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PREVALENCE OF ANISAKID NEMATODE LARVAE INFECTING SOME MARINE FISHES FROM THE LIBYAN COAST

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Abstract

This study examined eight hundred ninety six marine fishes belonging to nine different fish species; *Synodus saurus*; *Merluccius merluccius*; *Trachurus mediterraneus*; *Serranus cabrilla*; *Mullus surmuletus*; *Diplodus annularis*; *Spicara maena*; *Siganus rirulatus* and *Liza ramada*. The fishes were bought from fish markets at five different sites on Libyan coast, from January to December 2013, for study the anisakids larvae among them. The results showed that 344/896 fishes (38.4%) were infected with Anisakids larvae. *S. saurus* was the highly infected (80.9%), followed by *T. mediterraneus* (77.5%) but, *S. cabrilla*, *S. maena*, *M. merluccius*, *M. surmuletus*, and *D. annularis* were least anisakid infected showed rates of 58.2%, 53.8%, 43.7%, 36.7% & 3.6%, respectively. No parasites were in *S. rirulatus* and *L, ramada*. Ten species of Anisakids larvae was detected during the present study. Two *Pseudoterranova* sp. Larvae, two types of *Anisakis* larvae, *Anisakis simplex* larva and *Anisakis* sp. Larva, two types of *Contracaecum* sp. Larvae and four *Hysterothylacium* larvae. Females showed higher prevalence than males. The number of anisakid larvae varied according to body length and weight of infected fish, without significant difference between prevalence and seasons, but, a significant difference was between prevalence and regions.

Key words: Libya, Nine Mediterranean Fish**,** Anisakids larvae

Introduction

Fishes have substantial social and economic importance as they act as a vital source of food for people. They are considered as a single source of high-quality protein, providing □16% of the animal protein consumed by the world's population. Fishes are also zoonotic important, since several diseases transmitted to human by fish parasites including Anisakiasis, in additions hundreds of fishes suffer due to infection by helminthes parasites, several species of fish parasites have been identified as harmful (Bilqees *et al*, 2003).

Marine fish are known to be infected by many different parasites. Some nematodes are endoparasites in marine mammals, sea birds and fish, there are four main Anisakids known to infect marine fish: *Anisakis*, *Pseudoterranova*, *Contracaecum and Hysterothylacium* (Berland, 2006). Anisakids spp. larvae are a worldwide distribution parasite commonly found in the flesh and the body cavity of many species of marine fishes as well as cephalopods that act as paratenic or transport hosts (Tantanasi, *et al*, 2012). Anisakids are ascaridoid nematodes dependent upon aquatic hosts for the completion of their life cycle, which generally involves an array of invertebrates and fish as intermediate or paratenic hosts, and marine mammals or fish-eating birds, reptiles and fishes as definitive hosts (Koinari *et al*, 2013). Larvae of Anisakid nematodes are a major problem for commercial fishing industries, and are potential human health hazards, both as causative agents of anisakiasis and as potential food-borne allergens (Daschner and Pascual, 2005).

Human Anisakiasis is seafood borne parasitic zoonosis caused by larval nematodes of the genus *Anisakis* (Arafa *et al,* 2009). Humans are accidental hosts of the nematodes; they become infected by consuming raw or undercooked seafood that harbor the nematode larvae in their flesh and muscle. Larvae do not further develop in humans; however, they can penetrate the gastrointestinal tract and form eosinophilic granulomas, often with pathologic consequences (Audicana and Kennedy, 2008).

The present study report on the occurrence and infection of Anisakid larvae in some commercial fish species in Libya, these fish species under study are routinely used in the diet of the local population.

Materials and Methods

Study area (The Libyan coast): The Libyan state has a vast coast line of 1970Kms. of the Mediterranean Sea. The Libyan coast extending from Ras Jadir on the Libyan-Tunisian border to the Libya-Egyptian border.The Libyan coast has a Mediterranean climate of the moderate wet winter and worm, dry summer. During the winter season the temperature may drop to less than 5^oC at night but the average temperature between $10\text{-}17^oC$, whereas the temperature was raised up to 38^oC at the mid-day during the summer season. Annual rainfall was 268mm.

