A QUANTITATIVE ANALYSIS OF GASTRO-INTESTINAL HELMINTHS (TREMATODA: DIGENEA) INFECTION IN RANID FROGS IN JAMMU

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Abstract

Trematodiasis due to a number of flukes is a serious malady in amphibians. The incidence of trematode infection in ranid frogs in Jammu, Jammu and Kashmir State at an elevation of 300m. above sea level has been reported before by earlier workers, which contains information on the taxonomy, morphometrical description and seasonal incidence of worms. This paper complements the earlier works in describing the the parasitic burden on two ranid hosts, *Hoplobatrachus tigerinus* and *Euphlyctis cyanophylctis* inhabiting Jammu waters. The study revealed that more than 70 per cent of frog specimens were found infected with seven entric trematode species. The quantitative description of the worms has been described and the data statistically analysed.

Keywords

Gastro-intestinal trematodes, *Hoplobatrachus tigerinus*, *Euphlyctis cyanophlyctis*, parasitic helminths

Introduction

Hoplobatrachus tigerinus (Daudin) and *Euphlyctis cyanophlyctis* (Schneider) are two important ranid frog species known to inhabit the low lying areas especially the irrigated fields, ponds, pools and ditches in Jammu (Verma *et al*., 1995). These amphibians not only constitute an important trophic link in the environment wherein they live but also help in maintaining the ecological balance. Their role in pest control is well known; frogs being instrumental in insect vector control to some extent. Also it is known that amphibians serve as intermediate, complementary or reservoir hosts for some of the helminths infecting domestic and wild animals (Prokopic & Krivance, 1975) besides harbouring the adult digenetic trematodes, *Pleurogenoides*, *Tremiorchis*, *Ganeo*, *Diplodiscus*; nematodes, *Cosmocerca*, *Rhabdias*; and the cestode, *Nematotaenia* (Sathyanarayana & Sampath, 1996).

It has been found that amphibians, particularly anurans in Jammu province of the state, harbour a number of digenetic trematodes namely *Diplodiscus*, *Haematolechus*, *Plagiorchis*, *Ganeo*, *Loxogenes*, *Phyllodistomum*, *Astiotrema* and *Mehraorchis* involving gastropods as the intermediate hosts in the completion of their life-cycles (Duda & Verma, 1996).

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The parasite community forms an integral part of an ecosystem and is superimposed upon free-living communities which form intermediate and definitive hosts (Volnna, 1974). In nature, several species of parasites infect invariably every amphibian species, thereby bringing a stress on the population of the latter (Duda & Verma, 1996; Sathyanarayana & Sampath, 1996; Yamaguti, 1975) such a stress necessitates analysis of the composition of the helminth fauna infecting the amphibians.

A perusal of available literature reveals that a lot more attention has been paid to the taxonomic studies of these worms. Very little effort has been made to ascertain the extent of damage and losses incurred due to helminths on the herpetofauna; and, the present knowledge of parasitic helminths from Jammu amphibians and reptiles is spotty (Soodan, 1978; Khar & Duda 1980; Verma, 1988; Verma & Duda 1988; Duda & Verma, 1996; Singh, 1993; Duda *et al*., 1999). The present study was, therefore, undertaken to assess the prevalence and intensity of gastro-intestinal digeneans collected from ranid hosts in Jammu, Jammu and Kashmir State.

Materials and methods

The collections of helminths and their hosts has been described in detail elsewhere (Singh, 1993; see Table 1 for a list of the host collected). Collection for ecological analysis of the enteric

