Investigation of the Genus *Anisakis* Among Atlantic Chub Mackerels (*Scomber colias* Gmelin, 1789) in Tripoli's Main Fish Markets, Libya

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Abstract

Parasitism is a common incident in the marine environment and all species of fish are susceptible to being infested by various parasites. Thousands of parasitic species of fish are described in adult or larval form, which mainly belong to the groups of protozoa, arthropods, platihelminths (trematodes and cestodes) and nematohelminths (nematodes). Anisakidosis is a zoonosis caused by the intake of the nematode of the genus *Anisakis* in its third larval stage (L3). Atlantic chub mackerel (*Scomber colias* Gmelin, 1789), has great importance, both ecologically and economically in the Libya. From the economic point of view, it is possible to mean that Atlantic chub mackerel is the one of most abundant and frequent medium-sized pelagic species of fishing in the Libyan coastline. Atlantic chub mackerel is one of the most commercial significant species
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for the Libyan fishery. Consequently, in the period between 1st March to 28th April 2018, a total of 83 Atlantic chub mackerels were collected from two main fish markets in Tripoli, Libya to evaluate the parasitosis by infective larvae of the genus *Anisakis* spp. by the dissection method. Among the all examined fish, 15 (18.07%) were infested in the abdominal cavity with nematodes parasites, and identified as a third stage larvae, belonging to the *Anisakis* simples. Statistical analysis revealed there is no correlation between abundance of the infection by *A. simplex* with the host length. Furthermore, values of the infection intensity were significantly higher in the female of *S. colias*. However, this study indicates the need to continue efforts for in-depth investigate this metazoan parasite in different fish and cephalopod species in the coast of Tripoli, to represent the adequate risk of anisakidosis, and to alert the possible chance of the initial man case of anisakidosis in Libya, particularly among people who are more occupationally susceptible; like the fishmongers, fish processors, as well as to some residents population who prefer consume the raw or lightly cooked fish.

**Keywords:** Anisakis simplex; Atlantic chub mackerel; consumer; public health; Anisakidosis; Tripoli, Libya

**Introduction**

The parasitic fauna among marine fish species in the Libyan coast are not fully identified. Therefore, it is extremely important to determine the parasitic species particularly which have zoonotic potential. Likewise, if one considers the inadequate hygienic-sanitary conditions during their preparation; or eating habit that some resident populations has of consuming fishery products without having undergone an adequate cooking process. The existence of anisakid larvae in fish with commercial value can be a problem in two respects. Globally, the consequence resulting from the increased occurrence of these parasites in the fish of commercial value (Vidaček *et al.*, 2009). Furthermore, there is a problem of public health where the risk of disease (Anisakidosis) may be directly due to ingestion of viable larvae in raw or insufficiently pickled, salted, smoked, and under-
cooked fishery products or indirect, when larvae are present. *Anisakis simplex*, responsible for allergic reactions. The term "Anisakidosis" refers to diseases caused by parasites of the family *Anisakidae* and the term "anisakiosis" means parasitic infection in the digestive tract caused by ingestion of larvae of the genus *Anisakis* (Kassai et al., 1988). The species most commonly associated with human infection are; *Anisakis simplex* (or herring worm) and *Pseudoterranova decipiens* (cod or seal worm) (Audicana and Kennedy, 2008). The presence of larvae *Anisakis* in human, can evolve into distinct clinical pictures: digestive tract infection (gastric or intestinal anisakiosis) or occasionally of other organs (ectopic) and allergic forms, when *A. simplex* larvae are existing (gastroallergic anisakidosis and allergy to *A. simplex*) (Audicana and Kennedy, 2008). Gastric anisakiosis is the most common clinical form of parasitism by *A. simplex* (Daschner et al., 2000). The inflammatory reaction is due to the perforation of the larvae in the wall of the digestive tract with formation of eosinophilic granuloma and manifesting as abdominal pain, nausea and vomiting within 12 hours of ingestion of raw fish (Daschner et al., 2000). More than 90% of cases of anisakiosis are by A single larva causes more than 90% of cases of anisakiosis, but cases of heavy parasitism are described (Daschner et al., 2000). Some patients have abdominal symptomatology accompanied by response of IgE-mediated allergic hypersensitivity reaction with symptoms of urticaria, angioedema or anaphylaxis.

