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Original Article

Determination of helminth parasites in abdominal cavity of *Alosa caspia* (Eichwald, 1838) from the southeast part of the Caspian Sea

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Abstract: *Alosa caspia* (Eichwald, 1838) belongs to clupeidae family, is considered as one of the main fish in the southern coast of the Caspian Sea. The aim of the present study was to evaluate the helminthic parasite infections in abdominal cavity of *A. caspia* from southeastern part of the Caspian Sea. In this regard, 30 fish were caught from Bandar-Torkaman and transferred alive to the parasitological laboratory. Then parasites specimens were fixed and transferred to the National Museum of Parasitology, Faculty of Veterinary Medicine at University of Tehran for identification. A total of two parasite species including *Anisakis simplex* and *Pronoprymna ventricosa* were isolated from the fish. 100% of the fish were infected with at least one helminthic parasite species. *Pronoprymna ventricosa* has the highest infection prevalence rate and was isolated from pyloric caeca, intestine and stomach of 93.33% of the fish specimens. *Anisakis simplex* is found in abdominal cavity of 33.33 % of the studied fish. Intensity of *A. simplex* and *P. ventricosa* was calculated as 8.4±5.31 and 91.4±21.46, respectively. Based on the statistical analysis, there were no significant differences in total parasites burden, parasite prevalence and parasite intensity between male and female of the studied fish (P>0.05).

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Introduction

Alosa caspia (Eichwald, 1838) belongs to clupeid, is considered as one of the main fish in the southern coast of the Caspian Sea. Due to lack of *A. caspia* farming (aquaculture) as well as difficulties with conducting research on marine environments, the study on diseases and parasitic infections of the fishes in these environments has not been taken into consideration. On the other hand, infected fish can act as a source of infection and easily be hunted by sea creatures and fish-eating birds or marine mammals. This fact helps the evolution of life cycle of some fish parasites. This type of study also is important from "public health" aspect as always there is the danger of transferring the disease and the infection to human. Depending on the host species, parasites in the abdominal cavity contribute to damage, pathogenicity and disruption of the natural activities of the body. The parasites found in the abdominal cavity of the fish during clinical studies are mainly helminthes parasites, such as cestodes, nematodes, trematodes and acanthocephalans (Nezafat Rahimabadi et al., 2008). Although many of the parasites, particularly nematodes and trematodes, have also zoonotic importance and eating raw or improperly cooked or processed fish is the main source of these infections for humans, and this has been reported from various geographical regions. For example, *Anisakis* species are important in terms of public health and can cause gastroenteritis in humans (Pahlavan et al., 2014).

Alosa caspia is a type of valuable edible fish which is caught from the sea, so studying and evaluating its diseases and parasitic infections have always been faced with problems and limitations and a few studies have been conducted in this field. During a study on parasitic infections of *A. caspia*, Jalali and Vatandoust (1989) for the first time separated monogenean parasite, *Mazocras alosae* from gills of the examined fish in Khezerabad, Mazandaran Province. Later, Kornijchuk and Barzegar (2006) reported the isolation

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of digenean *Pronoprymna ventricosa* from the intestine of *A. caspia* from Shahid Madani center. During the evaluation of the parasitic infection of Iranian herrings, Bozorgnia et al. (2012) found *M. alosae* on the gills and metacerceria of *Diplostomum spathaceum* from the eyes of the fish as well as an unknown nematode species of *Hysterothylacium* sp. from the intestine of the examined fish in the Khazarabad.

Undoubtedly, in order to manage the rebuilding and preservation of natural resources, as well as to manage health and quarantine of the fish farms, having precise information about the status of fish health in each region is a key and valuable factor. The main objective of the present study was to investigate the prevalence and intensity of parasitic infestation of *A. caspia* from the southeast coast of the Caspian Sea.

Materials and Methods

A total number of 30 specimens of *A. caspia* were caught from the southeast coast of the Caspian Sea, Bandar-Torkaman and immediately transported alive in oxygen-filled plastic bags to laboratory. Identifying of the fish species was carried out in accordance with Naderi Jolodar and Abdoli (2001). The sex of the fish was determined by examination of the gonads after opening of the abdominal cavity. Then, abdominal cavity and gastrointestinal tract of the fish were monitored to find the helminthes parasites using a stereomicroscope.