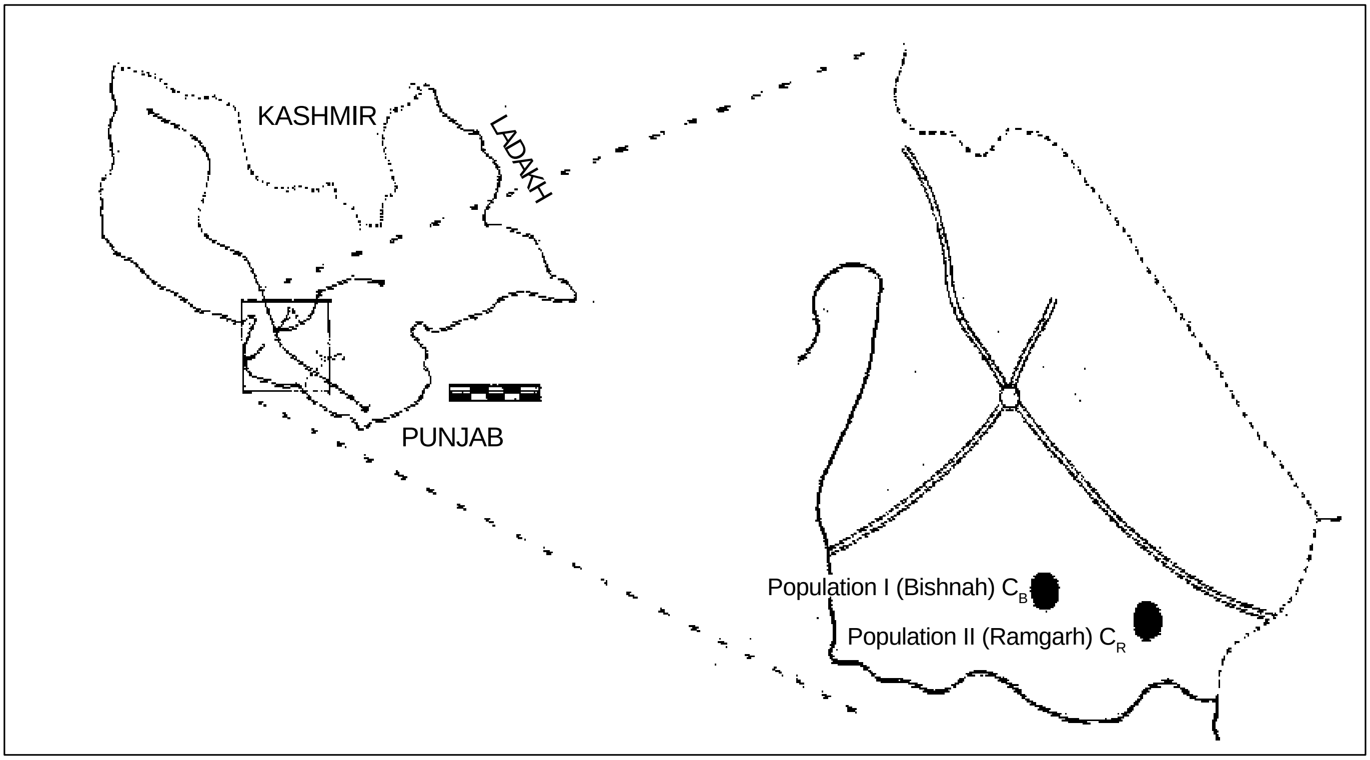

Figure 1. Map of Jammu District showing collection sites

helminths were made over a short period, from April to August 1998, in order to minimize the effect of seasonal variations on the helminth infra-community structure existing within these frog hosts.

The present data is based on 3,266 individual worms recovered from 283 frogs of the two species, *Hoplobatrachus tigerinus* and *Euphlyctis cyanophlyctis* out of a total 395 host individuals collected and screened for infection from two populations, Bishnah Population - C_B (Station-I) and Ramgarh Population - C_R (Station-II), two well defined and discrete sites lying about 25km. apart (Fig. 1). The terminology used is after Margolis and Arther (1979) and significance taken at $P < 0.05$ unless noted otherwise.

Prevalence is the percentage of infected frogs of each species; mean intensity is the number of worms per infected frog and the values expressed as mean S.D./S.E.; species-richness is the number of helminth species per frog.

Table 1. Overall collection data of the two hosts from two populations.

