

The infection of *Eustrongylides* spp. in Pike Perch (*Sander lucioperca* L., 1758) (Teleostei: Percidae)

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Abstract: *Eustrongylides* sp. is the most significant freshwater fish zoonotic nematode and has a great importance for the environment, fishing and human health. The importance of this nematode is the ability of the capacity to infect carnivorous organisms and humans that feed on them. In this study, *Eustrongylides* spp. infection was determined from 51 samples of pike perch (*Sander lucioperca* L., 1758) between October 2015 and February 2016 from Lake Marmara (Manisa, Turkey). Each sample was observed and the prevalence of the infection was detected 100%. The parasite samples were examined in Carl Zeiss 300 VP scanning electron microscope. The capsulated and encapsulated parasites were found in various muscles, abdominal cavity and on the internal organs. Furthermore, one sample was detected in the swim bladder without any harm. From scanning electron microscopy overviews, lips and papillae circles were detected on the anterior portion of the body as characteristics for diagnosis of the genus *Eustrongylides*.

Keywords: *Eustrongylides* spp., eustrongylidosis, food-borne parasite, nematodosis, *Sander lucioperca*.

Sudak Balıkları (*Sander lucioperca* L., 1758) (Teleostei: Percidae)'nda *Eustrongylides* spp. Enfeksiyonu

Öz: *Eustrongylides* spp., çevre, balıkçılık ve insan sağlığı açısından tatlı su balıklarının önemli bir zoonotik nematodudur. Bu nematodun önemi karnivor organizmaları ve onlarla beslenen insanları enfekte edebilme niteliğine sahip olmasıdır. Bu çalışmada Göl Marmara (Manisa, Türkiye)'dan Ekim 2015 - Şubat 2016 tarihleri arasında örneklenen 51 adet sudak (*Sander lucioperca* L., 1758) balığında *Eustrongylides* spp.'nin neden olduğu enfeksiyon tespit edilmiştir. Her bir örnekte enfeksiyonun prevalansı belirlenmiş ve incelenen örneklerin % 100'ünde parazit görülmüştür. Parazit örnekleri Carl Zeiss 300 VP taramalı elektron mikroskobu (SEM) ile görüntülenmiştir. Kapsüllü ve kapsülsüz parazitler çeşitli kaslarda, karın boşluğunda ve iç organlarda tespit edilmiştir. Ayrıca hava kesesinde de herhangi bir lezyona neden olmayan parazit tespit edilmiştir. Taramalı elektron mikroskobu görüntülerinde *Eustrongylides* cinsinin teşhisi için vücudun ön kısmında dudaklar ve papilla halkaları tespit edilmiştir.

Anahtar sözcükler: *Eustrongylides* spp., eustrongylidosis, gıda kaynaklı parazit, nematodosis, *Sander lucioperca*.

INTRODUCTION

Lake Marmara which is located in the immediate proximity of Manisa City, Turkey has an importance in the area with industrial fishing that population benefitted as a valuable food resource. According to İlhan & Sarı, (2013) 15 taxa were reported belonging to the Atherinidae, Cyprinidae, Cobitidae, Percidae, Gobiidae, Poeciliidae families in the lake and pike-perch (*Sander lucioperca* L., 1758) is one of the most commercial species with extensive economic value. The infection of *Eustrongylides* spp. being paratenic hosts on pike-perch due to its predator feeding regime (Bjelic-Cabrilo et al., 2013) while benthos-eating fish species, e.g. *Gambusia neogobius* and Cyprinidae family become intermediate hosts (Pazooki et al., 2007). From Danuba Canal, Serbia this parasite was registered from pike-perch meanwhile some reported cases from eel (*Anguilla anguilla*) (Romania), *Carassius carassius* (Iran), European catfish (*Silurus glanis*) (Serbia), sturgeon (*Acipenser persicus*) (Azerbaijan) and brown trout (*Salmo trutta*) (Norway) are known. Also its 3rd and 4th stages larvae were detected from various species of fish, aquatic birds, reptiles (Fauna Europaena, 2017; Molnar et al., 2006; Moravec, 1994), frog (Saglam & Arikan, 2006) and dice snake (Carlsson et al., 2011).

The first-stage larvae passed by infected bird faeces and oligochetes such as *Tubifex tubifex*, *Lumbriculus variegatus* or *Limnodrilus* sp. (Bjelic-Cabrilo et al., 2013). Into the water, the egg hatches and finds the first host then fish feed on infested oligochetes, parasite reach 3rd and 4th stages larvae that would encyst on organs, peritoneal serosa and into the muscle (Urdes et al., 2015). The lesions caused by *Eustrongylides excisus* in the proventriculus wall could be heavy in order to the pathogenic effects of the nematodes coiled in a capsule that protects from the serosal surface (Bjelic-Cabrilo et al., 2013).

