Survey of Anisakids larvae as a food-borne allergen source in some commercially important fish in Iran

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ABSTRACT

Anisakiasis is a fish-borne zoonosis caused by ingestion of larval stages of nematodes belonging to the Anisakidae family. The aim of the study was to determine the prevalence and intensity of Anisakidae larvae in the some commercially important fish in Iran. Between spring 2011 to winter 2012, parasitological examination of Scomberomorus commerson (n=100), Otolithes rubber (n=60) and Acipenser persicus (n=20) from Iran shores of the Persian Gulf and Caspian Sea were made for anisakids. Sampling was taken from different parts of a fish by routine methods. Results showed that abdominal cavity was the most infested organ of these samples. Two anisakid species (Anisakis larva and Contracaecum sp.) were identified morphologically. The percent of contamination of Contracaecum sp. ranged from 33.3% (O. rubber) and 10.0% (A. persicus) to 3% (S. commerson). The percentage of contamination of Anisakis larva in S. commerson was %16 in O. rubber %10 and 10% in A. persicus respectively.

These results indicated that anisakiasis could be a health hazard for people who consume it.

Key words: Anisakids Larvae, prevalence and intensity, Commercially Fish, Contracaecum sp., Iran

INTRODUCTION

Anisakiasis is caused by the ingestion of nematode larval belonging to the genera Anisakis, Pseudoterranova, Contracaecum, Hysterothylacium and Terranova in raw or undercooked cured and seafood. With the increased popularity of eating undercooked or raw fish dishes, the number of anisakiasis cases have been increased⁴. Anisakis and Contracaecum are common nematode parasites which are present in many marine and freshwater fish⁴. Adult parasites live in the stomach, intestine, viscera organs and also in the skeletal muscles of definitive hosts. Eggs shed in the faeces from the final hosts hatch in the water where the larvae are consumed by crustacean (usually Euphausiids), which in turn are consumed by fish. Several species of marine fish and cephalopods act as a second intermediate host, or as a paratenic host⁵. Incidental parasitism of a human host usually results in re-encystment of the juvenile worm.
Anisakis can cause different diseases in human by eating cured or pickled sea foods, crustaceans and squid. In acute anisakidosis, inflammatory and allergic response in the digestive tract mucosa along with abdominal pain, fever, diarrhea, vomiting and mental stupor were observed. It can also induce IgE mediated reactions with various clinical manifestations ranging from urticaria and angioedema to hypersensitivity reactions. Also, in chronic form of anisakidosis, eosinophilic granulomas with abscesses were observed. Results showed, anisakis threat human health through intestinal infection with worms from eating of under processed fish, and also, through allergic reactions to chemicals left by the worms in fish flesh.

The Persian Gulf is located in the south of Iran, is an extension of the Indian Ocean. This Golf is the most important fisheries resources, and also has very important ecological effects on fish parasites. The Caspian Sea is the largest continental water body on earth, surrounded by Iran, Russia, Kazakhstan, Turkmenistan and Azerbaijan.

Fish are the main meal of the people in south and north of Iran. *S. commerson* and *O. rubber* are one of the commercially important species in the Persian Gulf and Oman Sea that consumed as fried or roasted especially in the rural areas. Also, *A. persicus* is an important species that live in the southern margin of the Caspian basin. Unfortunately, in the last decade, fishery has seen a remarkable decline in the fishing yields of the Iranian Caspian Sea due to over-fishing and habitat and this fish has was listed as an endangered species in 1990.

The main objective of this study is to determine the prevalence and intensity of anisakids larvae in some commercially important fish in Iran.

**MATERIALS AND METHODS**

**Sampling**

In this study, between spring 2012 to winter 2013, parasitological examination of *S. commerson* (n=100), *O. rubber* (n=60) and *A. persicus* (n=20) from Iran’s shores of the Persian Gulf and Caspian had been done for anisakids.

**Parasite examination**

The fish were transported to the central laboratory and each fish was measured before observation. To determine the presence of parasites, fish were dissected and intestine, liver, spleen, gonads, muscles and body cavity were observed carefully. The collected nematode larve were washed using saline solution and fixed in 70% ethanol, then cleared in glycerin and finally survied using light microscope. The photograph was taken from parasite specimens with the aid of a light microscope that was equipped with micrometers and a camera lucida. The parasite specimens were identified by using the reference keys.

**Statistical analysis**

The data were subjected to statistical analysis using the SPSS software version no. 18. Results of this study were analyzed statistically using One way ANOVA (P< 0.05). Also, mean intensity was determined by dividing the total number of recovered parasites to the number of infected fish samples. Prevalence was also calculated by dividing the number of infected fish samples by the total number of examined ones and expressed as a percentage.

**RESULTS**

Majority of caught *S. commerson* were 40-69 cm in length and less than 5 kg in weight during the dissection.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Average length (cm)</th>
<th>Average weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. rubber</em></td>
<td>38.23±1.25</td>
<td>800-1000</td>
</tr>
<tr>
<td><em>A. persicus</em></td>
<td>164.6±0.96</td>
<td>31.4± 0.156</td>
</tr>
</tbody>
</table>

The obtained results indicated that abdominal cavity was the most infested organ of these fish. No external visible signs of disease was observed in the examined fish.
No parasite larva was observed in the livers, spleens and gonads of fish. Also, no significant differences in parasitisation were observed between both sexes from each fish. Two anisakid species (Anisakis sp. and Contracaecum sp.) were identified based on morphologically characterization. The prevalence and mean intensity of anisakids larvae in examined fish is shown in Table 2. The percentage of contamination of Contracaecum sp. ranged from 33.3% (O. rubber) and 10.0% (A. persicus) to 3% (S. commerson). The percentage of contamination of Anisakis larva in S. commerson was %13 in O. rubber %10 and 10% in A. persicus, respectively. Also, in this study, 3 larvae of Anisakis were found in the flesh of S. commerson. The highest parasite burden were found in S. commerson of more of 5 cm in length (Fig. 1).