Host species	Bishnah population C_B (Station-I)			Ramgarh Population C_R (Station-II)		
	Sex	No.	Total	Sex	No.	Total
Hoplobatrachus tigerinus	M	68		M	61	
	F	57	125	F	70	131
Euphlyctis cyanophlyctis	M	138		M	108	
	F	168	306	F	134	242
Total number of hosts collected *			431			373

* Of the total sample sizes from the two populations of the host from the two sites, only 125 hosts of *H. tigerinus* and 270 of *E. cyanophlyctis* from the two populations were included for the analysis.

Table 2. Intestinal trematode species found parasitising the frogs,* Euphlyctis cyanophlyctis *and* Hoplobatrachus tigerinus *in two populations.

Trematode species	C_B Population				C_R Population			
	E. cyanophlyctis		*H. tigerinus*		*E. cyanophlyctis*		*H. tigerinus*	
	Male	Female	Male	Female	Male	Female	Male	Female
Diplodiscus mehrai	+	+	+	+	+	+	+	+
Ganeo tigrinus	+	+	+	+	+	+	+	+
Gorgoderina elliptica	+	+	-	-	+	+	-	-
Indopleurogenes yamaguti	+	+	-	-	+	+	-	-
Loxogenes jammuensis	-	+	-	-	+	-	-	-
Mehraorchis ranarum	+	+	-	-	-	-	+	-
Prosotocus himalayai	-	+	+	+	-	+	+	+

Chi-square (X^2) analysis for two sample comparison was used to determine significant differences in the prevalence and intensity, respectively, of the dominant helminth species across nested variables of the host sex and maturity status.

Chi-square (X^2) was estimated according to the formula

$$\frac{[(Fo - Fe) - 0.5]^2}{Fe}$$

Where, Fo is the observed frequency, Fe is the expected frequency and 0.5 is the Yates correction for continuity.

Chi-square test was also used for studying association between presence and absence of various pairs of helminth species.

Results and Discussion

One hundred and eighty-nine frogs of *Euphlyctis cyanophlyctis* (70% of the total number of the frog examined) and 94 frogs of *Hoplobatrachus tigerinus* (75.2% of the total number examined) from the two populations (C_B and C_R) were found infected with one or more of the seven intestinal fluke species (Table 2). While *E. cyanophlyctis* was found infected with all the seven trematode species, *H. tigerinus* was found infected by only four, which therefore constituted the shared species. However, all the species of the trematodes described herein were never found simultaneously infecting the same host individual. Only about 1 to 5 different species were, if ever found parasitizing the same individual.

In all 3,266 gastro-intestinal flukes were recovered from 283 infected frogs, of which 1,141 (about 35%) were recovered in the two hosts from C_R populations at an overall relative intensity (RI) of 8.5 and the remaining 2,125 (about 65%) in the two host species from C_B population (Table 3) at an overall relative intensity of 14.24. The helminths recovered from the two ranid species in C_R population were less evenly distributed (Table 4) in their host

Table 3. Sex-wise data on the number of intestinal flukes obtained from the two populations of the ranid hosts along with relative intensity (RI) of the flukes in each host.

Host	C_R Population			C_B Population				
	Sex (n)	Worms recovered	Relative Intensity	Sex (n)	Worms recovered	Relative Intensity	Total worms recovered	Overall Intensity
Euphlyctis cyanophlyctis	M (39)	188	4.82	M (36)	183	5.0	371 (n=75)	4.90
	F (46)	318	6.9	F (68)	839	12.3	1157 (n=114)	10.1
Hoplobatrachus tigerinus	M (23)	390	16.9	M (23)	832	36.1	1222 (n=46)	26.3
	F (26)	245	9.4	F (22)	271	12.3	516 (n=48)	10.75
Total	134	1141	8.5	149	2125	14.24	3266 (n=283)	11.54

Table 4. Overall distribution pattern of worm load in the two ranid hosts from two populations

Hosts	CR population (n=134)		CB population (n=149)		Total		
	Worm load (%)	n	Worm load (%)	n	Worm load (%)	n	Absolute intensity
Euphlyctis cyanophlyctis	506 (44.34)	85	1020 (48.00)	104	1526 (46.80)	189	8.08
Hoplobatrachus tigerinus	635 (55.66)	49	1103 (51.98)	45	1738 (53.30)	94	18.49

