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Olgu Sunumu / Case Report

***Anisakis* spp. infection in Atlantic mackerel (*Scomber scombrus*, Linnaeus 1758) from the Sea of Marmara**

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ABSTRACT:

This study was aimed to determine the parasite infection of fish samples. Ten Atlantic mackerel (*Scomber scombrus*), which were suspected with parasite infection, were sent to the laboratory by the fisherman at the Sea of Marmara, Turkey. The skin, fins, eyes, buccal cavity, and gills were examined regarding ectoparasites, while internal organs were investigated concerning endoparasites. Seven of 10 fish were infected with endoparasites. A total of 27 nematode larvae were detected from the abdominal cavity of fish. The detected nematode larvae were rinsed in 0.9 % isotonic saline and preserved in glycerine-alcohol until examined. After that the anterior and posterior end of samples were cleared in lactophenol. The larvae had a prominent boring tooth at the anterior end. The body features of the larvae were measured (body width, oesophagus length, ventriculus length and mucron length) on a light microscope (4x-10x-40x). Morphological analysis showed that all samples were identified as *Anisakis* Type I larvae (L3). It is known that anisakiasis is one of the important fish-borne zoonotic diseases. Also, this nematode species had been previously reported in Turkish water from different fish species. The present case contributes valuable information about one of the intermediate hosts of *Anisakis* spp. in Turkey.

Marmara denizi Atlantik uskumrularındaki (*Scomber scombrus*, Linnaeus 1758) *Anisakis* spp. enfeksiyonu

ÖZET:

Bu çalışmada balık örneklerinde paraziter enfeksiyonların belirlenmesi amaçlandı. Marmara denizi balıkçıları tarafından paraziter enfeksiyon şüpheli on adet Atlantik uskumrusu (*Scomber scombrus*) laboratuvara gönderildi. Balıklara ait deri, yüzgeçler, gözler, ağız boşluğu ve solungaçlar ektoparazitler yönünden incelenirken, iç organların da endoparazitler yönünden araştırmaları yapıldı. Gönderilen on adet balığın yedisinin enfekte olduğu saptandı. Balıkların karın boşluğunda toplam 27 nematod larvası tespit edildi. Saptanan nematod larvaları % 0.9'luk izotonik tuzlu su ile yıkandı ve gliserin-alkol içinde saklandı. Sonrasında, örneklerin anterior ve posterior uçları laktofenolde şeffaflandırıldı. Larvaların ön kısmında belirgin bir kesici diş (boring tooth) görüldü. Larvaların vücut özellikleri (boy uzunluğu, özefagus uzunluğu, ventrikül ve mucron uzunluğu) ışık mikroskopu altında ölçüldü (x-4-x10-x40). Morfolojik analizler sonucu tüm örneklerin *Anisakis* Tip I larvaları (L3) olduğu saptandı. Anisakiasis balık-kaynaklı (fish-borne) zoonotik hastalıkların en önemlilerinden biri olarak bilinmektedir. Ayrıca, bu nematod türlerinin daha önce Türk sularında farklı balık türlerinde de bulunduğu bildirilmiştir. Bu çalışma ile, *Anisakis* spp.'nin Türkiye'deki ara konaklarından biri hakkında değerli bilgi elde edilecektir.

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1. Introduction

Anisakis spp. (Dujardin, 1845) is found in the digestive tract of marine mammals (cetaceans) and fish-eating birds. The parasite occurs in the Atlantic ocean, the Pacific ocean and Mediterranean sea in a wide range of marine mammals species (12). Although, there are few studies on parasites of sea mammals in Turkey (8, 27, 33), *Anisakis* spp. were detected in 6 striped dolphins (*Stenella coeruleoalba*) on the Eastern Mediterranean coast of Turkey (8).

The larvae of *Anisakis* spp. infect the aquatic invertebrates (cephalopods) and non-mammalian vertebrates (fish species) as an intermediate host or paratenic host. The crustaceans are the first intermediate hosts of anisakids (21, 22). According to Berland (10) morphological features of *Anisakis* spp. larvae can be distinguished as Type I and Type II. *Anisakis* Type I includes; *Anisakis simplex* complex (*A. simplex* sensu stricto (s.s), *A. pegreffii*, *A. simplex* C), *A. typica*, *A. ziphidarum* and *A. nascetti*, besides Type II includes; *A. paggiae*, *A. physeteris* and *A. brevispiculata* (21, 22, 24). Inside of them, *A. simplex* s.s, *A. pegreffii* from Type I and *A. physeteris* from Type II are also reported in humans (6, 23). Humans can be infected by ingesting raw or undercooked fish or squids as a paratenic hosts. It is one of the important pathogens associated with human and animal health. The third stage of larvae can penetrate the mucosa of the gastrointestinal tract, producing acute or chronic abdominal syndromes (abdominal pain, vomiting, diarrhea and mild fever) in humans. Also in some cases, serious allergic reactions such as urticaria, angioedema and anaphylaxis can occur (7, 29).

