Seasonal Incidence and the Existence of a Competition Between Echinostome and Gymnocephalus Cercariae in Lymnaeid Snails

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ABSTRACT

Seasonal incidence of echinostome and gymnocephalus cercariae in naturally infected lymnaeid snails (Gastropoda: Lymnaeidae) was investigated during the period from July/2001–June/2004. The lymnaeid snails, Lymnaea auricularia var rufescens and Lymnaea luteola were collected from the natural pool of water surrounding the Bangladesh Agricultural University, Mymensingh, and were examined for the presence of cercariae. Both snail species were infected either with echinostome or gymnocephalus cercariae. However, cercarial infection was more common in L. auricularia (28.8%) than in L. luteola (26.6%). Further, among the infected snails, 28.8% L. auricularia and 25.9% L. luteola were infected with echinostome cercariae, while gymnocephalus cercariae were recorded only in 0.7% L. auricularia and 0.66% in L. luteola. A relatively higher incidence of echinostome cercariae was recorded in summer (94.9%, 70%) followed by winter (86.6%, 65%) and monsoon (79.1%, 63.1%) in L. auricularia and L. luteola respectively. On the other hand, incidence of gymnocephalus cercariae was observed relatively higher in monsoon (94.9%, 70%) followed by winter (86.6%, 65%) and summer (79.1%, 63.1%) in L. auricularia and L. luteola respectively. We also observed that, both the lymnaeid snails harboured only single infection either with echinostome or gymnocephalus cercariae and dual infection with these cercariae was not seen under natural conditions. Based on the field observations, mature L. auricularia snails were infected experimentally either singly or concomitantly with echinostome and Fasciola miracidia to determine the existence of cercarial competition if any. Snails infected singly with Fasciola miracidia released gymnocephalus cercariae by 36-49 days post-infection at room temperature (24-26°C) while snails concomitantly infected with both the miracidia or exposed first to Echinostoma miracidia and then to Fasciola miracidia at an interval of 48 hours and vice versa released only echinostome cercariae by 16-25 days post-infection. These findings confirm the existence of a strong competition between echinostome and gymnocephalus cercariae in L. auricularia snails.

Keywords: Lymnaeid snails, Seasonal incidence, Gymnocephalus cercariae, Echinostome cercariae, Cercarial competition

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INTRODUCTION

Fascioliasis, caused by liver fluke (Fasciola spp.), results in major economic losses in cattle, buffaloes, sheep and goats all over the world. The losses are in terms of condemnation of livers, decreased meat and milk production, loss of weight and poor carcass quality and/or death. Diarrhoea, jaundice and bottle jaw are predominant features in chronic cases, while sudden death in peracute, acute and subacute cases are not uncommon (Soulsby, 1982). The disease is widespread throughout the Bangladesh in particular where extensive irrigation facilities have been developed for rice cultivation and in the low-lying areas containing enormous numbers of vector snails, Lymnaea spp. (Kendall, 1954). Like fascioliasis in ruminants, echinostomiasis in ducks caused by Echinostoma spp. is widely distributed all over the Bangladesh. Interestingly, echinostomiasis is not very harmful in ducks unless the young ducks are heavily infected (Soulsby, 1982). Briefly, paedogenesis of Fasciola and Echinostoma occur within the same snail intermediate hosts Lymnaea spp. (Rahman et al. 1997). But there are some evidences that usually only one type of fluke either Fasciola or echinostome develops in the snail intermediate hosts at a time. Snails infected with echinostome become refractory to infection with Fasciola gigantica. Competition of Echinostoma from ducks and other aquatic birds with Fasciola in the snails hampers the development of Fasciola (Suhardono et al. 1997) which may be an effective biological control measure of fascioliasis. Considering the facts, the present research work was undertaken to study the incidence and seasonal dynamics of echinostome and gymnocephalus cercariae in naturally infected lymnaeid snails and developmental antagonism of Echinostoma and Fasciola in experimentally infected Lymnaea auricularia var rufescens in the laboratory.