In the case of parasitic infections, the parasites were carefully separated and counted and the results were recorded. After separation and washing with normal saline (0.6% NaCl), trematodes were fixed in 70% alcohol and finally were stained by acetocarmen (Georgiev et al., 1986). While the nematodes were fixed in 10% formalin, then they were transferred to 70% alcohol and sent to the Museum of Parasitology in Faculty of Veterinary Medicine, University of Tehran for accurate identification. Parasites were stained with lactofenol after clearing with glycerin (Moravec, 1994) and they were mounted with Entellan on the slide and examined microscopically. Identification of the genus and species of parasites was carried out using identifying keys (Hanek and Fermando, 1972; Roberts, 2001). In order to do a parasitic evaluation, the following equations were used:

Percentage of parasitic infections: Prevalence rate = (The number of parasitic infected fish/the total number of examined fish) × 100

The average intensity of parasitic infection: The average intensity of parasitic infection = The number of counting parasites/ the total number of parasites of infected fish

Average frequency of parasite: Average frequency of parasite = The total number of parasites/ the total number of examined fish

Average parasitic burden: Average parasitic burden = The total number of counting parasites from all examined fish

Statistical calculations: Data of evaluating of the parasites were expressed as mean±standard deviation. In order to analyze the data, software SPSS version 16 was used. In this regard t-test was used to compare the parasitic burden of male and female fish. Furthermore Chi-square test and Fisher and s exact test was used to compare the prevalence and the intensity of parasitic infection in both male and female and the values of $P<0.05$ were considered as significant.

Results

Specimens of *A. caspia* with an average length of 28.4±3.92 cm and average weight of 363±36.2 g were examined (Table 1) and a total of two parasite species including *A. simplex* and *P. ventricosa* were isolated from the examined fish (Table 2). 100% of the fish samples were infected at least by one of the parasites species. The highest prevalence rate of parasite infection was belong to *P. ventricosa*, which was isolated from the pyloric area, intestine and stomach of 93.33% of the examined fish while *A. simplex* were found on the intestine, mesenteric and liver (abdominal area) of 33.33% of the infected fish (Table 2). According to the results, 33.33% of the intestine, 30% of the liver and 3.33% of the mesenteric were infected with *A. simplex* and 26.66% of the stomach, 93.33% of the pyloric ceca and 36.67% of the intestine

Table 1. Biometric characteristics of *Alosa caspia* from the southeast part of the Caspian Sea.

	Standard deviation±averaging	Minimum	Maximum
Weight (g)	363±36.2	245	423
Total length (cm)	28.4±3.92	24	31
Standard length (cm)	24.3±13.2	21.5	26

Table 2. Prevalence, infection intensity, range and frequency of parasites of *Alosa caspia* from the southeast part of the Caspian Sea.

	Prevalence (%)	Infection intensity	Range	Frequency
Parasite infection	100	114±2.2	4-121	114.22±2.2
A. Simplex	33.33	8.5±4.31	6-18	2.8 1±0.17
P. ventricosa	93.33	94.21±4.46	4-121	89.22±11.34

Table 3. Prevalence, infection intensity, range and frequency of parasites in different organs of *Alosa caspia* from the southeast part of the Caspian Sea.

	Parasite species	Prevalence (%)	Infection intensity	Range	Frequency
Intestine	*A. simplex*	33.33	8.43±4.61	7-18	2.1±31.1
Intestine	*P. ventricosa*	36.67	57.8±13.4	73-4	17.5±56.2
Pyloric	*P. ventricosa*	93.33	102±16.5	121-25	98.2±16.3
Stomach	*P. ventricosa*	26.66	15.1±6.24	5-36	4.2±42.0
Liver	*A. simplex*	30	8.28±3.64	7-15	2.1±48.3
Mesenteric	*A. simplex*	3.33	6±0	6	0.2±0

Table 4. Relationship between sex and parasite load in *Alosa caspia* from the southeast part of the Caspian Sea.

