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Histopathological alterations and distribution of *Adenoscolex oreini* in intestines and their seasonal rate of infestation in three freshwater fishes of Kashmir

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Abstract
On examination of 444 *Schizothorax* spp. revealed 144 (32.43%) were found to be infected with the *A. oreini*. Out of 224 spp. examined from the Dal Lake 71 (31.69%) were found infected with *A. oreini.* Host-wise distribution of the parasite was significantly varied ($p<0.01$) which showed *S. niger* (31.57%), *S. esocinus* (28.57%) and *S. curvifrons* (35.21%). Out of 220 spp. examined from River Jhelum 73 (33.18%) were infected with *A. oreini* which included *S. niger* (28.94%), *S. esocinus* (32.87%) and *S. curvifrons* (38.02%). All the three parasites showed higher prevalence during summer and least during winter. Overall the mean intensity of the cestodes was higher in River Jhelum. The parasitic infections were prevalent more in male compared to females. *A. oreini* showed positive correlation with pH of water. The presence of the parasites had reduced the condition coefficient of the infected fishes in both the water bodies. Histopathologically, parasite induced various intensities of enteritis coupled with hyperplastic goblet cells with increased acid mucopolysaccharide concentrations.

Keywords: Parasites; Cestodes; Enteritis; Correlation; Water

1. Introduction

Infections with helminths are quite common in both feral and cultured fish [1]. Fish harbor a variety of parasites viz, protozoa, cestodes, trematodes and acanthocephalans [2] and the degree of damage by infection is influenced to a large extent by the type and numbers of parasites present [3]. The distribution of parasites varies not only in different species of fish but also seasonally and from one water body to other. The pathogenicity of parasitism has been reported to cause extensive damage to the host leading to the lower production of the fish [4]. A positive relationship has been reported between eutrophication and fish parasitism [5]. It is observed those helminth parasites which inhabit the intestine mostly cause several pathogenic, biochemical and physiological changes [6]. The physical connection between parasite and host constitutes the micro-environment and the host's environment can be seen as the macro-environment of the parasite [7]. In certain studies the parasite has been found to be responsible for the death of the host [8]. The present study attributes towards the rate of infestation in intestines of fish and their prevalence, mean intensity along with abundance in different seasons. Extensive damage caused by helminth parasites on fish organs indirectly effect its growth, development and reproduction and thus, may leads to further decline in the population of the host fish. Heavy infestation of endoparasites interrupts the normal growth of fish. Injured fishes carry heavy parasitic infection which detoriate their food and that may be the ultimate cause of their mortality [9]. The aim of this study was therefore to determine the incidence of helminth parasite, *Adenoscolex oreini* in fishes and their correlation with water quality parameters and to assess histopathological changes caused by *A. oreini* in the liver and intestines affected fishes.

2. Material and Methods

2.1 Examination of live helminth parasites of fishes and preliminary treatment of the fish: For this purpose a survey of fishes of the Dal Lake and River Jehlum was conducted from August 2014 and August 2015 to determine prevalence of parasitic infection.
The fishes were first given serial number and then the total length (cm) and sex of each fish

were recorded. After collection, the three Fish species of Schizothoracine (*Schizothorax niger*, *S. esocinus* and *S. curvifrons*) were subjected to helminthological examination and then preserved in ice. Fishes were dissected in the laboratory. Intestine was removed and examined for gastrointestinal helminth parasites.

2.2 Isolation of helminth parasites

The parasitological examination of fishes was carried out as per the methodology of Scharperclaus [10]. The parasites were processed and identified with the help of keys provided by Manwell [11] and Yamaguti [12]. The cestodes were removed from the host without any form of treatment prior to preservation.

The regular record of the collection was maintained and the prevalence of cestodes were carried out by the following formulas and such ecological terms are studied as per Margolis *et al.* [13] and Gudivada *et al.* [14]

$$\text{Prevalence} = \frac{\text{Total No. of hosts infected}}{\text{Total No. of hosts examined}} \times 100$$

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

$$\text{Abundance or relative density} = \frac{\text{Total No. of parasites}}{\text{Total No. of hosts examined}}$$

3. Pathology

3.1 Gross pathology: Fishes were systematically subjected to detailed macroscopic examination with special emphasis on liver, intestine and the lesions were recorded.