MATERIALS AND METHODS

Collection and examination of snails

The lymnaeid snails, Lymnaea auricularia var rufescens and Lymnaea luteola were collected from the low lying areas, ponds, canals, pools and rice fields surrounding Bangladesh Agricultural University, Mymensingh, where ducks and cattle are reared in the same premises. Snails collected by scooped net or manually. They were brought to the laboratory and washed in tap water to remove the debris attached with the shell and identified by their characteristics shell characters (Hubendrick 1951 and Malek and Cheng 1974). After identification, snails were placed individually in the test tube containing water and left for 24 hours at room temperature for the emergence of cercariae. The snails that did not release cercariae were crushed in normal saline and the
visceral organs were examined under dissecting microscope to detect the presence of immature cercariae or other developmental stages if any. The cercariae were identified following the classification of Luhe as described by Dawes (1968) and Cheng (1964). The population of cercariae emerged from an individual snail was counted under a dissecting microscope. To study seasonal incidence of cercariae, the year was divided into three seasons viz. Summer (March–June), Monsoon (July–October) and Winter (November–February).

**Laboratory maintenance of snails**

The mature lymnaeid snails (L. auricularia var rufescens and L. luteola) collected from the fields as described above were reared in the plastic bowls. For collection of snail eggs and hatching of juvenile snails the standard method was followed (MAFF 1971). The young snails were properly washed in tap water and transferred in to a new bowl. Boiled immature mango leaves (Magnifera indica) were used as feed for the snails (Malek and Cheng 1974). Each species of L. auricularia var rufescens and L. luteola was separately reared in plastic bowls containing chlorine free tap water. The bowls were marked for their identification. Twenty snails were maintained per 2 litres of water in each bowl (Cohen at al. 1980. The bowls were examined daily, dead snails were removed and their faeces were washed by means of a gentle stream of water. The snails were exposed to miracidial infection after 3 weeks of rearing (MAFF 1971).

**Collection and incubation of parasite eggs**

The mature fertile eggs of Fasciola were collected from the gall bladder of infected cattle slaughtered at abattoirs in the local market. The gall bladder was opened in a 500 ml beaker containing phosphate buffer saline (PBS), washed thoroughly and left for 30 minutes to settle down the eggs. The supernatant was decanted. This process was repeated for five times to remove the bile. For echinostome eggs, adult Echinostoma spp. were collected from the small intestine of ducks. After collection, the worms were washed in PBS and the eggs were collected by dissecting the gravid uteri of the parasites in petridishes. At different intervals Fasciola and Echinostoma eggs were incubated in separate petridishes (10 cm in diameter) containing chlorine free tap water at room temperature (24-26°C) until the miracidia hatched (Fried 1985). At one week
post-incubation the petridishes were examined daily under dissecting microscope to observe the development of miracidia in eggs.

**Infection of snails**

The laboratory reared snails were divided into five groups namely A, B, C, D, and E each with 20 snails. Snails in group A and B were infected by Fasciola and Echinostoma miracidia respectively. The snails in group C were infected concomitantly by Fasciola and Echinostoma miracidia. D group snails were first exposed to Echinostoma miracidia and then to Fasciola at 48 hours post-exposure. The snails in group E were first exposed to Fasciola miracidia then to Echinostoma miracidia at 48 hours post-exposure. For infection in each case, a single snail was placed in a petridish (10 cm) quarter filled with water. With the help of a dissecting microscope 1-2 miracidia were taken up in a fine pipette and released in the petridish and left for 1-3 hours to allow miracidia to penetrate into the snails. From time to time the petridishes were examined to prevent the snails climbing out of the water. Each group of snail was returned to separate plastic bowls and reared accordingly.

**Examination of experimentally infected snails for cercaria**

The snails were examined first at one-week post-infection and onwards for the presence of cercariae. As soon as the cercariae were observed in a bowl, the snails were transferred individually to separate small plastic cups (7.25 cm in diameter at top, 4.5 cm at bottom and 7 cm in depth) and numbered properly. The snails were supplied with small pieces of boiled mango leaves as mentioned above. The time of releasing cercariae of the individual snails were recorded. For mixed infection cases, the snails that released one type of cercariae were reared up to 10 weeks. At 10 weeks post-infection, the snails were crushed and examined under dissecting microscope as described above.

