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Original Article

The prevalence and pathology of intestinal cestode infection in freshwater catfish in the reservoir dams of West Azerbaijan, Iran

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ABSTRACT

In the present study a total of 180 *S.glanis* (60 fish for each area) were collected from Aras, Mahabad and Zarrineh (shahid kazemi) dam reservoirs in the one-year survey of 2018. The seasonal prevalence of infection to *Siluris glanis* in Aras, Mahabad and Zarrineh were as spring (60%, 56%, 59%), summer (70%, 66.5%, 69%), Autumn (63.3%, 66%, 64%) and winter (45%, 39%, 41%) respectively. Maximum infection intensity (Mean ± SE) of *P.osculatus* was determined in Aras (6.61 ± 0.5), Mahabad (5.82 ± 0.61) and Zarrineh (5.7 ± 0.56) dams in summer. The numbers of parasites were ranged from 1 to 17 in different seasons. The prevalence and intensity (Mean ± SE) of infection were significantly different in summer and winter with the other seasons ($p<0.05$). Data analysis had demonstrated that infection rate in affected fish mostly depended on seasons. Also, there was no significant difference in infection to this parasite between male and female European catfish. Also, pathological study demonstrated that scolex adhesiveness to the intestine of *Siluris glanis* caused mechanical damages on it. According to the results, infection of freshwater catfish to *Protocephalus osculatus* enjoyed the highest prevalence in the study area.

شیوع آلودگی و آسیب شناسی عفونت با سستود روده ای در گربه ماهی آب شیرین در سدهای مخزنی آذربایجان غربی، ایران

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چکیده

در مطالعه حاضر در مجموع ۱۸۰ قطعه *S. glanis* (۶۰ ماهی برای هر منطقه) از مخازن سد ارس ، مهاباد و زرینه (شهید کاظمی) جمع آوری شد. شیوع فصلی عفونت *Siluris glanis* در سدهای ارس ، مهاباد و زرینه به ترتیب به ترتیب بهار (۶۰، ۵۶، ۵۹ درصد)، تابستان (۷۰، ۶۶/۵، ۶۹ درصد) ، پاییز (۶۳/۳، ۶۶، ۶۴ درصد) و زمستان (۴۵، ۳۹، ۴۱ درصد) بودند. حداکثر شدت آلودگی (Mean ± SE) سستود روده ای *پروتوسفالوس اسکولاتوس* در سدهای ارس (۰/۵ ± ۶/۶۱) ، مهاباد (۰/۶۱± ۵/۸۲) و زرینه (۰/۵۶ ± ۵/۷) در تابستان تعیین شد. تعداد این انگل در فصول مختلف از ۱ تا ۱۷ بود. شیوع و شدت عفونت در تابستان و زمستان با سایر فصول تفاوت معنی داری داشت ($p <$ ۰/۰۵). تجزیه و تحلیل داده ها نشان داده است که میزان آلودگی در ماهیان مبتلا بیشتر به فصول بستگی دارد. همچنین، اختلاف معنی داری در آلودگی به این انگل بین گربه ماهی نر و ماده وجود نداشت. همچنین، مطالعه پاتولوژیک نشان داد که چسبندگی اسکولکس به روده *Siluris glanis* باعث آسیب های مکانیکی به آن می شود. طبق نتایج ، آلودگی گربه ماهی آب شیرین به *Protocephalus osculatus* از شیوع بالایی در منطقه مورد مطالعه برخوردار است.

واژه های کلیدی: سستود روده ای، گربه ماهی آب شیرین، آذربایجان غربی، ایران

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INTRODUCTION

Infections caused by various pathogens among fishes in natural and aquaculture systems are harmful for fish health and growth [9]. The parasitic infections are sometimes very fatal and cause high mortalities when their life cycles are well supported by intermediate hosts. The helminthic parasites mainly found in freshwater fishes are belongs to Trematoda, Cestoda, acanthocephalans and nematoda that complete their life cycles through intermediate hosts like snails and piscivorous birds [19]. For successful prevention and elimination of such parasitic infections, it is extremely important to obtain early and correct data about the prevalence and intensity of a particular parasitic infection in specific group of fishes. Iran, regarding to its geographical position and climatic condition with different water resources, enjoys various types of fish species, that each one can be hosted of different kinds of parasites. Parasitological studies on fish in Iran have been initiated f since decades ago, however, considering variations of fish and different climatic conditions, different varieties of fish parasites are expected.

