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Zoonotic helminthic parasites in abdominal cavity of pike- perch, *Sander lucioperca*, from southeastern part of the Caspian Sea

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ABSTRACT

Some parasites can be transferred from fish to human via eating of contaminated meat and make serious problems in consumers. In the present study zoonotic parasites of abdominal cavity in pike-perch, *Sander lucioperca*, in south eastern part of the Caspian Sea have been surveyed. In this regard, 30 fish were caught by purse seine net and abdominal cavity, digestive tract and other organs (kidney, spleen, liver, gonads and mesentery) of specimens were fully examined for helminthic parasites. Totally two zoonotic nematodes including *Anisakis simplex* and *Eustrongylides* sp were isolated from pike-perch. According to the result, 46.67 % of specimens were infected at least with one type of mentioned parasites. In this study the prevalence of *Anisakis simplex* and *Eustrongylides* sp infections in pike-perch were recorded as 36.67 % and 23.33 %, respectively. Mean intensity of parasite infection were calculated as 12.13 ± 5.8 and 2.57 ± 1.51, respectively for *Anisakis simplex* and *Eustrongylides* sp in pike-perches. This study described that the pike-perches in south-eastern part of the Caspian Sea are infected with mentioned zoonotic parasites, so health care of consumers in this area should be considered.

1. Introduction

Considering eating habits and environmental pollution, fish can be intermediate host of different parasites. Over times, this can lead to an increase in the presence of fish endoparasites in natural ecosystem (Alves and Luque, 2001). Although in aquatic environments, it has been demonstrated that individual fishes may be infected by different parasites (Faliex and Morand, 1994). Some fish species have higher potential to be intermediate or definitive host of parasite species (Al-Zubaidy, 2010). Some parasites can be transferred by fish to human via eating of contaminated flesh. These zoonotic parasites including acanthocephalans, cestodes and nematodes are more important for human health care (Petersen et al., 1993). Some of these parasites are more important because of serious problems that they can cause in human. *Anisakis* spp and *Eustrongylides* spp are two important zoonotic nematodes which have been reported globally. Larvae of these parasites penetrate the

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intestinal wall of fish and migrate into tissues, so it is possible to be observed in the flesh and internal organs (Karl and Leinemann, 1995). Adult anisakids are located in gastrointestinal tract of the marine mammalians as a definitive host. Female worms produce eggs and pass through the feces. By the first stage, larvae hatch from the eggs, and develop into second-stage larvae in crustaceans of Euphausidae family. These larvae can be transferred to fish or squid as a second intermediate host and released through the gastrointestinal tract. Some of these parasites penetrate gastrointestinal wall and enter the mesentery, viscera or flesh of the fish. When humans consume this infected fish, the larvae attack to the gastrointestinal tract and cause disease (Adams et al., 1997).

Eustrongylides spp is another zoonotic parasite that can be transferred to human by infested fish. The first larval stage occurs in the eggs. These larvae are eaten by aquatic oligochaetes as the first intermediate host; develop into second- and third-stage larvae. If fish, as a second intermediate host, consume these infected oligochaetes, the third-stage larvae develop into fourth-stage larvae, and the birds are definitive hosts. Predatory fish, which eat contaminated fish, will be infected by the parasite (Spalding et al., 1993). Humans who have consumed raw or undercooked infected fish to 4th larval stage of this parasite may show clinical signs such as severe pain and gastritis or inflammation of stomach and intestinal. Sometimes these patients need surgery treatment to remove these worms (Deardorff and Overstreet, 1991; Cole, 1999).

Pike-perch, *S. lucioperca*, is the most popular fish of the Caspian Sea. This fish is a predator that feeds another small fish and crustaceans. This feeding habit can increase probability of infection with parasites. Most parasites will be killed during cooking or freezing process but eating raw or uncooked fish would result in the certain human parasitic infections, so it depends on feeding habits of consumers. Because of fresh fish consumption habit in the north of Iran, zoonotic parasites of this fish are important for health care. So in this study we tried to isolate the zoonotic endoparasites of pike-perch from southeastern part of the Caspian Sea.