3.2 Histopathology: Representative tissue samples from the liver, intestine affected by parasites were collected in 10% formalin. The tissue samples were processed for routine paraffin embedding technique and 5u thin section were stained with Haris haematoxylin and Eosin (Bernet *et al.*) [15]

3.3 Histochemistry: Parallel tissue section selected on the basis of histopathological examination was stained for following histochemical observation.

- Acid and neutral mucin were determined by combined alcian blue Periodic-acid Schiff (PAS) stain as per Bancroft and Gamble [16].
- Mast cells were determined by toluidine blue staining protocol as per Gandalfo *et al.* [17]

3.4 Statistical analysis

SPSS for Windows 17.0 (SPSS Inc.) was used for statistical analysis of the data. Post hoc test should be used to interpret the data. One-way analysis of variance (ANOVA) was used to see the significant differences in intensity and abundance within two water bodies. Non-parametric analysis i.e. Mann-whitney test was used to compare the condition factor of uninfected and infected fishes from the same populations.

$$U = n_1 n_2 + \frac{n_2(n_2+1)}{2} - \sum_{i=n_1+1}^{n_2} R_i$$

Pearson's correlation (r) was used to assess associations between different physico-chemical parameters and parasitic infection. The mathematical formula for computing ***r*** is:

$$r = \frac{n\sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2) - (\sum x)^2}\sqrt{n(\sum y^2) - (\sum y)^2}}$$

Where *n* is the number of pairs of data.

4. Results

4.1 Host-wise prevalence of *Adenoscolex oreini* in Dal Lake and River Jhelum

Examination of 444 fish specimens revealed 144 (32.43%) were found to be infected with the *A. oreini*. Out of 224 specimens examined from the Dal Lake 71 (31.69%) specimens were found infected with *A. oreini*. Host-wise distribution of the parasite was significantly varied (p<0.01) which showed *S. niger* (31.57%), *S. esocinus* (28.57%) and *S. curvifrons* (35.21%). Out of 220 *Schizothorax* spp. examined from River Jhelum 73 (33.18%) were infected with *Adenoscolex oreini* which included *S. niger* (28.94%), *S. esocinus* (32.87%) and *S. curvifrons* (38.02%). The overall mean intensities of *A. oreini* in *Schizothorax* spp. of Dal Lake and River Jhelum were 5.22 and 7.46 having an abundance of 1.65 and 2.4 respectively (Table 1).

Table 1: Overall prevalence of *Adenoscolex oreini* in various host species

Host	Dal Lake							River Jhelum						
	No. Examined	No. infected	Prevalence (%)	No. of parasites	Mean Intensity	Abundance	P-value	No. Examined	No. infected	Prevalence (%)	No. of parasites	Mean Intensity	Abundance	P-value
S. niger	76	24	31.57	95	3.95	1.25	< 0.01	76	22	28.94	180	8.18	2.36	< 0.01
S. esocinus	77	22	28.57	145	6.59	1.88	< 0.01	73	24	32.87	182	7.58	2.49	< 0.01
S. curvifrons	71	25	35.21	131	5.24	1.84	< 0.05	71	27	38.02	183	6.77	2.57	> 0.05
Total	224	71	31.69	371	5.22	1.65	< 0.01	220	73	33.18	545	7.46	2.47	< 0.01

4.2 Seasonal prevalence

Seasonal estimation of *A. oreini* infection revealed definite variation in the prevalence of infection in *Schizothorax* spp. with highest rate of infection in summer and lowest in winter. There was a gradual increase in the prevalence rate from spring to summer and decreased with the onset of autumn and least observed prevalence was observed during winter season (Table 2).