(44.3% in *E. cyanophlyctis* and 55.6% in *H. tigerinus*) than those in the two ranids of the C_B population (48.02% in *E. cyanophlyctis* and nearly 52% in *H. tigerinus*), although in either populations, *H. tigerinus* was found to show a relatively higher intensity than the other host species. Of the total 385 frogs examined from the two populations, 283 (71.65%) were found infected with gastro-intestinal flukes.

Of the seven enteric trematode species found in frogs in the two stations, only four species namely *Diplodiscus mehrai*, *Ganeo tigrinus*, *Prosotocus himalayai* and *Mehraorchis ranarum* were recovered from the two populations of *H. tigerinus*. Of these *M. ranarum* was obtained only once during the study period from a single male *H. tigerinus*, whereas the remaining species infected both sexes of this frog in C_B and C_R populations.

Of the seven species of enteric trematodes in the two frog species in C_B and C_R population, only four were found infecting both sexes in both population of *E. cyanophlyctis*; these were, *Diplodiscus mehrai*, *Ganeo tigrinus*, *Indopleurogenes yamaguti* and *Gorgoderina elleptica*, where as *Mehraorchis ranarum* was found infecting both the sexes of *E. cyanophlyctis* only in C_B population. This worm was not recovered in this frog host species in C_R population. The infection pattern of *Loxogenes jammuensis* was also ecologically bizzare in infecting only one male and one female frog from the two populations.

While *Prosotocus himalayai* was found as a parasite equally compatible in the intestines of both sexes of *H. tigerinus* in the two populations, this worm was recovered only in the female of *E. cyanophlyctis* in either population and never from its male members. *I. yamaguti* and *G. elliptica*, likewise, were found parasitising only *E. cyanophlyctis* but never *H. tigerinus* in either population despite their sympatry.

Prevalence of Infection

One thousand five hundred and twenty eight worms belonging to the trematode genera *Diplodiscus*, *Ganeo*, *Gorgoderina*, *Indopleurogenes*, *Loxogenes*, *Mehraorchis* and *Prosotocus* constituting 46.73 per cent of the total number of worms were found parasitic in the intestines of 189 specimens of *E. cyanophlyctis* in the two populations of the host. One thousand seven hundred and thirty eight trematode worms belonging to *Diplodiscus*, *Ganeo*, *Loxogenes* and *Mehraorchis* constituted 53. 27 per cent of the total number of trematodes obtained from the intestine of only 94 frogs of the other ranid host *H. tigerinus* drawn from similar habitats (C_B and C_R) during this study (Table 5).

Obviously, of the two host species, *E. cyanophlyctis* hosted a comparatively broader variety of trematode taxa (species richness) in its intestine in comparison to *H. tigerinus*, although both are sympatric and, therefore, equally exposed to similar set of factors and infections. However, the latter host species showed a relatively higher abundance (Table 5) and a higher mean intensity of worms (Table 6). Against 18.5 enteric worms infecting a frog of *H. tigerinus*, only 8.07 worms were found infecting an individual of *E. cyanophlyctis*; the difference of these mean intensities of the trematodes in the two host species from the two populations was found to be statistically significant (X^2 cal. = 4.3; X^2 tab. = 3.84 at $P < 0.05$).

While the male *E. cyanophlyctis* in the two host populations (C_B and C_R) shared an overall mean intensity of 4.94 at a more or less comparable intensity of 4.82 in the C_R population and 5.08 in the C_B population, the overall mean intensity of the intestinal

Table 5. Combined data on overall prevalence and abundance of infection in the two ranid hosts in two populations.