2. Materials and Methods

2.1. Fish samples

Specimens have been collected from south east part of the Caspian Sea. In this study the total number of 30 pike-perch (*Sander lucioperca*) with average weight of 295.91± 19.16 g and total length of 30.64 ± 2.38 cm (Table1) were caught by purse seine net by fishermen. Before examination, biometric parameters such as length and weight of specimens were recorded and internal examinations were conducted on abdominal cavity (digestive tract, liver, gonad, and mesentery) and parasites fauna were studied for zoonotic agents in February 2014. Then fishes were rinsed and abdominal cavities of specimens were opened, the digestive tract and other organs (kidney, spleen, liver, gonads and mesentery) were fully examined for parasites according to the standard parasitological methods described by Robert (2001). All isolated parasites were washed in normal saline, fixed in 4% formaldehyde, preserved in 70 % ethanol, transferred to fixative solution and sent to Iranian National Parasitology Museum, Faculty of Veterinary Medicine of Tehran University, for identification. The parasites were cleared in glycerin and stained by lacto phenol (Moravec, 1994). After staining the parasites were mounted in entellan (Merck Co. Germany). The parasite's specimens were deposited in the collection of fish parasite in museum. Identification was basically according to those described by Moravec (1994) and Roberts (2001) Bykhovskaya-Pavlovskaya et al. (1962).

Prevalence of infection (%), mean intensity and mean abundance of infection and the limit number of infection were calculated according to the following formula (Bush et al., 1997).

Prevalence of parasites (%) as calculated as the number of infected fish/ the total number of examined fish×100.

Mean intensity of infection = the number of counted parasites/ the total infected fish

Mean abundance of infection = the total number of parasites/ the total number of surveyed fish

2.2. Statistical analysis

Data were analyzed, separately using the SPSS18 and T-test used to determine the

presumptive differences between the genders. Data were presented as mean ± SD and P<0.05 was considered as significance.

3. Results

3.1. Sample collection

In the present study two zoonotic nematode parasites including *Anisakis simplex* and *Eustrongylids* sp were isolated from the caught pike-perch in southeastern part of the Caspian Sea. The prevalence and intensity of infections to mentioned parasites are shown in Table 2. According to the results, 46.67 % of specimens were infected with zoonotic parasites. *Estrongilides* sp was isolated from liver and mesentery of the specimens. In some infected fishes *Eustrongylides* attacked to gonads and penetrated this tissue that shows larval migration in this fish. In the present study the prevalence of infection with *Eustrongylides* sp was calculated 36.67%. *Anisakis simplex* was isolated from the liver, mesentery and intestine of 23.33% of specimens (Table 2). There wasn't any significant difference on parasite load and parasite kinds between genders in this study (Table 3 and 4). Intensity of infection with *A. simplex* and *Euustrongylides* sp in this study were recorded as 12.13 ± 5.8 and 2.57 ± 1.51, respectively.

Table 1. Biometric characters of examined *S. lucioperca* (mean ±SD) from the south eastern part of the Caspian Sea

Variables	Mean±SD	Minimum	Maximum
Weight (g)	295.91± 19.16	250.00	320.00
Total length (cm)	30.64 ± 2.38	27.00	34.00
Standard length (cm)	26.45 ± 1.97	24.00	29.00

Table 2. Type and prevalence of parasites in examined *S. lucioperca* (mean ±SD) from the south eastern part of the Caspian Sea

Kind of infestation	Prevalence (%)	Intensity (mean ±SD)	range	Mean abundance of infection±SD
Total parasite infection	46.67	11.06 ± 6.25	2 - 21	7.28 ± 7.52
A. simplex	36.67	12.13 ± 5.83	3 - 21	7.28 ± 7.52
***Eustrongylides* sp**	23.33	2.57 ± 1.51	1- 5	0.72 ± 1.35

Table 3. Relationship between sex and parasite burden in examined *S. lucioperca* from the south eastern part of the Caspian Sea.

Fish Host	Sex	No. examined	No. infected	parasitic load	Significance (P=0.05)
S. lucioperca	F	17	8	105	Negative
	M	13	6	69	(P> 0.05)

F = female. M = male.

