

## Prevalence of *Fasciola Cercariae* in Snail (Intermediate host) and its Correlation with Various Meteorological Factors in Bahawalpur, Pakistan

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### المخلص

لم تستكشف مجموعة حيوان القواقع و أمراض الطفيليات المصاحبة لها في بهاولبور. جمعت القواقع عشوائياً من النباتات و قيعان القنوات و المناطق المحيطة بها لكل مركز بهاولبور في الفترة من فبراير إلى أكتوبر من عام 2013. أُستبين وجود ذنائب المتورقة في تلك القواقع بواسطة فاعلية طرح الذنائب في الماء. فُحصت الذنائب تحت المجهر، بعد تعريض القواقع للضوء العادي لمدة 24 ساعة، ووضع قطرات من الماء على شريحة مجهرية. جُمعت 4012 قوقعة، من بينها وُجد أن قوقع ليمينا (360) فقط هي التي تؤوى ذنائب المتورقة بمدى إنتشار كلي 11.7%. إحصائياً، وجد أن تأثير درجة الحرارة (16.1-35°C) ومعدل الأمطار (0-53.6 مم) على إنتشار المرض غير جوهرياً ( $P>0.05$ ) بينما كان تأثير الرطوبة النسبية (35%-68.6%) جوهرياً ( $P>0.05$ ). ختاماً، أُستنتج أن غزو المتورقة ذو إنتشار هام و من المحتمل أن تصاحبه خسارة إقتصادية مقدرة.

### Summary

Bahawalpur has been unexplored for its snail fauna and parasitic diseases. Snails were collected on random basis from the vegetation, canals bottoms and areas surrounding canals from all Bahawalpur district between February and October 2013. These were examined for presence of *Fasciola cercariae* by cercariae shedding activity in water. A total of 4012 snails was collected, of which only *Lymnaea* snails (360) were found to harbour *Fasciola cercariae* with overall prevalence rate of 11.7%. Statistically, an insignificant effect ( $P>0.05$ ) of temperature (16.1-35 °C) and rainfall (0-53.6 mm) on the disease prevalence was found, while a significant effect ( $P<0.05$ ) was recorded between the disease prevalence and humidity (35-68.6 %). It is concluded that, in Bahawalpur district, infestations of *Fasciola* have a significant prevalence, with probably a significant related economic loss.

### Introduction

The most significant zoonotic water-borne helminthic diseases are either snail-borne, copepod-borne or transmitted by water intake contaminated with faeces. Snail-borne helminthiasis, including schistosomiasis, cercarial dermatitis, fasciolosis and fasciolopsiasis, are caused by digenetic trematodes (Platyhelminthes) that undergo complex life cycles involving various species of aquatic snails (Nithiuthai *et al.*, 2004). In all of these infections, the snails (Gastropoda) act as intermediate hosts during the life cycle of these trematode parasites infecting livestock and humans as well (Mushtaq *et al.*, 2011). Fresh water snails greatly influence prevalence of fasciolosis, because they are highly abundant and widely distributed. Furthermore, their enormous production of cercariae is another factor which is involved in

their transmission of this disease (Wongsawad and Wongsawad, 2011).

Higher snail prevalence causes an increased diversity of trematodes. The conditions that influence the proliferation of snails would also favour the existence of trematode parasites in their intermediate host. Some riverine and other marshy habitats provide physical and chemical conditions that favour the prevalence of freshwater snail population. For this reason, environmental conditions of different habitats in different climatic zones may support the trematode life cycle in different ways. Trematodes show a distinct and direct relationship with the temperature and other abiotic factors in their transmission and completion of their life cycles (Poulin, 2005). The life cycles of the parasite and intermediate host, are closely interrelated; therefore,

the control of these diseases and the studies on snail's fauna make an important step (Roberts, 1996).

