

FANDING OF PARASITIC NEMATODES OF FISHES PRESENT IN THE MARKET

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Abstract

When placing the fish and fish products on the trade it is necessary to pay attention to the presence of zoonotic parasites that can lead to infection of people especially if the fish is consumed raw or undercooked. Epidemiologically the most important are helminths from the group of *Nematoda*. The most important are *Anisaxis* spp. and *Eustrongylides excisus*. Anisakiasis is a serious zoonotic disease with a dramatic increase in prevalence throughout the world in the last two decades. The larvae are found in marine fish species most frequently in sardines, herring and mackerel. Eustrongylidosis is a disease that occurs primarily in freshwater fish species: catfish, zander and pike, a cause is a parasite of the genus *Eustrongylides* of which the most important species is *Eustrongylides excisus*. In this paper, the nematodes that occur in fish that are present in the market in the Republic of Serbia are present. Investigations of *Anisaxis* spp. were carried out at the Institute of Veterinary Medicine Novi Sad in the period of 2000-2013. Total of 2414 samples of imported marine fish was inspected. In 25 (1.29 %) samples of herrings (*Clupea harengus*) and mackerels (*Scomber scombrus*) was identified *Anisakis* spp. Investigations of *Eustrongylides* spp. were carried out in the period 2010-2013 at the Danube- Tisa -Danube Canal. Samples were collected from zander (*Sander lucioperca*) and European catfish (*Silurus glanis*) in which the presence of nematodes was found in the abdominal cavity, muscles, the lumen of the stomach and gastric wall where the parasites were encapsulated. Individuals of zander were examined during the 2013 at the Veterinary Institute Novi Sad, where the presence of larvae *Eustrongylides* spp. u muscle was detected. In order to avoid infecting people with parasites of fish it is necessary to carry out continuous control and monitoring. Fresh

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fishes and traditional fish products must be inspected for the presence of parasites before they find on the trade. Continuous education is a key factor in combating zoonotic diseases. Avoid consumption of raw or poorly cooked fish is still the best preventive procedure.

Key words: zoonotic nematodes, *Anisakis* spp., *Eustrongylides excisus*, fish, market

NALAZENJE PARAZITSKIH NEMATODA KOD RIBA NA TRŽIŠTU

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Kratak sadržaj

Prilikom stavljanja u promet i pregleda ribljeg mesa i proizvoda od ribe treba obratiti pažnju na prisustvo zoonotskih parazita koji mogu dovesti do inficiranja ljudi naročito ako se riba konzumira sirova ili termički nedovoljno obrađena. Sa epidemiološkog aspekta najveći značaj imaju helminti iz grupe *Nematoda*. Najznačajnije su *Anisaxis* spp. i *Eustrongylides excisus*. Anisakijaza je ozbiljno zoonotsko obolenje sa dramatičnim porastom prevalencije širom sveta u poslednje dve decenije. Larve se mogu naći kod morskih vrsta riba najčešće kod sardine, haringe i skuše. Eustrongilidoza je obolenje koje se javlja kod slatkovodnih riba prvenstveno grabljivica: soma, smuđa i štuke, a izazivaju je paraziti iz roda *Eustrongylides* od kojih je najznačajnija vrsta *Eustrongylides excisus*. U ovom radu prikazane su nematode koje se javljaju kod riba koje su prisutne na tržištu u Republici Srbiji. Istraživanja prisustva *Anisaxis* spp. su sprovedena na Naučnom institutu za veterinarstvo Novi Sad u periodu 2000-2013. Ukupno je pregledano 2414 uzoraka riba iz uvoza od čega je u 25 (1.29%) utvrđen *Anisakis* spp., kod haringe (*Clupea harengus*) i skuše (*Scomber scombrus*). Istraživanja prisustva *Eustrongylides* spp. su sprovedena u periodu od 2010-2013 godine na kanalu Dunav-Tisa-Dunav. Prikupljeni su uzorci konzumnog smuđa (*Sander lucioperca*) i soma (*Siluris glanis*) kod kojih je utvrđeno prisustvo nematoda u abdomenu, muskulaturi, lumenu želuca i želudačnom zidu gde su paraziti bili inkapsulirani. Jedinke smuđa pregledane se tokom 2013. godine i

