature herring from Washington were infected with *Anisakis* and *Contracaecum* larvae; the average worm burden was 27/fish. In addition, Abd El-Wahed (1997) recorded that the *Saurs tumbil* fish was infected with *Contracaecum* larvae (15.2%). Rosenthal (1967) isolated *Contracaecum sp.* from the peritoneal cavity of *herring* larvae where they were coiled around the gut causing reversible distortions and compression which prevented feeding and defaecation leading to death of the host after about 11 day. After feeding on the intermediate hosts (copepods containing *Contracaeum* larvae), fishes may serve as paratenic host, in which larvae typically occupy specific sites such as mesentery, liver or muscles depending on the species of larvae and host (Kinne, 1984). Moreover, Myjak *et al.* (1994) found that infection with *Contracaecum* larvae was mainly in the liver, but also in body cavities. On the other hand, Abd El-Wahed (1997) isolated *Contracaecum* sp. larvae from the gastro-intestinal tract of *Saurs tumbil* fish. Cheng (1982) stated that Contracaecum sp. can cause human anisakiasis. Also, Myjak *et al* (1994) mentioned that *Contracaecum* osculatum is one of the nematode larvae associaated with human anisakiasis. *Anisakis* and *Contracaecum* larvae can invade the gastro-intesinal tract of man, causing an eosinophilic granuloma syndrome (Woo, 1995). Adequate cooking of *Anisakis* sp. at 60^{o}C for 10 minutes and freezing at-20^{o}C for 24 hours, respectively, were lethal to those worms (Margolis, 1977 and Hui *et al.*, 1994). Moreover, Woo (1995) stated that freezing of fish for 24 hours or heating the processed fish to 65^{o}C can kill the *Anisakis* larvae.

MATERIALS AND METHODS

A total number of 50 fresh Sardine fish (*Sardina pilchardus*) of Red Sea was collected during November 1997 to January 1998. The fish samples were collected in an ice container, and each fish was placed in a plasttic bag and examined macroscopically for freshness according to Syme (1966). Then, the fish were dissected according to Roberts (1978) to detect and localize any nematode larvae and their predilection sites. Two methods for detecting nematode larvae in fish have been used: candling procedure (pressing by compressorium) and digestion technique (pepsin digestive solution) according to Jackson *et al.*, (1981). The recovered nematode larvae were isolated and placed in glacial acetic acid to straighten the larvae. Then, fixed in 70% ethyl alcohol and 5% glycerine, mounted in glycerine gelly preparation according to Ash and Orihel (1987). The morphometric measurements in millimeters were carried out on the twenty specimens, and the drawing has been done with the aid of camera lucida. The specimens were identified according to Yamaguti (1958) and Moravec (1994).

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Application of physical methods for controlling the detected larval nematodes included frying (for 3 and 5 minutes) on each side, grilling at (80^{o}C for 10 and 15 minutes) on each side, and freezing at (-18^{o}C for 24-48 hours) were carried out on the viability of larval nematodes using the digestive solutions (Oshima *et al.*, 1966).

RESULTS

In the present study, two species of *Contracaecum* larvae were isolated and identified, the description was based on 10 speciemens from each *Contracaecum* sp. Larvae.

Super family : *Ascaridoidea* (Railliet and Henry, 1915).
Family : *Anisakidae* (Railliet and Henry, 1912).
Genus : *Contracaecum* (Railliet and Henry, 1912).

The first species:

Contracaecum rudolphii (Hartwich, 1964) (Fig. 1-3 and plates 1-3)

The body was variable in size. The total body length was 0.38-0.78 mm and the maximum width 0.01-0.02 mm. At its anterior end, three lips and a cone-shaped larval tooth were present. The excretory pore was situated at the level of base of lips. The length of the oesophagus was 0.05-0.07 mm, ventriculus was small. The length of ventricular appendix was 0.05-0.03 mm. The intestinal cecum was 0.07-0.11 mm. long. It was relatively broader than the ventricular appendix and it ran anteriorly at nearly half or more of the muscular oesophagus. Tail was conical with blunt tip.