The *Anisakis* spp. larvae were detected from throughout the world involving Africa, Australia, Oceania, Europe, North America and South America in a wide range of fish species (1). Besides, larvae of *Anisakis* spp. had been reported in Turkish coastal waters from different researchers and fish species (Table 1).

Table 1: Data on the distribution of *Anisakis* spp. larvae in Turkish coastal waters

Tablo 1: Türkiye kıyı sularında *Anisakis* spp. larvalarının dağılımına ilişkin veriler

References	Fish species	Origine	Number of examined fish (n)	Diagnostic methods		Number of infected fish (n/ %)	Number of detected larvae (n)
				Morphological	Molecular		
(3)	Red mullet (<i>Mullus surmuletus</i>)	AS	142	<i>A. simplex</i>	-	4/ 2.8	7
(4)	Chup mackerel (<i>Scomber japonicus</i>)	AS	110	<i>A. simplex</i>	-	40/ -	163
		M	122			9/ -	14
(19)	Atlantic mackerel (<i>Scomber scombrus</i>)	SM	20	<i>A. simplex</i>	-	2/ 10.0	21
(5)	Conger eel (<i>Conger conger</i> L.)	AS	26	<i>A. simplex</i>	-	4 / 15.38	14
(32)	Horse Mackerels (<i>Trachurus trachurus</i>)	BS	-	-	<i>A. pegreffii</i> <i>A. simplex</i>	-	6
(2)	11 different species	AS	235	<i>A. simplex</i>	-	33/ -	160+>100
(20)	<i>Merlangius merlangus</i>	BS	3	-	<i>A. simplex</i>	3/ -	10
(30)	<i>Trachurus mediterraneus</i>	BS	56	<i>A. pegreffii</i>	-	1/ 2	11
(25)	10 different species	AS	70	<i>Anisakis</i> Type I	<i>A. pegreffii</i> <i>A. typica</i> Hybrid genotypes	33/ 47.1	569
(34)	7 different species	M	50	<i>Anisakis</i> Type I	<i>A. pegreffii</i>	23/ 46.0	197
	John Dory (<i>Zeus faber</i>)	M	1	<i>Anisakis</i> Type I	<i>A. pegreffii</i>	1/ -	15
		BS					
(26)	23 different species	AS	475	<i>Anisakis</i> Type I	-	-	102
		M					

BS: Black Sea, SM: Sea of Marmara, AS: Aegean Sea, M: Mediterranean Sea

Atlantic mackerel (*Scomber scombrus*, Linnaeus 1758) is represented by the Scombridae family of the Actinopterygii class. It is one of the widely distributed migratory pelagic fish species and lives in North-Atlantic

including Mediterranean (18). It has been assessed as the least concern (LC) in The Red List of Threatened Species since 2011 by The IUCN (15). In Turkey, Atlantic mackerel were reported in the Black Sea, Sea of Marmara, Aegean Sea, and the Mediterranean Sea (11). The 46.6-728.2 tons of Mackerel caught in-between years of 2009 and 2018 from these waters (31).

The aim of the present case was to investigate the presence of the Anisakid larvae in Atlantic mackerel from Sea of Marmara, Turkey.

2. Case Story

Ten Atlantic mackerel were sent by the fishermen in the Sea of Marmara, Turkey. Fish were stored individually, and transported with ice-box to the Ankara University, Faculty of Veterinary Medicine, Parasitology laboratory. Fish were investigated with parasitological examination. The skin, fins, eyes, buccal cavity and gills were examined in terms of ectoparasites, while internal organs were investigated regarding endoparasites. The nematode larvae were observed on 7 of 10 fish (3.85 ± 3.38 ; min: 1, max: 10). Total of 27 nematode specimens were collected from the abdominal cavity. The samples were rinsed in isotonic solution 0.9 % and fixed in glycerine-alcohol after that anterior and the posterior end of the larvae were clarified in lactophenol. The samples were evaluated under the light microscope (x4-x10-x40) and the body features were measured (body width, length of oesophagus, ventriculus, and mucron) (Table 2). Morphological identification was conducted according to Berland (10). The larvae were white and cylindrical shape. The larvae had a prominent boring tooth, excretory pore opened ventrally at the anterior end and the distinct mucron was located at the caudal end. The morphological analysis confirmed that all examined samples were *Anisakis* Type I larvae (Figure 1).