Table 4. Relationship between sex and parasite species' burden in examined *S. lucioperca* from the south eastern part of the Caspian Sea

Parasite Species	Sex	No. examined	No. infected	Total parasitic load	Significance (p=0.05)
A. simplex	F	17	7	89	Negative (P> 0.05)
	M	13	5	48	
***Eustrongylides* sp**	F	17	4	10	Negative (P> 0.05)
	M	13	3	8	

F = female. M = male.

4. Discussion

Despite reports on *Anisakis* and *Eustrongylides* infection in different bony fish of the Caspian Sea, there are little reports about these parasites from south eastern part of the Caspian Sea (Pazooki and Masoumian, 2012). These parasites can make serious problems in human (via consuming raw or uncooked infected fish). Anisakiasis in human was described in 1955 and recorded in 1960 from Netherland (Van Thiel et al., 1960), and after that have been

widely reported from other countries. The larvae of this parasite may produce gastrointestinal and allergic disease. In some cases, after ingestion of infected fish, larvae pass through gastro intestinal and were excrete in feces, but *Anisakis* can attack and penetrate digestive tract and be observed in intestinal wall, tongue, pharynx wall, lung, lymphatic ganglia or pancreas in human (Rosales et al., 1999). Several clinical signs in human due to infection by Anisakis such as allergic reaction, stomach pain, vomiting, nausea and gingivostomatitis has been described by different researchers (Smith and Wooten, 1978; Ancillo et al., 1997; Audicana et al., 2002). Clinical signs in human can be observed few hours after infection. In an experimental study, during 3rd stage, larvae penetrated intestinal wall and were observed in pancreas, liver, and intestinal mesentery 8 hours after infection of guinea pigs (Myers, 1963; Dziekoska-Rynko, 2004). Allergic reaction symptoms of anisakiasis, in human, may be observed in three aspects: urticaria, angioedema and anaphylaxis following fish consumption. The high level of serum specific IgE has been seen after infection to *A. simplex* (Audicana et al., 2000).

Eustrongylides is another zoonotic parasite which has been separated from perch in this study.

Life cycle of this parasite includes an aquatic oligochaetes and fish as intermediate host and piscivorous birds as definitive host (Lichtenfels and Stroup, 1 985). Accidental infection in human, by eating raw infected fish can cause gastritis and intestinal perforation which results in severe pain in abdominal cavity (Deardorff and Overstreet, 1991; Cole, 1999). First time natural human infection with *Eustrongylides* sp was reported by Guerin et al., (1982). These parasite larvae can result serious damages to patients. For example Perry (1982) explained long ranged migration of larvae which resulted in surgical intervention for treatment in an incidental human case that had eaten a small fish.

Pike- perch is carnivorous and infestation with these mentioned parasites was expected, because these parasites were isolated from wide range fish species of the Caspian Sea, but most reports are belonged to southern and southwestern parts of Caspian Sea. Finding these zoonotic parasites in the costal line of the Golestan province is important from several aspects. Beside human health problems, these parasites can make problems in other creatures too. For example Cole (2009) described high mortality in fish eating bird nestlings younger than 4 weeks due to infection with *Eustrogylides*. On the other hand, sometimes these creatures can act as intermediate host and infect human who feed them. Another concern is about possibility of post-mortem larval migration from the viscera into the surrounding flesh in storage conditions (Karl et al., 2011). So it is recommended to remove the abdominal cavity viscera and digestive tract of fish immediately after fishing.

According to the FDA (2001) report, freezing and heating are the best effective methods for killing parasites of flesh. In freezing method the larvae cannot survive in temperature below -20°C for 24 h (Audicana et al., 2002), but some places like north of Iran, peoples like to consume fresh fish. Heating can kill parasites too; in this regard the internal temperature of flesh should reach a minimum of 63°C longer than 10 minutes. For cooking with microwave and oven a temperature more than 77°C is recommended to kill parasites (Miller et al 1994). In barbequed or roasted meat, temperature of central part of flesh depend on its thickness scarcely can reach to 50°C.

Conclusion:

In this study two serious zoonotic parasites has been isolated from pike- perch. According to the life cycles of these parasites and carnivorous diet habit of this fish, probably it can be said that other fishes in this food chain are infected too. So it is strongly recommended to freeze or completely cook fish flesh before consumption, and just roast fish after at least 24 hours freezing (avoid to roast fresh fish) in this areas.

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Refereces

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