Eustrongylides spp. has great importance because of their capacity to infect carnivorous organisms and humans that feed on them (Mohammad et al., 2011). The infection of *Eustrongylides* spp. occur after ingestion of raw or poorly cooked fish meat (Ljubojevic et al., 2015). The nematodes cannot reach maturity in man, but remain in the fourth stage of larval development (Bjelic-Cabrilo et al., 2013). It causes gastritis and the perforation of intestines and the only possible way to cure this infestation is surgical removal of the larvae (Cole, 2009). Some reports from human cases are known that infected by *Eustrongylides* (Guerin et al., 1982; Eberhard et al., 1989).

The aim of the present work is to distinguish the presence of the parasite which is very important for human health in order to consume fish with an economic value in that area. It is substantial to increase public awareness for consuming fish meat containing infectious parasitic hazards.

MATERIAL and METHOD

51 samples of pike perch (*Sander lucioperca*) were collected from Lake Marmara, Manisa, Turkey (38°37'02.5"N, 28°01'03.1"E) between October 2015 and February 2016 and transferred to Izmir Katip Celebi University Faculty of Fisheries Fish Health Laboratory (Figure 1).

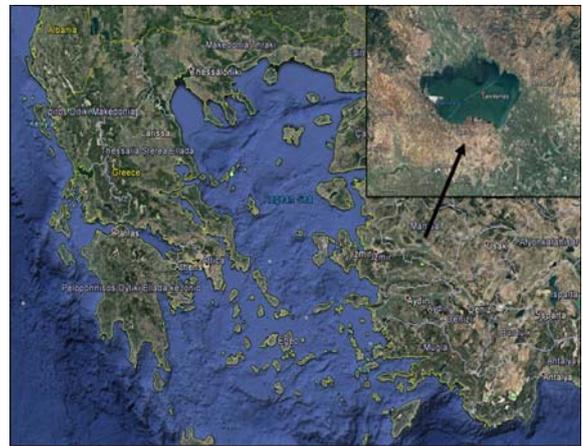


Figure 1. Lake Marmara.

The fish were measured 25.8 - 41.6 cm - and weighted 142.0-626.0 g and examined for parasites with the clinic and parasitological examination according to standard protocols (Hoffman, 1999).

After dissection, abdominal cavity and internal organs of each sample was examined. Nematodes were counted and collected from abdominal cavity, muscles, external and internal sides of the organs then identified according to Moravec 1994 and Anderson 2000.

After examination, they were fixed with 4% formaldehyde and stored. Samples were dehydrated according to Eisenback, (1986) and sputtered with gold with Quorum Q150 Res, examined in Carl Zeiss 300 VP scanning electron microscope from Izmir Katip Celebi University Central Research Laboratory.

RESULTS

From the examined fish, *Eustrongylides* spp. was determined from all the samples. The parasite was observed from bright red to reddish in colour. *Eustrongylides* infection was diagnosed in the muscle surrounding the abdominal wall, abdominal cavity, fatty tissue, dorsal muscle, swim bladder and between internal organs (Figure 2, 3, 4). The prevalence of the infection was detected at 100%. From every sample, parasites were detected from various parts of the body. In addition, capsulated parasites were located in various muscles on the contrary of encapsulated samples which were mainly found in the abdominal cavity and on the internal organs.



Figure 2. *Sander lucioperca*-body general appearance, capsuled larvae in muscle and in fatty tissue.



Figure 4. *Eustrongylides* spp. in swim bladder.



Figure 3. General appearance of *Eustrongylides* spp., free larvae in abdominal cavity and on liver.

Interestingly the parasite that was found in the swim bladder cause no harm to the tissue and the swim bladder looked undamaged. The intensity of *Eustrongylides* spp. was ranged between 1-10 parasite/fish. From scanning electron microscopy overviews, lips and papillae circles were detected on the anterior portion of the body as characteristics for diagnosis of the genus *Eustrongylides* (Anderson, 2000; Lezama & Sarabia, 2002) (Figure 5, 6).

