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RESEARCH ARTICLE
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Prevalence of snail's intermediate host infected with different trematodes cercariae in and around Ranchi

M. N. Tigga[1], R. K. Bauri[1], A. R. Deb[2] and S. S. Kullu[3]

1. Division of Veterinary Parasitology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India; 2. Division of Veterinary Parasitology, Ranchi Veterinary College, Ranchi, Jharkhand, India; 3. Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India.

Corresponding author: Ram Krishna Bauri, email: drrkbauri@gmail.com, MNT: marynisha28@gmail.com, ARD: asitranjandeb@gmail.com, SSK: singrayishere@gmail.com

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Abstract

Aim: The objective of this study was to determine the prevalence of snail's intermediate host affected with larval stages of different trematodes.

Materials and Methods: Snails were collected from ponds, tanks, ditches, canals and crop fields in and around Ranchi. They were transported to the laboratory in water containers and maintained in small aquaria, supplemented with natural food stuff. Then, snails were screened randomly for their infection with different trematodes by cercarial shedding method.

Result: A total of 600 snails of different species mainly *Indoplanorbis*, *Gyraulus*, *Lymnaea* spp. and *Vivipara* were screened by cercarial shedding method out of which 44 (7.33%) were found positive for different trematodes cercariae. The percentage of infection in *Indoplanorbis* spp., *Lymneae* spp. and *Gyraulus* spp. were 7.22%, 8.60%, and 14.67%, respectively. Prevalence was higher in *Gyraulus* whereas, *Vivipara* did not show any infection with trematodes cercariae.

Conclusion: The present study reveals that *Indoplanorbis* spp., *Lymnaea* spp. and *Gyraulus* spp. are common snails found in and around Ranchi. These snails act as intermediate hosts having infective stages of parasites.

Keywords: intermediate host, snails, *Lymneae*, *Indoplanorbis*, *Gyraulus*, *Vivipara.*

Introduction

Snails belong to a large and highly diverse group of invertebrate known as the phylum - *Mollusca*, class - *Gastropoda* and order - *Stylommatophpra*. Snails act as intermediate hosts of different trematode parasites, in which several developing larval stages such as sporocysts, rediae and cercariae are set up [1]. The proportion of snails that release cercariae (prevalence of infection) and the number of cercariae released from each infected snail (intensity of infection) play important roles in the transmission of trematodes from the snail host. Only *Lymnaea* group of snails is involved in establishing of life cycle in at least 71 species of trematodes [2]. Other species of snails also transmit various trematode parasites of livestock and birds. For example, *Indoplanorbis exustus* is responsible for the transmission of *Schistosoma nasale*, *Schistosoma spindale* and *Schistosoma indicum* as well as other trematodes such as *Echinostoma* spp. and some spirorchids [3]. Age and size of snails, light conditions, temperature ranges, depth of water are some of the factors that appear to affect the prevalence and intensity of digenetic trematode infections in the snail intermediate hosts [4,5]. Among various water snails, such as *Lymnaea, Gyraulus, Vivipara* and *Indoplanorbis* spp. are the common snails found in and around Ranchi district.

Therefore, the present work was undertaken to determine the prevalence of snails intermediate host affected with different trematode larval stage in and around Ranchi.

Materials and Methods

Study area

The investigation was carried out in Ranchi district, located in the state of Jharkhand, India. Ranchi lies at 23°21′N 85°20′E. Ranchi has a humid subtropical climate, its location and the forests surrounding it combine to produce the unusually pleasant climate for which it is known. Summer temperatures range from 20°C to 42°C, and winter temperatures from 0°C to 25°C. December and January are the coolest months, with temperatures dipping to the freezing point in some areas. The annual rainfall is about 1430 mm (56.34 inches). From June to September the rainfall is about 1100 mm.

Collection of snails

The snails were collected month-wise from ponds, tanks, ditches, canals and crop fields in and around Ranchi. The collected snails were kept in the aquarium for further study.

Identification of snails

The collected snails were identified up to the genus according to their morphological characters as described by Gupta *et al.* [6] and Rao [7].

***Lymnaea* spp**

Spiral, not very much pointed and less acuminate, Shells are more ovate with a narrow ovate aperture, the outer lip not very much expanded and almost straight in outline.