**Statistical Analysis**

Cercarial infections in different lymnaeid snails were compared by Pearson’s chi-square test (Petrie and Watson, 1999). A logistic regression analysis was conducted for testing whether the season had any significant influence on the development of the cercarial infections in the lymnaeid snails (Hosmer and Lemeshow, 1989).

**RESULTS**

In this study the lymnaeid snails, *Lymnaea auricularia* var *rufescens* and *Lymnaea luteola* (Fig. 1a, b) collected from the low lying areas, ponds, canals, pools and rice fields
were shown to be infected either with echinostome or gymnocephalus cercariae (Fig. 2a, b) but mostly with the echinostome. There was marked variation in the incidence of both the cercarial infections in the two species of snails. Cercarial infection was more frequently encountered in *L. auricularia* (28.8%) than in *L. luteola* (26.6%). Among the infected snails, 28.1% *L. auricularia* and 25.9% *L. luteola* harboured echinostome cercariae, while gymnocephalus cercariae were detected only in 0.7% *L. auricularia* and 0.66% *L. luteola* (Table 1). Marked seasonal variations of cercarial infection were also observed in lymnaeid snails irrespective of their species. In *L. auricularia*, a relatively higher incidence of echinostome cercariae was recorded in summer (94.9%) followed by winter (86.6%) and monsoon (79.1%). Similarly, cercarial burden was also relatively higher in summer (138 ± 1.1) followed by winter (129 ± 1.4) and monsoon (123 ± 0.8) (Table 2). Although the infection rate with echinostome cercariae in *L. luteola* was relatively lower than that in *L. auricularia* but the seasonal trend of cercarial infection and load was similar. In *L. luteola*, a relatively higher incidence of echinostome cercariae was recorded in summer (70%) followed by winter (65%) and monsoon (63.1%).
Figure 1a. The intermediate host snail, *Lymnaea auricularia* var rufescens.

Figure 1b. The intermediate host snail, *Lymnaea luteola*.

Figure 2a. Echinstome cercaria with head collar and spine (330X).

Figure 2b. Gymnocephalus cercariae with anterior and ventral suckers (330X).
**Echinostome and Gymnocephalus Cercariae in Lymnaeid Snails**

Table 1. Prevalence of Gymnocephalus and Echinostome cercariae with the test of difference in lymnaeid snails

<table>
<thead>
<tr>
<th>Lymnaeid snails</th>
<th>Cercariae identified (%)</th>
<th>Overall (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gymnocephalus cercariae</td>
<td>Echinostome caercariae</td>
</tr>
<tr>
<td><strong>Lymnaea auricularia</strong> (n= 7928)</td>
<td>59 (0.7)</td>
<td>2226 (28.1)</td>
</tr>
<tr>
<td><strong>Lymnaea luteola</strong> (n= 2880)</td>
<td>19 (0.66)</td>
<td>747 (25.9)</td>
</tr>
<tr>
<td>Pearson’s Chi-square with continuity correction</td>
<td>0.109 (P&gt;0.05)</td>
<td>4.75 (P&lt;0.05)</td>
</tr>
</tbody>
</table>

Table 2. Seasonal incidence of cercarial infections in *L. auricularia* snail

<table>
<thead>
<tr>
<th>Cercariae recorded</th>
<th>Seasons of the year</th>
<th>Chi-square value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monsoon (n=2616)</td>
<td>Winter (n= 2462)</td>
</tr>
<tr>
<td>Echinostome caercariae</td>
<td>79.1 (123 ± 0.8)</td>
<td>86.6 (129 ±12.4)</td>
</tr>
<tr>
<td>Gymnocephalus cercariae</td>
<td>0.4 (880 ± 2.64)</td>
<td>0 ( 0 )</td>
</tr>
</tbody>
</table>

