

DISEASES CAUSED BY LARVAL NEMATODES AMONG SOME FISHES IN RED SEA

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ABSTRACT

This study was conducted on 1000 marine fish of different species. Each species was represented by 100 fishes including *Siganus rivulatus*, *Lutjanus spp*, *Lethrinus nebulosus*, *Pagellus acarne*, *Mulloides flavolineatus*, *Saurida undosquamis*, *Sardinella spp.*, *Restrelliger kanagura*, *Trachurus indicus*, *Antherinidae* collected from Suez Canal water of Red Sea. Fishes were apparently healthy and no pathognomonic signs except abdominal distension, hemorrhagic area on abdomen and emaciation. PM lesions were in the form of enlargement and congestion in liver, excessive mucus secretion and swelling in intestine, petechial hemorrhage on stomach and presence of nematodes on liver and intestine. The isolated parasitic larval nematodes were (*Anisakis simplex*, *Hysterothylacium fabri*, *Contracaecum ovaes*, *Raphidascaris acus*, *Cucullanus truttae*, *Eustrongylides sp.*, *Paraquimperia sp.*, *Cystidicala farions*). The morphological description of the isolated nematodes was recorded. Also, the total and seasonal prevalence were investigated and discussed.

INTRODUCTION

Marine fish act either final or intermediate host for parasites which may cause injuries and even death to these fishes (*Shih and Jeng, 2002*). Parasitic nematodes constitute one of the earliest known groups of helminthes in fishes. They infest marine, brackish and fresh-water fishes. Also, they can infest almost all organs in a fish; the majority of the known species have been described from intestine. Most

nematodes infest fish as adults, but a large proportion of them occur as larval stages (*Eissa, 2002*). Marine fish nematodes represent an important public health problem because they may be a source of serious problems in man.

The present study is planned to investigate larval nematodes in some marine fishes in Red sea and dealing with their prevalence.

MATERIAL AND METHODS

Fishes:

A total of 1000 specimens from 10 different species of marine fishes were used in this study. Each was represented by 100 of these species (*Siganus rivulatus*, *Lutjanus* sp, *Lethrinus nebulosus*, *Pagellus acarne*, *Mulloides flavolineatus*, *Saurida undosquamis*, *Sardinella* sp., *Restrelliger kanagutra*, *Trachurus indicus* and *Antheridia* sp.). Identification of fish species was adopted according to **Randall (1983)**.

Specimens were collected seasonally from Suez Canal from October 2007 till the end of September 2008 by the aid of fishermen and fishing gears. They were transported to laboratory alive in polyethylene bags containing 1 / 3 of its volume water where the remaining volume was filled with air.

Aquaria:

Fully prepared glass aquaria (100 x 50 x 50 cm.) were used for holding fish. They were supplied with sea water and continuous aeration was maintained in each aquarium using an air pump. (Elmassy, Model EM-198).

Clinical picture:

Clinical picture was done on the live fish or freshly dead ones according to the methods described by **Amlacker (1970)**.

Parasitological Examination:

The collected larvae from each site were washed in physiological saline, then relaxed and fixed in hot alcohol - glycerin 5% until all alcohol evaporated and the specimen remains in nearly absolute glycerin. They were cleared in lactophenol and mounted in glycerin-gelatin according to **Meyer and Alsen (1992)**, left to dry and examined microscopically.

Identification of the isolated larval nematodes:

Nematode larvae identification was adopted according to **Deardorff et al. (1982) and Moravec (1994)**.

RESULTS

Clinical examination of Examined fishes :

The clinical signs in the examined fishes (*Siganus rivulatus*, *Lutjanus* sp, *Lethrinus nebulosus*, *Pagellus acarne*, *Mulloides flavolineatus*, *Saurida undosquamis*, *Sardinella* sp, *Restrelliger kanagutra*, *Trachurus indicus* and *Anthriniidae* sp.) revealed no pathognomic clinical abnormalities, some *Siganus rivulatus* fishes showed abdominal distension and hemorrhagic areas on nostrile, base of abdomen and base of dorsal fin. *Mulloides flavolineatus* showed abdominal distension with slight emaciation, while *Trachurus indicus* showed abdominal distension and hemorrhage at base of gill cover. (**Plate 1**).

