# <u>Original Article</u> Effects of climatic factors on prevalence of developmental stages of *Fasciola gigantica* infection in *Lymnaea* snails (*Lymnaea auricularia* var *rufescens*) in Bangladesh

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#### ABSTRACT

The present study was conducted to investigate the prevalence of developmental stages of *Fasciola gigantica* infection in *Lymnaea* snails and their populations in Sylhet region of Bangladesh. A total of 1865 *Lymnaea* snails were collected and examined, of which 56 (3%) were found to having infected with developmental stages of *Fasciola gigantica*. Only 4.08% infected of 515 snails were collected from Biswanath Upazilla followed by 3.16% of 443 from Beanibazar, 2.53% of 396 from Balaganj then 2.40% of 292 Jaintapur Upazilla and the lowest 1.83% of 219 from Sylhet Sadar. Month-wise data, the prevalence of snail infections was observed to be the highest in May (5.06%) and August (5.61%) and the lowest in March (0.74%) and February (0.68%). However there was no infection observed through November to January. Seasonal prevalence of the developmental stages of *F. gigantica* infection in *Lymnaea* snails was also studied. Highest prevalence (4.63%) was recorded during rainy season and lowest prevalence (0.76%) was recorded during winter season. The study revealed that the developmental stages of *F. gigantica* infection in snail populations decreases from November to January and increases from February to October and highest in August and September in Sylhet region of Bangladesh.

Keywords: Prevalence, snails, sporocyst, cercariae, Fasciola gigantica, Bangladesh

#### **INTRODUCTION**

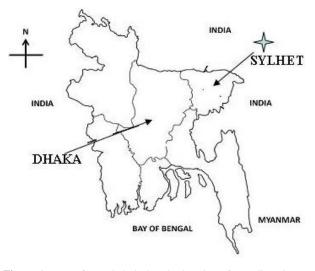
Fasciolosis is a worldwide zoonotic disease (WHO 2007, Singh & Singh 2009) caused by liver flukes of the genus *Fasciola* (Dalton 1999). It has been considered as the veterinary problem, particularly with intensive sheep, goat and cattle production, leading to

high economic losses(Torgerson& Claxton 1999). In Asia and Africa, *Fasciola gigantica* is regarded as one of the most common single helminth infection of ruminants. Snails have been known to play an important role as intermediate hosts for helminth parasites of men and animals. A *Lymnaea* snail acts as an intermediate host of *Fasciola gigantica* in all over the world including Bangladesh which carries Gymnocephalous group of the cercariae (Kandell 1954). The common Lymnaea snails are Lymnaea auricularia var rufescens which appears to be the most suitable intermediate host for Fasciola gigantica in Bangladesh (Qadir 1982) and in nearby countries(Mathur 1986, Morel & Mahato 1987). The adequate amount of rainfall, relative humidity and favorable temperature are required for the development of different larval stages of Fasciola gigantica within the snail and the reproduction of snails. The proper temperature ranges from 22-25 °C where development within snail takes place in an efficient manner similarly humidity ranges from 55-70% is adequate for the development of the snail and the parasite (Sarwar 1949, Sarwar 1953, Buriro & Chaudhry 1981, Parveen 1977, Akhtar & Khan 1989, Mughal 1993, Akhtar 1990, Alim 1997, Rahman et al 1997). Rainfall is very important for the spread of the disease. These factors are also responsible for the level of infection and prevalence in the snail populations. Incidence of Fasciola cercariae in Lymnaea auricularia var rufescens has been reported by Qadir (1982) and studies on different fresh water snails have been carried out only in Savar Upazilla under the district of Dhaka, Bangladesh (Chowdhury et al 1994). The geo-climatic conditions together with the water logged and low lying areas in Sylhet are highly favorable places for the development and multiplication of Fasciola gigantica and their intermediate hosts. Although, Sylhet region of Bangladesh have wide water resources of large irrigated agro-ecological zones adjacent to river Surma, flowing water within the tea garden lakes, hilly area and fresh water there is no attention on snails study in this area. The present study was designed to study the climatic factors affecting the prevalence of different developmental stages of Fasciola gigantica in snails and their populations in Sylhet region of Bangladesh.

## MATERIALS AND METHODS

**Study area.** This study was conducted in Sylhet region of Bangladesh. Sylhet region is located in North-

East part of Bangladesh between 24°30' North latitude and 91°40' East longitudes (Figure 1). The division has an area of 3490.40 square kilometers. More than three quarter of the division consists of mostly tea garden, hilly, water logged and low lying areas. The average maximum and minimum temperatures are 31°C and 21 °C, respectively. The annual average rainfall is 3854.2 mm and humidity is 68.92%. Climatic data regarding temperature (°C), rainfall (mm) and relative humidity (%) of the selected areas were collected from the meteorological station which located within the Sylhet City Corporation, Sylhet, Bangladesh to determine the effect of climatic change on infection of different developmental stages of Fasciola gigantica in Lymnaea snails and prevalence of Lymnaea auricularia var rufescens.



