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Prevalence of *rhabdochoniasis* in snow trout of river Swat and river Panjkora, Khyber Pakhtunkhwa province, Pakistan

Prevalência de rabdoconíase em trutas das neves do rio Swat e do rio Panjkora, província de Khyber Pakhtunkhwa, Paquistão

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ABSTRACT

This research was aimed to explore the helminth parasitic diseases in *Schizothorax plagiostomus* (the snow trout) from river Swat and river Panjkora, Pakistan. Collection of 360 fish specimens have done from the lower, middle and upper reaches of both the rivers through gill nets, cast nets, dragon nets and hooks. All the samples were examined in the University of Malakand, Zoology Department for helminth parasites during the months from January 2015 to December 2016. Of the total examined fish samples 21.9% (n=79) were infected with *Rhabdochona spp* including 17.7% (n=32/180) in river Swat and 26.6% (n=47/180) in river Panjkora. Highest month-wise prevalence (p=0.09878,p<0.05) was reported in May (30%), then by February and October each (26.6%) while the lowest during August (13.3%). Highest prevalence (p=0.9723, p<0.05) was reported in summer season (26.6%), while lowest in the winter season (20%). Adults were highly infected (p=< 0.0001) in prevalence (63.7%) followed by sub-adults (13.2%) while no infection was found in juvenile specimens. Females fish samples had higher (p=0.0277, p>0.05) prevalence (28.8%) than males (16.6%). Fishes of the lower reaches had highest (p=0.0029, P>0.05) prevalence (31.7%) followed by middle reaches (16.5%) while the lowest prevalence was observed in samples of fish collected from upper reaches (9.87%). Present study address that *Rhabdochona spp* in the intestine of snow trout has a long term relationship and call as a natural infection in cyprinids and zoonotic risk to human.

Keywords: snow trout, fish parasitic diseases, nematode infection, prevalence, zoonosis.

Resumo

Esta pesquisa teve como objetivo explorar as doenças parasitárias por helmintos em Schizothorax plagiostomus (a truta das neves) do rio Swat e do rio Panjkora, Paquistão. A coleta de 360 espécimes de peixes foi feita nos trechos inferior, médio e superior de ambos os rios por meio de redes de emalhar, de lançamento, de dragão e anzóis. Todas as amostras foram examinadas na Universidade de Malakand, Departamento de Zoologia, para helmintos parasitas durante os meses de janeiro de 2015 a dezembro de 2016. Do total de amostras de peixes examinadas, 21,9% (n = 79) estavam infectados com Rhabdochona spp, incluindo 17,7% (n = 32/180) no rio Swat e 26,6% (n = 47/180) no rio Panjkora. A maior prevalência no mês (p = 0,09878, p <0,05) foi relatada em maio (30%), depois em fevereiro e outubro cada (26,6%), enquanto a menor em agosto (13,3%). A maior prevalência (p = 0,9723, p <0,05) foi relatada no verão (26,6%), enquanto a menor no inverno (20%). Os adultos foram altamente infectados (p = <0,0001) na prevalência (63,7%), seguidos por subadultos (13,2%), enquanto nenhuma infecção foi encontrada em espécimes juvenis. As amostras de peixes do sexo feminino tiveram maior (p = 0,0277, p> 0,05) prevalência (28,8%) do que o masculino (16,6%). Os peixes do curso inferior tiveram maior (p = 0,0029, P> 0,05) prevalência (31,7%) seguida do curso médio (16,5%), enquanto a menor prevalência foi observada em amostras de peixes coletados do curso superior (9,87%). O presente estudo aborda que Rhabdochona spp no intestino da truta das neves tem uma relação de longo prazo e pode ser considerada uma infecção natural em ciprinídeos e risco zoonótico para humanos.

Palavras-chave: truta da neve, doenças parasitárias de peixes, infecção por nematóides, prevalência, zoonose.