***Indoplanorbis* spp**

Shells are large, whorls are rounded at periphery. Shells are usually not more than 5 mm in diameter.

***Gyraulus* spp**

Maximum diameter is rarely above 5 mm, whorls 4 or 5 rounded and all the whorls can be clearly seen from above, suture well-defined periphery subangulate closely and obliquely striate.

***Vivipara* spp**

Shells are thin more or less smooth with three or spiral bands, Embryonic shells are delicate and thin with three primary rows of chaetae, low ridges are well developed, secondary ridges bearing chaetae may develop between the primary ones.

Identification of infective stage of the parasite in the intermediate hosts

The collected snails were washed and kept in a test tube of 1″ diameter half filled with tap water and tubes were exposed to direct sunlight for 1-4 h in the day time for observing the emergence of phototrophic cercariae. The non-phototrophic cercariae emerging in the absence of sunlight were also being searched after keeping them in shed under laboratory condition. In a cloudy day, snails were placed against a glowing 200 w electric bulb for 4-5 h and snails were examined up to 5 days. After emergence of different types of cercariae, a small number of each species of cercariae was fixed in 10% formalin and then stained with the borax carmine for microscopic examination as per the method developed by Jithendran and Krishna [8] (Figure-1).

Statistical analysis

Raw data were entered into a Microsoft excel spreadsheet and descriptive statistics were used to summarize the data. The prevalence was calculated for all data as the number of infected individuals divided by number of individuals examined and multiplied by 100 to express in percentage. Chi-square test was used to assess the association of risk factors on the prevalence of parasites. The data were analyzed statistically using SPSS software (IBM Software Company, USA) to determine the prevalence.

Result

A total of 600 snails was identified on the basis of morphological characters belonged to the Genus of *Indoplanorbis* spp., *Gyraulus* spp., *Lymnaea* spp. and *Vivipara* spp. Out of 600 snails, 44 (7.33%) were found infected with different cercariae of trematodes parasites.

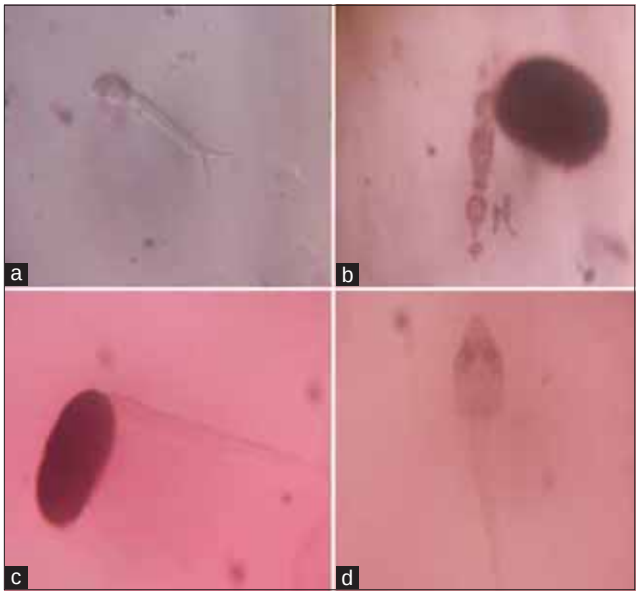

Figure-1: Different types of cercariae found in snail's body.

The overall prevalence of the cercarial emergence from different species of snails in and around Ranchi, were in *Indoplanorbis* spp. (7.22*%),* in *Gyraulus* spp. (14.67%) and in *Lymnaea* spp. (8.60%) (Table-1).

The overall month-wise prevalence of cercarial infection in *Indoplanorbis* spp. were in May (3.70%), June (2.86%), July (7.14%), August (6.67%), September (12.12%) and October (9.76%). Data indicated that there was no significant difference in the prevalence of infection between different months (Table-2). In *Gyraulus* spp. percentage of infection were in May (0%), June (0%), July (11.11%), August (12.50%), September (10.71%) and October (25.00%). However, there was no significant difference in the prevalence of infection between different months (Table-3). In *Lymnaea* spp. percentage of infection were in May (2.56%), June (4.35%), July (5.13%), August (10.87%), September (12.90%) and October (25.00%). Data revealed that there was significant difference in prevalence of infection and month of October showed higher (25%) infection rate, whereas month of may showed less number of infections (Table-4).