Table 2: Infection dynamics of *Adenoscolex oreini* recorded of *Schizothorax* spp. from Dal Lake and River Jhelum

Season	Host	Dal Lake							River Jhelum						
		No. Examined	No. infected	Prevalence (%)	No. of parasites	Mean Intensity	Abundance	P-value	No. Examined	No. infected	Prevalence (%)	No. of parasites	Mean Intensity	Abundance	P-value
Spring	*S. niger*	14	6	42.85	29	4.8	2.07	> 0.05	15	5	33.33	36	7.2	2.4	> 0.05
	S. esocinus	19	5	26.31	14	2.8	0.73	> 0.05	20	8	40	54	6.7	2.7	> 0.05
	S. curvifrons	16	7	43.75	38	5.4	2.37	> 0.05	19	7	36.84	35	5	1.84	> 0.05
Summer	*S. niger*	18	9	50	42	4.6	2.33	> 0.05	16	6	37.5	80	13.3	5	> 0.05
	S. esocinus	17	8	47.05	92	11.5	5.41	> 0.05	18	7	38.88	42	6	2.33	> 0.05
	S. curvifrons	19	9	47.36	56	6.2	2.94	> 0.05	15	9	60	39	4.3	2.6	> 0.05
Autumn	*S. niger*	25	6	24	18	3	0.72	<0.05	20	7	35	52	7.4	2.6	> 0.05
	S. esocinus	19	5	26.31	27	5.4	1.42	> 0.05	19	6	31.57	65	10.8	3.42	> 0.05
	S. curvifrons	18	7	38.88	29	4.1	1.61	> 0.05	22	7	31.81	80	11.4	3.63	> 0.05
Winter	*S. niger*	19	3	15.78	6	2	0.31	< 0.01	25	4	16	12	3	0.48	< 0.01
	S. esocinus	22	4	18.18	12	3	0.54	< 0.01	16	3	18.75	21	7	1.31	< 0.05
	S. curvifrons	18	2	11.11	8	4	0.44	< 0.01	15	4	26.66	29	7.2	1.93	> 0.05
	Total	224	71	31.69	371	5.2	1.65	< 0.01	220	73	33.18	545	7.4	2.47	< 0.01

4.3 Gender wise prevalence of *Adenoscolex oreini*

Gender-wise distribution of *A.oreini* in *Schizothorax* spp. of Dal Lake revealed that the overall prevalence of the parasite in males was 32.83% whereas it was 30% in females. In River Jhelum the overall prevalence in males was 36.75% while as in females it was 23.3%. The overall mean intensities of the parasite in males and females of *Schizothorax* spp. were 5.7 and 4.3, with an abundance of 1.8 and 1.3 respectively in Dal Lake. In river Jhelum the overall mean intensities of the parasite in males and females of S*chizothorax* spp. were 8.0 and 8.2 with an abundance of 2.9 and 1.5, respectively (Table 3).

Table 3: Gender-wise infection dynamics of *Adenoscolex oreini* recorded of Schizothorax spp. from Dal Lake and River Jhelum

Season	Host	Dal Lake							River Jhelum						
		No. Examined	No. infected	Prevalence (%)	No. of parasites	Mean Intensity	Abundance	P-value	No. Examined	No. infected	Prevalence (%)	No. of parasites	Mean Intensity	Abundance	P-value
S. niger	Male	43	16	37.20	63	3.9	1.46		33	14	42.42	101	7.2	3.06	< 0.05
	Female	33	8	24.24	32	4	0.96	> 0.05	43	8	18.60	79	9.8	1.83	
S. esocinus	Male	39	13	33.33	97	7.4	2.48		47	17	36.17	120	7	2.55	> 0.05
	Female	38	9	23.68	48	5.3	1.26	> 0.05	26	7	26.92	62	8.8	2.38	
S. curvifrons	Male	52	15	28.84	94	6.2	1.80		37	12	32.43	125	10.4	3.37	> 0.05
	Female	19	10	52.63	37	3.7	1.94	> 0.05	34	9	26.47	58	6.4	1.70	