Fish sampling: A total of eight hundred and ninety six of marine fishes belong to nine genera were collected from the Libyan coast with the help of fisherman and bought from fish markets at different cities, collected fishes were transferred in icebox to the laboratory of zoology department, Faculty of Science, Omar Al-mukhtar University, Libya from January to December 2013. Collected fishes were first identified by the experience of fisherman with their local common names. Scientific identification was done according to Golani *et al.* (2006). All fish species belonged to Class: Osteischthyes, Families and genera to which the different species belong to following species: *Trachurus mediterraneus* (80); *Merluccius merluccius* (87); *Synodus saurus* (110); *Serranus cabrilla* (110); *Mullus surmuletus* (120); *Diplodus annularis* (110); *Spicara maena* (80) *Siganus rirulatus* (110) and *Liza ramada* (89).

Laboratory examination of fish for parasites**:** Total body length was measured by meter and body weight was measured by gram using an electronic balance. Fish sexes was determined on dissection.

Parasite diagnosis: Fishes were dissected. The whole body cavity, muscles, internal organs and gonads of each sample were carefully examined at first by naked eye for the presence of Anisakids larvae .Collected larvae were washed with in isotonic saline solution for several times to remove any attached mucous. Anisakid larvae presserved in 5% glycerin in 70 % ethanol to straighten up the nematodes body during fixation and for preventing the dryness of the larvae and kept in small plastic tubes with a label carrying the most important information**.** The anisakid larvae were carefully washed in distilled water. They were cleared by gradual evaporation of glycerin alcohol for several days, cleared in glycerin and permanently mounted in glycerin. Then left to dry on hot plate at 30-50°C for 30 minutes, and examined microscopically.

Identification of Anisakids larvae: Anisakids larvae were identified based on the morphological characters (Moravec, 1994 and Choi *et al*., 2011), and based on the assistance of Prof. Dr. Ali Al-Zubaidy, Department of Marine Biology & Fisheries, Faculty of Marine Science and Environment, Hodeidah University, Yemen.

Statistical Analysis: Data were analyzed using SPSS version-17 software. Pearson's Chi square test has been used to measure statistical significance of results. In order to consider a result to be statistically significant 95% CI and p-value < 0.05 has been taken.

Results

The results showed that out of the total examined fish, 344 (38.4%) were infected with L3 Anisakids larvae parasites. Morphological examination revealed that all larvae specimens examined belonged to the family Anisakidae. Larvae were found free in the body cavity and encapsulated (coiled in a thin walled cyst) on wall of stomach, liver, and muscles (Tab. 1).

Table 1: Overall prevalence of larval anisakid parasites in fishes:

Status	No. infected	Percentage (%)
Non- infected	552	61.6
Infected	344	38.4
Total	896	100.0

Synodus saurus was commonest (80.9%), followed by *T. mediterraneus* (77.5%). While, *S. cabrilla*, *S. maena*, *M. merluccius, M. surmuletus* and *D. annularis* showed infection rates of 58.2%, 53.8%, 43.7%, 36.7% & 3.6%, respectively. None was detected in *S. rirulatus* and *L .ramada*. There was a significant difference between prevalence and fish species (P= 0.000).

Table 2: Prevalence of larval anisakid parasites in fish species:

Fish species	No. examined	Non-infected	Infected
Trachurus mediterraneus	80	22.5% (18)	77.5% (62)
Merluccius merluccius	87	56.3% (49)	43.7% (38)
Synodus saurus	110	19.1% (21)	80.9% (89)
Serranus cabrilla	110	41.8% (46)	58.2% (64)
Mullus surmuletus	120	63.3% (76)	36.7% (44)
Diplodus annularis	110	96.4% (106)	3.6% (4)
Spicara maena	80	46.2% (37)	53.8% (43)
Siganus rirulatus	110	100% (110)	0.00 (0)
Liza ramada	89	100% (89)	0.00 (0)
Total	896	552	344

Table 3: Prevalence of larval anisakid parasites in examined fishes and sexes:

Sex	No. examined	Non-infected	Infected
Males	180	65% (117)	35% (63)
Females	716	60.8% (435)	39.2% (281)
Total	896	552	344

Anisakid larvae were 22.1%, 37.9%, 67.3%, 24.1% & 16.7% at body lengths 7-12.5cm, 13-18.5cm, 19-24.5cm, 25-30.5cm and more than 30.5 cm respectively. Fishes with length 13-18.5cm and 19-24.5cm showed higher prevalence. There was a significant difference between prevalence and body length (P= 0.000).