Prevalence data indicated that the percentage of infections in different species of snails was in May (2.00%), June (3.00%), July (5.00%), August (9.00%), September (11.00%) and October (14.00%). Chi-square value showed prevalence rate was significantly higher in the month of October, whereas, in the month of May less number of infections were observed (Table-5).

Discussion

The present study was undertaken to record the prevalence of snail's intermediate host infected with different trematodes cercariae in and around Ranchi. An overall prevalence of snail's intermediate host infected with different trematodes cercariae was recorded as

Table-1: Percentage of parasitic infection in different snails in and around Ranchi.

Months	Snails	Number of snails examined	Number of snails positive	Percentage of infection
May	*Indoplanorbis* spp.	27	1	3.70
	Gyraulus spp.	2	-	-
	Lymneae spp.	39	1	2.56
	Vivipara spp.	32	-	-
	Total	100	2	2.00
June	*Indoplanorbis* spp.	35	1	2.86
	Gyraulus spp.	-	-	-
	Lymnaea spp.	46	2	4.35
	Viviparas spp.	19	-	-
	Total	100	3	3.00
July	*Indoplanorbis* spp.	28	2	7.14
	Gyraulus spp.	9	1	11.11
	Lymnaea spp.	39	2	5.13
	Vivipara spp.	24	-	-
	Total	100	5	5.00
August	*Indoplanorbis* spp.	30	2	6.67
	Gyraulus spp.	16	2	12.50
	Lymnaea spp.	46	5	10.87
	Vivipara spp.	8	-	-
	Total	100	9	9.00
September	*Indoplanorbis* spp.	33	4	12.12
	Gyraulus spp.	28	3	10.71
	Lymnaea spp.	31	4	12.90
	Vivipara spp.	8	-	-
	Total	100	11	11.00
October	*Indoplanorbis* spp.	41	4	9.76
	Gyraulus spp.	20	5	25.00
	Lymnaea spp.	36	5	13.89
	Vivipara spp.	3	-	-
	Total	100	14	14.00
	Grand total	600	44	7.33

Table-2: Overall month-wise prevalence of *Indoplanorbis* spp. having infective stages of parasites in and around Ranchi.

Month	Number of snails collected	Number of snails found positive	Percentage of prevalence	χ^2 value
May	27	1	3.70	3.085 NS
June	35	1	2.86	
July	28	2	7.14	
August	30	2	6.67	
September	33	4	12.12	
October	41	4	9.76	
Total	194	14	7.22	

χ^2 test of significance for overall month wise comparison of *Indoplanorbis* spp. **p<0.01, *p<0.05, NS=Non significant

Table-3: Overall month wise prevalence of *Gyraulus* spp. having infective stages of parasites in and around Ranchi.

Month	Number of snails collected	Number of snails found positive	Percentage of prevalence	χ^2 value
May	2	0	0	2.257 NS
June	0	0	0	
July	9	1	11.11	
August	16	2	12.50	
September	28	3	10.71	
October	20	5	25.00	
Total	75	11	14.67	

χ^2 test of significance for overall month wise comparison of *Gyraulus* spp. **p<0.01, *p<0.05, NS=Non-significant

Table-4: Overall month wise prevalence of *Lymnaea* spp. having infective stages of parasites in and around Ranchi.

Month	Number of snails collected	Number of snails found positive	Percentage of prevalence	χ^2 value
May	39	1	2.56	11.342*
June	46	2	4.35	
July	39	2	5.13	
August	46	5	10.87	
September	31	4	12.90	
October	20	5	25.00	
Total	221	19	8.60	

χ^2 test of significance for overall month wise comparison of *Lymnaea* spp. **p<0.01, *p<0.05, NS=non-significant

7.33%. Islam *et al*. [9] supports our findings where they reported that 5.3% snails were found infective with different trematodes cercariae. Whereas, Jayawardena *et al*. [10] reported higher prevalence rate, and their found that 16% snails were infected with different trematodes cercariae in Srilanka. Chontananarth and Wongsawad [11] also found similar findings, about 17.27% in fresh water snails infected with different trematodes cercariae in Chiang Mai province. Imani-Baran *et al*. [12] also reported a high prevalence rate of cercarial infection 74.56 % in *Lymnaea auricularia* snails. Whereas, Sharif *et al*. [13] reported a low prevalence rate of cercarial infection in snails, he observed only 3.9% *L. gedrosiana* was infected by different trematodes cercariae.