**Figure 1.** Map of Bangladesh that the location of sampling site at Sylhet Division (star mark).

**Collection of snails.** A total of 1865 *Lymnaea* snails were collected randomly with hand-picked from five different Upazilla in Sylhet region of Bangladesh during the period March 2013 to February 2014 (Figure 3A and Figure 3B). Laboratory analysis of collected *Lymnaea* snails was carried out at the Department of Parasitology, Faculty of Veterinary and Animal Science, Sylhet Agricultural University, Sylhet, Bangladesh between March 2013 and February 2014.

Different areas of Sylhet region were selected on the basis of irrigated agro-ecological zones such as Sylhet Sadar, Balaganj, Beanibazar, Biswanath and Jaintapur Upazilla (Figure 2).



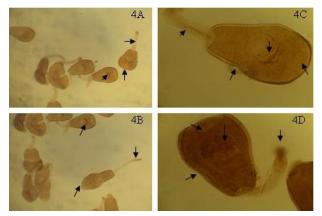
**Figure 2.** Map of Sylhet district that summarizes the locations of sampling sites at Sylhet Sadar, Balaganj, Beanibazar, Biswanath and Jaintapur Upazilla (star mark).



**Figure 3.** Collection and identification of *Lymnaea* snails (*Lymnaea auricularia* var *rufescens*) is the intermediate host of *F. gigantica* (A-B) and Microscopic features of a sporocyst (developmental stages of *F. gigantica*) 4X (C) & 10X (D).

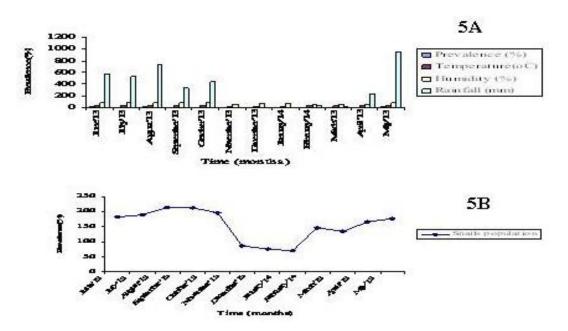
**Identification and examination of snails.** The collected snails were washed thoroughly, cleaned from mud, debris and cittiates. Identifications were done according to the shell morphology of the snails (Figure 3A and Figure 3B) as described by El-gindy (1960) and Malek (1984). *Lymnaea* snails were kept individually in test tubes containing distilled water and exposed to a

100-W light bulb at a distance of 15 cm for 4-6 hours for cercarial shedding (Faltynkova et al 2008). The snails which did not shed cercariae on the first exposure were re-exposed on the second day. The water of these tubes was examined against the light, with naked eye or with a hand lens, every hour interval during the day and on the following. The cercariae were characterized by morphological and biometrical examinations under a stereo-microscope as described by Dawes (1968) and Frandsen and Christensen (1984). The infected snails were segregated from the rest as described by El-gindy (1960) and Malek (1984). The snails those could not shed cercaria under the artificial light; were crushed and their internal contents were examined for the presence of other developmental stages of Fasciola gigantica (Shaikh & Rahman, 1968). The various types of cercariae were identified by (Cable 1950).



**Figure 4.** Microscopic feature of a cercaria (well develop ventral sucker, oral sucker, spines and a projectile long tail-arrow) 10X (A-B) & 40X(C-D).

Statistical analysis. The prevalence of developmental stages of *Fasciola gigantica* infection in *Lymnaea* snails populations on the basis of different areas in Upazilla, month and season were analyzed by logistic regression using software SPSS statistical program (version 15.2, SPSS Inc., Chicago, IL, USA). Values of p<0.05 were considered as significant at 95% confidence interval. Meteorological data including maximum and minimum temperature (°C), relative humidity (%) and rainfall (mm) were obtained from meteorological station; their correlation with the



**Figure 5.** Month-wise prevalence (%) of different developmental stages of *F. gigantica* infection in *Lymnaea* snails in relation to temperature ( $^{\circ}$ C), relative humidity (%) and rainfall (mm) in Sylhet region of Bangladesh (A) and Month-wise population of *Lymnaea* snails (*Lymnaea auricularia* var *rufescens*) in Sylhet region of Bangladesh (B).

prevalence of infection was carried out by using Pearson's correlation.