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1. Introduction

Pakistan is one of the deficient country in protein production derived from fish. Inland fisheries can have a significant role in the propagation of fish. In Pakistan 179 species of fresh water fishes are survived. Amongst them only 31 species are economically important. In the fresh water reservoirs of Malakand division, almost 40 species of the fish fauna are found. The famous game fish: *S. plagiostomus* has a wide range of distribution found in all the rivers, and tributaries of Himalaya uptoborders of China, Afghanistan, Pakistan, Turkistan, Nepal, Tibet, Bhutan and north-eastern India. *S. plagiostomus* is the chief edible fish of the Himalayan region in all over India, including Kashmir, Himanchal Pradesh, Uttarakhand, the Uttar Pradesh foothills and Assam (Day, 1888).

Due to increasing in rate of fishing and inundation of spawning ground *S. plagiostomus* is becoming rare. Further this species is indigenous and is facing anthropogenic, environmental and natural hazards. Diseases free fish is the need of today and tomorrow. Parasites effect on fishes, weakening the size, fish become not attractive for selling and making them not suitable for human consumption. Parasites present in fishes can transmit to human and cause diseases, and even death. Fish is one of the aquatic animals studied for helminth infection. Fish disease is one of the leading health problem and great attention is being paid to get more and more attention. About 1000 species of fish (both marine and fresh waters) are found in Pakistan. Most of them are edible and very few are studied for helminth parasites (Cheng and Cooperman,1964).

Nematode of the genus *Rhabdochona* Railliet, 1916 parasitizes fish fauna in all the zoogeographical regions of the world. This genus of nematode parasitizes more than 150 known species distributed worldwide. Ninety two species of the genus *Rhabdochoana* were considered valid (Moravec et al., 2008). A good number of studies have been undertaken on the taxonomic spectrum of *Rhabdochona* species as: *R. denudata* (Dujardin, 1845); *R. fortunatowi* Dinnik, 1933; *R. grandipapillata* Rahemo & Kasim 1979; *R. hellichi* (Sÿramek, 1901); *R. mesopotamica* Fattohy, 1975; *R. mesopotamica* Rahemo & Kasim 1979; *R. similis* Moravec and *R. tigridis* Rahemo 1978 (emend.); *R. tigrae* Rahemo 1978; Fattohy, 1975; Rahemo, 1978; Rahemo & Kasim, 1979; Ali et al., 1987; Moravec et al., 1991; Saraiva et al., 2007). However, *R. mesopotamica* Rahemo & Kasim, 1979 has been synonymized with *R. Denudata* by Moravec and Scholz (1991), whereas *R. mesopotamica* Fattohy, 1975 is an invalid name according to the International Code of Zoological Nomenclature (González-Solís et al., 1997). However, broad geographic range and high host specificity of *Rhabdochona* spp., result massive kills of fishes is perhaps the most important pathogen of fresh water fishes throughout the world.

Keeping in mind the problem in beef consumption, diseases of mad cow, foot-mouth disease in sheep and goats, bird flu in in poultry, acquired more demand today for fish than before. Healthy fish is the demand of today and tomorrow. It is therefore needed to understand and recognize the prevalence of parasitic diseases of snow trout from river Swat and river Panjkora, in order to take steps for the control.

2. Materials and Methods

This research was carried out on the year round infection of *Rhabdochona species* in snow trout of both the rivers in Swat and in Panjkora, Khyber Pakhtunkhwa, Pakistan. The fish samples were collected from the lower, middle and upper reaches of both the rivers through gill nets, cast nets, dragon nets and hooks during the months from January to December 2015 and 2016. The collected fish specimens were kept in containers containing preservatives (10% formalin) and transported University of Malakand, where they were necropsied for parasites.

Measurements of fish were made by ruler and Venire caliper. Body length of each fish sample was measured by china tape and steel scale. Each sample of fish was weighted with the help of precise balance model No.18220 Switzerland. The sexes of the fish were determined by using the procedure: (1) the abdomen of each rip fish sample was pressed as to extrude whitish milt (represent male) or eggs (represent female). (2) Each sample of fish was dissected and ensure the presence or absence of testes or ovaries. Presence of testes evidenced maleness and presence of ovaries considered that the fish was a female. (3) During dissection gonads were removed and examined under the microscope for immature eggs or milt. Fish samples were identified by the keys provided by Talwar and Jhingran (1991), Jayaram (1999), Mirza and Sandhu (2007).