Table-5: Overall month-wise prevalence of different snails having infective stages of parasites in and around Ranchi.

Month	Number of snails collected	Number of snails found positive	Percentage of prevalence	χ^2 value
May	100	2	2.00	16.678**
June	100	3	3.00	
July	100	5	5.00	
August	100	9	9.00	
September	100	11	11.00	
October	100	14	14.00	
Total	600	44	7.33	

χ^2 test of significance for overall month-wise comparison of different snails. **p<0.01, *p<0.05, NS=Non-significant

Data indicated that the percentage of infection in snails was higher in July to October. Yadav *et al*., [14] also reported that the prevalence of the infection in snails was highest (4.18%) during the rainy season (July-October) whereas; it was lowest (0.76%) during the summer (March-June). Singh *et al*. [15] also reported similar result; the number of snails shedding cercariae was highest during the rainy season (8.31%), followed by winter (6.87%) and summer (2.31%). No furcocercus cercariae were found in *I. exustus* during the summer. Loy and Haas [16] also support our findings with the mean prevalence of most cercarial species increased from May-June to August-October. The results of our study are in agreement with that of these workers. However, it is not in agreement with the findings of Pfukenyi *et al*. [17] reported Amphistome cercariae were recorded from both the highveld and lowveld areas with peak prevalence during the post-rainy season (March to May). Likewise, prevalence of Fasciola infection in *L. auricularia* of tarai region was highest in May (18.06%) and September, (17.84%) and lowest in April (0.8%) and November (1.18%) reported by Garg *et al*. [18]. Prevalence rate was high in our observation, and it may be due to the snails being infected by the parasitic stage, i.e., cercarial stage during mid-rainy season when the eggs containing miracidium, are being carried by water to low-lying areas where snails breed. The snails get infected by the parasites in about 1-1½ months before and in the next 1-1½ months the natural cercariae emerge out from the snails. The maximum emergence of cercariae from snails is noted in early winter months. The percentage of cercarial emergence during winter months have also been reported by Prasad [19], Sahai [20], Rajkhowa *et al*. [21] and Kumar [22] from different regions of the country.

Conclusions and Recommendations

Several snails' species were found in and around Ranchi that act as intermediate hosts of different trematodes that affect our livestock and birds. In this study we identified the prevalence of trematodes cercariae on the basis of cercarial shedding, but this traditional method has several problems; first this method is tedious and time consuming and second it does not gives actual prevalence rate because this method depends upon cercarial shedding whereas in the case of prepatent infection it gives a false result. Due to these drawbacks parasitologists utilize molecular techniques for detection and characterization of parasites within their intermediate and final hosts. The development of a molecular approach for cercarial detection in infected snails is necessary and will be useful, such as polymerase chain reaction (PCR). Various conventional PCR assays have been developed to detect *Haplorchis taichui* DNA in feces, definitive hosts and intermediate hosts. Van *et al*. [23] separated larvae and adult stages of *H. taichui* and *Haplorchis pumilio* using ITS-2 gene sequencing. Kozak and Wedrychowicz [24] developed a PCR assay for the detection of *Fasciola hepatica* in *Galba truncatula* and found the overall prevalence of *F. hepatica* infection was 26.6%. Caron *et al*. [25] also optimized the multiplex PCR for the detection of *Fasciola* spp. in lymnaeid snails and found 6.25% snails were positive for *Fasciola* spp. Molecular approaches for sensitive and specific detection of the parasite species in the snail hosts are not being used routinely in our country; with only a few reports on the use of PCR and PCR-restriction fragment length polymorphism in the field detection of trematode infected snails being available. Therefore, it is recommended to use molecular techniques to diagnose the actual prevalence of snail's intermediate hosts infected with cercariae of different trematodes.

Author's Contributions

ARD and RKB designed the work, MNT carried out the work. SSK helped in manuscript preparation; ARD and SSK collected materials for manuscript. MNT and RKB collected samples. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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