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Research Article
Occurrence of Helminth Parasites in *Schizothorax plagiostomus* and *Cyprinus carpio communis* from Nallah Sukhnag, Kashmir

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Abstract

Background and Objective: Fish are an important group of vertebrates and are frequently challenged by different types of infectious disease, some of which are caused by helminth parasites. The main aim of this study was to study the prevalence of helminth parasites in *Schizothorax plagiostomus* and *Cyprinus carpio communis* which is an important source of dietary protein for people in Kashmir and plays an important role in this region's local economy. **Materials and Methods:** This study was conducted to study the helminth infection of 768 fishes belonging to 574 *Schizothorax plagiostomus* and 194 *Cyprinus carpio communis* fishes collected from different sites of Nallah Sukhnag in Budgam, Kashmir. **Results:** Helminths recovered from *S. plagiostomus* were *Diplozoon kashmirensis* (trematoda), *Adenoscolex oreini* (cestoda) and *Pomphorhynchus kashmirensis* (acanthocephalan) with prevalence of 10.27%, 11.84% and 12.54% respectively. Helminth parasites reported from *C. carpio communis* were *Bothriocephalus acheilognathi* (cestoda) with prevalence of 4.63% and *P. kashmirensis* (acanthocephalan) with prevalence of 6.18%. Gender wise and size wise prevalence in *S. plagiostomus* and *C. carpio communis* showed maximum prevalence percentage of helminths in males and larger fishes in comparison to females and smaller ones, respectively. **Conclusion:** Based on the observations as gathered in the present study, the occurrence of maximum infection in larger fishes could be due to accumulation of larvae from year to year. This study also concluded that helminth prevalence in fish is mainly dependent on fish feeding habits, pollution of water body, type of fish as well as on their defence system.

Key words: Helminths, fish, prevalence, acanthocephalan, trematodes, *Pomphorhynchus kashmirensis, Schizothorax plagiostomus*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish is taken as a high quality food throughout the world[1] and is considered a valuable source of proteins, lipids, vitamins, oil and minerals[2]. Fish is also a main source of economy to many countries of the world as well[3] and has a beneficial role in diminishing cardiovascular disorders and cholesterol level in blood due to presence of prostaglandins, thromboxane and Omega-6 fatty acid[4] in their muscle tissues. Important byproducts like fish meal, fish glue and fish oil etc., are obtained from fish[5]. Fish oil play an effective role in proper functioning of the brain, heart and immune system due to presence of omega-3-essential fatty acids[6]. The major fish fauna present in the fresh water bodies of Kashmir comprises of mainly indigenous fish (*Schizothorax* spp.) and exotic fish[7]. Unfortunately fish resources are diminishing now due to degradation of water quality, parasites and diseases[8]. About 30,000 species of helminth parasites are reported in fish throughout the world[9]. Thirty one species of helminth parasites have been reported from Kashmir which cause severe damage to fish health[10]. Extensive damage has been reported due to pathogenicity of parasitism in fish and is considered an important cause for lowering their production[11]. The occurrence of helminth parasites in fishes have been studied extensively in various water bodies of Kashmir, but no work has been carried out on helminth parasites in *Schizothorax* and *Cyprinus carpio* species of Nallah Sukhnag. Hence, a comprehensive work was undertaken to study the helminths in the fishes of Nallah Sukhnag.

MATERIALS AND METHODS

Nallah Sukhnag is one of the important tributary of River Jhelum and is among the five major inflows of the Wular Lake (Ramsar site). This Nallah runs through Budgam district and originates both from spring ′Sukhnag spring′ (Sokha Nag, the spring of solace) and high altitude glacier called Damdam in Damsar near Tossa Maidan. Nallah Sukhnag finally merges with the outlet of Hokersar wetland (Ramsar site) at Sozeith Narbal. This Nallah not only is a source of water for domestic and agricultural purposes, but also provides natural environment to aquatic biota particularly fish. The fish hosts were collected at 4 sites from Nallah Sukhnag from Feb, 2017-Jan, 2019 and brought alive to the Parasitological Research Laboratory by using small containers containing water. The sites were selected on the basis of flow of water, altitudes and the level of pollution. Sites selected were as:

- **Beerwah:** This site is located at 74°35'0"East longitude and 34°1'0"North latitude. This is the upper portion of Nallah Sukhnag located at high altitudes. This site is least inhibited by people with clear, transparent water having fine pebbled bed and is highly torrential in nature
- **Makhama:** This site is located at 74°35'0"East longitude and 34°4'0"North latitude. This site is below Site I and inhibited by small human population. The velocity of water here is slow in comparison to Site I and the bottom includes boulders, gravel and sand
- **Kanihama:** This site is located at 74°36'0"East longitude and 34°6'0"North latitude. This site is below Site II and is inhibited by large human population. Water flows slowly and the water body is highly polluted at this site due to domestic garbage thrown directly into the water body
- **Narbal:** This site is located at 74°39'0"East longitude and 34°7'0"North latitude. This site is located in lower reaches of Nallah where the velocity of water is less in comparison to first 2 sites, but is more in comparison to Site III. This site has human settlements also

The fish hosts were identified as *Schizothorax plagiostomus* Heckel, 1838 and *Cyprinus carpio communis,* Linnaeus, 1758. The sample size was calculated by using the formula given by Thrustfield[12]:

$$n = \frac{1.962^2 . \ Pexp\,(1\text{-}Pexp)}{d^2}$$

Where:

n = Required sample size
Pexp = Expected prevalence = 50%
d = Desired absolute precision = 5%

Hence, d = 0.05 and p = 0.5 (50%)

As no work was done on the selected study area, so the expected prevalence in the study area was taken as 50%[13]. Thus, the minimum desired sample size was calculated to 768. The fishes were then killed by a blow on head. Immediately after killing, fishes were examined externally and then thorough examination of the whole body surfaces was done with the help of stereomicroscope. For internal examination, fishes were dissected mid ventrally and whole body cavity was scanned for helminth parasites. Then visceral organs like alimentary canal, liver kidney and gall bladder were removed and scanned in separate petridishes containing normal saline

Fig. 1: Cestodes in *Schizothorax* intestine

Fig. 2: Acanthocephalan in *Schizothorax* intestine

for monogenetic and encysted digenetic trematodes. Few menthol crystals were added to the normal saline containing the parasite adhered to the intestinal wall which helped in their easy detachment from the intestinal wall.

In order to identify the recovered parasites (Fig. 1-3) permanent slide preparation of trematodes, cestodes and acanthocephalan worms was carried out. For this purpose

Fig. 3: Cestodes in *Cyprinus*

worms were fixed in carnoy's fixative, stained with acetoalum carmine, dehydrated in ascending grades of ethanol, cleared in Xylene and mounted in DPX. Photomicrography was taken with DP-12 Digital Camera attached to Olympus Research Microscope in the Department of Zoology, University of Kashmir, Srinagar. Identification of helminth parasites was then carried out by the keys given by Yamaguti[14]. Prevalence and mean intensity of helminth infection was calculated using formulas given by Margolis *et al.*[15] and Gudivada *et al.*[16] :

$$\text{Prevalence} = \frac{\text{Total number of hosts infected}}{\text{Total number of hosts examined}} \times 100$$

$$\text{Mean intensity} = \frac{\text{Total No. of parasites}}{\text{Total No. of infected hosts examined}}$$

Data analysis: Data was fed into Microsoft excel and then analyzed by using Minitab Version 13. The effect of size, gender on the level of parasitism in fish hosts was analyzed by employing Chi-square test. The p-value is <0.05 (at 5% level of significance) was considered as significant.

RESULTS

Helminth parasites recovered during the current study were *Diplozoon kashmirensis, Adenoscolex oreini* and *Pomphorhynchus kashmirensis* from *S. plagiostomus*, *Bothriocephalus acheilognathi* and *Pomphorhynchus kashmirensis* from *Cyprinus carpio communis* (Fig. 4).