Libya has the longest Mediterranean coastline among African nations. The Atlantic chub mackerel (*Scomber colias* Gmelin, 1789), is a cosmopolitan pelagic species inhabiting temperate and subtropical waters globally at depths ranging from near the surface down to 300 m (Collette and Naun 1983; Collette 1986). Tripoli is the capital city of Libya (32° 54' North latitude and 13° 11' East longitude), located in the northwest of the country on the periphery of Sahara, on the point of rock-strewn ground salient into the Mediterranean Sea. It is the most populous city, both locals and foreigners. Atlantic chub mackerel (colloquially named: “cawalli” according to the local dialect), is one of the most available and preferred for consumption fish among its inhabitants. This species of fish is a resource of high demand in the Libyan fish markets, due to their nutritional properties, so it finds in second place of importance fishing among pelagic species. Moreover, the studies performed on metazoan parasites fauna of *S. colias* along the Libyan coast are scarce, and a little considered in-depth. Hence,
the present study aims to investigate the occurrence of infective larvae of nematodes of *Anisakis* spp. in the Atlantic chub mackerels from the main fish markets in Tripoli.

**Materials and methods**

**Sampling**

During the period from 1 March till 28 April 2018, the parasitological study was performed on eighty-three specimens of Atlantic chub mackerel intended for human consumption. These fishes were collected from two main fish markets in Tripoli; The Central Fish (Bab El-Baher) Market, and Souq Alhoot Market. Each sample was introduced in a sterile bag and immediately transferred in portable thermoelectric coolers to the Microbiology lab at Biotechnology Research Centre (BTRC)/ Tripoli, Libya, where the parasitological analysis was performed. All the fish samples were individually identified by recording the total weight and length.

**Parasitological procedures**

The presence of infective larvae (third stage [L₃]) of *Anisakis* spp. was investigated for body cavities and somatic musculature by using the dissection method. Subsequently, opening of the abdominal cavity where viscera, peritoneum and muscle were examined visually. The sex of the fishes was identified and recorded. Magnifying glass and handheld were used to improve visualization. The viscera were removed for a detailed macroscopic inspection, as a complement to this inspection; the digestive system was opened to verify its contents. For the data collection, a questionnaire especially designed for these purposes was used. The larvae of *Anisakis* spp. were determined, based on the description of the morphological characteristics of the anisakid according to the criteria mentioned by Cheng (1986). Prior to the microscopic observation, the larvae were clarified, placing them in lactophenol solution for a minimum of 24 hours, in order to appreciate the internal structures that help their morphological identification.

As fundamental characteristics of the third larvae of the anisakid genus, specific structures of their anatomy were taken into account to achieve the exact identification. The morphological criteria used for the identification of larvae present in fish are: the shape and size of the esophageal ventricle and
the presence or absence of ventricular appendix and intestinal blind. The presence of an appendix, if it is previously projected, is recognized as an intestinal blind and if it is projected posterior to the ventricle area, it is known as a ventricular appendix. The position of the excretory pore is essential for determination (Cheng 1986). In addition, the colour of the larvae contributes to more recognition.

The morphological characteristics considered for identification of the third larva of the genus *Anisakis* were:

i. Ventral cuticular tooth.

ii. Excretory pore located between the bases of the sub ventral lips.

iii. A ventricle.

iv. Absence of ventricular appendix.

v. Absence of intestinal blind.

vi. Whitish colour.

vii. Ventricular-oblique bowel plane (*Anisakis simplex*).

viii. Conical back end ending in a mucron.

For the determination errand, stereoscopic microscope was used.

**Statistical procedures**

The epidemiological parameters (prevalence, mean intensity and mean abundance) were estimated as described by Margolis *et al.*, (1982). For the statistical analysis, the software SPSS® 16.0 was used. Spearman’s rank correlation coefficient ($r_s$) was used to analyze the association between abundance of infection and length of the fish (Siegel and Castellan, 1988). Whereas, Wilcoxon rank-sum test ($z$) was used to compare the mean intensity of infection by the sex of fish (Zar 1996). A statistical level of $P \leq 0.05$ was considered significant.