Postmortem Examination :

The examination was performed on the freshly dead fishes and it revealed that the internal organs of infested fishes were generally congested, had poetical hemorrhage.

In some cases of *Lethrinus nebulosus*, there were pale livers with severe congestion in their edges and showed hemorrhagic area on liver, in some cases of *Pagellus acarne*, there were severe bloody hemorrhage in liver. (Plate 2).

Morphological descriptions of isolated larval nematodes :**Family: Anisakidae****1- *Anisakis simplex*, Rudolphi, 1809.**

These are long larvae collected from liver surface of *S. undosquomus*. The body length of the third stage larvae was 22 – 36mm, and width was up to 0.60 mm. Cuticle was characterized by fine transverse striation. Anlagen of lips 0.039mm. Length of esophagus was 1.80 – 2.66mm. The distance of nerve ring from anterior extremity was 0.326 – 0.394mm. Ventriculus was fairly long (1.05 – 1.52 mm) with width 0.204 – 0.245mm. The tail was conical, about 0.120 mm long ending in sharp cuticular spike 0.012–0.015mm long. (Plate 3).

2- *Hysterothylacium Fabri*, Gehan and El Ashram, 2007.

The present specimens were collected from intestine of *Lutj. sp*, *L nebulosus*, *M Flavolineatus*, *S*

undosquamis and *R. kanagutra*. The detected third stage larva body length is 10.84 – 20.16mm and 0.41 – 0.49mm width. Cuticle is thick and smooth. Head end is separated by cuticular constriction, provided with minute larval tooth and small mouth papillae. Lips are ill-developed. The length of esophagus is 1.19-2.20mm. The size of ventriculus is 0.15 - 0.18 by 0.18 by 0.18- 0.27mm, ventricular appendage is narrow and long (0.75–0.88mm). Tail is conical with broadly rounded tip (Plate 3).

3- *Contracaecum ovaes*, Rudolphi, 1809.

These larvae were isolated from stomach and intestine of *Lutj sp*, *P. acarne*, *S. undosquamis*, *R .kanagutra*, *Sardinella sp*. The body length was 3.82-4.13mm, with maximum with 0.21-0.25mm. Anlagen of lips 0.049mm. Esophagus about 0.6mm long and about 0.04-0.05 X 0.03 - 0.04mm. Length of ventricular appendix is 0.68 – 0.80mm, while that intestinal caecum is long (about 213 of esophagus length).

Tail Conical and bluntly rounded (Plate 3).

4- *Raphidascaris acus*, Bloch, 1779.

These larvae were collected from stomach and intestine of *R. kanagutra*, *Sardinella* and *Anthrina sp*. They were characterized by medium sized nematodes with firm, densely transversely striated cuticle, body brownish, and elongated tap-

ering to both ends. Mouth surrounded by three well developed lips, one dorsal and two ventrolateral, interlabia absent. Esophagus almost cylindrical, muscular, ending in small globular ventriculus provided with long posteriorly directed ventricular appendix. Nerve ring encircling esophagus approximately at border of its first and second third.

Excretory pore situated slightly below nerve ring level. Intestine straight, rectum surrounded by three elongated unicellular rectal gland. Tail is conical and short (**Plate 3**).

Family: Cucullanidae

5- *Cucullanus truttae*, Fabricius, 1794.

This nematode parasite was collected from anterior part of intestine of *Lutj sp* and *S undosquamis*. It characterized by medium sized nematodes, whitish in colour with firm, almost smooth cuticle. Body length was 5.04 – 5.52mm and width was 0.29mm. Head end rounded and dorsally bent. Mouth opening was slit – like, surrounded by narrow cuticular flange armed at its inner base by row of numerous small teeth. Mouth was surrounded by two pairs of large mouth papillae. Esophagus was cylindrical ended with a huge cell nucleus. The tail is conical with a distinct small rounded process at its tip (**Plate 3**).

Family: Dioctophymatidae

6- *Eustrongylides sp.*, Jagerskiold, 1909.