4.4 Influence of sex and condition factor on the level of infection

Insignificant relationship existed between gender and helminth infection. Condition factor (k) was significantly higher in uninfected fish in winter (p=0.03), spring (p=0.03) and summer (p= 0.02). Overall, condition factor showed significant differences in infected (p=0.00) and uninfected fish (p=0.00) when compared among the seasons (Fig. 1). Analysis of the condition factor of uninfected and infected S*chizothorax* spp. of Dal Lake and River Jhelum revealed significant differences (p<0.05) with higher in River Jhelum. The condition factors for various *Schizothorax* spp. in the two water bodies by Mann-Whitney test revealed S. niger (U=13, p<0.01), *S. esocinus* (U=45, p>0.05) and for *S. curvifrons* (U=34, p>0.05) of Dal lake While as in River Jhelum it was *S. niger* (U=3, p<0.01), *S. esocinus* (U=3, p<0.01) and for *S. curvifrons* (U=16, p<0.05).

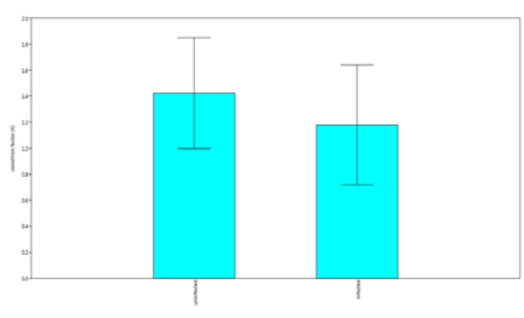

Fig.1: Overall comparative analysis of condition factor of infected and uninfected fish in two water bodies with different water quality

4.5 Correlation between prevalence and water quality in two water bodies: Temperature was the most important abiotic factor that affected the parasites at all life cycle stages. A positive correlation existed between water temperature and parasitic prevalence in Dal Lake (Pearson correlation, r=.722**, p<0.01; Spearman's correlation, r=.664*, p<0.05) and River Jhelum (Pearson correlation, r=.922**, p<0.01;

Spearman's correlation, r=.729**, $p<0.01$).Prevalence of infections showed insignificant positive correlation in Dal Lake ($P>0.05$) with Carbon dioxide ((Pearson correlation, r=.598*, $p>0.05$; Spearman's correlation, r=.629* and River Jhelum (Pearson correlation, r=.579*, $p>0.05$; Spearman's correlation, r=.581*, $p>0.05$) (Table 4).

Table-4: Correlation between environmental variables and prevalence of *Adenoscolex oreini*

Environmental variables	Prevalence of *Adenoscolex oreini*			
	Dal Lake		River Jhelum	
	Pearson's coef.	Spearman's rho	Pearson's coef.	Spearman's rho
Temperature	.802**	.699*	.810**	.592*
Oxygen	-.755**	-.643*	-.105	-.084
pH	.487	.601*	-.111	-.182
Carbon dioxide	-.007	-.035	.529	.322

4.6 Histopathology of *Adenoscolex oreini* infections of *Schizothorax niger*

The fishes infected with *Adenoscolex oreini* were anemic and the abdomen appeared slightly pot bellied. On opening the intestine necrotic debris was present on the surface and numerous parasites were recovered (Figs.2 & 3). During spring enteritis characterized by inflammatory cells in the lamina propria and lamina epithelialis of intestine was seen. Goblet cells hyperplasia was seen with positivity for acid mucopolysaccharide. During summer severe chronic enteritis was seen with infiltration of lymphocytes and fibroblasts in the lamina propria. Mast cell infiltration was evident with scattered metachromatic granules (Fig. 4 & 5). The necrotic villi were completely denuded of epithelial mucosa. Alcian blue PAS staining revealed goblet cells hyperplasia with presence of acid mucopolysaccharide (Fig. 6). During autumn necrosis of some villi was seen represented by fibrillar networks (Fig 7). During winter eosinophiles granules were seen in lamina propria Mast cells were evident. Liver cells were swollen showing vacuolar degeneration and Kupffer cell hyperplasia (Figs. 8 & 9).

