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PREVALENCE AND TREMATODE INFECTION OF FRESHWATER SNAILS WITH EMPHASIS ON FASCIOLOSIS IN PUNJAB, PAKISTAN

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ABSTRACT

Many snails act as an intermediate host to spread parasitic diseases in humans and animals. Studies on parasitic infections in freshwater snails are rare in Pakistan. Freshwater snails were collected on monthly basis from the northeastern areas of Punjab for the period of one year (June 2005 to May 2006). Snails were exposed to light and also crushed to assess the presence of larval Trematodes in them. Larvae were identified by microscopy. A total of 23,922 snails, comprising eight genera, were collected from six water bodies and among these, prevalence of the genus *Indoplanorbis* was found highest (23.4%), followed by *Bulinus* (21.5%), *Oncomelonia* (15.4%), *Lymnaea* (14.9%), *Bellamya* (10.6%), *Gyraulus* (7.6%), *Melanoides* (3.8%) and *Physa* (2.8%). All snails were examined for Trematode infection, from which, 3541 (14.8%) showed the presence of Trematode larvae. Four morphologically distinguishable types of *Cercariae* were identified. Most Trematode infections were recorded from *Lymnaea*, which harboured three morphologically different *Cercariae*. Amphistome was the commonest types of c*ercariae* (67.07%) recorded, while Xiphidiocercaria cercariae (0.82%) was the least common. No trematodal *cercariae* were recovered from *Oncomelonia*, *Bellamya*, *Melanoides*, *Gyralus* and *Physa*. The only intermediate host for *Fasciola* cecariea were *Lymnaea* snails. Infection of these snails with *Fasciola* cecariae was influenced by temperature and rainfall.
Keywords: Trematode infection, freshwater snails, fasciolosis, Pakistan

INTRODUCTION

Many snails act as intermediate host of parasites and spread parasitic diseases in humans and animals (Mas-Coma and Bargues, 1997; Bargues et al., 2001). For example, snails of genus *Filopaludina*, *Melanoides*, *Lymnaea*, *Bulinus* and *Planorbis* were reported as intermediate hosts of *Echinostoma spp*. *Fasciola spp*. and *Schistosoma spp.* and play important role for their transmission (Isaac, 2009; Chai et al., 2011; Aldhuun et al., 2012; Niaz et al., 2013).

Temperature, rainfall (water), contact with trematode eggs containing cattle faeces and other ecological factors such as vegetation, altitude and nature of substratum may affect the development of parasites within snails. Susceptibility of snails to trematodes may be dependent to some extent on the environmental conditions at the time of infection (Chen, 1993; Madsen, 2003).

In Pakistan, researchers worked on prevalence and ecology of fresh water snails (Akhtar and Khan, 1989; Akhtar, 1990; Mughal, 1993; Arshad et al., 2011), but studies on larval trematode infections in freshwater snails, still needs attention.

The present study addresses the infection of freshwater snails with larval trematodes and emphasizes to assess intermediate snail host of *Fasciola spp*. larvae, in Punjab, Pakistan.

MATERIALS AND METHODS

Snail collection

Snails were collected from six water bodies of Punjab for the period of one year (June 2005 to May 2006); as Upper Chenab canal Sheikhupura, Nullahdek canal Muridke, Wali Poota Farm Kamonki, Nandipur canal Gujranwala, River Ravi adjacent to Shahdra and Noor pur canal Kasur (Fig 1). Snails were collected with net scoop and by hand and brought to laboratory in glass jars covered with cotton plugs to allow aeration. During transit, natural vegetation found at collection site was used as food for snails, while spinach leaves were provided as a food in the laboratory and kept at room temperature (25.0±2.0^{o}C) in earthen pots of 1320 cm^2 with 2.5liter ordinary tap water (Tanveer and Khan, 1989). They

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were identified to genus level as described by Brown (1994).