These larvae specimens were collected from abdominal cavity of *M. flavolineatus*, *S undosquamis* and *R kanagutra* larval body thread like, 20.0- 30.0mm long, with maximum width 0.090 – 0.190mm. Two lateral rows of papillae extending a long body. Head end was rounded, provided with 12 papillae arranged in two circles of 6 papillae. Buccal cavity 0.093mm long.

Length of cylindrical esophagus 2.46 – 4.54mm. Nerve ring encircling esophagus 0.092 – 0.108mm from head end. Anal opening terminal Tubular genital primordium of male representing about 117 – 118 of body length. Female genital primordium in posterior part of larval body representing 113 of body length (**Plate 3**).

Family: Quimperiidae

7- *Paraquimperia sp.*, Baylis, 1934.

This larva was Isolated from intestine of *Lutj.sp.*, *M.flavolineatus* and *S.undosquamis*. It was characterized by medium sized nematodes with anterior end of body dorsally bent. Esophagus composed of three portions, first, short and strongly muscular portion forming, pharynx 0.030- 0.039mm long, this being followed by narrow part of esophagus. Esophagus opening into intestine through valves Nerve ring encircling esophagus slightly anterior to

its middle. Excretory pore lying below nerve ring level. Tail conical sharply pointed and had a drop-like extension at the tip (Plate3).

Family: Cystidicolidae

8- *Cystidicola farionis*, Fischer, 1798.

These larval nematodes were recovered from stomach and intestine of *L.nebulosus*, *S.undosquamis*, *R.kanagutra* and *Sardinella sp.* It was characterized by whitish, medium sized nematodes with almost smooth cuticle, body slightly tapering to both ends. Mouth provided with two rudimentary lateral pseudolabia. Mouth surrounded by 4 small dorso-and ventrolateral papillae and 2 lateral amphids.

Oral opening oval and dorso-ventrally elongated Esophagus very long, distinctly divided into short

and narrow muscular part and much longer and wider glandular part.

Nerve ring encircling muscular esophagus approximately at its mid-length. Excretory pore localized near junction of muscular and glandular parts of esophagus-Tail is conical, somewhat rounded at its end and usually forming a small terminal protuberance (Plate3).

Prevalence of larval nematodes in examined fish species:

The total prevalence of detected nematodes larvae were *Anisakis simplex* (3.7%), *Hysterothylacium fabri* (6.3), *Contracaecum ovales* (22.6%), *Raphidascaris acus* (6.1%), *Cucullanus truttae* (1.5%), *Eustrongylides sp.* (3.4%), *Paraquimperia sp.* (8%) and *Cystidicola farionis* (5.6%) (Table 1).

Table (1): Prevalence of larval nematodes in examined fish species.

Fish species	No. of Examined ish	Anisakis Simplex %	Hysterothylacium Fabri%	Contracaecum Ovales %	Raphidascaris acus %	Cucullanus truttae %	Eustrongylides sp %	Paraquimperia sp %	Cystidicola farionis %
<i>S.rivulatus</i>	100								
<i>Lutj.sp</i>	100		5	28		11		7	
<i>L.nebulosus</i>	100		21					25	3
<i>P.acarne</i>	100			8					
<i>M.flavolineatus</i>	100		28				17	37	
<i>S.undosquamis</i>	100	37	3	32		4	9	11	30
<i>Sardinella sp</i>	100			17	33				12
<i>R.kanagutra</i>	100		6	32	25		8		11
<i>T.indicus</i>	100			49					
<i>Anthrina sp</i>	100			60	3				
Total	1000	37	63	226	61	15	34	80	56

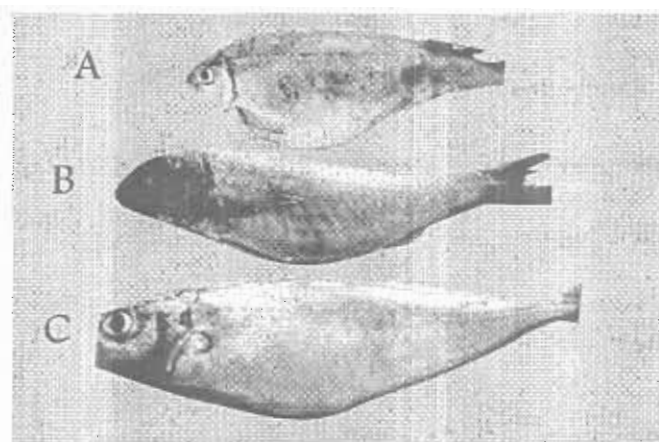
Seasonal prevalence of larval nematodes among examined fish species:

Concerning the seasonal prevalence of larval nematodes from investigated fish, it was found that prevalence of *Anisakis simplex* was highest in autumn (10%) and the prevalence was 4.8% in spring and 0% in winter and summer. *Hysterothylacium fabri* prevalence was 7.2% in winter, while in spring and autumn prevalences were 6.4%, and in summer it was 5.2%. *Contracaecum Ovale*. Showed the highest prevalence in winter (45.2%), while in summer the prevalence was the lowest (10.4%). *Raphidascaris acus*, the highest

prevalence was (20%) in autumn, then (3.2%) in winter, (1.2%) in spring and 0% in summer. *Cucullianus truttae*, prevalence was 4.4 and 1.6% in autumn, and summer respectively but was 0% in winter and spring. *Eustrongylides sp.*, the highest prevalence was 6.8% in autumn and spring. The lowest prevalence was 0% in winter and summer. *Paraquimperia sp.*, the prevalence was 12.4, 9.6, 8 and 2% winter, autumn, spring and summer respectively. *Cystidicola farionis*, the highest prevalence was recorded in winter (14%), then in spring (7.2%), in autumn (1.2%) and the lowest prevalence was (0%) in summer (Table 2).

Table (2): Seasonal prevalence of larvae nematodes among examined fish species.

Parasite / Season	No. of Examined fish	<i>Anisakis simplex</i>	<i>Hysterothylacium Fabri</i>	<i>Contracaecum Ovale</i>	<i>Raphidascaris acus</i> %	<i>Cucullianus truttae</i> %	<i>Eustrongylides sp.</i> %	<i>Paraquimperia sp.</i> %	<i>Cystidicola farionis</i> %
Autumn	250	25 (10)	16 (6.4)	23 (9.2)	50 (20)	11 (4.4)	17 (6.8)	24 (9.6)	3 (1.2)
Winter	250	-	18 (7.2)	113 (45.2)	8 (3.2)	-	-	31 (12.4)	35 (14)
Spring	250	12 (4.8)	16 (6.4)	64 (25.6)	3 (1.2)	-	17 (6.8)	20 (8)	18 (7.2)
Summer	250	-	13 (5.2)	26 (10.4)	-	4 (1.6)	-	5 (2)	-
Total	1000	37 (3.7)	63 (6.3)	226 (22.6)	61 (6.1)	15 (1.5)	34 (3.4)	80 (8)	56 (5.6)

**Plate (1):**

A- *Siganus rivulatus* showing abdominal distension and hemorrhagic area on abdomen and nostril.

B- *Mulloidus flavolineatus* showing abdominal distension

C- *Trachurus indicus* showing abdominal distension.

**Plate (2):**

A- Showing pale liver with congested edges and attached larva in *Lethrinus nebulosus*.

B- Showing sever hemorrhage in liver of *Pagellus acarne*.