In Iran, Catfish family consists of two species, *Siluris glanis*, which is native to aquatic territories of Khazar and Urmia lakes, and *Parasilurus triostegus* which is native to Tigris, Karoon river [2,3,18]. Freshwater catfish enjoys a broad distribution and mostly observed in European water resources, Asia Minor and Central Asia. Besides the economical revenue in natural waters, they are also considered as cultured fish and recreational fishing [8]. Also, regarding the shortage of availability for suitable pituitary gland, the pituitary gland of this fish can be used as an artificial spawning inducer in cyprinid fish [15]. Due to nutritional protein values and also export importance of *Siluris glanis*, many investigations are conducted to study its artificial propagation and larviculture in pools and other water resources. The aim of this study was to determine seasonal intestinal infection prevalence and pathological effects of *Protocephalus osculatus* in freshwater catfish (*Siluris glanis*) from West Azarbaijan dams.

MATERIALS AND METHODS

A total of 180 *S. glanis* (60 fish for each area) were collected from Aras, Mahabad and Zarrineh dam reservoirs during of 2018. For parasitic examination, the intestinal and gastric contents of the fish were washed in a 100 micron sieve, and then examined by a stereomicroscope. Parasite scolex was isolated from intestine mucus of fish carefully by two clips. Isolated samples were washed in 6% salt solution and then fixed by70% alcohol on the slide glass, carmine stained and mounted by Canadian adhesive [1,5]. Prepared slides were photographed and measured by microscope equipped with camera Lucida. Parasitic identification was carried out based on the scolex shape and existing or lack of bands. The slides were prepared using some pieces of parasite's body such as genital organs, opening parts in genital organs and type of genital organ in bonds according to identifying keys such as [6,7,11]. Pathological study was performed after tissue processing and staining samples by H&E. All statistical analyses were performed by Chi-square and Kruskal-Wallis tests using SPSS software version 17 (SPSS, Inc., Chicago, IL). $P < 0.05$ was considered significant.

RESULTS

Seasonal prevalence of *P.osculatus* infection *of S.glanis* in West Azarbaijan dams was determined as follows: Winter (41.6%), spring (58.3%), summer (68.5%) and autumn (64.4%), respectively. The highest intensity (Mean ±SE) was due to summer (6.14±0.46) and the least one was observed in winter (4.6±0.55), respectively. Statistical analysis of seasonal prevalence (Mean ±SE) and intensity (Mean ±SE) of parasites in different areas were illustrated in Tables1 and 2, respectively.

Significant seasonal infections were observed within group of the sampling sites ($p>0.05$).The prevalence (Mean ±SE) and intensity (Mean ±SE) of infected samples in studied dams were significantly different in summer and winter compared with the other seasons ($p<0.05$). The comparison of infection percentage of *S.glanis* in different seasons in study sites were shown in Figure 1. The intensity and prevalence of P.osculatus in S. glanis was high in studied dams (Figure 1). Severe intestine infection of *S.glanis* by *P.osculatus* was illustrated in Figure 2.

This parasite contains a scolex with evolved frontal sucker and four clear stabulom whose strobilus has a lot of clear bonds of proglotides. The final bonds are longer than others (Figure 3).

Comparing the fish infection by Chi-square test in different seasons revealed a significant difference ($p<0.05$). Comparing the infection percentage among the seasons were showed that spring had a significant difference with summer ($p<0.05$), there was no significant differences in fall and winter seasons ($p<0.05$). Also, summer and fall had not any significant difference ($p>0.05$), while winter showed a significant difference ($p<0.05$), finally, fall and winter were not shown any significant difference ($p>0.05$). Comparing the infection intensity in total fish in different seasons by Kruskalwallis test, showed significant differences ($p<0.05$). Thus, comparing the infection intensity in infected fish by Mann-Whitney test in different seasons, revealed that spring had a significant difference with summer ($p<0.05$), while it had not any significant difference with other seasons ($p>0.05$). Summer had significant difference with fall and winter ($p<0.05$), but fall and winter were not shown any significant difference ($p>0.05$). Furthermore, according to the obtained data there were not any significant differences between male and female *S. glanis* in mentioned parasite infection ($p>0.05$).