Eeach fish samples was washed with distilled water, dissected and screened for parasites. By using the surgical dissecting incisors each sample of fish was cut alongside the mid ventral line. The visceral organs were exposed and placed in separate dishes. Hand lens was used to examine the encysted larvae, parasites embedded on the visceral organs, body cavities and membranes. The recovered specimens of parasites were washed properly in sodium chloride solution and then fixed in alcohol- formol acetic acid (Olurin and Somorin, 2006). Each of the nematode were preserved in vials labeled containing 1:1 parts alcohol (70%) pure glycerin. The specimens were placed on a glass slide in a small drop of fixative and put a coverslip. Excess fixatives were removed with tissue paper, the cover slip sealed with Vaseline and observed under a microscope first under (10x4) and then (10x40) power lenses.

The nematode parasite specimens were identified up to the species level, through keys provided by Yamaguti (1963), Quentin and Petter (1976), Schmidt (1986), Baker (1987), Anderson (2000). Observation on differential morphology was noted.

Differences of the number of individuals examined and the number infected regarding months, seasons, age (length and weight), sex and localities between the host *S. plagiostomus* and the parasite *Rhabdochona spp* were calculated. The statistical analysis was done with Graph pad Prism- 5. A statistically significant difference was considered, if $P > 0.05$.

3. Results

A total of 360 specimens of *S. plagiostomus* were screened. The rate of infection rate was calculated on the basis of months, seasons, age (length plus weight), sex and locality of the fish. The highest infection 30% was noted in the fish collected in May followed by February and October each 26.6% while minimum infection 13.3% was noted in the fish collected in the month of August. However, (P>0.005) was noted between the examined and the infected individuals of both the rivers (Table 1).

The fish samples collected in summer were highly 26.6% infected while in winter season the fish samples were found least 20% infected with *Rhabdochona spp*, however no significant association (P>0.005) was found between the fish samples examined and that of infected from both the rivers (Table 2).

Adults' fish specimens were found highly 63.7% infected followed by sub-adults 13.2% while no infection was observed in juvenile fish specimens. The relationship between the host examined and that of infected was significantly associated (P< 0.0001). Female fish were more infected than females but the infection rate was not significantly associated with the host examined (Table 3).

Regarding locality the fish collected from lower reaches were found highly 31.7% than middle 16.5% while least infection rate 9.87% was found in the fish collected from upper reaches of both the rivers. The significant difference (P= p=0.0029) was noted between the examined and the infected fish specimens collected from both the rivers (Table 4).

Table 1. Month wise (Jan- Dec. 2016) prevalence (%) of *Rhabdochona spp* in *S. plagiostomus* of river Swat and river Panjkora KPK, Pakistan.

Factor	River Swat			River Panjkora			Total examined	Total infected	Prevalence (%)
	No. examined	No. infected	Prevalence (%)	No. examined	No. infected	Prevalence (%)			
January	15	3	20	15	4	26.6	30	7	23.3
February	15	4	26.6	15	4	26.6	30	8	26.6
March	15	1	6.66	15	6	40	30	7	23.3
April	15	2	13.3	15	4	26.6	30	6	20
May	15	4	26.6	15	5	33.3	30	9	30
June	15	2	13.3	15	3	20	30	5	16.6
July	15	3	20	15	2	13.3	30	5	16.6
August	15	1	6.66	15	3	20	30	4	13.3
September	15	3	20	15	4	26.6	30	7	23.3
October	15	3	20	15	5	33.3	30	8	26.6
November	15	2	13.3	15	3	20	30	5	5 (16.6)
December	15	4	26.6	15	2	13.3	30	6	6 (20)
Total	180	32	17.7	180	47	26.1	360	79	79 (21.9)
P Value	0.9665			0.9832			0.9878		

Table 2. Season wise prevalence of *Rhabdochona spp* in *S. plagisotomus* of rivers Swat and river Panjkora, Pakistan (spring: March to May; Summer: June to August; autumn: September to November; winter: December to February).