The present study aimed at investigating the prevalence of *Fasciola cercariae* in their respective snail intermediate host and at showing their role in the transmission of fasciolosis. Furthermore, the impact of various abiotic factors like temperature (°C), humidity (%) and rainfall on the *Fasciola* infection was also assessed.

### Materials and Methods

#### Snails collection and identification

A total of 4012 snails was collected on random basis from the vegetation, canals bottoms and the adjacent areas, Bahawal Canal LalSuhanara, surroundings of Satluj River, Bahawalpur, Desert branch, Yazman, Ahmadpur Canal branch, Ahmadpur, Bahawal Canal, Khairpur and various Tobas, Hasilpur.

The snails were collected from these areas with scoop net (Frandsen and Christensen, 1984). The collected snails were then transferred in small polythene bags to the Laboratory of Zoology, Department of Life Sciences, The Islamia University of Bahawalpur, for proper identification to determine the number of *Lymnaea*. The snails were washed with water and identified to genus level based on their morphology and various taxonomic characters like shell shape, count of the whorls, height and width of the shell and mouth of the shell (sinistral or dextral) (El-Gindy, 1960).

#### Examination of cercarial infection

To determine the prevalence of cercarial infection, the snails were placed in separate beakers containing 5 ml of 0.3 % saline water at room temperature. They were exposed to ordinary light for 24 hrs. Cercariae shedded in water were studied by taking few drops of water from the beakers on glass slides to which a few drops of 1% methylene blue were added, and examined using a light microscope at 10x40 magnification. Cercariae were identified using the key of Dawes (1968).

#### Meteorological data and statistical analysis

The monthly meteorological data was obtained from Meteorological Department of Bahawalpur. The data was properly tabulated using Microsoft excel. For statistical analysis, SPSS version 16 was used. As the data was found to be categorical, the best test considered was Chi-square ( $\chi^2$ ). For correlating fasciolosis with the different abiotic factors [rainfall (mm), humidity (%) and temperature (°C)] linear regression was applied and significance was found by applying Pearson's correlation test. The confidence interval was taken at 95%.

### Results

#### Prevalence of *Fasciola cercariae* in snails

Overall 4012 snails belonging to genera *Indoplanorbis*, *Lymnaea*, *Physa*, *Bulinus*, *Gyrulus*, *Oncomelania* and *Bellamaya*, were identified and subjected for detection of cercariae presence. Almost all genera were infected with different types of trematode cercariae. However, only the genus *Lymnaea* was found to harbour *Fasciola cercariae*. Of 360 *Lymnaea* snails from different areas of Bahawalpur, the overall prevalence of *Fasciola cercariae* was 11.7% (Table 1).

Monthwise overall infection in *Lymnaea* snails was highest in September (25.86%) and no infection was recorded in May. Chi<sup>2</sup> ( $\chi^2$ ) test showed highly significant ( $P < 0.05$ ) difference in September, August, February and October as compared to May. Areawise, infection of *Lymnaea* snails was highest in Yazman (13%) followed by Bahawalpur (11.8%), Khairpur (11.4%), Hasilpur (11.1%) and lowest in Ahmadpur (9.3%). Statistically, Yazman showed significant difference ( $P < 0.05$ ) compared to Bahawalpur and Khairpur, while in Hasilpurmon insignificant difference ( $P > 0.05$ ) was recorded (Table 1).

Seasonwise data showed that prevalence rate of *Fasciola cercariae*, in *Lymnaea* snails, was high during autumn (September-October) (19.6%), then winter (February) (12.5%), and summer (May-August) (8.7%) and the lowest in spring (March-April) (4.2%) (Table 2). Chi-square test showed a

highly significant difference ( $P < 0.05$ ) in autumn and summer, while the difference was less significant in winter as compared to spring. All areas showed the same type of pattern of prevalence of *Fasciola cercariae* in snails (Table 2).

#### Correlation of various meteorological factors with prevalence of *Fasciola cercariae* in snails of Bahawalpur

Mean maximum temperature recorded in June was 35°C, while mean minimum 16.1°C was in February. Similarly, mean highest humidity was found in September (68.60 %), while the mean lowest in May (35%). Mean highest rainfall (53.6 mm) was found in July while no rain in October (Fig. 1).