From microscopic point of view, when the number of *P.osculatus* was high in intestine, it showed an intense dilution that might cause a blockage in intestine. But from the tissue losses viewpoint, *Protocephalus* parasite by its boteria that was existed on head of parasite, stacked to fish intestine and each boteria evolved one or two intestine villi. The adhesiveness of Scolex to the intestine caused mechanical damages to the intestine tissue. Necrotic bottoms appeared where boteria had

Table 1: Seasonal prevalence of infected *S.glanis* to *Protocephalus osculatus* in different dams

		Sampling area		
seasons	variable	Aras	Mahabad	Zarrineh
spring	Infected %	60	56	59
	No-observed%	40	44	41
summer	Infected %	70	66.5	69
	No-observed%	30	33.5	31
Autumn	Infected %	63.3	66	64
	No-observed%	36.7	34	36
winter	Infected %	45	39	41
	No-observed%	55	61	59

impacted on underlying layer due to impacts of parasite boteria on intestine mucus. Mucus secretions were not high on adhesive areas, but lymphocytes increased in mucus layer, and inflammation indices were indicated under layer of mucus. Spot hemorrhages in connecting areas were observed in some cases, but were limited to these areas. The Figures 4 and 5 show the cross section of *S. glanis* intestine invaded by *P.osculatus*

DISCUSSION

Parasite is an object that lives on or inside another named host. It feeds from host and shows degrees of compatibility with it, and often do not make any serious damages on it. Therefore, parasite uses the host as a nutritional source and shelter to protect

Table 2: Intensity (Mean ±SE) of *P.osculatus* in *S.glanis* of 3 dams of West Azerbaijan

		Sampling area		
seasons	variable	Aras	Mahabad	Zarrineh
spring	N	36	34	34
	Mean	5.83	5.62	5.41
	SEM	0.61	0.59	0.57
	Minimum	2	1	1
summer	N	42	39	40
	Mean	6.61	6.13	5.69
	SEM	0.5	0.46	0.43
	Minimum	2	2	2
Autumn	N	38	36	33
	Mean	5.55	5.31	5.08
	SEM	0.46	0.44	0.42
	Minimum	1	1	1
winter	N	27	36	28
	Mean	5.05	5.83	4.27
	SEM	0.6	0.61	0.5
	Minimum	1	2	1

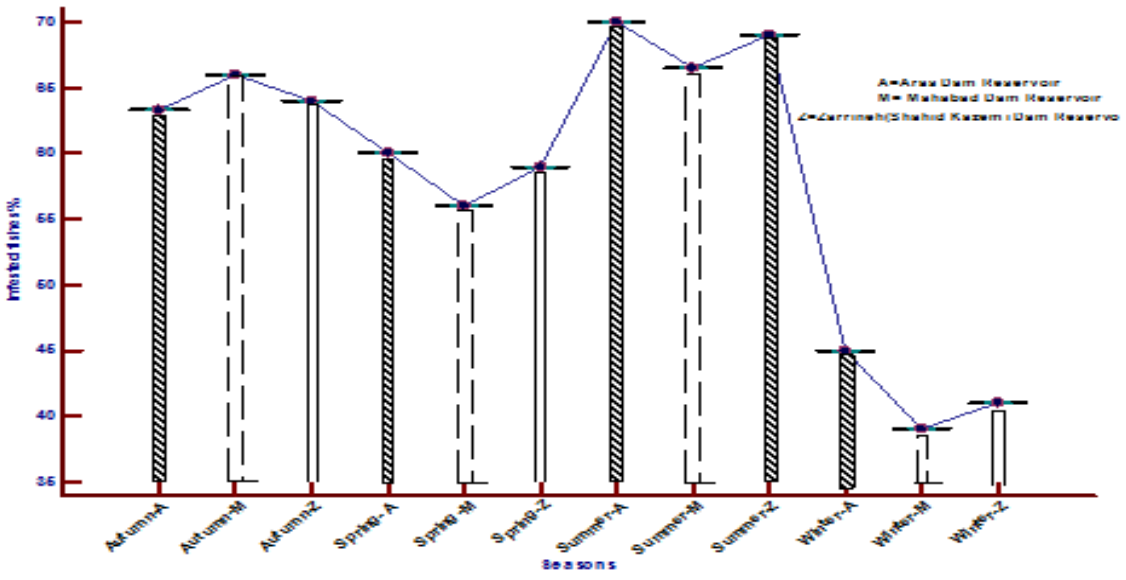

Figure 1. Comparing infection percentage of *S.glanis* in different seasons in study sites

