

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

Milad Adel¹, Armin Abedian Amiri^{1,2*}, Keyvan Farahmandi³, Ahmad Reza Safian⁴ and Elham Khalili⁵

¹Department of Aquatic Animal Health and Diseases, Caspian Sea Ecology Research Center, Sari, Iran

²Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Department of Aquatic Animal Health and Diseases, Tehran, Iran

³Department of Medical Sciences, School of Public Health, Tehran University of Medical Sciences, Iran

⁴Department of Clinical Sciences, Faculty of Veterinary Medicine, University Shahrekord, Shahrekord, Iran

⁵Department of Health and Food Quality Control, Faculty of Veterinary Medicine, University Shahrekord, Iran

*Corresponding Author E-mail: abedian_a637@yahoo.com

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 ruber* (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. ruber*) and 10.0% (*A. persicus*) to 3% (*S. commerson*). The percentage of contamination of *Anisakis larva* in *S. commerson* was %16 in *O. ruber* %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 be increased¹.

Anisakis and *Contracaecum* are common nematode parasites which are present in many marine and freshwater fish¹. 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), whiche in turn are consumed by fish. Several species of marine fish and cephalopods act as a second intermediate host, or as a paratenic host². 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 observed³. 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 observed². 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 flesh³.

The Persian Gulf is located in the south of Iran, is an extension of the Indian Ocean. This Gulf 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 areas⁴. 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 1990⁵.

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^{6,7,8}.

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 1. Average fish length and weight in the study

Fish	Average length (cm)	Average weight (g)
<i>O. rubber</i>	38.23±1.25	800-1000
<i>A. persicus</i>	164.6±0.96	31.4± 0.156

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 2: The prevalence and mean intensity±SD of anisakids larvae collected from selective fishes in Iran

Fish species	Anisakids larvae			
	<i>Anisakis</i> sp.		<i>Contracaecum</i> sp.	
	Prevalence (%)	Mean intensity±SD	Prevalence (%)	Mean intensity±SD
<i>S. commerson</i>	3	2.2± 1.6	13	3.7±4.6
<i>O. rubber</i>	10	3.0±3.12	33.3	6.4±4.8
<i>A. persicus</i>	10	5.4±4.26	10	4.1±2.38

Fig. 1. Macroscopic Anisakidae helminth parasite isolated from intestine of *Scomberomorus commerson*



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 area³.

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 Iran¹⁰. 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 *Esox lucius*, *huso huso*, *Rutilus frisii kutum* and *A. persicus* from Caspian Sea^{1,11,12} and Persian Gulf^{12,13} as well as Atash, Sobhanieh, Al-hai and Houfelloagoons of Khuzestan¹⁴, have been reported to be infected with *Anisakis* sp. and *Contracaecum* sp.

The larvae of *Contracaecum* spp. was reported from Acipenseridae^{11,15} *R. frisii kutum*¹², *Capoeta damascina*¹⁶, *Iranocichla hormuzensis* from Iran¹⁷. 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 larvae was reported in different sturgeon species including *A. Persicus*, *Acipenser guldenstaedti*, *Acipenser stellatus* and *Huso huso* in previous study^{11,15}.

The results of the present study indicated that the most numerously represented 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^{15,18}.

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 consum 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

1. Eslami, A., M. Anwar and Sh. Khatibi. Incidence and intensity of helminthose in pike (*Esox lucius*) of Caspian Sea (northern Iran). Riv. It Piscic Ittiop, 7: 1-14 (1972)
2. Gani, F., Lombardi C., Senna G. and P. Mezzelani. Anisakiasis: a borderline disorder. Medical, **92**: 6-605 (2001)
3. Murrell, K.D. Food and water-borne parasitic zoonoses in the 21st century. Trends Parasitology, **17**: 163-164 (2001)
4. McPherson, G.R. Age and growth of the narrow barred Spanish Mackerel (*Scomberomorus commerson* Lac'ep'ede 1800) in north-eastern Queensland Waters. Australian Journal Marine Freshwater Research, **43**: 1269-1282 (1992)
5. Bahmani M., Kazemi R., Donskaya P., A comparative study of some hematological features in young reared sturgeon. Fish Physiology and Biochemistry, **24**: 135-140 (2001)
6. Koyama, T. Anisakidae larvae. 1. Morphology and classification. Fish and anisakis (The Japanese Society of Fisheries Science). Koseisha koseikaku, **1**: 9-19 (1979)

7. Rocka A. Nematodes of the Antarctic fishes. *Polish Polar Research*, **25**: 135-152 (2004)
8. Shamsi S., Norman R., Gasser R., Beveridge I., Redescription and genetic characterization of selected *contracaecum* spp. (nematoda: Anisakidae) from various hosts in australia. *Journal of Parasitology Research*, **104(6)**: 1507-1525 (2009)
9. Van Thiel P.H., Kuipers F.C., Roskam R.T., A nematode parasitic to herring causing acute abdominal syndrome in man. *Tropical Geogria Medical*, **2**: 97-113 (1960)
10. Eslami A., Sabokroo H., Ranjbar- Bahadori, SH. Infection of Anisakids Larvae in Long Tail Tuna (*Thunnus tonggol*) In North Persian Gulf. *Iran J Parasitol*, **6**: 96–100 (2011)
11. Mokhayer, B., A list of parasites of eusturgeon (Acipenseridae) in Iran. *Journal Veterinary Faculty, University Tehran*, **29**: 11- 12 (1973)
12. Eslami, A. and M. Kohneshahri. Study on the helminthiasis of *Rutilus frisii kutum* from the south Caspian Sea. *Acta Zoologica et Pathologica Antverpiensia*, **70**: 153-155 (1978)
13. Radfar, M.H. and A. Eslami. Study on the helminth infections of *Epinephlus tauvina* of Persian Gulf, Iranian shores. In the Proceedings of the 1th Congress Diseases Aquaculture Ahwaz, Iran, pp: 11-14 (2000)
14. Farahnak, A., I. Mobedi and Tabibi, R. Fish anisakid helminthes in Khuzestan province, south west of Iran. *Iranian Journal Public Health*, **31**: 129-132 (2002)
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)
16. Pazooki, J., F. Nazari Chamk and Masoumian, M.. New host records for fish nematodes from Iran. *Journal of Cell and Animal Biology*, **6**: 15-20 (2012)
17. Ansary, T.H., N. Moghaddar and Esmaeili, H.R. *Iranocichla hormuzensis* (Coad 1982), a new paratenic host of *Contracaecum* sp. and *Phocanema* sp. (Nematoda: Anisakidae). *Comparative Clinical Pathology*, **19**: 335-337 (2010)
18. Peyghan R., Hoghoghi Rod N., Mesbah M., Rastkerdar M., Frequency of helminthic infestation in Tiger Tooth croaker, *Otolithes rubber*, Black pomfret, *Parastromateus niger*; Javelin grunter, *Pomadasys kaakan* and Malabar blood snapper, *Lutjanus malabaricus* of Persian Gulf, Iran. *Iranian Veterinary Journal*, **12(10)**: 81-87 (2006)