Abiotic environmental factors and infection of *Fasciola gigantica* in vector snail *Lymnaea acuminata.*

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Abstract: Abiotic factors influence the infection of *Fasciola* larva in vector snail *Lymnaea acuminata.* Every month during the year 2011-2012, the rate of infection of *Fasciola* larva (sporocyst, redia and cercaria) in snail *Lymnaea acuminata* and simultaneous measurement of abiotic environmental factors viz temperature, pH, dissolved oxygen and free carbon dioxide in Mahesara Lake were studied. Each infected snail were dissected in glass petri dish containing 10 ml of dechlorinated water. After dissection the sporocyst, redia and cercaria were separated in different petri dish and counted with the help of Sterio-microscope. The highest infected snails were noted in month of June to November. On the basis of this observation, it was noted that abiotic environmental factors significantly affect the infection rate of *Fasciola* larva in snail *Lymnaea acuminata.* Highest infections of sporocyst (38/snail), redia (2782/snail) and cercaria (6357/snail) were noted in September to October month. There was significant ($p<0.05$) negative correlation between number of *Fasciola* larva (sporocyst, redia and cercaria) and dissolved oxygen concentration of water in different months of the year 2011-2012. A positive correlation was noted in between different larval stages and temperature/ pH /free carbon dioxide in water.
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1. Introduction:

Fascioliasis is an important helminth disease caused by two trematodes *Fasciola hepatica* and *F. gigantica* (Mas-Coma *et al.,* 2005). Fresh water snail *Lymnaea acuminata* is the intermediate host of *F. gigantica* (Kumar & Singh, 2006). Fascioliasis caused high economic losses in the animal husbandry industry (Singh *et al.,* 2005). Human fascioliasis was considered a secondary disease until the end of 1990s. Worldwide estimates of human fascioliasis increased from the 2000, cases in between 1960-1970 (Chen & Mott, 1990) to 2.4 million people (WHO, 1995) and 17 million people at present (Mas-Coma *et al.,* 2009). Human fascioliasis has been reported in 51 different countries from five continents (Mas-Coma *et al.,* 2005). Incidence of endemic animal fascioliasis is very common in the eastern region of the state of Uttar Pradesh in India (Singh & Agarwal, 1981; Sunita & Singh 2011; Kumar *et al.,* 2011). Development of larval digenetic trematodes is complex process involving initial infection of the snail host by the free-swimming miracidium, its sequent transformation to a parasitic primary sporocyst stage, followed by asexual reproduction and release of secondary sporocyst or redia, and finally the eventual formation and release of cercaria the next free-swimming stage in the life cycle. An effective method to reduce the incidence of fascioliasis is to control the population of vector snail or by breaking the life cycle of the developing larva of *F. gigantica* (Singh *et al.,* 2005; Kumar & Singh, 2006; Shukla *et al.,* 2006). Change in abiotic environmental factores alters pathogen transmission in freshwater habitats (Hakalahti, *et al.,* 2006; Marcogliese, 2008). Climate change affected the distribution and abundance of freshwater diseases of both medical and conservation significance (Pascual *et al.,* 2006; Atkinson & La Pointe, 2009; Johnson & Paull, 2010). However, the multifaceted interaction between environmental factors makes the response of freshwater diseases challenging (Ibelings *et al.,* 2011). Environmental change will leads to both direct (i.e.; physiological) and indirect (i.e.; interspecific interactions) effects on parasite transmission, some of which many increase disease while other will reduce infection or pathology. Temperature can act directly on disease by altering the susceptibility of host, the virulence of parasites and the growth rates of both hosts and parasites, which can in turn influence host pathology and disease emergence (Cairns *et al.,* 2005; Raffel *et al.,* 2006). The objective of this study was to explore the possibility that seasonal changes in abiotic environmental factors temperature, pH, dissolved oxygen and free carbon dioxide may influence the rate of infection of *F. gigantica* larva in the vector snail *Lymnaea acuminata*.

2. Methods and Materials

2.1 Animal

Adult snail *Lymnaea acuminata* (2.6 ± 0.30 cm in length), is a know vector of fluke *F. gigantica*, (Sunita & Singh, 2011) which cause endemic fascioliasis in animal of eastern Utter Pradesh. The test animals *L. acuminata* were collected from Maheshra Lake of Gorakhpur. On the basis of morphological identification, uninfected and infected snail *L. acuminata* were identified by the shape, size, colour of shell and locomotion (Sunita & Singh, 2011). Infected snail is comparatively larger in size due to swelling of the body and colour of the shell appeared yellowish. The infected snails were allowed to acclimatize for 24 hours in laboratory condition. Each infected snail was dissected in a glass petri dish containing 10 ml of dechlorinated water at 22^0C-24^0C. The pH of the water was 7.1-7.3, and dissolved oxygen, free carbon dioxides and bicarbonate alkalinity were 6.5-7.2 mg/l, 5.2- 6.3 mg/l, and 102.0-105.0 mg/l, respectively. After dissection sporocyst, redia and cercaria were separated in a different petri dish containing 10 ml of dechlorinated water. The methods for counting larva (sporocyst, redia and cercaria) were done by the method of Sunita & Singh (2011).