na Naučnom Institutu za veterinarstvo Novi Sad gde je utvrđeno prisustvo larvi *Eustrongylides* spp. u muskulaturi. Kako ne bi došlo do zaražavanja ljudi zoonotskim parazitima riba neophodno je sprovesti stalnu kontrolu i monitoring. Sveže meso ribe i tradicionalni riblji proizvodi pre nego što se nađu u prometu moraju biti pregledani na prisustvo parazita. Kontinuirana edukacija je ključni faktor u borbi sa zoonozama a izbegavanje konzumiranja sirovog ili termički slabo obrađenog ribljeg mesa i dalje najbolja preventivna procedura.

Ključne reči: zoonotske nematode, *Anisakis* spp., *Eustrongylides excisus*, ribe, tržište

INTRODUCTION

Zoonotic nematodes infect freshwater and marine fish species. Pathology normally occurs within the intestines but can affect all organs. Nematodes can infect fish as adults but larval stages of nematodes infecting piscivorous birds, mammals or reptiles, or less frequently predatory fish, can also infect fish species (Roberts and Janovy, 2000). Some nematodes are zoonotic. Among these parasite *Anisakis* spp. has the highest medical importance because of the severe allergic reactions and gastrointestinal symptoms it causes in humans after eating or handling infected fish or crustaceans (Lima dos Santos and Howgate, 2011). These symptoms are especially prevalent in countries where it is common to eat raw or undercooked fish. Anisakiasis a serious zoonotic disease with a dramatic increase in prevalence throughout the world in the last two decades. People who are usually accidental hosts infected when ingest the third larval stage of the parasite that can be found in the internal organs and muscles of a large number of marine and anadromous fish (sardines, herring, salmon, tuna, mackerel, etc.) (Bullini, 1997; Oliva, 1999). Definitive host of *Anisakis* species are marine mammals commonly dolphins and whales in which intestines are adult forms of the parasite. The first intermediate hosts are marine crustaceans and second intermediate hosts are cephalopods and fishes. *Anisakis simplex* is present in Atlantic, Pacific, Mediterranean Sea, Norwegian Sea and Barents Sea. The highest prevalence of anisakiasis is found in north Asia and western Europe (Netherlands, Germany, France and Spain) (Chai et al., 2005). In Serbia *Anisakis* can be present in imported marine fish.

The most significant freshwater fish zoonotic nematodes which occurs in Serbia are *Eustrongylides* species (Ljubojević et al., 2012; Novakov et al., 2013). They have complex life cycles involving a definitive host and two intermediate hosts. Definitive hosts include aquatic birds mostly from order Ciconiiformes family Ardeidae, Anseriformes, Gaviiformes and Pelecaniformes (Spalding

and Forrester, 1993; Measures 1988). First intermediate hosts for *Eustrongylides* spp. are aquatic oligochaetes (Spalding et al., 1993). Second intermediate hosts are planktivorous and benthivorous fishes that could pass the infection on to fishes (paratenic hosts) and finally on to fish-eating birds (Moravec, 1994). Such exposure is usually common in larger fish species, like channel catfish - *Ictalurus punctatus* or pike-perch - *Sander lucioperca*, which, as predators, become infected with *Eustrongylides* spp. nematodes. In fish, these parasites are conspicuous as long, red, coiled individuals located in the body cavity or embedded in the muscle (Mitchum 1995, Overstreet 2003).

In humans who have consumed raw or undercooked fish, *Eustrongylides* spp. have produced gastritis and intestinal perforation (Deardorff and Overstreet 1991; Cole 1999). Guerin et al., (1982) were the first to report a natural (accidental) human infection with *Eustrongylides* spp.

The goal of this paper is to distinguish the presence of *Anisakis* spp. and *Eustrongylides* spp. nematodes in marine and freshwater fish species that are present in the market and inland waters of Serbia and to indicate the need for adequate preparation of fish meat.