Host	Number examined	Number infected	Prevalence (%)	Number of worms	Abundance
Euphlyctis cyanophlyctis	270	189	70.0	1528	5.66
Hoplobatrachus tigerinus	125	94	75.2	1738	13.90

Table 6. Data on intensity and prevalence of enteric trematode infection in the two ranid host populations

		Ramgarh population (C_R) *E. cyanophlyctis* (85) *H. tigerinus* (49)			Bishnah population (C_B) *E. cyanophlyctis* (104) *H. tigerinus* (45)			Overall		Combined percentage	
Host (No. examined)	**Sex**	**Intensity**	**Host infected**	**Prevalence (%)**	**Intensity**	**Host infected**	**Prevalence (%)**	**Mean Intensity**	**Mean Prevalence**	**Intensity**	**Prevalence**
E. cyanophlyctis (n=270)	M	4.82	39	20.6	05.08	36	19.00	4.94	26.5	8.07*	66.78
	F	6.9	46	24.33	12.3	68	35.97	10.15	40.28		
H. tigerinus (n=125)	M	16.9	23	24.46	36.1	23	24.66	26.56	16.25	18.5*	33.22
	F	9.4	26	27.65	12.30	22	23.4	10.75	16.96		
Total (n=395)		8.51	134	47.34	14.24	149	52.65	11.53	100	11.53	100

(* =calculated = 4.3; tabulated = 3.84 at $P < .05$)

trematodes (Table 6) in the females of this host in the two populations was nearly twice as much (=10.15). Moreover, at population level, the mean intensity of infection in female *E. cyanophlyctis* from C_B population was significantly higher (Table 6) than that observed in the male in the same population.

Quite a reverse of such a situation regarding the intensity of infection was observed in *H. tigerinus* in the two populations. An overall intensity of only 10.75 was recorded in female hosts in the two populations which is favourably comparable to the overall intensity of infection in female of the other host species *E. cyanophlyctis*. In male *H. tigerinus*, on the other hand the overall mean intensity of infection was significantly higher being of the order of 26.56 (Table 6).

The higher intensity of infection in males than in females of *H. tigerinus* was recorded even separately for the host in the two populations. Against a mean intensity of 9.4 worms in female *H. tigerinus* in C_R population, the mean intensity of infection was found to be 12.3 (Table 6) in C_B population. On the contrary, the difference in the mean intensities of infection in male and female *H. tigerinus* was wider and statistically significant. Table 6 reveals that against a mean intensity of 12.3 in female *H. tigerinus* in C_B population, an intensity of 36.1 was recorded for the male of this ranid host species in the same population.

No significant sex-related difference was found in the prevalence of *D. mehrai*, *G. tigrinus* and *P. himalayai* between the male and female *H. tigerinus* from C_B population. In sympatric *E. cyanophlyctis* population, no gender related significant difference in the prevalence of *D. mehrai* and *G. elliptica* was found. However, the prevalence of *G. tigrinus* and *I. yamaguti* was significantly higher in female host of *E. cyanophlyctis* than in its males in C_B population.

In C_R population no significant difference was found in the prevalence of *D. mehrai* and *P. himalayai* between the infected female and male hosts of *H. tigerinus*. Although seemingly the prevalence of *G. tigrin*us was markedly higher in females than in males of *H. tigerinus*, yet the difference in the prevalence in the two sexes of the host in this population did not turn out to be statistically significant.

In *E. cyanophlyctis* in this population (C_R), no significant difference in the prevalence of *G. tigrinus* and *I. yamaguti* was found between the infected males and females of the host. Likewise, the prevalence of *D. mehrai*, though markedly higher, was not statistically significant in males than females of the host, whereas statistically significant gender related prevalence (73.4%) of *G. elliptica* infection was recorded in the females than in males of *E. cyanophlyctis*.

Combes (1972) has attributed the diversity of parasitic community structure to difference in the behaviour of the sexes of the hosts. While higher prevalence, intensity and abundance of dominant helminth species in specific sex of the host, (*Gorgoderina elliptica* in this case) could be attributed to behavioural differences between the male and female members of the host (Bush & Holmes, 1986a,b), immunological difference between the two sexes of the hosts cannot be excluded (Wassom *et al*., 1973; 1986).

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