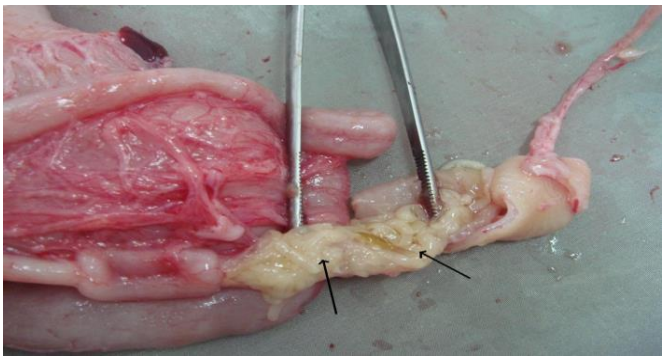

Figure 2. Severe intestine infection of *S.glanis* by *P.osculatus*

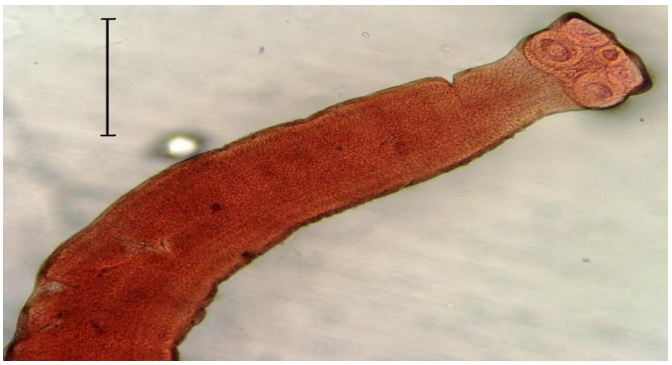

Figure 3. *P.osculatus* identified by scolex with stablom (magnification, ×40)

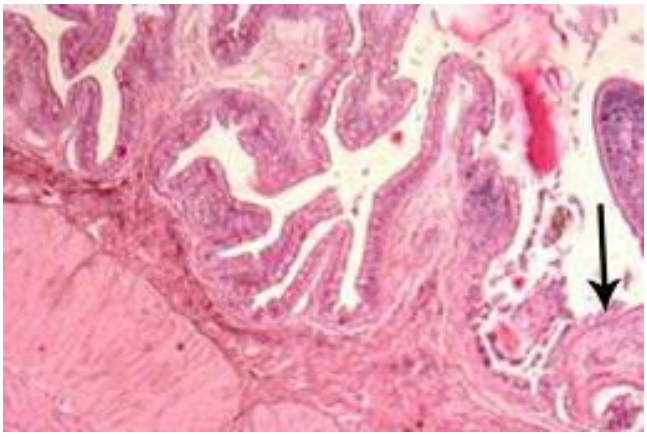

Figure 4. Cross-section of infected intestine by *S. glanis*. Intestinal mucosal layer invaded by *P.osculatus* adhesion (arrow) (H&E stain, ×400).

himself [16]. In natural environments, parasites and their hosts are compatible in a way that their existence would be continued and thus host's life would not be threatened so much. In these situations, the parasites that have indirect life cycle with one or two intermediate hosts will be dominant [12]. In most countries, the prevalence of parasitic diseases in native fish population has made the researchers to investigate source of the diseases and control them through biological methods. This has led to complete recognition of species diversity, life cycle and infectious transmission characteristic of parasitic diseases [16]. A lot of studies on fish parasites have been done in Iran and all over the world. Based on these studies, physicochemical variations in water, presence or absence of intermediate hosts of some parasites, and