**Results and discussion**

Occurrence of *Anisakis* spp. infection in Atlantic chub mackerels from Tripoli fish markets was investigated. The third larvae of *Anisakis simplex* was the only pathogenic species for man found in this study. Prevalence rate was 18.07% (i.e., 15 individuals were infected from 83 examined fishes). All the detected larvae were found in the abdominal cavity. Table (1) shows
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the prevalence, mean intensity and mean abundance of 3\(^{rd}\) larvae of *A. simplex* among *Scomber colias* in Tripoli fish markets.

Table 1. Prevalence, mean intensity and mean abundance of third larvae of *Anisakis simplex* among *Scomber colias* in Tripoli fish markets.

<table>
<thead>
<tr>
<th>Number of fish infected/ investigated</th>
<th>Prevalence (%)</th>
<th>Mean Intensity ± S.D.</th>
<th>Mean Abundance ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>3(^{rd}) larvae of <em>Anisakis simplex</em></td>
<td>15/83</td>
<td>18.07</td>
<td>12.84 ± 3.51</td>
</tr>
</tbody>
</table>

S.D. – standard deviation

There is no correlation founded between abundance of the infection with *A. simplex* and the host length of examined fishes by using Spearman's rank correlation coefficient. Table (2) illustrates intensity of *A. simplex* larvae infection among *Scomber colias* according to their length class.

Table 2. Prevalence and mean abundance of third larvae of *Anisakis simplex* larvae infection according to the length class of examined fishes in the current study.

<table>
<thead>
<tr>
<th>Host length class (cm)</th>
<th>Number of fish infected/ investigated</th>
<th>Mean Abundance ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.0 – 18.9</td>
<td>3/14</td>
<td>11.45 ± 2.84</td>
</tr>
<tr>
<td>19.0 – 20.9</td>
<td>2/13</td>
<td>10.72 ± 3.45</td>
</tr>
<tr>
<td>21.0 – 22.9</td>
<td>3/14</td>
<td>9.91 ± 3.14</td>
</tr>
<tr>
<td>23.0 – 24.9</td>
<td>2/14</td>
<td>10.75 ± 4.19</td>
</tr>
<tr>
<td>25.0 – 26.9</td>
<td>3/14</td>
<td>12.07 ± 3.36</td>
</tr>
<tr>
<td>≥ 27</td>
<td>2/14</td>
<td>9.72 ± 3.95</td>
</tr>
</tbody>
</table>

Correlation between intensity and host length

Not Sig

\(r_s = -0.074\)

\(P = 0.681\)

S.D. – standard deviation, Not Sig – not significantly different.

Furthermore, by using Wilcoxon rank-sum test, the females were more affected than males. It would be related to their behavior as need more
nourishing feed-in prepare for spawning season, which could increase the chance of catching the infection. Table (3) describes the relationship between the prevalence and mean intensity of third larvae of *A. simplex* according to the sex of *Scomber colias* in the current study.

Table 3. Prevalence, mean intensity and mean abundance of third larvae of *Anisakis simplex* according to the sex of examined fishes in the current study.

<table>
<thead>
<tr>
<th>Epidemiological parameter</th>
<th>Female</th>
<th>Male</th>
<th>Significance between two sex (female/male)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence (%)</td>
<td>21.42</td>
<td>14.63</td>
<td></td>
</tr>
<tr>
<td>Number of fish infected/ investigated</td>
<td>(9/42)</td>
<td>(6/41)</td>
<td>* Sig</td>
</tr>
<tr>
<td>Mean Intensity ± S.D</td>
<td>14.61 ± 2.74</td>
<td>9.81 ± 1.91</td>
<td>$z = -5.352$, $P = 0.000$</td>
</tr>
</tbody>
</table>

S.D. – standard deviation, * Sig – significantly different.

Unlike *A. physeteris*, the prevalence of *A. simplex* in other latitudes varies, and can reach up to 100% in teleost fish from the Norwegian coast (Strømnes and Andersen 2000); in the United Arab Emirates parasites 40 commercial species, in some cases associated with *Hysterothylacium* spp. (Kardousha, 1992). This discrepancy would be explained by the differences in the time of the study; our research was carried out between March and April 2018 under apparently normal conditions, unlike the several studies which examined the genus of *Anisakis* among various fish species during different seasons. As well as, the variation in the findings could be due to the sensitivity of diagnostic method used. However, we have no valid arguments to explain these differences, although it is possible to speculate that it is due to the climatic changes produced in the marine ecosystem.