MATERIAL AND METHODS

Investigations of *Anisaxis* spp. were carried out at the Institute of Veterinary Medicine Novi Sad in the period of 2000-2013. Total of 2414 samples of imported marine fish was inspected. Each fish was eviscerated and carefully examined. In some cases muscles of fish were checked by method of artificial digestion.

Diagnostics and investigations of *Eustrongylides* spp. larvae were conducted from 2010-2013. Fifty two fish samples of European catfish (*Siluris glanis*) weighing 250-450 g and twenty one samples of zander (*Sander lucioperca*) were collected from different locations on Danube-Tisa-Danube Canal. Fishes were parasitologically examined through cutting the body and the abdominal part, digestive tract and other ventral organs. Samplaes of zander was also examined at the Institute of Veterinary Medicine Novi Sad during 2013. Collected nematodes were were fixed in 70% ethanol for 24 h and cleared in lactic acid for morphological observation. Relative parametars were measured and identification was performed using Bauer (1987), Moravec (1994) and Anderson (2000) keys.

RESULTS AND DISCUSSION

Anisakis spp was first isolated in the laboratory of Veterinary Institute of Novi Sad in 2003. On a total of 2414 samples of imported marine fishes, in 25 (1.29%) was found *Anisakis* spp. Presence of *Anisakis* spp. in samples of imported fish from 2000-2013 are shown in table 1 and chart 1. Coiled nematodes were present in herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) (Fig. 1). Common to all positive samples is the presence of the viscera, because fishes were not eviscerated after fishing. Number of parasites ranged from 2-3 to 52 nematodes per fish. In two samples (0.08%) the presence of larvae in fish muscles was performed by the method of artificial digestion.

Table 1. Presence of *Anisakis* spp. in samples of imported fish.

Years	Number of inspected fish	Number of positive fish	% of positive fish
2000	12	0	0
2001	12	0	0
2002	17	0	0
2003	66	3	4.54
2004	268	3	1.2
2005	239	4	1.67
2006	212	1	0.41
2007	151	3	2
2008	177	3	1.69
2009	394	5	1.3
2010	440	2	0.45
2011	240	0	0
2012	148	0	0
2013	38	1	2.63

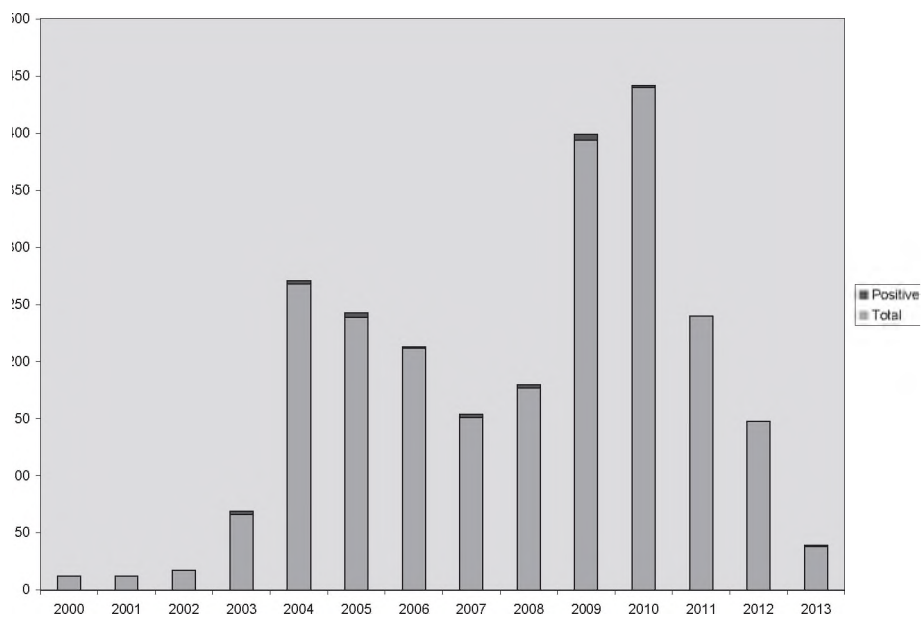


Chart 1. Presence of *Anisakis* spp. in samples of imported fish.