	Sex	Number of samples	Number of infected fish	Parasitic load
A. simplex	Female	13	5	43
	Male	17	6	54
P. ventricosa	Female	13	12	1310
	Male	17	16	1742

of the fish were infected with *P. ventricosa* (Table 3). The mean intensity of *A. simplex* and *P. ventricosa* in *A. caspia* is presented in Tables 2 and 3. Accordingly, the highest rate of intensity of infection was related to infection of the pyloric ceca with *P. ventricosa*. The prevalence, infection intensity, range of parasite number and average frequency of parasites of the examined fish are presented in Table 3. At the same time, no significant difference was found in total parasitic burden and isolated parasites between males and females ($P<0.05$) (Table 4).

Discussion

Parasites are a large group of pathogens infecting various fish species and seafood (Woo, 2006). Many parasites may infect other vertebrates, including humans, in addition to aquatic animals. In fact, the fishes act as carriers of several parasites transferring them to other vertebrates. Several studies have been carried out on parasitic infections of the Caspian Sea fish species in Iran, which is mainly related to the Iranian coasts. Information about fish infestation is scarce in the southeast coast of the Caspian Sea. In this study, two parasitic species including *A. simplex* and *P. ventricosa* were isolated from *A. caspia* in the southeast part of the Caspian Sea. The migration of fish between the northern and southern parts of the Caspian Sea, and reproduction and feeding patterns increase the risk of exposure to various parasites (Naderi Jolodar and Abdoli, 2004). In this survey, all samples were infected by at least one parasite species. In the other words, 100% of samples were infected with parasites. At the same time, no differences were found between the male and female individuals which is probably due to the type of feeding and the same immigration behavior of the male and female.

Despite the numerous reports of parasitic infections in the Caspian Sea, the relationship between trematode

infections is very limited. *Pronoprymna ventricosa* is commonly reported from the pyloric and intestine of various fish species, and particularly from Clupeidae family in the world. For example, this parasite has been reported from various species of the *Alosa* spp. in the north Atlantic ocean, the Mediterranean Sea (Bray and Gibson, 2000) and the Black Sea (Gaevskaya and Kornijchuk, 2003; Popjuk, 2009; Ozer et al., 2013). In Iran, although reports are limited in this regard, this parasite has been reported only from the Clupeidae in the Caspian Sea. For example, Shamsi and Dalimi (1996) isolated *Pseudopentagramma symmetrica* which is synonymous for *P. ventricos* from *C. engrauliformis*, *C. cultriventris* and *C. grimmi*. In another report, Varshoie et al. (2010) again found this trematode from 53 to 58% of various species of kilka (Sprat) in the Caspian Sea. The *P. ventricosa* parasite was first isolated in 2005 from *A. caspia*, (Kornijchuk and Barzegar, 2005). However, in two separate studies, Yousefi et al. (2011) and Barzegar et al. (2012) isolated and reported the parasite from *A. caspia* in the Caspian Sea. In the recent review of Barzegar et al. (2012), 100% of the samples studied in the Caspian Sea were infested with *P. ventricosa*. In the present study, 96.66% of the fish were infected with this parasite. In general, due to the similar nutritional and migration habits among clupeids, it is not surprising to expect the infestation of this parasite in other members of the family in the Caspian Sea. The trematode *P. ventricosa* is one of the parasites that can be detected by the naked eye. This parasite is not important in terms of the public health. Meanwhile, in the case of mild to moderate infection in the fish host, it is also not very problematic, and serious damage occurs only when the intensity of the infection and the number of parasites are increased (Jalali, 1998).

Another parasite isolated from *A. caspia* is nematode *A. simplex* from Anisakidae family. Crustaceans are the first intermediate host of the nematode, and the fish mainly act as the second intermediate and sometimes the final hosts for the parasite. When fish are infected by infectious larval stages of the parasite, the larvae can be found as cyst or free organism in the gastrointestinal tract, abdominal cavity, muscles and other organs. Serious damages will occur if the intensity of the infection is high and due to the migration of the parasite to other organs (Jalali, 1998). So far, this parasite has been reported from various fish species on the northern and southern coast of the Caspian Sea. The larvae stages of *A. simplex* are isolated from sprats (Shamsi et al., 1998), *Acipenser stellatus* (Mokhayer, 1974) and *Rutilus kutum* (Eslami and Kohneshahri, 1978). It seems that this is the first official report on infections of young *A. caspia* with this parasite in Iran.