Fig.2: Intestinal mucosa showed necrotic debris and numerous parasites were pesent

Fig.3: Numerous *Adenoscolex oreini* were recovered from the intestines

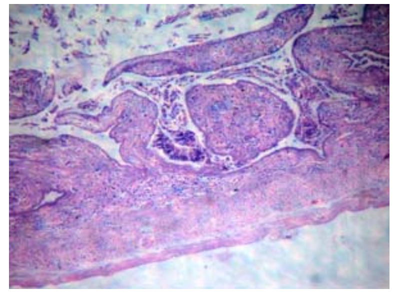

Fig.4: During summer severe chronic enteritis was seen with infiltration of lymphocytes and fibroblasts in the lamina propria. H & E X 28.

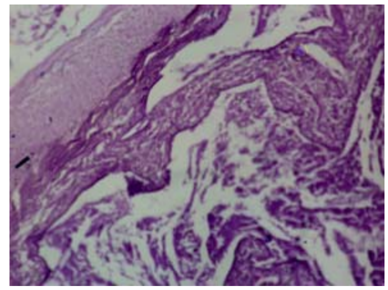

Fig.5: During summer necrotic villi were completely denuded of epithelial cells. Alcian blue PAS X 40.

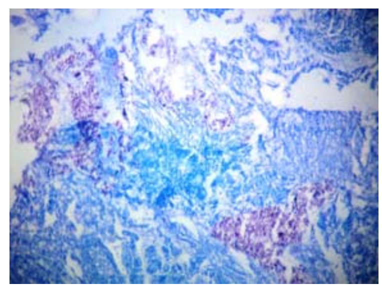

Fig.6: During summer mast cell infiltration was evident with scattered metachromatic granules. Toluidine blue stain X 25.

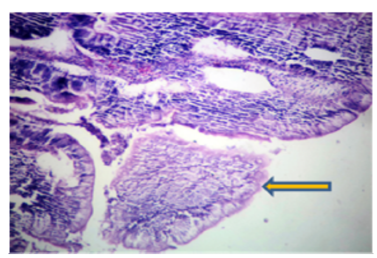

Fig.7: During autumn Necrosis of some villi was seen represented by fibrillar networks (arrow). H & E X 30.

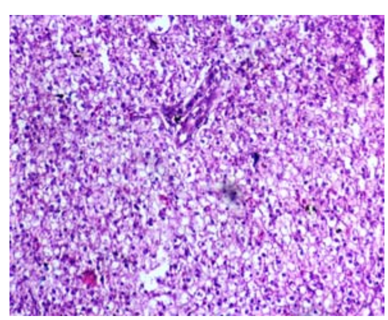

Fig.8: During spring cells were swollen showing vacuolar degeneration. H & E X 57

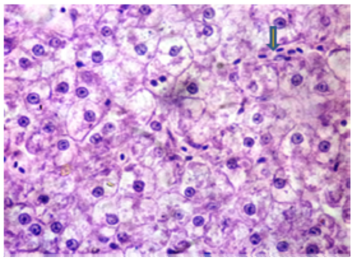

Fig.9: Higher magnification of the same showing Kupffer cell hyperplasia (arrow). H & E X 160.