Table 2: The comparative body measurements of *Anisakis* Type I larvae (mm)

Table 2: *Anisakis* Type I larvalarının karşılaştırmalı vücut ölçümleri (mm)

	Present study	<i>A. simplex</i> (28)	<i>A. pegreffii</i> (34)	<i>A. pegreffii</i> x <i>A. simplex</i> (ss) (17)	<i>A. typica</i> (16)
Total body length	9.12- 25.36	12.75-29.94	12.80-24.65	9.0-17.5	21.05-24.97
Length of oesophagus	0.95-1.79	1.18-2.58	0.98-1.87	0.56-1.75	1.50-1.70
Ventriculus length	0.39-0.74	0.90-1.50	0.53-0.74	0.20-0.40	0.89-0.94
Mucron length	0.01-0.02	0.02-0.03	0.01-0.02	0.01-0.03	0.005-0.010

3. Discussion and Conclusion

Anisakiasis is a fish-borne zoonotic parasitic disease and were detected throughout the world in humans (13). *Anisakis* Type I larvae, including *A. simplex* sensu stricto and *A. pegreffii* have been reported as agents of human anisakiasis (9, 14). It is well-known, *Anisakis* larvae reported surviving in various fish species (13). The larvae of *A. simplex* and *A. pegreffii* had been reported in Turkish coastal waters from different researchers (25, 26, 32). *Anisakis* spp. larvae were detected in Aegean (25) and the Sea of Marmara (19) from Atlantic mackerel as previously reported. In the present case, the higher rate of infected Atlantic mackerel (7/10) might be assessed as a potential intermediate host of anisakiasis for Turkish waters.

Despite the extensity of this nematoda in Turkish coast, there is no reported case in human from Turkey. Because of symptoms of human anisakiasis are usually not specific and the disease is thought to be frequently misdiagnosed and underdiagnosed (9).

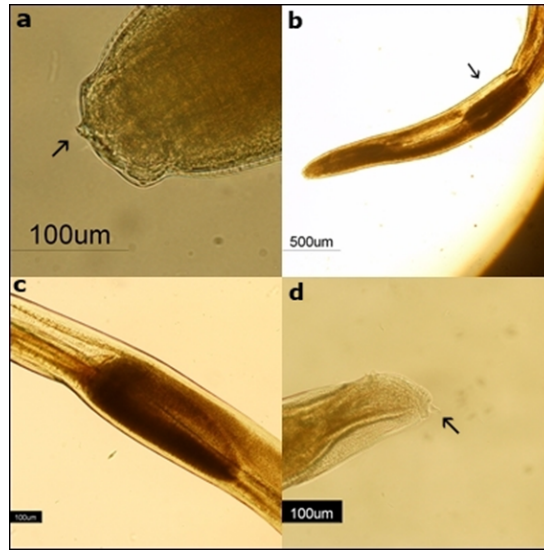


Figure 1: *Anisakis* Type I larvae (a) anterior end, larval tooth (arrow) (x40), (b) anterior end, ventriculus (arrow) (x4) (c) ventriculus (x10), (d) caudal end, mucron (arrow) (x20)

Şekil 1: *Anisakis* Tip I larva (a) ön nihayet, larval diş (ok ucu) (x40), (b) ön nihayet, ventriculus (ok ucu) (x4) (c) ventriculus (x10), (d) arka nihayet, mukron (ok ucu) (x20)

According to Berland (10), larval morphological features including the absence of a ventricular appendage and an intestinal caecum are useful for the distinction between several anisakid genera. *Anisakis Type I or Type II* larvae can be identified based on ventriculus length and the presence of a tail spine (or mucron), however, these measurements insufficient for species identifications. For this purpose, the latest studies were conducted via molecular approaches. Although our preservation methods of samples were useful for PCR protocol, we couldn't obtain sufficient amount of DNA. Therefore, the identification of larvae samples could not be performed via PCR.

Although five hundred twelve marine fish species were previously identified from the Turkish coasts (11), the *Anisakis* spp. larvae were detected in only 23 fish species in Turkey so far (2, 25). Due to the rich variety of Turkish marine fish species, further studies are required. Every new data is essential to understand the epidemiology of anisakiasis.

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