The second species

Contracaecum species (Fig. 1-2 and plate 1-2)

The body length was 0.5-0.9 mm with a maximum width of 0.01-0.4 mm. At the anterior and, three large lips were present, two large latero-ventral and one dorsal. The dorsal one was somewhat short. The muscular oesophagus was 0.08-0.11 mm in length. The ventricular appendix length was 0.03-0.06 mm. The intestinal cecum measured 0.08-0.21 mm, long, it ran anteriorly at nearly half or more of the muscular oesophagus. The tail was short and thumb-like with retractile tip, bearing small spines at its posterior end.

Table 1 shows that the incidence of *Contracaecum* larvae infection and the pattern of their distribution in the muscles and organs of *Sardina pilchardus* fish. The prevalene rate was proved to be 100%. The highest incidence of their distribution was recorded in the liver of infested fish (100%), while, the lowest was observed in their muscles (22%).

Table 2 shows that the average number of *Contracaecum* larvae in the muscle and organs of examined fish, where the abdominal muscles were only infected, the highest number of larvae was in liver tissues.

Thermal procedures as frying and grilling were sufficient to kill all *Contracaecum* larvae in the infected fish. Frying for not less than 5 minutes on each side and grilling for not less than 15 minutes on each side at 60-80^{o}C were quite sufficient. Moreover, the fresszing procedure at -18^{o}C for 48 hours proved to be sufficient to kill *Contracecum* larve in the infested fish.

DISCUSSION

The present study revealed a high rate of *Contracaecum* larvae infection in *Sardina pilchardu* fish collected from Red Sea, where it reached 100% (Table 1). This result is in agreemet with that obtained by Woo (1995), but in disagreement with that recorded by Abd El-Wahed (1997), who stated that 15.2% of *Saurs tumbil* fish of Red Sea was infected with *Contracaecum* larvae. This differences in the prevalence may be attributed to the influence of the seasonal variation of the water temperature, and also, due to the host-parasite interaction (Kennedy, 1982). Moreover, the same table showed that the highest infection with *Contracaecum* larvae was in the liver and body cavity, reaching 100% and 92%, respectively, while, the lowest infection was in the muscles (22%). These results supported those of Rosenthal (1967) and Myjak *et al.* (1994). Table 2 showed that the intensity of *Contracaecum* larvae was also high in the liver and low in the abdominal muscles, where it reached 8.7 and 0.53/g, respectively. In this regard, Myers (1979) reported that, the number of Anisakid larvae was higher in the viscera than in the muscles. The rate of the distribution and the intensity of *Contracaecum* larvae were high mainly in the liver, due to the fact that these larvae are restricted to visceral organs, mainly the liver (Facerhalm, 1982), or *Contracaecum* larvae typically occupy specific sites, depending on the species of larvae and host (Kinne, 1984).

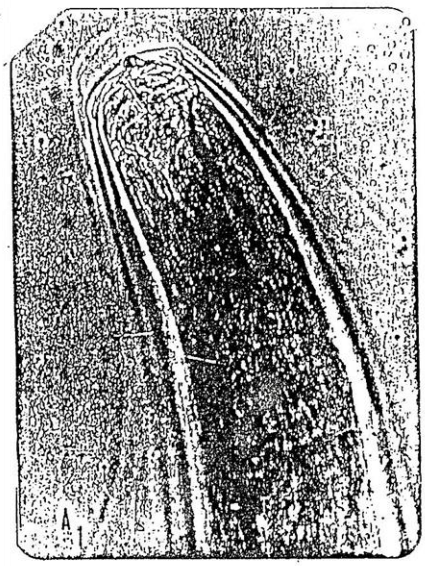
The morphological characters of the obtained *Contracaecum* larvae in this work are identical with those of the original description (Hartwich, 1964 and