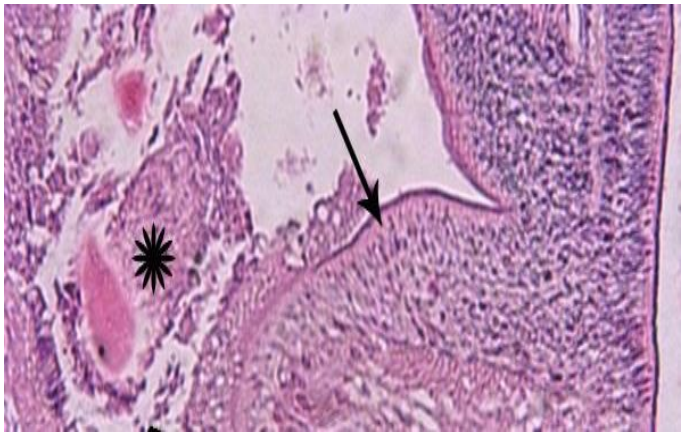

Figure 5. Cross-section of the infected intestine by *S. glanis*. Scolex boteria of *P.osculatus* stacked in intestinal villi (arrow) and destroyed mucosal glands so that the necrotic villi with cellular debris (asterisk) are visible in right upper area of the figure. (H&E stain, ×1000).

different factors might have roles on appearing parasite variations [17]. These variations are of a significant importance and could be the main topic of some parasitological studies on different areas. In Iran, in last decade these studies focused on freshwater fish parasites mostly limited to Ciprinidae species that establish about 70% freshwater fish in Iran But in the case of catfish, it has a high fishing value and not only has economic benefits in natural waters, but also belongs to the recreational fishing group. Due to protein nutritional values of *S. glanis* and its export importance, investigations have been conducted to make artificial propagation of *S.glanis* in pools and other water resources. *S.glanis* has special importance in neighboring countries particularly northern and west-northern countries of Iran and because of this it can be considered as an exportable species. The first report about parasite prevalence in European catfish (*Siluris glanis*) was presented by Sefidkar Lankroodi in 1996. He reported the *Cuculanus sphaerocephalus* nematode from *Siluris glanis* intestine in Anzali marsh. Then, Mokhayer, B. in 1980 introduced *Silorodiscoides silory* parasite from *S.glanis* bronchia of sephidrood [14]. In 2005 Soylu, worked on some metazoan parasites of freshwater catfish in Durusu Lake in Turkey [20]. Also in Sudan, De Chambrier *et al.* reported the infection rate of catfish intestine to *P.osculatus* parasite as 73% and parasite range in 1-10 numbers [4]. According to the data from this study, the infection to *P.oscolatus* in *S. glanis* was observed in Iran. It had a high prevalence (58.3%) and the infection intensity and percentage of parasite in fish were reported be affected by season and water temperature. The results demonstrated that in summer, fall and winter, the temperature condition is suitable for parasite reproduction, proliferation and transmission to the new hosts, and infection percentage in fish increases as well. As is indicated in Table 1 and Figure 1, in fall and winter the maximum infection was observed and then it was decreased in spring and winter. It is assumed that some long term and general processes form parasitic structure communities but habitation or seasonal effects can cover the effects of these processes or reinforce them causing some differences among the various populations. Interpreting the data from seasonal changes in infection to *P.osculatus* parasite has demonstrated that this parasite in all seasons presented in *S. glanis* intestine (Table 1) and this could be attributed to indirect life cycle of parasite. Thus it would be inevitable to assign strategic prevention in

intensive culture of these species in artificial pools, and also perform prevention instructions on parasites such as *Protocephalus* particularly in winter [13]. Investigating the pathological lesions of *P.osculatus* parasite carried out for the first time in Iran. Pathogenesis of this parasite in intestine is often related to its Scolex structure and presence of four sucker organs (boteria) in scolex. Also, the size of these organs can determine the pathogenesis intensity of parasite. As showed in pictures 3 and 4, scolex of parasite penetrates into the intestine and each botridy evolves one or two crinkles of intestine and causes inflammation in this area. Following the intestine inflammation, epithelial lesions, hyperplasia of epithelial cells and necrosis can be observed in attachment point of parasite to host intestine.

CONCLUSION

These results indicated that fish in storage may be more exposed to parasites (intermediate host) than individual's lives in rivers and natural waters. Also, the relationship between infection intensity and parasitic pathogenesis is very important. In the high prevalence of infection, fish growth is decreased due to consumption of the nutrients which are readily uptaken by parasite. However, intense dilation can be observed if there is a high infection rate and increased the number of parasites in intestine. Even it might result in blocking the intestine making some holes in it (Figure.1). With the further development of the disease, the intestine mucus is completely destroyed leading to fish death.

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ETHICS

All ethical standards have been respected in this study

CONFLICT OF INTEREST

None declared.

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