Examination of cecariea

All snails were carefully washed. Each snail was isolated in glass tubes 3x1" about 3/4th filled with tap water and exposed them for few hours to light source e.g., by placing bottles under a gooseneck lamp. Then from each bottle, water was examined, in petri dish under dissecting microscope for the presence of cercariae. Cercarial morphology was studied by transferring them with a pipette to a glass slide and covering them with a cover slip. Slight thumb pressure was exerted to remove extra water and to slow down their activity for observation under microscope (10x10). Identification of cercariae was carried out as described by Roberts and Janovy (2000).

Meteorological Data:

Meteorological data including maximum and minimum temperature(C), relative humidity (%), rainfall (mm) and pan evaporation (mm) was obtained from meteorological station, Lahore for all snail collection sites.

Statistical Analysis:

Prevalence of snails and cercariae was calcualated in percentage (%) and tabulated area, month and season wise. Chi-square (χ^2) test was used for statistical analysis at 95% Confidence Interval (C.I) and probability (P value) less than 0.05 ($P<0.05$) was considered significant for comparison of these parameters. Correlation between climatic factors and prevalence of *Fasciola spp*. cercarae in snail hosts was found out by linear regression.

RESULTS

Overall Prevalence of snails' genera

A total of 23,922 snails of eight genera were collected from six fresh water bodies of Punjab. Prevalence of *Indoplanorbis* was found highest (23.4%), followed by *Bulinus* (21.5%), *Oncomelonia* (15.4%), *Lymnaea* (14.9), *Bellamya* (10.6%), *Gyralus* (7.6%), *Melanoides* (3.8%) and *Physa* (2.8%) (Fig 2a). Over all area wise prevalence was highest in Kamonki (20.16%) followed by Muridke (18.1%), Kasur (16.0%), Shahdra (15.88%), Gujranwala (14.97%) and Sheikhupura (14.44%) (Fig 2b).

In monthly data, over all prevalence of snails was highest in May (12.82%) and lowest in January (0.79%). Overall monthly prevalence's of snails genera were observed significantly variable in a year (P=0.048) (Fig 2c).

In overall season wise data highest prevalence of snails was observed in summer (45.9%) followed by spring (20.3%), autumn (19.28%), and lowest in winter (14.53%). Overall seasonal prevalence was also significantly variable (P=0.00) (Fig 2d).

Prevalence of trematodal cercariea:

All snails were examined for trematode infection. Only 14.8% showed presence of Trematode larvae (Table 1). Four morphologically distinguishable types of cercariae were identified. Most Trematode infections were recorded from *Lymnaea*, which harboured three morphologically different cercariae. Amphistome (67.07%) was the commonest types of cercaria recorded, followed by gymnocephalus *Fasciola cercaria* (22.11%), *Schistosoma furcocerus* ccercariae (9.99%) while *Xiphidiocercariea* (0.82%) were the least common. No Trematodes were recovered from *Oncomelonia*, *Bellamya*, *Melanoides*, *Gyralus* and *Physa* (Table 1 and 2).

Prevalence of *Fasciola* cecaria in *Lymnea* snail host:

The only intermediate host for *Fasciola* cecaria was *Lymnaea* snails. Overall, 3565 *Lymnea* snails were collected and *Fasciola sp*. cercariea was found in 22.0% (Table 1).

Area wise

Over all area wise infection of *Lymnea* snails was highest in Kamonki (27.97%) followed by Muridke (26.65%), Shahdra (22.71%), Kasur (21.36%), Gujranwala (18.8%) and Sheikhupura (12.62%). Chi square (χ^2) test showed overall non significant difference between prevalence of all areas (χ^2= 6.90, df=5, P=0.23) (Table 3).

Month wise

In month wise data, over all infection snails was highest in July (40.11%) and lowest in January (0.0%). Significant variation was noted in overall monthly prevalence of all genera (χ^2= 44.14, d.f=11, P=0.00), (Table 4).