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Anisakis larvae</th>
<th>Contracaecum larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence (%)</td>
<td>Mean intensity±SD</td>
</tr>
<tr>
<td>S. commerson</td>
<td>3</td>
<td>2.2±1.6</td>
</tr>
<tr>
<td>O. rubber</td>
<td>10</td>
<td>3.0±3.12</td>
</tr>
<tr>
<td>A. persicus</td>
<td>10</td>
<td>5.4±4.26</td>
</tr>
</tbody>
</table>

DISCUSSION

Food-borne parasitic zoonoses cause death and serious problems in humans and they have public health significance and socioeconomic importance. The reason for the emergence of the zoonotic helminth depends on a variety of factors, including: the number of infected hosts, the number of transmissive stages excreted, host behaviour and activity, geographic distribution, food sources and supplies and the climate and hydrogeology of the area. Anisakis is known as an important human health risk because of its tendency to infect fish which may be eaten uncooked or lightly cured, such as herring. It is also difficult to detect due to its small size and lack of color. Because anisakidae are not host specific at the larval stage, they may be found in a wide range of different available host species. Since the first case study in the Netherland in 1960 by Van Thiel et al. (1960), several lawsuits have been reported from the five continents of Asia (Korea, Japan), Europe (The Netherlands, France, the United Kingdom, Spain, Germany, Italy), Africa (Egypt), and the Americas (Alaska, Hawaii, Canada) and South American countries.
No human anisakiasis is yet reported from Iran. This issue could be attributed to the cooking habit of fish in the areas we studied as well as other parts of the country. In Iran, several species of fish including Esoc lucius, Huso huso, Rutilus frisii kutum and A. persicus from Caspian Sea and Persian Gulf as well as Atash, Sobhanieh, Al-hai and Houfellagoons of Khuzestan, have been reported to be infected with Anisakis sp. and Contracaecum sp. The larvae of Contracaecum spp. was reported from Acipenseridae R. frisii kutum, Capoeta damascina, Iranocichla hormuzensis from Iran. Results of Farahnak et al. (2002) showed that Barbus spp., Cyprinus carpio, Liza abu, Aspius vorax act as paratenic host for Anisakis sp. in south west of Iran. Results of this study showed the presence of Anisakis larve in Acipenser persicus. The occurrence of this larve was reported in different sturgeon species including A. Persicus, Acipenser gudzenstaedti, Acipenser stellatus and Huso huso in previous study.

The results of the present study indicated that the most Numerous representated anisakid species in Iran was Contracaecum sp., while the most infected fish species among investigated fish was O. rubber. Results showed that eating raw, semi raw O. rubber, could be the source of infection with Contracaecum sp. larvae of humans in southern parts of Iran. It is believed that eating S. Commerson, O. rubber and A. persicus in southern and northern of Iran could be a threat to human health.

This study is the first report of Anisakis sp. and Contracaecum sp. in S. Commerson in Iran, but infection to these nematodes in O. rubber and A. persicus have been reported in previous studies.

The presence of Anisakis sp. and Contracaecum sp. in several commercially important fish in the Persian Gulf and Caspian Sea has been demonstrated, enforcing the necessity of a design for a good manufacturing practice protocol in the processing industry to minimize the presence of this parasite as food-borne allergen source in seafood products.

According to an old belief of northern and southern parts of Iran, it is important to consume raw fish in order to cure the jaundice, therefore it is essential that doctors consider Anisakis sp. and Contracaecum sp., in patients with signs of abdominal pain and Phlegmonous Enteritis.

The control of anisakiasis, requires the consideration of several factors including: the thorough understanding of parasite life cycles, biology, transmission and epidemiology as well as the hygiene habits of the population, climate conditions and the culture and customs of different nationalities. Finally, an educational campaign and executive measures need to be implemented. The center for disease control recommends cooking fish at 60 °C for 5 min or freezing at -20 °C at least 60 h before consumption to kill juvenile anisakid worms. In conclusion, there are little information about distribution of larval anisakid nematodes among Iranian fish species, so, comprehensive and complete studies needed to assess the distribution of larval anisakid nematodes among commercially important fish in Iran.

Acknowledgment

The authors wish to thank Dr. Hamid Reza Azizi and Mr. Nader Ahmadi for their kind assistance.

REFERENCES
11. Mokhayer, B., A list of parasites of eusturgeon (Acipenseridae) in Iran. Journal Veterinary Faculty, University Tehran, 29: 11- 12 (1973)
15. Sattari, M., B. Mokhayer, A. Eslami and Bokaei S. Parasites of *Acipenser persicus* (chondrostei: Acipenseridae) from south-west of Caspian Sea. Journal of the Faculty of Veterinary Medicine, University of Tehran, 55: 19-24 (2000)