The most important thing about *A. simplex*, along with the possible damage to the host fish is its pathogenicity to human. *Anisakis* species are able to infect humans who eat raw or undercooked fish and may causes serious allergies and digestive problems (Robert, 2001). Jeddy et al. (2012) surveyed the parasitic infestation of the Kilka fish in the Caspian Sea during whole year period. They examined 64 fish per season and a total of 4 parasites including *Pseudopentagrama symmetrica*, *Bunocotyle cingulate*, larval stages of *Anisakis* sp. and *Contracaecum* sp. were found from the examined fish. Their results shows that *P. symmetrica* (*P. ventricosa*) had the highest percentage rate of infection and the most intensity of infection among other parasites. They also found that the parasites induced no serious damages in the fish, but only reduced their growth; also no significant effects were found on the fish reproduction. The researchers reported that the highest levels of parasite infection was in the gastrointestinal tract, followed by abdominal cavity and gonads while in the present study, the highest prevalence rate of parasites was observed in pyloric area.

According to the present results, it is concluded that the infection of helminthes parasites in abdominal cavity of *A. caspia* from the southeast part of the Caspian Sea is significantly high, but the parasitic diversity is low. At the same time, due to the isolation of the zoonotic parasite *A. simplex* from the examined fish, consideration should be given to the consumption of the fish. Therefore, consuming of the raw or semi-cooked fillet of this fish is strictly forbidden.

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چکیده فارسی

شناسایی انگل‌های کرمی محوطه شکمی شگ ماهی زالون (*Alosa caspia* (Eichwald, 1838 در سواحل جنوب شرقی دریای خزر

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چکیده:

شگ‌ماهی دریای خزر (*Alosa caspia*) از اعضاء خانواده شگ‌ماهیان و یکی از ماهیان اصلی در سواحل جنوبی دریای خزر به‌شمار می‌آید. هدف از تحقیق حاضر بررسی وضعیت آلودگی انگل‌های کرمی محوطه شکمی شگ‌ماهی زالون (*A. caspia*) در سواحل جنوب شرقی دریای خزر می‌باشد. بدین منظور تعداد ۳۰ نمونه شگ‌ماهی از منطقه بندر ترکمن صید و به آزمایشگاه منتقل شدند و پس از جداسازی انگل‌ها جهت شناسایی نهایی به موزه انگل‌شناسی دانشگاه تهران منتقل شدند. در بررسی حاضر ۱۰۰ ٪ نمونه‌ها حداقل به یک نوع انگل مبتلا بودند؛ و در نهایت ۲ گونه انگلی شامل *Anisakis simplex* و *Pronoprymna ventricosa* از محوطه شکمی ماهیان مورد مطالعه جداسازی گردید. بر اساس نتایج بالاترین شیوع آلودگی انگلی متعلق به *P. ventricosa* بود که از زوائد پیلوریک، روده و معده‌ی ۹۳/۳۳ درصد نمونه‌ها جدا شد و انگل *A. simplex* نیز از محوطه شکمی ۳۳/۳۳ درصد ماهیان مورد بررسی جدا شد. شدت آلودگی انگل‌های *A. simplex* و *P. ventricosa* به‌ترتیب ۸/۴±۵/۳۱ و ۹۴/۴±۲۱/۴۶ برای ماهیان آلوده محاسبه گردید. در عین حال بر اساس بررسی‌های آماری هیچ اختلاف معنی‌داری برای بار انگلی کل، شیوع و شدت انگل‌های جداسازی شده بین دو جنس نر و ماده در این مطالعه مشاهده نگردید ($P>0/05$).

کلمات کلیدی: انگل‌های کرمی، *Alosa caspia*، زالون، دریای خزر.