In snails, the highest prevalence was found in September (25.7%) when the

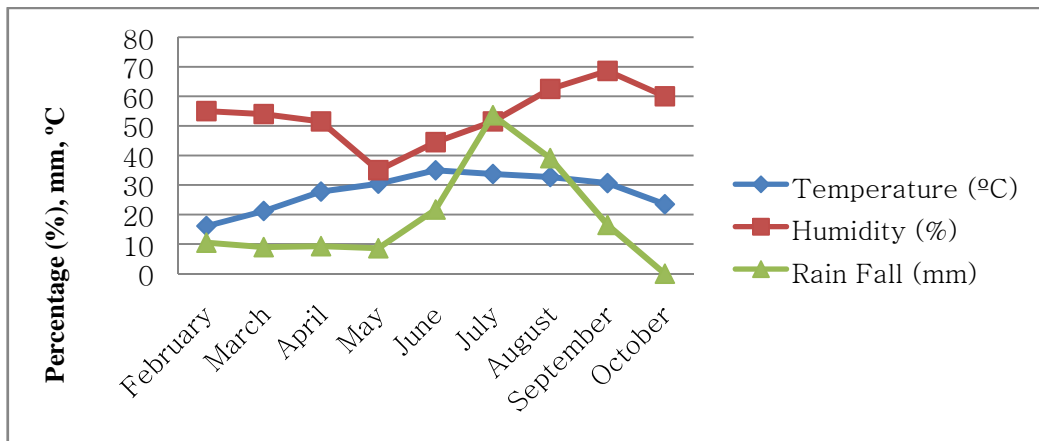
average temperature, humidity, and rain fall were 30.35 °C, 68.6%, and 16.5mm, respectively and no infection was found in May when the average temperature, humidity, and rainfall were 32.5 °C, 35 °C and 8.5mm, respectively. The results showed that a decrease in temperature associated with increase in humidity and rain in September favoured the increased incidences of the infection in snails. Statistically Pearson's correlation gave an insignificant ( $P > 0.05$ ) positive correlation between temperature and cercarial infection prevalence. The correlation between rainfall and cercarial infection prevalence was also found to be positive but insignificant ( $P > 0.05$ ), while a significant ( $P < 0.05$ ) positive correlation was recorded between cercarial infection prevalence and humidity.

**Table 1: Monthwise prevalence rate of infected snails of genus *Lymnaea* in different areas of Bahawalpur between February and October, 2013**

Month		Infected/ Total snails (%)					Overall
		Yazman	Bahawalpur	Khairpur	Hasilpur	Ahmadpur	
February	Collected	13	11	8	5	3	40
	Infected	2	2	1	0	0	5
	% infected	15.38	18.18	12.5	0	0	12.5
March	Collected	12	10	5	4	8	39
	Infected	1	1	0	0	0	2
	% infected	8.33	10	0	0	0	5.12
April	Collected	10	8	7	3	3	31
	Infected	1	0	0	0	0	1
	% infected	10	0	0	0	0	3.22
May	Collected	9	7	5	5	1	27
	Infected	0	0	0	0	0	0
	% infected	0	0	0	0	0	0
June	Collected	7	6	9	5	2	29
	Infected	1	0	1	0	0	2
	% infected	14.28	0	11.11	0	0	6.89
July	Collected	9	8	8	7	4	36
	Infected	1	0	1	1	0	3
	% infected	11.11	0	12.5	14.28		8.33
August	Collected	15	10	7	9	5	46
	Infected	2	1	1	2	1	7
	% infected	13.33	10	14.28	22.22	20	15.21
September	Collected	18	13	12	8	7	58
	Infected	4	4	3	2	2	15
	% infected	22.22	30.76	25	25	28.57	25.86
October	Collected	16	12	9	8	9	54
	Infected	2	2	1	1	1	7
	% infected	12.5	16.66	11.11	12.5	11.11	12.96
Total	Collected	108	85	70	54	43	360
	Infected	14	10	8	6	4	42
	% infected	12.96	11.76	11.42	11.11	9.3	11.66