Seasonal incidence is expressed as percentage; n, number of snails examined

** P<0.001
The load of echinostome cercariae was 94\pm 2.2, 89\pm 2.3 and 87\pm 1.8 in summer, winter and monsoon respectively (Table 3). Unlike echinostome cercarial incidence in *L. auricularia* the incidence of gymnocephalus cercariae was recorded almost similar in monsoon (0.4%) and summer (0.36%), but in *L. luteola* it was relatively higher in monsoon (4.6%), followed by summer (1.7%). Surprisingly no gymnocephalus cercaria was found in winter. Whereas, in both the lymnaeid snails, cercarial burden was recorded higher in summer than in monsoon. The estimated number of gymnocephalus cercariae in *L. auricularia* was 960\pm 1.3 in summer and 880\pm 2.6 in monsoon while *L. luteola* harboured 542\pm 1.9 cercariae in summer and 520\pm 1.7 in monsoon. To study the population dynamics it was noted that larger sized snails harboured more cercariae than the smaller sized snails.

This study revealed that *Fasciola* eggs hatched by 20-21 days at room temperature (24–26ºC), while *Echinostoma* eggs took 22-23 days for hatching under experimental conditions. This study also showed that *L. auricularia* snails of groups A and B previously infected with *Fasciola* and *Echinostoma* miracidia developed infections with gymnocephalus and echinostome cercariae respectively. The time required for the release of mature gymnocephalus cercariae ranged from 36 to 49 days while for echinostome cercariae ranged between 16 and 25 days (Table 4). Notably, snails concomitantly infected with both the miracidia developed infection only with echinostome cercariae (Group C, Table 3). More interestingly, snails having an earlier exposure to *Echinostoma* miracidia followed by an exposure to *Fasciola* miracidia or vice versa were found to harbour only echinostome cercariae (Groups D and E). In these cases snails developed infections and released cercariae within 17-25 days (Table 4).

**DISCUSSION**

The overall higher incidence of cercarial infection in *L. auricularia* than in *L. luteola* observed in the present study is in conformity with the earlier findings. Rahman *et al.* (1997) recorded 12.6% cercarial infection in *L. auricularia* and 7.3% in *L. luteola*. The reasons behind higher incidence with echinostome cercariae but relatively lower incidence of gymnocephalus cercarial infection in *L. auricularia* than in *L. luteola* are difficult to explain but it may be due to some unexplored physiological interactions between the respective parasitic developmental stages and the vector snails. In both the snails, relatively high incidence of echinostome cercarial infection and the cercarial load as well in summer than in winter and monsoon might be due to more concentration of both ducks and snails in the dry season and comparatively less chance of infection to snails in monsoon, as rain washes away the duck faeces and also removes the snails. On the other hand, relatively higher incidence of gymnocephalus cercarial infection both in
Table 3. Seasonal incidence of cercarial infections in *L. luteola* snail

<table>
<thead>
<tr>
<th>Cercariae recorded</th>
<th>Seasons of the year</th>
<th>Chi-square value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monsoon (n=685)</td>
<td>Winter (n= 784)</td>
</tr>
<tr>
<td>Echinostome cercariae</td>
<td>63.1 (87 ± 1.8)</td>
<td>65.0 (89 ± 2.3)</td>
</tr>
<tr>
<td>Gymnocephalus cercariae</td>
<td>4.6 (520 ± 1.7)</td>
<td>0 ( 0 )</td>
</tr>
</tbody>
</table>

Seasonal incidence is expressed as percentage; n, number of snails examined

** P<0.001

Table 4. Experimental infection of *L. auricularia* snails with *Echinostoma* and *Fasciola* miracidia