Factor	River Swat			River Panjkora			Total examined	Total infected	Prevalence (%)
	No. examined	No. infected	Prevalence (%)	No. examined	No. infected	Prevalence (%)			
Spring	45	7	15.5	45	15	33.3	30	7	23.3
Summer	45	6	13.3	45	8	17.7	30	8	26.6
Autumn	45	8	17.7	45	12	26.6	30	7	23.3
Winter	45	11	24.4	45	10	22.2	30	6	20
Total	180	32	17.7	180	47	26.6	360	79	21.9
P Value	0.6931			0.5960			p=0.9723		

Table 3. Age and sex wise prevalence (%) of *Rhabdochona spp* in *S. plagisotomus* of rivers Swat and river Panjkora KPK, Pakistan

Factor	River Swat			River Panjkora			Total examined	Total infected	Prevalence (%)
	No. examined	No. infected	Prevalence (%)	No. examined	No. infected	Prevalence (%)			
Age									
Adult	39	26	66.6	52	32	56.1	91	58	63.7
Sub-adult	80	6	7.5	78	15	19.2	158	21	13.2
Juvenile	61	0	0	50	0	0	111	0	0
Total	180	32	17.7	180	47	26.6	360	79	21.9
P Value	< 0.0001			< 0.0001			< 0.0001		
Sex									
Male	106	13	12.2	98	21	21.4	204	34	16.6
Female	74	19	25.6	82	26	31.7	156	45	28.8
Total	180	32	17.7	180	47	26.6	360	79	21.9
P Value	0.0551			0.2327			0.0277		

Table 4. Locality wise prevalence (%) of *Rhabdochona spp* in *S. plagisotomus* of rivers Swat and river Panjkora KPK, Pakistan

Factor	River Swat			River Panjkora			Total examined	Total infected	Prevalence (%)
	No. of fish examined	No. of fish infected	Prevalence (%)	No. of fish examined	No. of fish infected	Prevalence (%)			
Upper reaches	43	05	11.6	38	03	7.89	81	8	9.87
Middle reaches	59	10	16.9	50	08	16	109	18	16.5
Lower reaches	78	17	21.8	92	36	69.2	170	54	31.7
Total	180	32	17.7	180	47	26.6	360	79	21.9
P value	0.4916			0.0054			0.0029		

4. Discussion

Present study provides information regarding the prevalence of a nematode parasite, *Rhabdochona spp* in the population of *S. plagiostomus* (the snow trout) from different localities in river Swat and river Panjkora, Pakistan. The species of the genus *Rhabdochona* have also been reported in *S. plagiostomus* inhabiting rivers Panjkora and river Swat, Pakistan (Ahmad et al., 2020; Khalid et al., 2020). The natural route of this nematode transmission in snow trout remain unresolved and it may include the possible involvement of an intermediate host, *Trichopteran* (caddis-fly) larvae of the genus *Hydropsyche* (*H. angustipennis* and *H. pellucidula*) as natural intermediate hosts (Moravec and Scholz, 1995) where the parasite becomes more infectious to the snow trout. This is due to the extreme flexibility in *Rhabdochona* species that can be induced by manipulating various hosts and culture conditions. If an intermediate host exists, then the results from this study indicate that its geographic range must be very expansive throughout the world. Even with the economic importance of snow trout, published information on its biology and fishery is scarce.

Ahmad et al. (2014) studied 115 snow trout from river Swat for the prevalence of internal and monogenean parasites and find out 93% infection including with *Rhabdochona* spp. parasites found inside the body of fish from other parts of the country have been studied by Ahmad et al. (2014) from Shall-Bagh and Kashmir; Ahmad et al. (2014) from river Swat, Pakistan; Ayaz et al. (2011) from three districts of KPK, Pakistan; Asmatullah et al. (2006) from Kharan Baluchistan. Thehigh values for prevalence and mean intensity of infection for the parasite (*Rhabdochona sp*) agree well with the data provided by the only study on parasites of *Labeogedrosicus* Zugmayer.