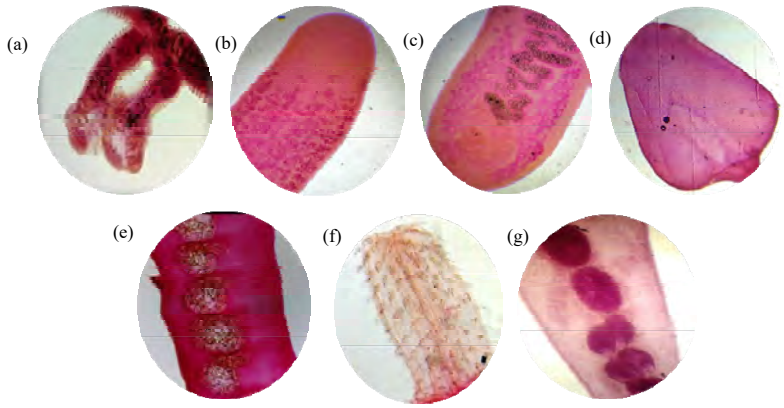

Fig. 4(a-g): Posterior part of *Diplozoon kashmirensis* (Opisthaptor), (b) Anterior region of *Adenoscolex oreini* with smooth scolex, (c) Posterior part of *Adenoscolex oreini* showing inverted A shaped ovary (d) Scolex of *Bothriocephalus acheilognathi* heart shaped, (e) Gravid proglottid of *Bothriocephalus acheilognathi*, (f) Scolex of *Pomphorhynchus kashmirensis* bearing hooks and (g) Posterior end of *Pomphorhynchus kashmirensis* showing testes of male and cement glands

Table 1: Gender wise prevalence of helminth parasites in *S. plagiostomus* and *C. carpio communis*

	S. plagiostomus			*C. carpio communis*		
Gender	No. of examined	No. of infected	Prevalence (%)	No. of examined	No. of infected	Prevalence (%)
Male	273	86	31.5	100	16	16
Female	301	59	19.6	94	5	5.31
Total	574	145	25.26	194	21	10.82
χ^2 (p-value)	6.392	(0.011)		χ^2 (p-value)	5.015	(0.025)

Table 2: Size wise prevalence of helminth parasites in *S. plagiostomus* and *C. carpio communis*

	S. plagiostomus			*C. carpio communis*		
Size	No. of examined	No. of infected	Prevalence (%)	No. of examined	No. of infected	Prevalence (%)
Larger	245	91	37.14	82	15	18.29
Smaller	329	54	16.41	112	6	5.35
Total	574	145	25.26	194	21	10.82
χ^2 (p-value)	18.742 (0.000)			χ^2 (p-value)	6.507 (0.01)	

Gender wise prevalence of helminth parasites in *S. plagiostomus* and in *C. carpio communis:* From February, 2017-January, 2019, out of 574 fish specimens of *S. plagiostomus*, 273 were males and 301 were females. Out of 273 males, 86 were infected with helminth parasites showing the prevalence of 31.5%. Out of 301 females, 59 were infected showing prevalence percentage of 19.6%. Prevalence of helminth parasites was higher in males than females. In *C. carpio communis* during the two years, out of 194 specimens, 100 were males out of which 16 were infected having prevalence of 16% and out of 94 females, 5 were infected giving the percentage prevalence of 5.31%. Significant differences were observed in both fish species in gender wise prevalence as p 0.05 (Table 1).

Size wise prevalence of helminth parasites in *S. plagiostomus* and *C. carpio communis*: During current study, out of 574 specimens of *S. plagiostomus,* 245 were in larger size and 329 were smaller. Out of 245 fishes of *S. plagiostomus,* 91 were infected giving the prevalence of 37.14% and out of 329, 54 were infected showing the prevalence percentage of 16.41%. Out of 194 specimens of *C. carpio communis*, 82 were larger in size and 112 were smaller. Out of 82, 15 were infected giving the prevalence of 18.29% and out of 112, 6 were infected giving the prevalence of 5.35%. Data is statistically significant at $p<0.05$ (Table 2).