2.2 Statistical analysis

Each experiment was replicated at least six times and values of temperature, pH, dissolved oxygen and free carbon dioxide are expressed as the mean of six replicates. Values and counting of sporocyst, redia and cercaria were expressed as mean ± SE. The product moment- correlation coefficient was applied to determine significant ($p<0.05$) difference between environmental factor such of temperature pH, oxygen, free carbon dioxides and number of sporocyst, redia and cercaria in infected snails in each month of the year 2011-2012 (Sokal & Rohlf, 1973).

3. Results

Highest numbers of infected snails were noted in month of October (55%) November (70%), followed by June to August-(45-50%) (Figure-1). Highest number of sporocyst (38/snail) was noted in the month of October, whereas highest number of redia (2782/snail) and cercaria (6357/snail) were observed in month of September (Table-1). The lowest infection of sporocyst (1), redia (1032) and cercaria (1670) were noted in month of May, February and April, respectively (Table-1).The highest temperature (34.3^0C), pH (8.9) dissolve oxygen (6.021 mg/l) and free carbon dioxide (29.93 mg/l) were observed in month of August, February, January and July, respectively. The lowest temperature (10.33), pH (6.04), dissolved oxygen (1.01) and free carbon dioxide (14.21) were observed in the month of December, June, July and March, respectively (Table-1). There was significant ($p<0.05$) negative correlation between number of *Fasciola* larva snail (sporocyst, redia and cercaria) and dissolved oxygen concentration in water in different months of the year. Positive correlations were noted in between the number of larva/snails and temperature/pH/ free carbon dioxide concentration in different months of the year 2011- 2012.

4. Discussion

The affect of abiotic environmental factors on infectious disease dynamic depends on the full spectrum of direct and indirect effect on host and parasite life histories. Importantly, these effects will extend beyond simple change in host or parasite geographical distribution to include significant shift in the physiology and temporal interaction between host and parasites which could alter disease dynamics in natural population (Johnson & Paull, 2010). It is clear from the above finding that in Maheshra lake, high infection percentages were observed in between June to November. Infection of *Fasciola* larva was highest (<70%) in month of November. Number of cercaria larva snail was highest in month of September to November (5877-6357/snail). This indicate that higher cercarial out put in these months provide maximum chance of contact in between the host *Lymnaea* and cercarial/metacercarial number in aquatic environment. Temperature ranges in these months are in between 22.16^0C to 24.66^oC. Numbers of sporocyst/ redia larva per snail were high in month of July to November. Temperature is one of the crucial factors for snail as well as larval growth of *Fasciola* (Njoku-Tony, 2011), so that July to November month is suitable period for infection of *Fasciola* larva in host snail.

Dissolved oxygen in different month of year 2011-12 range in between 1.01 to 6.02 mg/l. Highest dissolved oxygen was noted in month of January (6.02 mg/l). Although dissolved oxygen is high in month of December to February, yet number of sporocyst/redia/cercaria larva is comparatively less than July to November. It indicates that oxygen above 1 mg/l is sufficient for snail as well as larval growth in between July to November (1.01-4.97). Snail respiratory pigment show affinity with oxygen 1-7% saturation and oxygen below 1% is fatal for snails (Wrignt, 1959; WHO, 1994; Njoku-Tony, 2011).

Table: 1 Variations in Maheshra lake abiotic factor in different months of year 2011-12 and infection of *Fasciola* larva in snail *L. acuminata.*

Months	Sporocyst Snail^{+}	Redia Snail^{+}	Cercaria Snail^{+}	Temperature $^{+}$	pH $^{+}$	D.O (mg/L)*	CO_2 (mg/L) $^{+}$
February (2011)	2±0.729	1032±1.729	1869±0.964	19.50±0.223	8.92±0.012	5.04±0.017	14.78±0.268
March	4 ±0.515	2730±0.645	2739±2.390	20.16±0.306	8.16±0.167	4.25±0.024	14.21±0.454
April	2±0.854	1283±0.964	1670±1.314	22.00±0.257	7.56±0.239	3.01±5.154	24.91±0.339
May	1±0.364	1362±0.964	1961±1.253	22.33±0.332	7.46±0.113	2.50±5.763	24.96±0.188
June	7±0.665	1674±8.563	2683±0.815	34.33±0.332	6.04±0.837	1.80±5.763	24.86±0.347
July	23±2.125	2652±1.349	3875±1.502	27.66±0.210	7.10±3.067	1.01±5.154	29.93±0.331
August	34±2.671	1397±3.330	2481±1.710	24.83±0.306	7.09±4.764	1.47±0.012	25.11±0.236
September	35±3.236	2782±1.181	6357±0.866	24.66±0.420	8.01±0.020	2.29±4.274	20.15±0.174
October	38±2.507	2272±2.003	5887±1.918	22.83±0.306	8.08±0.011	3.00±6.135	20.51±0.368
November	20±3.111	2493±3.838	5996±1.724	22.16±0.306	8.08±4.208	4.97±0.013	19.86±0.177
December	3±0.364	1461±1.524	2486±0.576	10.33±0.332	8.75±7.624	5.04±0.019	14.98±0.210
January (2012)	3±0.652	1180±1.288	2351±0.576	11.50±0.340	8.95±0.035	6.02±0.023	14.98±0.047

Each experiment was replicated six times. Sporocyst redia cercarias were counted in each month in infected snail. Value of temperature, pH, dissolved oxygen and dissolved free carbon dioxide is the mean of six replicate are the mean ± SE of six replicate.