Length and weight (represents age) are directly related to the prevalence of parasites. According to Omeji et al. (2014) parasitic infection increase with the increasing of length and weight, they had been studied 5 species of nematodes from the fish *Malapteruru selectricus*. These nematodes were *Camallanus* sp., *Capillaria* sp., *Contracaecum* sp., *Eustrongylides* sp and *Caenorhabditisbriggsa*. The overall prevalence of infection noted was 47%. Present findings were in accordance with the studies conducted by Bichi and Yelwa (2010), they had studied the fish *Clarias gariepinus* from Nigeria and observed a nematode *Procamallinus laevionchus* with 28% rate of infection. The incidence was found to increase as the fish grew.

Present study reveals that female fish were found more infected28.8% than male individuals16.6% infection rate.

Findings of the present research agreed with Amare et al. (2014), they were studied 412 fish from the Lago lake Nigeria and recorded species of the genus *Clinostomum* from nematode, further they concluded their results as females were found more infected than male fish. Omeji et al. (2014) was noted that female fish were highly infected with 57.5% infection rate and male with 42.4%. They have studied *Malapteruruselectricus* fish from Nigeria. Bichi and Yelwa (2010) investigated a total of 645 fish *Clariasgariepinus*, out of the examined 235 female fishes with 75.5% infection rate and 78 male with 24.9% infection rate were observed. All the infected fish were found infected with a nematode *Procamallinuslaevionchus*, they concluded that female fish had higher incidence rate of infection than males.

Species wise variation is there in reports of other researchers throughout the world. In the findings of the present research 79 fish with 21.9% infection rate out of the total examined, were infected with a nematode Rhabdochona spp Ahmad et al. (2014) studied *S. plagiostomum* and *S. labiatus*and reported two species of *Rhabdochona*as *R. schizothoracis* with 22.8% and *R. charsaddiensis* with 10.8% infection rate. *R. denudata* with 66.6% infection rate was reported in *Glypothoraxsilvae* from Iran by Raissy et al. (2010). In another study conducted by Rafique et al. (2002) 37.5% of the fish *Mystusvittatus* was infected with *R. magna*. According to Leela and Rao (2014) studied goldfish in India and reported *R. garuaiin* with 40.6% infection rate. Their result indicates 3.14 mean intensity and 1.40 relative density.

Present study reveals that fish samples collected in summer were highly 26.6% infected while in winter season the fish samples were found least 20% infected with *Rhabdochona spp.* Ayaz et al. (2011) studied the fishes of river Punjkora, Pakistan and reported 7.5% of the infection with *R. magna*. In another study conducted 251 by Aydoğdu et al. (2011) observed *R. denudate* with 86.6% prevalence in three species of fish including *Capoetaantalyensis*, *Aphaniusmento* and *Pseudophoxinusbattalgi*.

The seasonal occurrence of helminth parasites in the fish may also be due to the age of fish host and life cycle of the parasites because as the length of fish host increases, the prevalence of infection also increases. Data reveals that the prevalence of infection is found in almost directly proportion to the body weight in where the weight is increasing, it record to increase the infection rate caused by the parasite. In case of sex, female fish were found more infected than male and these differences in the incidence of infestation could be due to the physiological status of the females and their increased rate of food intake to meet their food requirements for the development of their egg might have exposed them to more contact with the parasites, which subsequently increased their chance of being infested. Infection between the small and big fish as related to their length and weight may be due to changes in their diet from weeds, seeds, phytoplankton and zooplankton to insect larvae, crustacean and worm. Ecological factors have been held widely responsible for the occurrence of the parasites.

Year round infection of *Rhabdochona spp* was noted in the *S. plagiostomus* (the snow trout) in river Swat and river Panjkora Pakistan for the first time. The snow trout of river Panjkora were more infected than the snow trout of river Swat. Current study enlighten the issue of introduction of parasites in freshwater fishes particularly in the fish fauna of river Swat as well as river Panjkora which are the main reservoirs of edible fish in this locality. These diseased fish must be strictly check for parasitic infection as to safeguard the ecosystem and diversity.

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