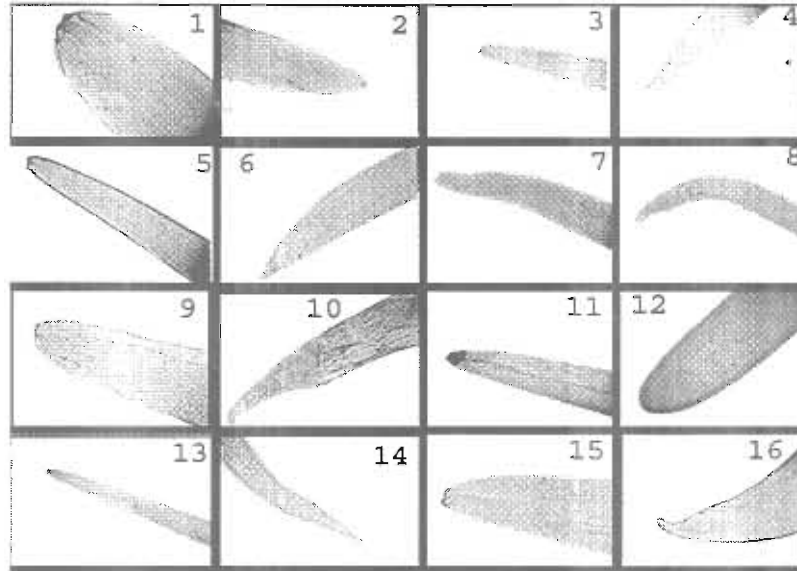


Plate (3):

Showing:

- *Anisakis simplex*
1 - Anterior end. 2 - Posterior end
- *Hysterothylacium fabri* .
3- Anterior end 4- Posterior end
- *Contraecaecum ovaes*
5- Anterior end 6- Posterior end
- *Raphidascaris acus*
7- Anterior end. 8- Posterior end
- *Cucullanus truttae*
9- Anterior end 10- Posterior end
- *Eustrongylides sp*
11- Anterior end. 12- Posterior end
- *Paraquimperia sp*
13- Anterior end 14- Posterior end
- *Cystidicola farionis*
15- Anterior end 16- Posterior end (X 100)

DISCUSSION

There was no characteristic clinical signs observed in infested fishes with parasitic nematodes.. The recorded signs were abdominal distension, hemorrhagic area at abdomen, base of gill cover and nostril, this result agree with that reported by **Maather El-Lamie (2007)** who reported that disease signs due to round worms are slight.

Regarding the postmortem examination, it was revealed in the form of enlarged pale liver and variable degrees of congestion' thick, excessive mucus secretions and swelling in intestine. This result agrees with **Maather El-lamie (2007)**.

The parasite isolated from liver Surface of *Saurida undosquamis*. It can be seen clearly that anatomical structure and body measurements are closely similar to that of the original description **Walaa Talaat (2009)**).

So the parasite is *Anisakis simplex*, **Rudolphi, 1809**.

The parasite isolated from intestine of *Lutjanus sp.* *Lethrinus nebulosus*, *Mulloides flavalineatus*, *Saurida undosquamis* and *Restrelliger kanagutra*. agrees with **Gehan shager and El-Ashram (2007)** in its morphological characters and measurement character. So it is *Hysterothylacium fabri*, **Gehan and El-Ashram, 2007**.

Another parasite had been isolated from stomach and intestine of *Lutjanus sp*, *Pagellus acarne*, *Saurida undosquamis*, *Sardinella sp.*, *Restrelliger kanagutra* and *Trachurus indicus* and *Anthrina sp.* disagree with **Moravec et al. (1995)** and agree with that described by **Gehan Shager and El-Ashram (2007)** in its general morphological and measurement characters. So it is *Contracaecum ovaes*, **Rudolphi, 1809**.

The parasite isolated from intestine of *Restrelliger kanagutra*, *Sardinella sp.* and *Anthrina sp.* was similar in measurements and morphological characters to that obtained by **Gehan Shager and El-Ashrom (2007)**. On the Contrary **Myjak et al. (2005)** obtained it from intestine of *Salmo salar*, *Barbatula barbatula* and herring, respectively. So it is *Raphidascaris acus*, **Bloch, 1779**.

This parasite has been isolated from intestine of *Lutjanus sp.* and *Saurida undosquamis*. This disagrees with **Moravec (1994)**. Its general morphological characters are similar to that reported by **Amany Ahmed (2007)**, so the parasite is *Cucullanus Truttae*, **Fabrieius, 1794**.

The parasite isolated from abdominal cavity of *Mulloides flavalineatus*, *Restrelliger kanagutra* and *Saurida undosquamis*. This result disagrees with **Ali et al.**

(2008). Its characteristics are similar to that reported by *Moravec et al.*, (1995). So the parasite under discussion is *Eustrongylides sp.*, *Jagerskiold*, 1909.

The parasite had been isolated from intestine of *Lutjanus sp.*, *Mullodies flavolineatus* and *S.undosquamis*. This disagree with *Conneely and Mearns (2006)* who showed that infection and intensity related to age and size of *Anguilla anguilla* so the parasite is *Paraquimperia sp.*, *Baylis 1934*.