<table>
<thead>
<tr>
<th>Snail groups†</th>
<th>Name of the miracidia infective to snails‡</th>
<th>Cercariae developed</th>
<th>Name of cercariae</th>
<th>Time required (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><em>Fasciola</em></td>
<td>Gymnocephalus</td>
<td></td>
<td>36–49</td>
</tr>
<tr>
<td>B</td>
<td><em>Echinostoma</em></td>
<td>Echinostome</td>
<td></td>
<td>16–25</td>
</tr>
<tr>
<td>C</td>
<td><em>Fasciola &amp; Echinostoma</em>, concomitantly</td>
<td>Echinostome</td>
<td></td>
<td>18–24</td>
</tr>
<tr>
<td>D</td>
<td><em>Echinostoma</em> then <em>Fasciola</em> at 48 h interval</td>
<td>Echinostome</td>
<td></td>
<td>17–25</td>
</tr>
<tr>
<td>E</td>
<td><em>Fasciola</em> then <em>Echinostoma</em> at 48 h interval</td>
<td>Echinostome</td>
<td></td>
<td>17–24</td>
</tr>
</tbody>
</table>

† Laboratory reared snails were grouped each with 20
‡ Miracidia were allowed to infect snails taken in a quarterly filled petridish
L. auricularia and L. luteola in monsoon than in summer might be due to some environmental factors. Fasciola eggs hatch out by 11-12 days at 25°C and the mature cercariae comes out from day 42 of infection up to 118-127 days at 23.5°C to 26.3°C (Kendall, 1954). So, the over wintered snails infected with Fasciola miracidia in the summer release mature cercariae in monsoon. The absence of gymnocephalus and the presence of echinostome cercariae in the winter reflects less contamination of pasture in monsoon by Fasciola gigantica infected cattle faeces as the animals are mostly confined in the stable yards in the rains. Apart from this, low environmental temperature hampers both the hatching of Fasciola eggs and their larval development in the snails. Moreover, in winter the snails undergo aestivation, which also keeps the snails away from Fasciola miracidial infection. The larger sized snails were mostly infected and harboured more number of cercariae of either species of trematodes. This finding is in agreement with the observation of Lim and Lie (1969) who reported that parasite population size or parasite mass was dependent on biomass of the snail host and not size of the infective dose of parasites.

Under experimental conditions, hatching of Fasciola eggs by 20-21 days and Echinostoma eggs by 22-23 days of incubation at room temperature (24–26°C) differs from the earlier reports. Fasciola gigantica eggs hatch in 17 days at 26°C and Echinostoma eggs hatch under favourable condition by 21 days (Soulsby 1982). According to Fried (1985) Echinostoma eggs hatch by 8-12 days at 26–28°C. In this study, incubation of eggs at room temperature (24–26°C) without a strict maintenance of certain temperature may be the causes of time variation for egg hatching. Hatching time of eggs may also vary due to the species and strain variation of the parasites involved. Fasciola larval stages developed and cercariae emerged by 36-49 days only in snails of group A. But echinostome cercariae emerged by 16-25 days from snails of group B, C, D and E. Development of no gymnocephalus but only echinostome cercariae in group C, D and E snails suggests that mixed infection of the snails with Echinostoma and Fasciola miracidia precluded subsequent development of Fasciola cercariae. Although the exact mechanism of this inhibitory phenomenon supported by histological and immunobiochemical studies were not searched in this study, but direct predation and ingestion of echinostome or schistosome cercariae by echinostome rediae have been observed in snails (Lie et al. 1967 and Heyneman and Umathey 1968) and double infections involving redia-producing species, especially echinostomes are rare (Boray 1967 and Vernberg et al. 1969). A snail host that already supports one species of parasite to its maximum capacity offers a diminished biomass for support of a second parasite, although some compensatory hypertrophy of the host may occur (Pan 1965). Suhardono, et al. (1997) reported that competition of Echinostoma from ducks with Fasciola in the
snails hampered the development of *Fasciola* and is an effective biological control measure of fascioliasis.

In conclusion, from field observations and laboratory experiment it is assumed that there is inhibitory and/or suppressive effect of developmental stages of echinostome on the gymnocephalus cercariae in the vector lymnaeid snails. Additionally the present findings may contribute to further understanding if competition between the gymnocephalus and echinostome cercariae to be used as a bio-control tool for fasciolosis in Bangladesh.

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