Table 4: Prevalence of larval anisakid parasites and body length of examined fishes :

Body length(cm)	No. examined	Non-infected	Infected
7 –12.5	235	77.9% (183)	22.1% (52)
13 –18.5	430	62.1% (267)	37.9% (163)
19 –24.5	171	32.7% (56)	67.3% (115)
25 –30.5	54	75.9% (41)	24.1% (13)
30.5More than	6	83.3% (5)	16.7% (1)
Total	896	552	344

Prevalence of anisakid larvae parasites varied according to fish body weight. High infection rate was with body weight 115-165gm 57% (73) followed by body weights 166-216gm 43.5% (20), 64-114gm 42.2% (116), 13-63gm 32.6% (125) and 217-267gm 20.7% (6), 268-318gm 15% (3), 319-369gm 12.5% (1), 370-420gm 0.00% (0), and more than 420gm 0.00% (0), with significant difference between infection rate and fish body weight (P= 0.000).

High infection rate was in Spring (45.2%) followed by Autumn (37.7%), Winter (34.2%) and Summer (34%), but without significant difference between infection rate and seasons (P= 0.390). Infection rate in Tripoli was (46.1%), in Benghazi (39.7%), and in Darna (21.7%) but none in Ras-Altten or Al-Tememi (0.0%), with a significant difference between infection rate and regions (P= 0.000).

Table 5: Prevalence of larval anisakid parasites and body weight of fishes.

Body weight (g)	No. of examined fish	Non-infected	Infected
63 –13	383	67.4% (258)	32.6% (125)
114 –64	275	57.8% (159)	42.2% (116)
165 –115	128	43% (55)	57% (73)
166 –216	46	56.5% (26)	43.5% (20)
217–267	29	79.3% (23)	20.7% (6)
268 –318	20	85% (17)	15% (3)
319 –369	8	78.5% (7)	12.5% (1)
370 –420	4	100% (4)	0.00% (0)
More than420	3	100.0% (3)	0.00% (0)
Total	896	552	344

Table 6: Prevalence of larval anisakid parasites in fishes and seasons.

Seasons	No. examined	Non-infected	Infected
Summer	150	66% (99)	34% (51)
Autumn	236	62.3% (147)	37.7% (89)
Winter	240	65.8% (158)	34.2% (82)
Spring	270	54.8% (148)	45.2% (122)
Total	896	552	344

Table 7: Prevalence of larval anisakid parasites in fishes and regions.

Regions	No. examined	Non-infected	Infected
Benghazi	476	60.3% (287)	39.7 % (189)
Tripoli	280	53.9% (151)	46.1% (129)
Darna	120	78.3% (94)	21.7% (26)
Ras-Altten	10	100% (10)	0.00 % (0)
Al-Tememi	10	100% (10)	0.00% (0)
Total	896	552	344

Ten species of Anisakids larvae were collected and identified. They were *A. simplex* and *Anisakis* sp., two types of *Contracaecum* spp. larvae, two types of *Pseudoterranova* spp. larvae & four *Hysterothylacium* spp. Anisakids larvae in 896 fishes were *Pseudo-terranova* sp. larva 1 (23.44%); followed by *Pseudo-terranova* sp. larva 2 (18%); *Anisakis simplex* larva (12.61%); *Contracaecum* sp. larva 1 (12.3%); *Hysterothylacium* sp. larva 3 (10.16%); *Anisakis* sp. larva (8.93%); *Hysterothylacium* sp. larva (8.26%); *Contracaecum* sp. larva 2 (3.91%); *Hysterothylacium* sp. larva 2 (2.9%) and *Hysterothylacium* sp. Larva 4 (2.68%). Commonest anisakids larvae in 344 infected fishes was *Pseudoterranova* sp. larva 1(61.10%) followed by *Pseudo-terranova* sp. 2 (46.80%); *Anisakis simplex* Larva (32.85%); *Contracaecum* sp. larva 1 (32%); *Hysterothylacium* sp. larva 3 (26.45%); *Anisakis* sp. larva (23.26%); & *Hysterothylacium* sp. larva 1 (21.51%); *Contracaecum* sp. larva 2(10.17%); *Hysterothylacium* sp. larva 2 (7.56%) and *Hysterothylacium* sp. larva 4 (6.98%).