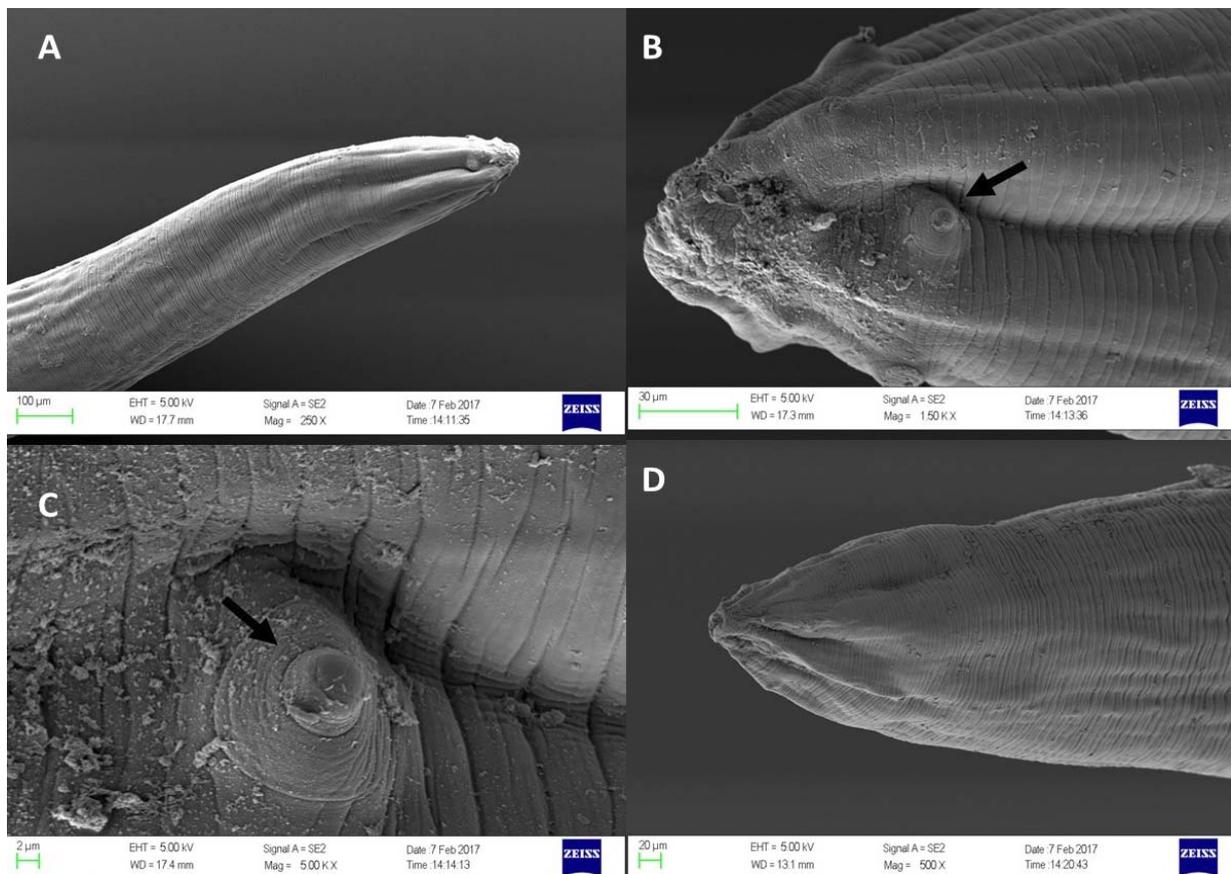


Figure 5. Scanning electron microscopy of the anterior portion of *Eustrongylides* spp. with different scales (A: 100 µm; B: 30 µm; C: 2 µm; D: 20 µm). Anterior portion showing lips and papillae circles.