The knowledge of the ecological viewpoints of helminths in marine species of direct consumer commercial interest affecting public health is significant in ascertaining the extent of infection and proposing some control measures. Among these pathogens are the larvae of nematodes *Anisakis simplex*, *A. physeteris* and *Pseudoterranova decipiens* which cause
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... a parasitic zoonosis named anisakidosis, characterized by an eosinophilic infiltration, that evolves in the formation of granulomas due to the intake of raw or undercooked fish stews marine or squid infected with these larvae (Kagei *et al*., 1978; Asato *et al*., 1991; Ishikura *et al*., 1992). Adults of these parasites infect the marine mammals (whales, dolphins and sea lions). Although the human is a paratenic host because the larva does not develop until the adult stage. The infective larvae of *A. simplex* could survive for almost two months within anchovies in vinegar, since the heat treatment may be insufficient and not uniform and, finally, imported culinary practices such as smoking. Traditionally in Libya, the fishes are mainly eaten either as fried, roasted or oven-baked (tagine), but without omission certain occasional recipes containing dried salted mackerels (called: *cawalli mokaddad*). Although there are unrest and conflicts in Libya, however there are quite peoples number from foreign workforce, students, as well as migrants and asylum seekers from different countries of several continents. The most common risk practices in our country are the elaboration of traditional dishes from raw fish and cephalopod, such as cebiche, sashimi, and sushi for the foreign residents mainly inhabit the study area, would be exposed to consequences of the infection. With respect to the prevention and control strategies of parasites of medical and ichthiopathological importance, the evisceration of fish should be evaluated before being frozen by artisanal fishers, as a possible strategy to reduce the risk of human infection.

In the current study, the identification of 3rd larval forms of anisakid was based on morphological characters. Nevertheless, this recognition means places some obstacles, which are also due to the similarity between the species. Admissible to verify that the methodologies using morphology were inadequate to identify "true" species within genera of *Anisakis* and *Pseudoterranova*. Moreover, the use of molecular techniques such as conventional PCR (Chen *et al*., 2008) are more reliable. The molecular genetics studies allowed us to identify seven species within the *A. simplex* complex, with structure genetics, biological cycle and geographical distribution distinctive and wide variety of hosts (Mattiucci *et al*., 2002, 2005): *A. simplex* s.s. in the North Atlantic, *A. simplex* C in the North Pacific and southern waters below 30º N, and *A. pegreffii* in the Mediterranean Sea (Mattiucci *et al*., 1997). *A. typica* in the Indian Ocean and Mediterranean Sea, *A. physeteris* in the Atlantic Ocean and

Mediterranean Sea, *A. brevispiculata* in the Atlantic Southeast and *A. zyphidarum* in the Southeast Atlantic and Mediterranean (Mattiucci et al., 2005). Within the *P. decipiens* complex, Paggi et al., (2000) identified four species, using genetic markers. The dynamics of current anisakids larval infection in fish from the Libyan coast are still unknown and need more reviewing research. Thus, for the objective of definitive identification, the present study suggest would it necessary achieve a further research of molecular techniques.

**Conclusions**

The findings of the current study demonstrates that a significant existence of anisakid infestation in fresh Atlantic chub mackerels marketed in Tripoli with possible of serious health hazards. Heretofore, the studies on fishery products in Libya are not yet developed, while the worldwide evidence of parasitization, as well as the increase in reported anisakidosis cases have caused growing concern and question the effectiveness of existing measures to ensure the safety of these products. The magnitude of the infection in humans can be known by cross-sectional studies, but considering the probability of occurrence in the fishmongers, fish processors and foreign residents, could be detected through sentinel surveillance. Longitudinal studies of infection by anisakid larvae in other commercial fish may give some light to raise adequate control measures.

**Authors' contributions**

Design and conception of the research (Mansour L E, Gerish E KH); data and specimens collection (Jalal M B, Al-Khallab E H, Saleh S M, Gerish E KH); lab processing and parasitological identification (Mansour L E, Elmeghirbi W M); statistical analysis (Atia A A); interpretation and manuscript preparation (Mansour L E, Gerish E KH); All the authors read and approved the final manuscript.

**Disclosure statement**

The authors declare that there is no conflict of interest.
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