**Table 2: Seasonwise Prevalence of infected snails of genus *Lymnaea* in different areas of Bahawalpur between February and October, 2013**

Season		Infected/ Total snails (%)					Overall
		Yazman	Bahawalpur	Khairpur	Hasilpur	Ahmadpur	
Winter	Collected	13	11	8	5	3	40
	Infected	2	2	1	0	0	5
	% infected	15.38	18.18	12.5	0	0	12.5
Spring	Collected	22	18	12	7	11	70
	Infected	2	1	0	0	0	3
	% infected	9.09	5.55	0	0	0	4.28
Summer	Collected	40	31	29	26	12	138
	Infected	4	1	3	3	1	12
	% infected	10	3.22	10.34	11.53	8.33	8.69
Autumn	Collected	34	25	21	16	16	112
	Infected	6	6	4	3	3	22
	% infected	17.64	24	9.04	18.75	23.07	19.64

**Fig.1:** The meteorological data during the study period in Bahawalpur district

### Discussion

The snails act as an intermediate host in which the cercarial stage develops. The cercaria leaves the snail host to water and swims around till it finds the next definitive host of its life cycle. Thus, it is the cercaria stage that transmits the infection from the snail, intermediate host, to the second intermediate host or the definitive host, which is a vertebrate host (Fingerut *et al.*, 2003).

Of 360 *Lymnaea* snails from different areas of Bahawalpur, overall prevalence of *Fasciola cercariae* was found to be 11.7%. Overall areawise infection of *Lymnaea* snails was highest in Yazman (13%) followed by Bahawalpur (11.8%), Khairpur (11.4%), Hasilpur (11.1%) and lowest in Ahmadpur (9.3%). Overall seasonwise prevalence of *Fasciola cercariae* in *Lymnaea* snails was found to be highest in autumn (19.6%), followed by winter (12.5%), and summer (8.7%) and lowest

in spring (4.2%). Our results get favour from the work of Qureshi *et al.* (2015), who reported the same eight different genera of fresh water snails from Lahore, Pakistan. According to them *Lymnaea*, harboured three types of *Cercariae* i.e. *Amphistome* followed by *Cercariae* and *Xiphidio cercaria*. Mufti (2011) also reported on snails prevalence from Potohar region, Pakistan, and the same genera, with various prevalence rates were documented. Devkota *et al.* (2011) reported nine freshwater snail species from Nepal in which six types of cercariae were found.

*Lymnaea* snails are intermediate hosts for *Fasciola* spp. and play important role in the epidemiology of fasciolosis (Dreyfuss and Rondelaud, 1997). In the present study, only *Lymnaea* snails were found to be infected with *Fasciola cercariae*. Kumar *et al.* (2012) also reported that *Lymnaea acuminata* is an intermediate host for *Fasciola* spp. Our results are

similar to the previous findings of Chaudhary *et al.* (1994) who reported infection of *Lymnaea acuminata* with *Fasciola cercariae*. Mufti (2011) also confirmed the presence of *Fasciola cercariae* in *Lymnaea acuminata* in Pakistan. Our results show that a decrease in temperature associated with an increase in humidity and rain in September, enhanced increased incidences of *Fasciola cercariae* in snails. Statistically Pearson's correlation showed an insignificant positive correlation among temperature, rainfall and disease prevalence. While a significant positive correlation was recorded between *Fasciola cercariae* and humidity. Niaz *et al.* (2013) reported a significant correlation amongst snails infection, humidity and rainfall, while it was insignificant with temperature. These findings strongly support our results, but the only difference in our findings is rainfall, which may be due to low precipitation level of this dry area.

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