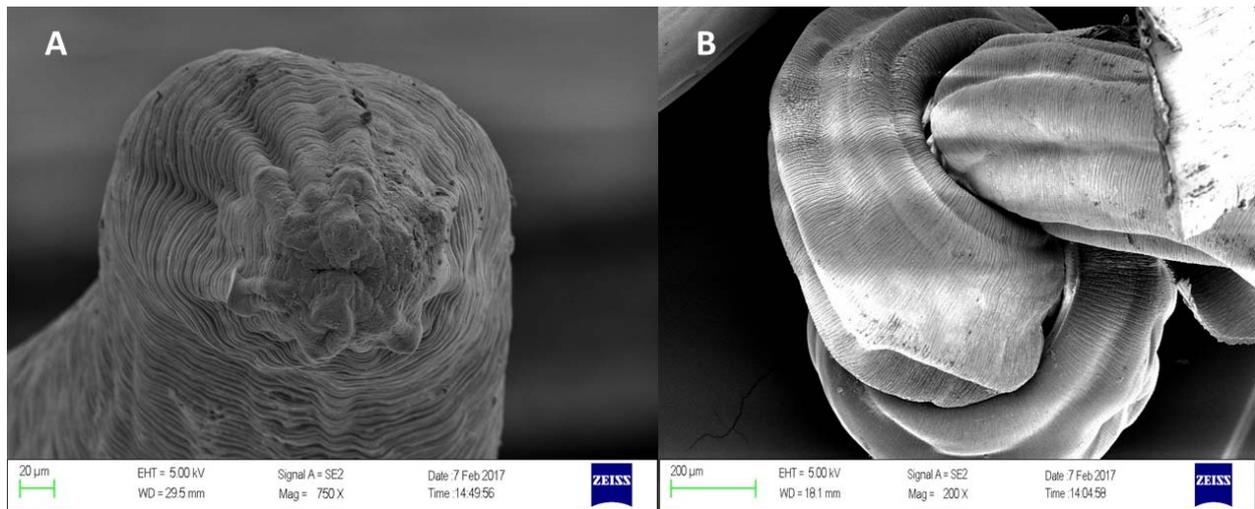


Figure 6. A: SEM overview of the anterior region of *Eustrongylides* spp. (20 µm); B: Capsulated nematode with 200 µm bar.

DISCUSSION and CONCLUSION

The genus *Eustrongylides* is reported from several geographical distribution such as Europe, Australia, Middle East, United States and Canada (Gora et al., 2013). From Turkey, it has declared from different locations and different species such as Eğirdir Lake in *Carassius gibelio*, *Cyprinus carpio*, *Sander lucioperca* and *Atherina boyeri* (Akcimen et al., 2014) and Lake Ulubat and Manyas in *Gobius fluviatilis* (Ozturk et al., 2001, 2002). In recent years the number of case reports referring to fish Eustrongylidosis not only has increased but also not paying any attention on the contrary to its importance. It is represented that in Beyşehir Lake (Konya, Turkey) *Sander lucioperca* is fed by *Atherina boyeri* (Apaydin Tarhan, 2012), which is a natural prey of perch and intermediate host of *Eustrongylides*. Our results demonstrated that this infection may occur by the feeding chain of pike perch based on the transmission of infection by feeding infected fish.

In Lake Marmara total pike-perch hunting was determined as 11655 kg in 2015 (Ministry of Food, Agriculture & Livestock, 2016) and according to Ilhan and Sari, (2013) fishing is the main source of living in that area. As a commercial species, pike-perch has significant importance for fishing and consuming of fish meat. The presence of parasitic hazards causes safety concerns related to the consumption of raw or poorly cooked fish meat. The presence of *Eustrongylides* spp. in pike-perch is harmful to both natural environment in case of being potential for more species and human health to cause incidental/paratenic host relationships.

Eustrongylides spp. is reported as the most significant freshwater fish zoonotic nematode (Novakov et al., 2013; Bjelic-Cabrilo et al., 2013). Fish have confirmed as intermediate hosts that the larvae develop in muscles, body cavity and on visceral organs (Bjelic-Cabrilo et al., 2013). In this study, *Eustrongylides* spp. larvae were mostly found in the abdominal cavity, abdominal wall, muscles, in fatty

tissue, between internal organs and unlike other reports in the swim bladder. Moravec, (1994) published that the larvae are capable of doing severe damage to various organs of the fish as large scars at the muscles, gastric and intestinal wall, complete destruction of the kidneys and inflammatory lesions.

Infected piscivorous fish are usually fed on infected prey and consumption of these fish seems to be becoming a considerable problem by its zoonotic potential (Urdeset et al., 2015). Guerin et al., (1982) and Eberhard et al., (1989) were reported two different human cases that were identified as fourth-stage larvae of *Eustrongylides*. It was confirmed that the patients consume raw minnows while fishing and after severe abdominal pain, a pair of worms were detected from the abdominal cavity of each man (Eberhard et al., 1989). Mitchell et al., (2009) also claimed in humans who have consumed raw or undercooked fish, *Eustrongylides* spp. have produced gastritis and intestinal perforation. In this case, fish act as intermediate and paratenic hosts in the development of parasites' lifecycle (Ljubojevic et al., 2015). FDA suggested freezing, heating and adequate combination of salt content and storage time or hot smoking to kill parasites (FDA, 2001). Also, Murrell, (2002) published that, environmental control of surface water, hygienic aquaculture and control or elimination of the first intermediate hosts could help to control parasite infection originating from freshwater.

In conclusion, *Eustrongylides* spp. which belong to the group of food-borne parasites has great importance for both fish health and human consumption. It recently becomes a considerable problem for the natural environment, commercial fishing and human health in Turkey but more studies are needed to eliminate and minimize this problem. Its geographical distribution, relations with hosts and the place in the food chain should be clarified in order to avoid the hazards to human health and environment.

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