Season wise

When data was tabulated season wise, highest infection of snails was observed in summer

(27.74%) followed by autumn (22.1%), winter (16.15%) and lowest in spring (10.24%) (Table 4). Statistically analysis revealed significant differences between infection in all seasons (χ^2= 9.47, df=3, P=0.024) as indicated in Table – 5.

Correlation between prevalence of *Fasciola* cecaria and Metreological factors: Overall average meteorological data of all study areas is shown in Table 6. Linear regression was calculated to find out correlation between infection and climatic factors. It was found significantly (P<0.05) positive with temperature and pan evaporation. Although rainfall also showed positive relation with snail infection but not at significant level, while non-significant negative relation was noted with relative humidity (Fig 3a, b, c & d).

Table 1: The prevalence (%) of Trematodecercarie in fresh water snails of Punjab, Pakistan

Snails' genera	No. of snails Collected	Types of Trematode cercarie	No. of snails infected (%)
Lymnaea	3565	• *Fasciola* cercariae (Gymnocephalous) • Amphistome • Xiphidiocercaria cercariae • **Overall**	783 (**22.0**) 310 (**8.7**) 29 (**0.8**) **1122** (**31.5**)
Indoplanorbis	5593	• Amphistome • *Schistosoma* cercariae (Furcocercaria) • **Overall**	1918 (**34.3**) 354 (**9.6**) **2272** (**40.6**)
Bulinus	3683	• Amphistome • **Overall**	147(4.0) **147** (**4.0**)
Oncomelonia	5138	None	-
Bellamya	2538	None	-
Gyraulus	1816	None	-
Melanoides	911	None	-
Physa	678	None	-
Total	**23922**	-	**3541 (14.8)**

Table 2: Overall prevalence (%) of trematode cercariae in fresh water snails Punjab, Pakistan

Type of Trematodecercarie	Prevalence **(%)** n*=3541
Amphistome	2375 **(67.07)**
Fasciola cercariae (Gymnocephalous)	783 (**22.11**)
Schistosoma cercariae (Furcocercaria)	354 (**9.99)**
Xiphidiocercaria cercariae	29 **(0.82)**

n*=total no. of infected snails

Table 3: Area wise prevalence of Fasciola infection in Lymnaea snails

Area	Total collected	Infected (%)
Sheikhupura	531	67 **(12.62)**
Gujranwala	682	128 **(18.8)**
Kamonki	740	207 **(27.97)**
Muridke	559	149 **(26.65)**
Shahdra	524	119 **(22.71)**
Kasur	529	114 **(21.5)**
Total	**3565**	783 **(22.0)**

Table 4: Monthly prevalence of *Fasciola* infection in *Lymnaea* snails

Month	Total collected	Infected (%)
June	650	119 (**18.31)**
July	713	286 (**40.11)**
Aug	89	27 (**30.3)**
Sep	79	19 (**22.78)**
Oct	75	15 (**20.0)**
Nov	71	16 (**22.53)**
Dec	26	4 (**15.4)**
Jan	6	0 (**0.0)**
Feb	58	5 (**8.62)**
March	293	37 (**12.63)**
April	722	70 (**9.7)**
May	783	190 (**24.3)**

Table 5: Seasonal prevalence of *Fasciola* infection in *Lymnaea* snails

Season	Total collected	Infected (%)
Summer	2235	620 (27.74)
Autumn	154	34 (22.1)
Winter	161	26 (16.15)
Spring	1015	104 (10.24)

Table 6: Overall average Meteorological data including temperature (^{o}C), relative humidity (%), rainfall (mm) and pan evaporation (mm) from June 2005-May 2006