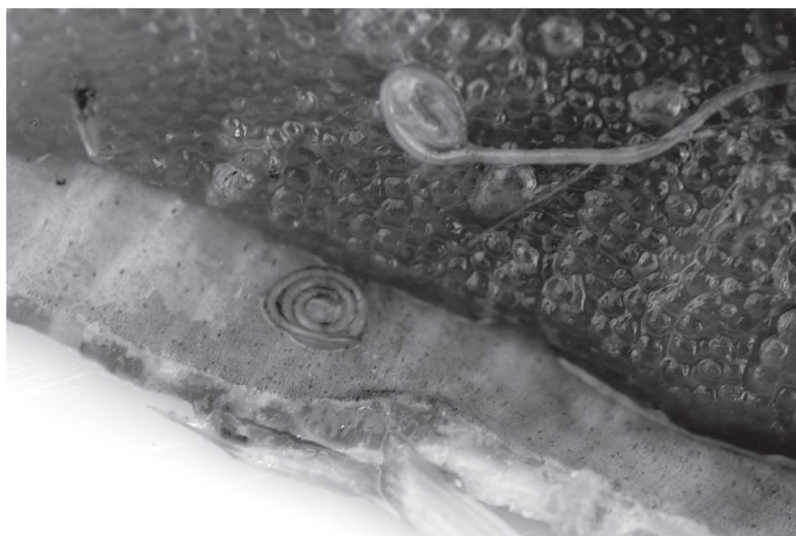


Figure 1. Appearance of *Anisakis* spp. larvae in herring (*Clupea harengus*).

Presence of *Eustrongylides* spp. nematodes was revealed in the abdominal cavity (Figure 2), musculature, in the lumen of the stomach and encapsulated

in stomach in 4 individuals of zander and 6 individuals of European catfish, what represented the prevalence of 14.26%, respectively 11.54%. During the 2013 at the Institute of veterinary medicine of Novi Sad larvae of *Eustrongylides* spp. were detected in the muscles of four samples of zander (Fig. 3). The number of parasites per fish ranged from a few up to the 256. This larva, was robust and pinkish red. Length of body was 27 – 60.5 mm, maximum width 0.49 – 0.58 mm.



Figure 2. *Eustrongylides* sp. in the abdominal cavity of European catfish (*Silurus glanis*).



Figure 3. *Eustrongylides* spp. in the muscles of zander (*Sander lucioperca*).

Fish parasites such as *Anisakis* spp. and *Eustrongylides* spp. are highly important because they are capable to infect humans (Mohammad et al., 2011). Murrell (2002) suggested several control measures for preventing parasitic infections originating from freshwater, such as environmental control of surface water, hygienic aquaculture, and the control or elimination of the first intermediate hosts. FDA (2001) indicated that the effective methods for killing parasites are freezing, heating, adequate combination of salt content and storage time or hot smoking. On the other hand, brining and cold smoking may reduce the parasite hazard in fish, but they do not eliminate or minimize it to an acceptable level (Murrell, 2002). While health education is a key factor in combating zoonotic infections, experience in various countries has shown that for successful implementation of control measures, it is necessary, as Hughes (1992) points out, to have formal and informal cooperation between medical and veterinary interests at all levels of government, and with the community. According to Okumura et al., (1999) and Chieffi et al., (1992), the recommendation to avoid consumption of raw or poorly cooked fish is still the best preventive procedure.

CONCLUSION

Zoonotic nematodes can lead to human infection and should pay special attention on them. Fresh fish meat and traditional fish product should be subjected to a visual examination for the purpose of detecting visible nematodes and other parasites before being placed in the trade. The consumption forms and the preparation of the fish food should be modified in a way that hazards to human health due to zoonotic parasites could be avoided. Health education is a key factor in combating zoonotic infections.

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