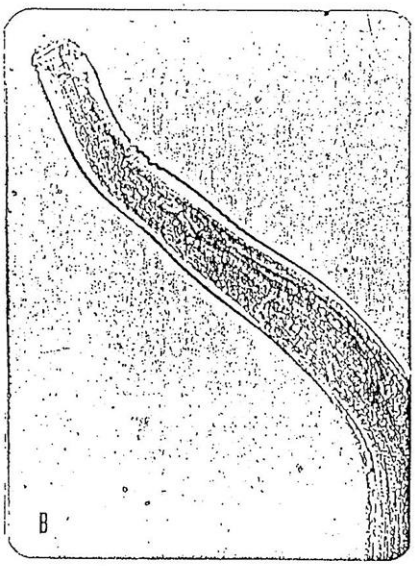
Table 1. Incidence of *Contracaecum* larval infection and pattern of distribution in the muscles and organs of examined Sardine fish.

Fish species	Total number of fish		Percentage %	Muscle		Body cavity		Liver		Gonads	
	Examined	Infected		No.	%	No.	%	No.	%	No.	%
Sardina pilchardus (sardine fish)	50	50	100 %	11	22%	46	92%	50	100%	18	36%

Table 2. Intensity of infestation with *Contracaecum* larvae/g of infested muscles and organs of examined Sardine fish.

Fish species	No. of *Contracaecum* larvae/g in fish muscle				No. of Contracaecum larvae fish				Average dimensions/cm		
	Abdominal muscle		Dorsal muscle		Body cavity		Liver		Liver	Length	Width	Depth
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range Mean			
Sardina pilchardus (sardine fish)	1-3	0.53	0	0	1-10	3.6	1-22	8.7	1-4 0.6	20.5	3.9	5.2





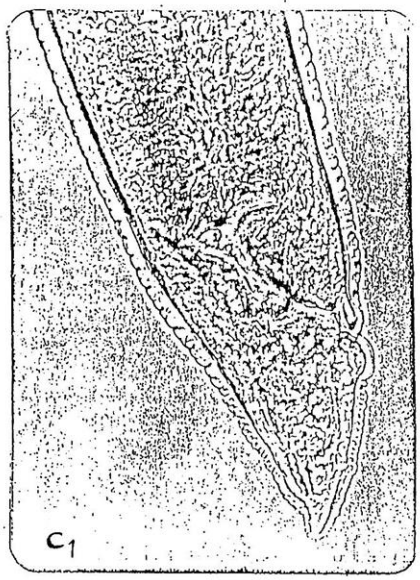


Fig. 1. *Contracaecum* Rudolphii
A1 - Anterior part x 400
B - Middle part x 100
C1 - Posteroir part x 400