Time (months)	Temperature (^{o}C)			Relative Humidity (%)			Rainfall (mm)	Pan evaporation (mm)
	Min	Max	Mean	Morning (Max)	Evening (Min)	Mean		
June	29.2	40.5	**34.85**	47	29	**38**	32.0	9.9
July	26.7	34.3	**30.5**	87	68	**77.5**	223.5	4.2
Aug	27.3	35.3	**31.3**	76	58	**67**	129.0	5.3
Sep	25.7	34.1	**29.9**	77	59	**68**	69.8	4.4
Oct	20.5	32.5	**26.5**	69	39	**54**	0.8	3.9
Nov	13.8	27.1	**20.45**	71	40	**55.5**	0.001	2.6
Dec	7.0	21.6	**14.3**	82	45	**63.5**	0.0	1.8
Jan	8.4	19.7	**14.05**	75	50	**62.5**	18.9	2.3
Feb	14.7	26.7	**20.7**	76	44	**60**	4.9	2.4
March	16.1	26.8	**21.45**	68	43	**55.5**	42.1	5.1
April	22.7	35.7	**29.2**	39	20	**29.5**	0.001*	7.4
May	27.4	39.5	**33.45**	51	28	**39.5**	22.4	7.8
Mean	**19.96±2.24**	**31.15± 1.9**	**25.55± 2.1**	**68.17± 4.3**	**43.58± 4.0**	**55.88± 4.0**	**45.28± 19.6**	**4.76± 0.72**

*0.001= rain in traces

Fig 1: Map showing snails collection areas.