Table 8: Prevalence of larval anisakid species parasites in examined (N = 896) and infected fishes (N = 344):

Parasite	% Out of fishes (896)	% Out of infected fishes (344)
Pseudoterranova sp. larva 1	23.44% (210)	61.10% (210)
Pseudoterranova sp. larva 2	18% (161)	46.80% (161)
Contracaecum sp. larva 1	12.3% (110)	32% (110)
Contracaecum sp. larva 2	3.91% (35)	10.17% (35)
Anisakis simplex larva	12.61% (113)	32.85% (113)
Anisakis sp. larva	8.93% (80)	23.26% (80)
Hysterothylacium sp. larva 1	8.26% (74)	21.51% (74)
Hysterothylacium sp. larva 2	2.90% (26)	7.56% (26)
Hysterothylacium sp. larva 3	10.16% (91)	26.45% (91)
Hysterothylacium sp. larva 4	2.68% (24)	6.98% (24)

Single anisakids larvae was 22.97% (79) and mixed infection was 77.03% (265), with significant difference between Prevalence and infection type (P= 0.000).

Table 9: Single and mixed infection of anisakid larvae in infected fishes:

Type of infection	No. infected (344)	(%)
Single infection	79	22.97
Mixed infection	265	77.03
Total	344	100.0

Females were higher than males. Females infection was 39.2% (281) and males infection was 35% (63), without significant difference (P= 0.168)

Discussion

In the present study the morphological description of anisakid third stage larvae agreed with Shih (2004).In the present investigation, the prevalence of totalexamined nine fish species reached to be 38.4%. This prevalence was higher than those previously reported by other authors, 20% (Al-Bassel and Hussein, 2012), 16% (Adel *et al*, 2013), 5.33% (Hassan *et al*, 2013) and 7.6% (Koinaria *et al*, 2013). On other hand, the present prevalence was lower than other studies; 97.7% (Mansour *et al*, 2003), 75% (Shamsi *et al*, 2010), 63.11% (Khanum *et al*, 2011) and 65.81% (Nada *et al*, 2013). Such variation in data could be due to fish health condition, affected by environmental, geographical distribution, water temperatures, and type of water supply, crowding of fishes, transport, and management (Kayis *et al*, 2009).

The present study revealed that out of nine examined fish species, seven were infected with Anisakid larvae and two fish species were not, this in agreement with previous studies which reported that not all examined fishes were infected with Anisakid larvae (Shamsi *et al*, 2010; Hassan *et al*, 2013; Koinaria *et al*, 2013).

In the present study, *S. sauruss* showed high rate (80.9%), followed by *T. mediterraneus* (77.5%), *S. cabrilla* (58.2%), *S. maena* (53.8%), *M. merluccius* (43.7%), *M. surmuletus* (36.7%) and *D. annularis* (3.6%). The same and other fish species were found infected with anisakid larvae recorded around the world (Shamsi and Aghazadeh-Meshgi, 2011; Sobecka *et al,* 2012).

The present study revealed that ten species of anisakid larvae belong to four genera were detected among examined nine fish species*;* they are two *Pseudoterranova* spp. Larvae*,* two *Contracaecum* spp. Larvae*, Anisakis simplex* Larva, *Anisakis* sp. Larva and four *Hysterothylacium* spp. larvae. The same anisakid larvae species were recorded from different fishes around the world (Nada *et al*, 2011; Shamsi and Aghazadeh-Meshgi, 2011, Sobecka *et al*, 2012; Adel *et al*, 2013**;** Koinaria *et al,* 2013). The detected prevalence of *Anisakis* sp. Larva in the present study was 8.93%. Such prevalence was lower than those reported by other previous studies 87.97% (Valero *et al*, 2006 a) and 62.4% (Abattouy *et al*, 2012) and higher than 2.4% (Quiazon *et al*, 2009).