Site wise prevalence of helminth infection: Out of 183 fishes belonging to *S. plagiostomus* collected from Site I (Beerwah), 34 were infected showing the prevalence percentage of

Table 3: Site wise prevalence of helminth infection

Sites	Examined	Infected	Prevalence (%)	χ^2 (p-value)
I	183	34	18.57	16.835 (0.001)
II	197	53	26.90	
III	194	58	29.89	
IV	194	21	10.82	
Total	768	166	21.61	

Table 4: Overall parasite wise prevalence and mean intensity of helminth infection

Parasite	No. of examined	No. of infected	No. of parasites	Mean intensity	Prevalence (%)	χ^2 (p-value)
Diplozoon kashmirensis	768	59	67	1.13	7.68	54.903 (0.000)
Adenoscolex oreini	768	68	1200	17.64	8.54	
Bothriocephalus acheilognathi	768	9	72	8	1.17	
Pomphorhynchus kashmirensis	768	84	839	9.98	10.93	

Table 5: Parasite wise prevalence in *S. plagiostomus* and *C. carpio communis*

Host	Trematode	Cestode		Acanthocephalans	
S. plagiostomus	*Diplozoon kashmirensis*	*Adenoscolex oreini*	*Bothriocephalus acheilognathi*	*Pomphorhynchus kashmirensis*	χ^2 (p-value)
No. of examined	574	574	574	574	236.137 (0.000)
No. of infected	59	68	0	72	
No. of parasites	67	1200	0	733	
Mean intensity	1.13	17.64	0	10.18	
Prevalence (%)	10.27	11.84	0	12.54	
C. carpio communis					
No. of examined	194	194	194	194	171.633 (0.000)
No. of infected	0	0	9	12	
No. of parasites	0	0	72	106	
Mean intensity	0	0	8	8.33	
Prevalence (%)	0	0	4.63	6.18	

18.57%. Out of 197 fishes from Site II (Makhama), 53 were infected showing the prevalence percentage of 26.9%. From Site III (Kanihama), 194 specimens of *S. plagiostomus* were collected, out of which 58 were infected showing percentage prevalence of 29.89%. From Site IV (Narbal), 194 fishes belonging to *C. carpio communis* were collected out of which 21 were infected with prevalence of 10.82%. Statistically significant differences were observed in site wise prevalence at $p<0.05$ (Table 3).

Overall parasite wise prevalence of helminth infection: During the current study, out of 768 fish specimens, 59 were infected with trematodes belonging to genus *Diplozoon* with a prevalence of 7.68% and mean intensity of 1.13, 68 infected with *Adenoscolex oreini* (cestode) with prevalence of 8.54% and mean intensity of 17.64, 9 were infected with *Bothriocephalus acheilognathi* (cestode) showing prevalence of 1.17% and mean intensity of 8 and 84 were infected with *Pomphorhynchus kashmirensis* showing prevalence of 10.93% and mean intensity of 9.98. The highest prevalence was of *Pomphorhynchus kashmirensis* and least was that of *Bothriocephalus acheilognathi*. Highest mean intensity was of *Adenoscolex oreini* followed by *Pomphorhynchus kashmirensis* then *Bothriocephalus acheilognathi* and lowest that of *Diplozoon kashmirensis*. Statistically significant differences were observed in parasite wise prevalence as well as mean intensity at $p<0.05$ (Table 4).

Parasite wise prevalence and mean intensity in *S. plagiostomus* and *C. carpio communis*: Table 5 showed the significant differences in parasite wise prevalence and mean intensity in *S. plagiostomus* and *C. carpio communis* at $p<0.05$. Out of 574 specimens of *S. plagiostomus,* 59 were infected with *Diplozoon kashmirensis,* 68 with *Adenoscolex oreini* and 72 with *Pomphorhynchus kashmirensis* showing prevalence percentage of 10.27, 11.84 and 12.54%, respectively. Mean intensities of *Diplozoon kashmirensis* was 1.13, *Adenoscolex oreini* 17.64 and *Pomphorhynchus kashmirensis* 10.18. Out of 194 fishes of *C. carpio communis*, 9 were infected with *Bothriocephalus acheilognathi* and 12 with *Pomphorhynchus kashmirensis* showed prevalence percentage of 4.63 and 6.18%, respectively. Mean intensities of *Bothriocephalus acheilognathi* and *Pomphorhynchus kashmirensis* in *C. carpio communis* was 8 and 8.33, respectively.