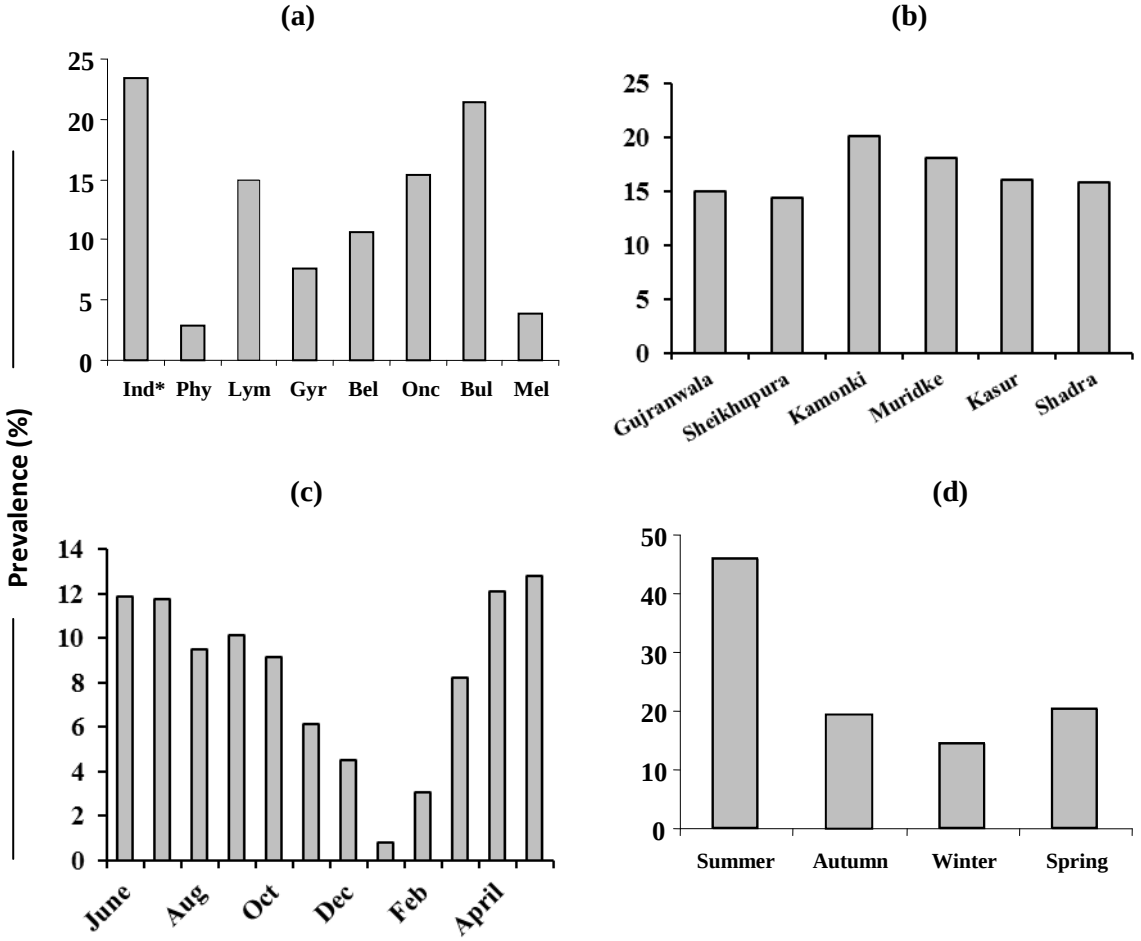

Fig 2: Overall Prevalence of snails in Punjab, Pakistan: (a) genus wise, (b) area wise, (c) month wise and (d) season wise.

*Ind=*Indoplanorbis*; Phy=*Physa*; Lym=*Lymnaea*; Gyr=*Gyraulus;* Bel= *Bellamya*; Onc=*Oncomelonia*; Bul=*Bulinus*; Mel=*Melanoides*