## RESULTS

The prevalence of both *Fasciola gigantica* cercariae and sporocyst infection in Lymnaea auricularia var rufescens in different Upazilla in Sylhet region of Bangladesh is shown in the Table 1. In the present study, a total of 1865 Lymnaea snails were collected randomly and examined during the period of one year, of which 56 (3.00%) snails were found positive for Fasciola cercariae and sporocyst. The highest prevalence was found in Biswanath Upazilla (4.08%) followed by Beanibazar (3.16%), Balaganj (2.53%), Jaintapur (2.40%) and Sylhet Sadar (1.83%) regions. The percent infection of Fasciola gigantica larvae varies 1.83 to 4.08 from March 2013 to February 2014 in different localizations of Upazilla of Sylhet region of Bangladesh. In the present study, both the sporocyst and cercariae of Fasciola gigantica were found in

Lymnaea snails during the period of study. Microscopically Fasciola sporocyst were found a sac like structure whose function is asexual multiplication then produces more sporocyst and finally rediae (Figure 3C and Figure 3D) and Fasciola cercariae is a large heart shaped body and a simple long tail. The body has a characteristically thick wall and is surrounded by minute spines all over its surface. The ventral sucker is well developed and larger than the oral sucker. The alimentary canal is rudimentary and consists of a mouth followed by pharynx surrounding the esophagus that leads to intestine. The later bifurcates into two simple's branches that extend around the ventral sucker to a level below the posterior border of the ventral sucker (Figure 4). During the present study, overall month-wise infection in Lymnaea snails were showed highest in May (5.06%) and when the average temperature, August (5.61%) average relative humidity and rainfall dropped to 29.55 and 29.60 °C, 78 and 80%, 958.2 and 727.2 mm, respectively. The prevalence of different developmental stages of Fasciola gigantica increasing from April to September, decreasing from February to March and no infection from November to January were observed (Table 2). Related to the seasonal variations the highest prevalence of developmental stages of Fasciola gigantica infection in snail populations were detected in rainy season (4.63%) followed by summer (1.92%) and then winter (0.76%), (Table 2). The present study also revealed a Pearson's correlation between snail infections and meteorological factors (temperature, relative humidity, and rainfall) which were found highly significant (Table 2 and Figure 5A). The different developmental stages of Fasciola gigantica infection in snail populations decreases from November 2013 to January 2014 and increases from February 2014 to October 2013 and highest in August 2013 and September 2013 (Table 2 and Figure 5B).

### DISCUSSION

L. auricularia var. rufescens belonging to genus Lymnaea act as intermediate hosts of F. gigantica infecting livestock populations mainly ruminants. Both human beings and livestock populations are infected by various species of trematodes (Platyhelminthes: Trematoda), which are required as intermediate hosts of snails (Class: Gastropoda). In the present study, the prevalence of infected snails was determined as 3%. The findings of both sporocyst and cercaria infection in Lymnaea snails is higher than that recorded by Chowdhury et al. (1994) and lower than the Qadir (1982). Chowdhury et al. (1994) examined 4149 L. auricularia var rufescens snails and Qadir (1982) examined only 730 of the same snails and incidence of Lymnaea snail infections with same cercariae were found as 0.31% and 3.70%, respectively. This finding is similar to other studies of Garg et al. (2009), Islam et al. (2012) and Imani-Baran et al. (2013) who reported the prevalence of developmental stages of F. gigantica (cercariae) in Lymnaea snails was 2.35%, 5.5% and 16.8%, respectively. The variation of infection rates in Lymnaea snails about the findings of different investigators were thought due to total number of Lymnaea snails examined and level of F. gigantica infection in the animals, sample size, duration of the study period, meteorological factors that govern the breeding, life span, infection of snails and development of different developmental stages within the snails. In Biswanath Upazilla, there were enough marshy areas which favor for the development of snails and the prevalence of helminths was high in flooded pasture than plain. The highest prevalence at Biswanath Upazilla indicated that the environmental conditions are more suitable for the snail breeding. The overall prevalence and prevalence in the five different areas in Upazilla were showed some differences with the findings of Qadir (1982); Mathur (1986); Alim (1997) and Garg et al. (2009) who reported the prevalence of Fasciola cercariae were 5.5%, 3.70%, 0.67% and 0.8%, respectively. This was due to difference in the environmental and managemental conditions. Similar observation was reported by Tanveer (1990a) and Rondelaud et al. (2007). The microscopic features of both sporocyst and cercaria of F. gigantica were observed and characterized by a sack like structure and large heart shaped body and a simple long tail, ventral sucker well develop than oral. These findings have similarities with the observations of Belding (1965) and Koie et al. (1977). Prevalence of different developmental stages of F. gigantica infection in the Lymnaea snails varied significantly in different months of the year. The different developmental stages of F. gigantica in the Lymnaea snails were found around the year, but the developmental stages both sporocyst and cercariae started to appear in the snails from April, reached a peak May to October then low in February to March and disappeared during November to January. In the collected Lymnaea snails, only sporocyst were found during the period of February to April were observed. Similarly, Qadir (1982) and Chowdhury et al. (1994) were observed too much higher incidence of Gymnocephalous cercariae (F. cercariae) in Lymnaea snails in different months except November to April. Lymnaea snails infested during the period when