DISCUSSION

In current study significant variations were observed in helminth infection between males and females. Maximum helminth infection was reported in males in comparison to females that is in conformity with the study performed by Goselle *et al.*[17], who reported maximum helminth infection in males of *Clarias gariepinus* and *Tilapia zilli* at Lamingo Dam, JOS, Nigeria. Mgbemena[18] found a higher infection in male of *C. lazera* Emere[19] reported differences in parasitic load between male and female fish and attributed it to differences in feeding habit of fishes as well as different degrees of resistance shown to infection. Bekele and Hussien[20] observed the highest infection rate of parasites in males of *Oreochromis niloticus and Clarias gariepinus* in Lake Ziway, Ethiopia. Our findings are in conformity with Gautam *et al.*[4], who also observed highest helminth infection in males of *Channa punctatus and Channa striatus.* Khan *et al.*[21] observed the seasonal variation of parasitic infections in fish *Johniuss dussumieri* where more males were infected as compared to female fishes that supports our results. However Adegbehingbe and Umezurike[22] noticed higher infection of helminth parasites in females of *Parachanna Obscura* than males that is in contrast to our study. Adikwu and Ibrahim[23] reported high infection in females as compared to male in *Clarias garienpinus,* Nigeria. According to Emere and Egbe[24], the highest infection in female fishes is attributed to physiological state of most gravid females that could have reduced resistance to infection by parasites.

According to our study maximum helminth infection was observed in large sized fishes of both *S. plagiostomus* and *C. carpio communis* in both years that is supported by Dar *et al.*[25] also reported that increase in prevalence in *Cyprinus* and *Schizothorx* species with larger size. Ibraq and Fayaz[26] observed the maximum infection in large size fishes of both *S. plagiostomus* and *C. carpio communis*. As the fish grows older, there is greater possibility of parasitic larvae to get accumulated from year to year and may lead to increased parasitism in larger fishes[27]. Maximum prevalence of parasites in larger fishes could be also attributed to the longer time of exposure to the surrounding environment by large body size[28]. Our findings clearly demonstrate that there exists a significant difference in the prevalence of infection in different study sites on the same water body (Table 3). The results of current study revealed the maximum prevalence of helminths in fishes at Site III (Kanihama) that may be due to pollution of water that have increased the development of intermediate hosts at that region and ultimately have increased the parasitic load in fishes[29]. This rise in prevalence infection at Site III is in agreement with Khurshid and Ahmad[30], who reported the higher parasitic prevalence at highly polluted site in comparison to other sites in Shallabugh wetland. Our findings are also in agreement with Qayoom *et al.*[31], who observed the maximum prevalence of helminth infection in cold water fishes of River Jhelum at polluted site.

During this study, significant differences were observed in overall parasite wise prevalence and mean intensities. Among acanthocephalans, only *Pomphorhynchus kashmirensis* was reported that showed highest prevalence which is inconsonance with the earlier findings[32,33]. Ecological factors including feeding behavior, diet of the host, water temperature[34] and the wide host range[35] are the main factors for influencing the parasitic prevalence. Significant differences were also reported in parasite wise prevalence and mean intensities in *S. plagiostomus* and *C. carpio communis* in both years as p-value is <0.05. In current investigation, *S. plagiostomus* was abundantly infested with acanthocephalans followed by cestodes and then trematodes that is in accordance to Yousuf *et al.*[7]. This differences in parasitism in above fish species is attributed to the different preferences of fishes for food at different phases of life[36] and the parasitic resistance of the exotic host i.e., *Cyprinus* species.

CONCLUSION

This study concluded that prevalence of helminths was maximum in large sized and male fishes in comparison to smaller and females. In *S. plagiostomus* and *C. carpio communis* maximum prevalence was shown by *P. kashmirensis.* This study showed that *S. plagiostomus* were abundantly infected with helminths than *C. carpio communis* and prevalence of helminths mainly depends on fish feeding habits, pollution of water, sex and size of fish.

SIGNIFICANCE STATEMENTS

To enhance the fish production, it is important to study their parasitic infestation as the pathogenicity of helminths is responsible for causing extensive damage to fish health. In order to prevent and eliminate such infections from the fish, it is extremely important to achieve early and correct diagnosis of the larval stages of the parasites. It is imperative to evaluate the parasitic load in fish as it serves as an essential tool that not only provides information concerning health status of fish, but also provides information on their own changes in diversity, prevalence and abundance related to different environmental stressors. The data of this study will give a baseline data for other studies.

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