Anisakid nematodes are widespread as a natural event and may cause fish-borne anthropozoonosis as a result of eating infested raw, undercooked or improperly processed food.

Pathogenesis

In humans the anisakiasis may manifest as an acute form with a predominantly gastric localisation, characterised by nausea, vomiting and epigastric pain that occur after 4-6 h after ingestion of fish parasitised.

We distinguish localised forms where it is possible discover Granulomas in the submucosa, with the presence of eosinophils (eosinophilic granulomas) and diffuse forms characterised by an edematous infiltration that affects the entire gastric or intestinal wall called eosinophilic Phlegmon (Mattucci et al., 2011).

Larval forms of A. simplex have been identified as responsible for IgE-mediated allergic reactions, with symptoms ranging from urticaria, to asthma up to anaphylactic shock (Audicana et al., 2002).
Life-cycle of Anisakid parasites

Anisakid nematodes are heteroxenous and their life cycles involve small crustaceans as first intermediate or paratenic hosts, where second stage larvae (L2) mutate to the third stage (L3), thus becoming potentially infective to their definitive hosts.

Taxonomy

The taxonomy of anisakids is mainly based on the morphology of adult specimens.

| ANISAKIDAE | 
| --- | --- |
| Anisakinae | Raphitascariniae |
| Genera | Genera |
| Anisakis | Hysterothylacium |
| Pseudoterranova | Raphidiocaris |
| Contracaecum | Phocascaris |

The most frequently recovered anisakid species in fish belong to *Anisakis*, *Pseudoterranova*, *Contracaecum*, *Phocascaris* genera.

Geolocalization

The geolocalization of the anisakid species benefits preventive actions because it allows us to make appropriate health decisions.

Purpose

This work is a part of a wider mapping scheme of the parasite in the Mediterranean sea, led by Italian "Centro di Referenza Nazionale per le Anisakiasi", aiming to indentify risk factors and subsequently come up with prevention measures.
Material and Methods

584 fish of five different commercial species caught in GFCM sub-area 16 were screened for Anisakid nematode.

Morphological data were collected after optical vision and Scanning Electron Microscope (SEM).

Molecular date were obtained by PCR, sequencing and sequence analysis.
Parasites collected from the fish samples were 6.318

Fish products received by the Reference Center were eviscerated and subjected to observation by stereo microscope for collection of larvae that maybe present in the viscera, organs and muscles.

Pepsinhydrochloric dye digestion

Artificial digestion of fish fillets for the isolation of Anisakidae larval stages

Genus Identification

The larvae collected were placed on a glass slide, clarified with glycerol and observed under optical microscope to perform the genus identification.
Anisakid larvae can be identified at genus level by light microscopy, mainly on the basis of the evaluating the front and terminal morphological appearance and the initial part of the digestive system.
Artificial fracture reveal double coat

Histology reveal a thick wall

The ascidian larvae is characterized by gut consisting of high columnar cells.
Molecular methods

After genus identification the 6318 larvae collected from the 584 fish were analyzed by molecular tools.

Molecular targets

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Target</th>
<th>Primers name</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anisakis</td>
<td>ITS1, 5.8SrRNA, ITS2</td>
<td>NC5/NC2</td>
<td>Zhu X., 1998</td>
</tr>
<tr>
<td>Anisakis</td>
<td>cox2</td>
<td>211F/210R</td>
<td>Nadler SA, 2000; Mattucci S, 2011</td>
</tr>
<tr>
<td>Anisakis</td>
<td>28SrDNA (LSU)</td>
<td>391/390</td>
<td>Nadler SA, 2005</td>
</tr>
<tr>
<td>Anisakis</td>
<td>ITS</td>
<td>ITSF/ITSR</td>
<td>Iniguez AM, 2009</td>
</tr>
</tbody>
</table>

Using NC5/NC2 primers (Zhu X., 1998), ribosomal genomic regions ITS1, 5.8S rRNA and ITS2 of DNA were amplified.

RFLP (Restriction Fragment Length Polymorphism) analysis with enzymatic reaction show the restriction profiles.
**RFLP analysis using HhaI**

1 to 15 = analyzed samples  
C1 = A. pegreffii  
C2 = A. simplex  
C3 = A. physeteris  
L = 100 bp Ladder

**Sequencing**

PCR products were sequenced and the sequences were analysed using a NCB online Blast tool.

Anisakids species were detected by comparing with the sequences present in the GenBank and by phylogenetic analysis.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scabbard fish</td>
<td>100%</td>
</tr>
<tr>
<td>European anchovies</td>
<td>15.3%</td>
</tr>
<tr>
<td>Hakes</td>
<td>25%</td>
</tr>
<tr>
<td>European pilchards</td>
<td>5%</td>
</tr>
<tr>
<td>Red mullets</td>
<td>4.2%</td>
</tr>
</tbody>
</table>

Results

Anisakid nematodes were found in 18.32% of fish. Within this percentage range, the distribution of larvae for each species varied.

Number of parasites in fish species. The number of parasites from scabbard fish reported in the graphic is limited to 25 to allow the graphic view of data concerning the others fish species.
Significance

The only anisakid species found in this study was *Anisakis pegreffii*.

The data suggest that *Anisakis pegreffii* infestation in fish in GFCM sub-area 16 is dominant.

Visual inspection of fish should be carried out by qualified operators to remove the parasite and avoid reaching consumers.

Control strategies of anisakid worm infestation to achieve reductions of pathogenicity were discussed both by the FDA (Food and Drug Administration) and the EFSA (European Food Safety Authority).

---

EFSA/FDA


For parasites other than trematodes the freezing treatment must consist of lowering the temperature in all parts of the product to at least:

(a) – 20 °C for not less than 24 hours; or
(b) – 35 °C for not less than 15 hours.

**FDA recommends that all fish and shellfish intended for raw (or semiraw such as marinated or partly cooked) consumption be blast frozen to:**

(a) -20°C or below for 7 days; or
(b) -35°C or below for 15 hours.

---

Risk assessment

**What?**

**How bad?**

**How often?**

---

Conclusion

*Anisakis pegreffii* therefore remains the main etiological infecting agent of pelagic and demersal fish in the whole Mediterranean sea.

Therefore, reported cases of human anisakiasis caused by *Anisakis pegreffii* in some Mediterranean countries, as well as our data, highlight the predictive clinical relevance for this specie.

**EFSA and FDA indicate in freezing the sure way to reduce the risk. At the same time, EFSA encourages the search for new methods to control the risk.**
Thanks for your attention