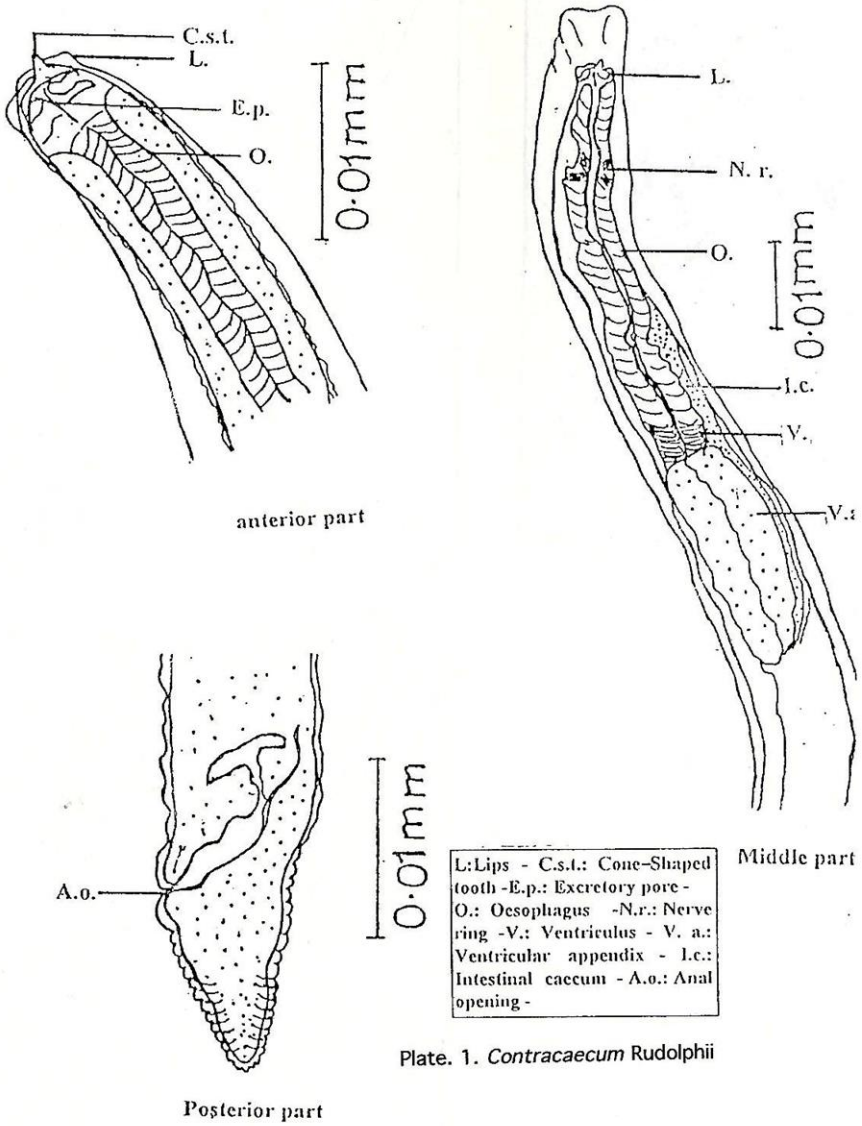
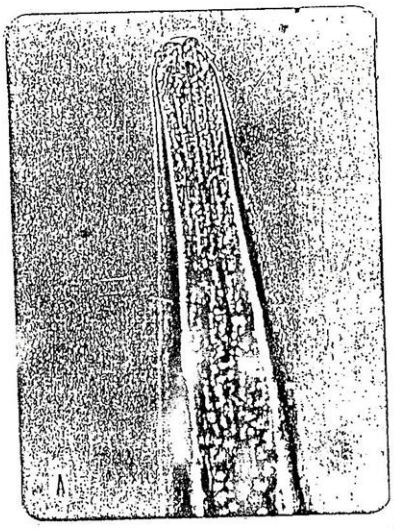


Plate. 1. *Contracaecum* Rudolphii



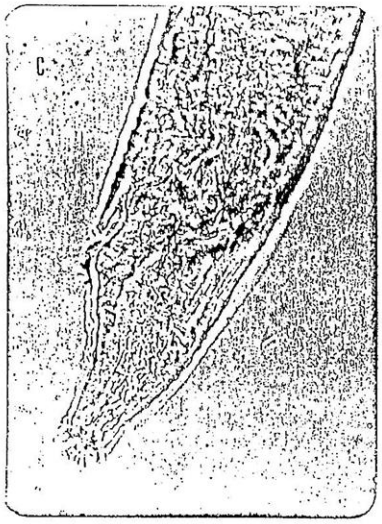
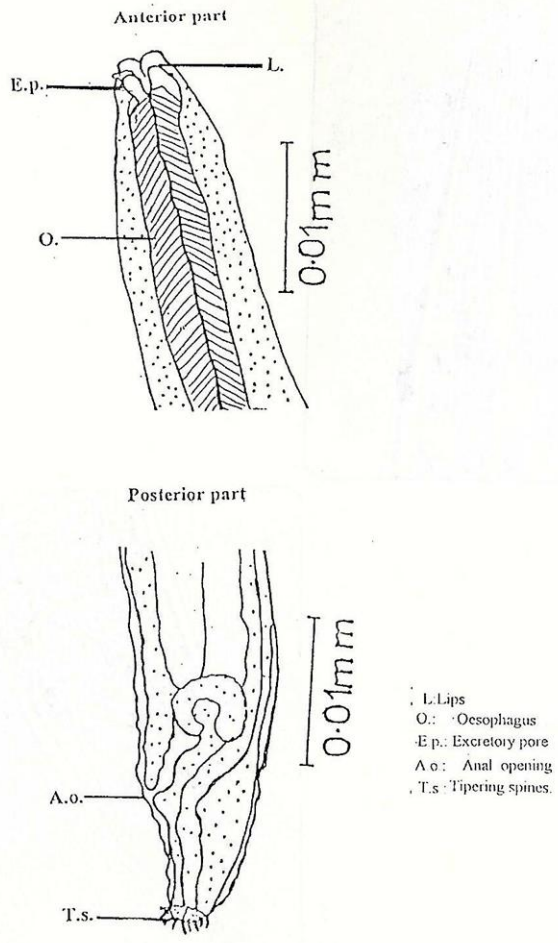