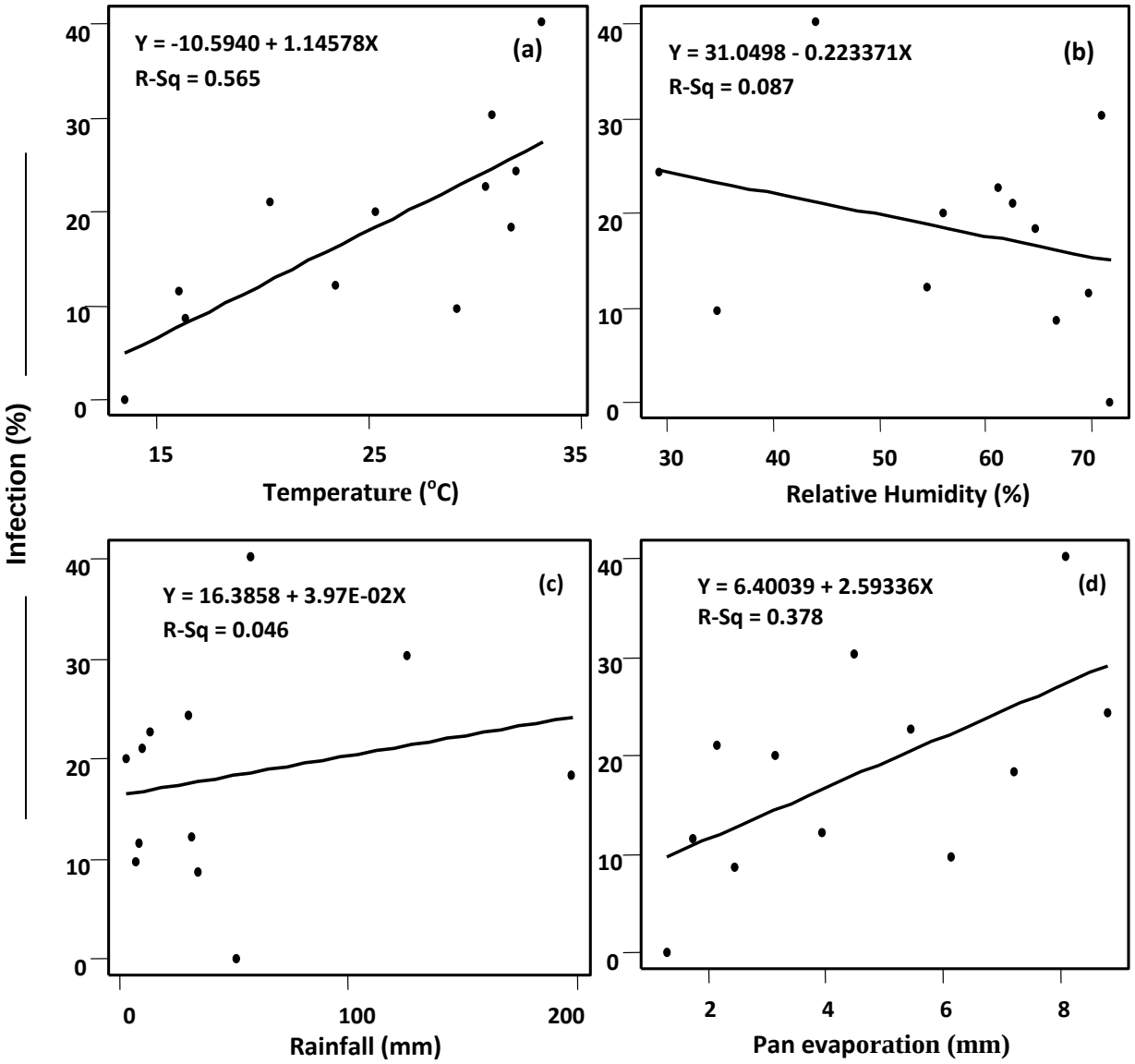

Fig 3: Linear Regression plot showing relationship between *Lymnaea* snail infection and metreological factors: (a) Temperature (°C), (b) Relative Humidity (%), (c) Rainfall (mm) and (d) Pan evaporation (mm).

DISCUSSION

One of the most important factors that influence the occurrence of fasciolosis in an area is availability of suitable snail host. Snails play very important role in the transmission of *Fasciola sp*. as they act as intermediate host in which three larval stages (sporocyst, rediae and cercariae) of life cycle are completed. In Pakistan ideal environmental conditions exists for the development of snails (Tanveer and Khan, 1989).

The snails belonged to genera *Indoplanorbis*, *Bulinus*, *Oncomelania*, *Lymnaea*, *Bellamya*, *Gyraulus*, *Melanoides* and *Physa* were found prevalent in present study. Other workers also reported the presence of these snails in Punjab, Pakistan (Tanveer and Khan, 1989; Maqbool et al., 1998, 2003; Khan et al., 2008)

In our study, area wise prevalence was highest in Kamonki followed by Muridke, Kasur, Shahdra, Gujranwala and lowest in Sheikhupura. Some other workers also reported variable prevalence of these snails in different areas of Punjab, Pakistan (Hussain et al., 1996;

Maqbool et al., 1998, 2003). The difference in area wise prevalence of snails was influenced by presence or absence of water reservoirs, canals, rivers and may be due to environmental and managemental conditions. Similar findings were reported from Pakistan and other countries (Tanveer 1990; Pfukenyiet al., 2005; Rondelaud et al., 2007).

Monthly data showed overall significant variation in prevalence of all snails genera. Higher number of snails were observed in May and then they start decreasing when temperature further increases in June (upto 40.5°C) and lowest prevalence was noted in January when temperature decreases below 20°C. Temperature plays very important role in snails growth rate. Below 20°C and above 40°C temperature found detrimental effect on breeding of different snails' genera (Tanveer and Khan, 1989). Seasonal data also showed in summer significantly higher number of snails. Almost all genera had highest prevalence in summer season except *Bellmaya* and *Oncomelania* which showed highest prevalence in winter and autumn, respectively while lowest prevalence of *Indoplanorbis, Physa* and *Oncomelania* was noted in winter, in *Bellmaya, Gyraulus, Bulinus* and *Melonoides* in spring and *Lymnaea* showed in autumn. Previous studies on monthly and seasonal variation also showed similar pattern of snails' abundance in Punjab, Pakistan (Tanveer and Khan, 1989; Maqbool et al., 1998; Khan et al., 2008).