Upazilla	No. examined	Different devel	opmental sta	Total Positive	Prevalence	
		Sporocyst	Redia	Cercaria	Total Fostive	(%)
Sylhet Sadar	219	0	0	4	4	1.83
Balaganj	396	1	0	9	10	2.53
Biswanath	515	3	0	18	21	4.08
Beanibazar	443	2	0	12	14	3.16
Jaintapur	292	1	0	6	7	2.40
Average	1865	7	0	49	56	3.00

**Table 1.** Prevalence of developmental stages of *F. gigantica* infection in *Lymnaea* snails

Table 2. Seasonal variation of snails and percentage of infection with co-relation of temperature, relative humidity and rainfal	11
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Season	Month	No collected	Positive	Prevalence	Seasonally	Temperature (°C)		Humidity	Rainfall
						Minimum	Maximum	(%)	( <b>mm</b> )
Rainy	June'13	183	09	4.92		25.8	33.9	75	567.8
	July'13	189	08	4.23	<sup>\$</sup> 4.63 <sup>a</sup> ±0.40**	25.8	33.3	78	534.1
	August'13	214	12	5.61		25.6	33.6	80	727.2
	September'13	212	08	3.77		25.3	32.8	78	337.1
Winter	October'13	197	06	3.05		24.6	31.5	74	444.1
	November'13	86	00	0.00	<sup>§</sup> 0.76	17.4	30.1	63	nill
	December'13	76	00	0.00	<sup>b</sup> ±0.76**	14.6	26.2	68	nill
	January'14	71	00	0.00		13.1	27.1	66	nill
Summer	February'14	148	01	0.68	<sup>\$</sup> 1.92	18.4	31.6	62	34.2
	March'13	135	01	0.74		19.7	34.2	58	15.4
	April'13	166	02	1.21	<sup>b</sup> ±1.05**	21.9	33.0	59	236.1
	May'13	178	09	5.06		24.7	34.4	78	958.2

 $\pm$  standard deviation; In a column among seasons with same alphabet do not differ significantly as per DMRT; Data were calculated at 95% level of significance and a *p* value <0.02 was considered significant. A Pearson's correlation of prevalence (%), temperature (°C), humidity (%) and rainfall (mm) were significant at the 0.01 level.

conditions were favorable for infestation to develop may have died by November and new snails had not become infested. During the post monsoon and winter months (November to January) development and hatching of eggs ceased or took a long time due to low temperatures. Thus, snails were not infested during this period and cercariae did not appear in the snails by April. There were favorable temperatures, humidity and rainfall from May to October onward for the development and hatching of F. gigantica eggs and development of different developmental stages of Fasciola within the snails. The development and hatching of F. gigantica eggs takes 16-30 days at 20 °C, 11-25 days at 25 °C and 12-24 days at 30 °C and development of cercariae stages of F. gigantica within the snails takes 75 days (Gosh 1985). Thus cercarial infestation in the snails began to increase from April and continued until October due to warm, wet and humid conditions. Lymnaea snails infested during the period when conditions were favorable for infestation

to develop may have died by November and new snails had not become infested, during the months November to January development and hatching of eggs ceased or took a long time due to low temperatures. Thus, Lymnaea snails were not infested during this period and cercariae did not appear in the snail by November to April, another developmental stages were found during February to April. The peak infection Lymnaea snails were recorded rainy followed by summer and winter season due to the results of the maturation of immature stages as the temperature, relative humidity and rainfall rises. These results were in agreement with Tanveer (1990b). Effect of climatic factors on the prevalence was found to be highly significant. These findings are in agreement with the findings off Imani-Baran et al. (2013). Related to the effect of meteorological factors on snail populations, it was determined that late summer providing optimum temperature required for their breeding and reproduction of snails. It was clear that the temperature had a great effect on snail populations. In this study, during the late summer a large number of mature snails oviposit and die consequently and gradually fall down. As the summer merges into rainy season an increase was observed in snails populations. These results were agreement with the findings of Qadir (1982) and Chowdhury et al. (1994). They reported that ova production of snails is not affected by increasing the period of day light and darkness has not harmful effect on it. These results were similar to other workers with Karimi (2003).

This study shows the presence of both *F. gigantica* cercariae and sporocyst in Sylhet region of Bangladesh and effects of climatic factors on prevalence of developmental stages. Further studies should be needed to control of snail populations according to climatic factors in this region

## Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

## **Conflict of Interest**

The authors declare that they have no conflict of interest.

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