Product moment correlation coefficient in between the different larval stages and abiotic factor indicate significant ($p<0.05$) (+) positive/ (*) negative correlation.

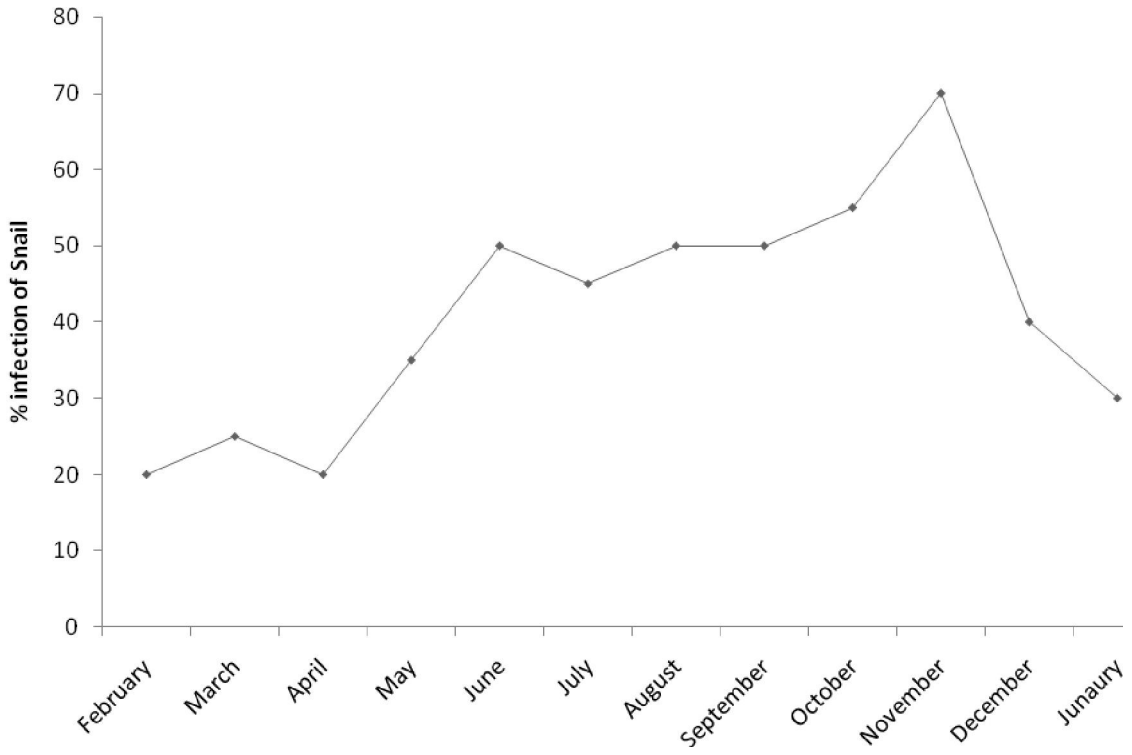

Fig.1 Per cent infection of *Fasciola gigantica* larva in snail *Lymnaea acuminata* collected from Maheshra lake in different month of the year (2011-2012).

Rise of temperature in month of June to November influences the high larval (sporocyst, redia and cercaria) infection and free carbon dioxide concentration was also high in June to November (19.86-29.93 mg/l). At higher temperature, the increasing rate of snail metabolism many release more CO_2, which effect the pH of water (Toews *et al.,* 1995; Berge *et al.,* 2006). This was evident from the elevated concentration of CO_2 which decreases in the pH of water during summer season (Singh & Singh, 2009). Recent finding have highlighted the relationship between infection of *Fasciola* larva and abiotic factors. Higher pH as noted in September to November is also favorable for the infection of *Fasciola*. Present study clearly indicates that *Fasciola* larvae are highly sensitive to temperature changes. The small size of larval may allow them to multiply more efficiently increasing, with temperatures than their temperature dependence of host. Parasite infection rates requires direct involvement of metabolic energy use (i.e. oxygen consumption rate), of abiotic environmental factors such as temperature, pH, O_2 and free CO_2 with respect to different larval stages (sporocyst, redia and cercaria) of the *F. gigantica* in host snail *L. acuminata*.

5. Conclusions

It can be conclusively noted from the present study that abiotic factors has crucial affect on the infection of *Fasciola* in host snail *Lymnaea acuminata*. Maximum infections were noted in between June to November months. So that the use of potent larvicides for *Fasciola* larva/ or molluscides for host snails in between the months of June to November will be effective in control to fascioliasis.

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