In current study only *Lymnaea spp*. snails were harbouring *Fasciola spp*. carcariae (gymnocephalus) indicating the only intermediate host in study areas. Previous studies from other areas of Punjab, also reported Lymnnaeid snails to be infected with cercariae of *Fasciola* species (Parveen, 1977; Hussain et al., 1996; Maqbool et al., 2003). Snails of genus *Lymnaea* were also reported as an intermediate host of *Fasciola hepatica* and *F. gigantica* in other parts of world (Abrous et al., 1999; Vignoles, 2001; Remigio, 2002; Tiwari et al., 2004; Cucheret al., 2006; Dreyfuss et al., 2007a, b).

Over all area wise frequency of presence of *Fasciola* cercariae infection of *Lymnea* snails varied from one place to another. This may be due to variations in local habitats and the availability of the hosts and infective stages at the time of exposure. The highest prevalence at Kamonki indicated that in this area, the environmental conditions are more suitable for snails breeding.

In relation to metreological factors it was noted that *Lymnaea* snails showed highest infection (40.11%) in July when average temperature was dropped to 30.5^{o}C from 34.85^{o}C, average relative humidity was 77.5%, pan evaporation 4.2 mm and highest rainfall was recorded i.e., 223.5mm in this month. Linear regression between snails' infection and these climatic factors showed that temperature and pan evaporation favour the infection significantly ($P<0.05$), rainfall had non-significant positive relation, while humidity also had non-significant but negative relation. It was reported that the favourable temperature for snails' survival was in the months of July and August (late summer), while in January temperature was noted detrimental for snails (Tanveer, 1990; Hussain et al., 1996).

Other type of cercariae found in *Lymnaea* snail were amphistome and xiphidocercaria. Presence of amphistome cercaria was also noted in *Indoplanorbis* and *Bulinus*, while *Indoplanorbis* was also harbouring *Schistosoma* cercariae (Furcocercous). The presence of amphistome cercariae of *Paramphistoma* in *Indoplanorbis* and *Lymnaea* snails was also reported previously in Pakistan (Khan et al., 2008) while in studies from other areas of world, *Bulinus* snails were also reported as intermediate host of this type of cercaria (Pfukenyi et al., 2005; Phiri et al., 2007). *Lymnaea* snails were also found infected with xiphidocercariae in our study. Presence of these cercariae was also reported from Southern Germany (Loy and Haas 2007), while presence of *Schistosoma* cercariae in *Indoplanobis* snails was also reported from Punjab and Sindh in Pakistan (Naeem, 1998; Arshad et al., 2011; Arijo et al., 2005; Niaz et al., 2013). Some workers from other parts of world also reported *Indoplanorbis* as an intermediate host of *Schistosoma* (Bungurn et al., 2005; Chiewet al., 2007; Samanta el al., 2007; Sri-Aroonet al., 2007). Host specificity in mollusk trematode relationships have been described by many malacologists. The attraction of miracidia to a specific snail host is influenced by physico-chemical factors present in the environment. The cycle of transmission for larval helminths in freshwater habitats, also depends its accessibility to definitive hosts (Upatham, 1972; Sturrockand Upatham, 1973; Mattison et al., 1995).

No trematodes were recovered from *Oncomelonia*, *Bellamya*, *Melanoides*, *Gyralus* and *Physa*. This findings agree with other studies (Phiri et al., 2007; Jayawardena et al., 2010), which reported absence of trematodes from these snails. Snails in which no larval trematodes were recorded could have been resistant to infection. Resistance of snails to trematode infection has been reported to play a role in determining infection rates (Bayneet al., 1989; Chingwenaet al., 2002).

This study provides preliminary information about the distribution and prevalence of *Fasciola* and other larval trematode species in snail population of some areas of Punjab. This data can be helpful to develop control strategies for snail-borne parasites of medical and veterinary importance, not only in study areas but also in other localities of similar climatic conditions. However, further study by PCR method would be able to detect the species of snails host and larval trematodes.

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