5. Discussion

Adenoscolex oreini was seen more in *S. niger* and *S. curvifrons* in Dal lake and River Jhelum during Summer and lowest during winter, respectively. The seasonal prevalence showed significant differences in prevalence. Clear seasonal trend was observed in Dal Lake and River Jhelum with maximum infection level during summer months and least in winter months. Significant differences (p<0.01) in prevalence were recorded vis-à-vis the season in both the water bodies which were in conformity with the results of Yufa and Tingbao [18] who concluded that the helminth species like monogeneans showed seasonal alterations associated with environmental changes. The abrupt increase in helminth infection from summer in both water bodies could be due to increased duration of life of the infective larva, and has been reported to assist in the transfer of helminth infection like Diplozoon infection from fish to fish [19]. In the findings of Genc *et al* [20] the parasite infection showed seasonal variations with the highest prevalence in summer season, as suggested that decrease in water volume during the dry season caused nutritional imbalance resulting in less production of fish food organisms and moreover thereby reducing the immune response in fish and making them more vulnerable to disease agents. Boping and Wenbin [21] studied the seasonal population of *Pallisentis* (*Neosentis*) *celatus* in the intestine of rice -field eel *Monopterus albus* and inferred that the prevalence of parasite undergoes a distinct seasonal trend being highest in spring and decreased corresponding to a fall in temperature. Further, no significant seasonal differences were found in mean intensities. Results of Singh and Sahay [22] indicated that all parameters i.e, prevalence, intensity, density and index of infection showed highest values in June. These results clearly suggested that they were related to the rise of water temperature and the availability of secondary host. Wani and Magray [23] reported highest prevalence of *Adenoscolex* during spring and winter seasons in river Jhelum and in winter in Anchar Lake.

Both the cestodes and the acanthocephalan infection were prevalent more in male fishes compared to females. This study is in full agreement with Machado *et al.* [24] Reports on the prevalence of parasites with respect to host sex are diversified. Records on higher prevalence in male hosts are supported by the work of Zelmer and Arai [25] who observed that the slope of relationship between number and abundance of *Bothriocephalus* species and *Proteocephalus* species was greater for male perch than for females. Takemoto and Pavanelli [26] reported that male hosts had significantly higher parasite intensity than females. The influence of sex on the susceptibility of animals to infections could be attributed to genetic predisposition and differential susceptibility owing to hormonal control.

The presence of the parasites had reduced the condition coefficient of the infected fishes in both the water bodies. However; the overall condition factor of fish in Jhelum River was higher. Condition coefficient was found to be lower in infected fish than in uninfected fish in both Dal Lake and River Jhelum. It might be due to the fact that parasites decrease the immune system of the hosts, which may lead to decreased growth of fish. Decreased growth may lead to decrease in condition coefficient [27, 28]. Parasites might also alter the physiological as well as reproductive functions of hosts. This might also lead to decreased growth of fish [29]. It has been reported earlier that condition factor tend to be higher due to higher food quality and availability [30]. In summary, season, condition factor and microhabitat seem to have a significant impact on the helminth infection.

The present study showed that some of the physic-chemical features showed a significant positive correlation with the prevalence. A positive correlation existed between the water temperature and pH with the prevalence of the cestode parasite while as it was negatively correlated with the DO. With regard to free Carbon dioxide, cestode showed positive correlation. Modu *et al.* [31] showed that there existed a significant correlation between Helminth infection and water quality parameters in a pond. A number of workers (Beer and German; Kennedy and Watt; Marcogliese; Lafferty and Kuris) [32, 33. 34, 35] have suggested that natural abiotic factors such as temperature, oxygen, salinity, hydrogen ion concentration and eutrophication have a positive influence on the occurrence of parasite populations. Evidence from the present study suggested that the water temperature played an important role in the progression of all the three parasitic infection. Dissolved O_2 had been found to be the predictor of habitat selection in monogeneans [36]. Our results, however, showed insignificant relationship between O_2 level and the parasites.

6. Concluding remarks

The present study was undertaken with the objectives to determine the incidence of helminth parasites in fishes with special reference to water quality parameters, and histopathological changes caused by the residues of these metals in the liver and intestines affected by cestode parasite. Cestodes were prevalent more in male fishes compared to females. The mean intensity and abundance of the cestodes was highest in summer and lowest in winter. The presence of the parasites reduced the condition factor of the infected fishes in both the water bodies. However, the overall condition factor of fish in Jhelum River was higher. A positive correlation existed between the water temperature and pH with the prevalence of all the three parasites while as it was negatively correlated with the DO. With regard to free carbon dioxide, cestodes showed positive correlation.

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