In the present study, two species of *Contracaecum* larvae were detected at prevalence rates 32% and 10.17%. This prevalence was higher than those reported in previous studies 3% (Adel *et al*, 2013), However lower than incidences reported by Lymbery *et al*. (2002) at 81-100%,Valero *et al*. (2006b) at 87.97%. The differences and similarities of the above results might be attributed to many factors such as the positive correlation of host-parasite interaction, the influence of regional ecological disturbance and the ontogenetically changes in the feeding behavior of fish (Sabas and Luque, 2003).

In the present study mixed infection was high than single infection in all fishes. This agreed with other studies abroad (Khanum *et al*, 2011; Aliyu and Solomon 2012; Yakhchali *et al*, 2012). Mixed infections had been reported among other helminthes parasitic infection in different fish species (Varjabedian, 2005).

The present finding revealed that females with higher prevalence than the males.The present finding agreed with previous studies (Ahmad and Ahmad, 2012; Olurin *et al*, 2012) and disagreed with previous studies (Dan-Kishiya *et al,* 2012, Aliyu and Solomon, 2012; Idris *et al*, 2013). Such variation in the obtained data could be due to males are known to be usually more sensitive to parasites than females due to testosterone synthesis which may exert a cost, decreasing immune competency (Bichi and Yelwa, 2010). The variation in prevalence between sexes could be due differential feeding or as a result of different levels of resistance to infection. It could be also due to physiological state of the female (Emere and Egbe, 2006).

The results revealed that no significant differences were detected between prevalence of anisakid larvae infection and body length of fishes. This finding agreed with Olurin *et al*. (2012) but disagreed with others (Khanum *et al*, 2011; Dan- Kishiya *et al,* 2012; Yakhchali *et al*, 2012; Esiest, 2013; Idris *et al*, 2013).

The relationship between parasites infection and host body length varied according to host and parasite (Hila Bu and Leong, 1999). This was attributed to variation of fish lengthes, and related to the feeding upon crustacean intermediate hosts or due to an accumulation of parasites in host in its life (Bussmann and Ehrich 1979) or to variations of fish diet (Valero *et al*, 2006b).

The body weight had effect on the prevalence of anisakid larvae parasites and total infected fishes. This finding agreed with Yakhchali *et al*, 2012). The prevalence increased with increasing the fish body weight may be due to the increase and growth of the internal organs of the hosts leading to the increase in the surface areas of infection as suggested by Hagras *et al*. (1995), or could be due to exposure time of infection (Muzzall *et al*, 1990).

In the present study, the seasonal variations of anisakids larvae infection rate was peaked in spring season in fishes. The result nearly agreed with Eissa (2002) who found that the thin-shelled eggs were laid, and passed out into seawater through the feces of infected final hosts as dolphins or whales (*Anisakis*), the first stage larva undergoes the first molt within the egg capsule where its development is strongly influenced by water temperature. Thus, water temperature has great role on enhancing the life cycle and increasing prevalence in the summer and spring. These results agreed with Choi *et al*. (2011) and Li *et al*. (2011).

Concerning the regions, high prevalence rates were recorded among fishes collected from Tripoli, Benghazi and Darna. But no infection was in fishes from Ras-Altten and Al-Tememi. Many studies gave variations in infection intensity among individual fish within a certain geographic area. Arthur *et al*. (1982) reported that the infection rate of anisakids larvae varied with geographic location. The infection dynamicwas strongly fish species and area specific (Rokicki *et al*, 2009). A higher prevalence of Anisakid infection depended on the hosts' availability in the region and the parasite ability to complete life cycle as well as its food and water column inhabited; bottom versus pelagic (Palm *et al*, 2007).

Conclusion

In Libya, none was published on the parasites of fish from the Mediterranean Sea. Generally speaking, the anisakiasis is one of the zoonotic parasite, and larvae of Anisakid is a major problem for fishing industries, and in home cooking.

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