Fig. 2. *Contracaecum* species
A - Anterior part x 400
C - Posteroir part x 400



Plat 2. *Contracaecum* species.

As the *Contracaecum* larvae have a public health hazard, Cheng (1982), Myjak *et al.* (1994) and Woo (1995) reported that the Contracaecum larvae can cause human anisakiasis. In the present study, the thermal procedures were including frying and grilling for not less than 5 minutes on each side and 15 minutes on each side at 60-80^{o}C, respectively. Moreover, the freezing procedure at-18^{o}C for 48 hours, proved to be sufficient to kill all *Contracaecum* larvae in the infected fish. These results are in agreement with the findings reported by Margolis (1977), Hui *et al.* (1994) and Woo (1995).

In conclusion, and as diseases of marine fish in their natural environment are more difficult to be controlled, the following points are recommended to minimize the risk of this problem:

1. Transfer of susceptible marine animals into epizootic areas, or marine animals from epizootic areas should be avoided.

2. Basic informations concerning the life history and ecology of the communicable diseases should be available.

3. Disposal of viscera or entire diseased fish into the sea should be prohibited.

4. Lastly, it is to be stated that the isolated *Contracaecum* larvae from Sardine fish (*Sardine pilchardus*) were very small in size and difficult to be seen. So, the danger of the infection could be almost eliminated by good cleaning of fish soon after capture with adequate cooking or freezing before consumption.

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بعض الدراسات على يرقات الديدان الاسطوانيه (الاسكاريديديا) فى أسماك السردين بالبحر الأحمر وعلاقتها بالصحه العامة

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قد اشتملت الدراسه على فحص ٥٠ سمكه طازجه من أسماك سردين البحر الأحمر. وكانت نسبه إصابه هذه الأسماك بيرقات الكنتراسيكم (١٠٠ ٪). وقد سجلت أنسجه الكبد أعلى معدل للإصابه باليرقات وكذلك أعلى شده إصابه حيث كانت (١٠٠٪ ، ٨,٧) على التوالى بينما كان أقل معدل وشده الإصابه بعضلات البطن، حيث بلغت (٢٢٪ ، ٠,٥٣) على التوالى.

وقد تم عزل نوعين من يرقات الكنتراسيكم والتى تم وصفها مورفوميتريا، ويعتبر عزل هذه اليرقات لأول مره فى مصر من أسماك سردين البحر الأحمر. بالإضافه الى ذلك تم مناقشه علاقتها بالصحه العامه، حيث تعتبر هذه اليرقات إحدى مسببات مرض (الأنيساكياسز) بالإنسان. كما تضمنت الدراسه أيضا طرق معالجة هذه اليرقات بالحراره مثل (القلى والشى) لمده ٥ دقائق، ١٥ دقيقة. بينما المعالجة بالتجميد عند درجه (١٨ ٥م) ولمده ٤٨ ساعه كانت كافيه لموت جميع يرقات الكنتراسيكم بالاضافه إلى مناقشه التوصيات الخاصه لمكافحه هذه اليرقات فى البيئه البحريه.