The parasite isolated from stomach and intestine of *Lethrinus nebulosus*, *Restrelliger kanagutra*, *Saurida undosquamis* and *Sardinella sp.*, disagree with *Moravec (2003)* who found it from stomach of *Salmo solar smolts* and *Salmo trutta fario*. Its general characterizes and measurements are nearly similar to that obtained by *Anna Rocka (2004)*. So it is *Cystidicola farionis*, *Fischer, 1798*.

Regarding the total prevalence of detected larval nematodes among examined fishes, It was (3.7%) for *Anisakis simplex*, *Hysterothylacium fabri* (6.3%), *Contracaecum ovales* (22.6%), *Raphidascaris acus* (6.1%), *Cucullanus truttae* (1.5%), *Eustrongylides sp* (4.1%), *Paraquimperia sp*, (7.3%) and *Cystidicola farionis* (5.6%). However *Romuk (1988)* reported prevalence for *Anisakis simplex* as 37.5% and *Abd-El-Maksoud (1992)* recorded prevalence with nematode larvae was 34.9%.

Regarding the seasonal prevalence of larval nematodes among examined fishes, it was found that *Anisakis simplex* was highest in autumn (10%) and 0% in winter and summer this result disagreed with *Carvajal and Cattán (1985)*.

Hysterothylacium fabri in winter showed the highest prevalence (7.2%) and in summer (5.2%), this result disagree with *Joaber et al. (2004)*. *Contracaecum Ovales* in winter showed the highest prevalence (45.2%) and the lowest in autumn was observed (9.2%) this result disagree with *Luque et al. (2003)*. *Raphidascaris acus*, highest prevalence was (20%) autumn, (3.2%) winter, (1.2%) spring and (0%) in summer, this result disagree with *Gehan Shager and El-Ashram (2007)*. *Cucullanus truttae*, highest prevalence was (4.4%) autumn and lowest (0%) in winter and spring, this disagreed with *Lanfranchi et al. (2004)*.

Eustrongylides sp, highest prevalence was (6.8%) in autumn and spring, this result disagreed with *Paperna (2006)*. *Paraquimperia sp* highest in winter (12.4%) and lowest in summer (2%), this result agreed with *Nie and Kennedy (2006)* and disagreed with *Saraiva and Eiras (1996)*. *Cystidicola farionis*, highest prevalence was (14%) in winter and lowest (0%) in summer.

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المخلص العربي

الأمراض الناجمة عن يرقات الديدان الاسطوانية في بعض أسماك البحر الأحمر

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أجريت هذه الدراسة علي عشرة أنواع من الأسماك البحرية وهي 100 من كل نوع) السيجان وللشخرم و الشعور و المرجان و البريوني و الحارت و السردين و الكسكمرى و الباغه و بساريا). وقد تم تجميعها عشوائيا من المياه المالحة لمدينة السويس في المواسم المختلفة. وقد تميزت الأسماك بالأحجام والأوزان المختلفة. أسفر الفحص الاكلينيكي للأسماك المصابة بيرقات الديدان الاسطوانية عن عدم وجود علامة مرضيه مميزة. بعض الأسماك المصابة أظهرت انتفاخ البطن والهزال ونزيف علي أجزاء من البطن والأنف والخياشيم كما أظهر فحص تلك الأسماك بعد نفوقها تضخم واحتقان في الكبد وزيادة الإفراز المخاطي وانتفاخ الأمعاء ونزيف في بعض الأعضاء الداخلية وفي بعض الحالات التصاق اليرقات علي الكبد مع شحوب في اللون. وقد تم التعرف علي يرقات الديدان الاسطوانية كالتالي وهي الانيساكس سمبلكس ، هستيرونلثيم فابري ، كونتراسيكم أوفال برافيراسكارس اكييس ، كوكولنيس تريبتيتا ، ايوسترونجيليدي باراكوميريا وسيتيدكولا فارونسي. كما تم رصد نسب الإصابة الكلية والموسمية لكل مرض وتم مناقشتها.