Investigation on Infection of Trematodal Larvae in Snails in Taunggyi and Ayetharyar Areas, Myanmar

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Abstract | During the study period, a total 1,632 snails belonging to eight species which act as intermediate host(s) of trematodes were collected by hand picking using the time-collection method from near watering points. Among them, 13.2% (216/1,632) snail samples were found to be infected with trematode larvae. Abundance of infected snails was higher in rainy season showing significant relationship with monthly temperature and monthly rainfall. Abundance of infected snails was higher in Taunggyi Township than in Ayetharyar Township.

Keywords | Snails, Trematodes' larvae, Intermediate host, Rainy season, Myanmar

INTRODUCTION

Freshwater snails play a significant role in life cycles of economically important trematodal infections like amphibostomosis and fasciolosis (Kaur et al., 2008). In addition to providing reproduction to these parasites, freshwater snails also act as carriers to transport them to their next final hosts. Acting as obligatory intermediate hosts for 18,000 trematode species, snails have a close relationship with trematodes (Mas-Coma et al., 2005; Zbikowska and Nowak, 2009). Depending on the trematode species, cercaria directly penetrates into the final host or metacercaria is formed. Metacercaria creates cysts in outer environment or penetrates into the second vertebrate or invertebrate intermediate host (including snails) (Mas-Coma et al., 2005; Zbikowska and Nowak, 2009; Kaur et al., 2009). The danger of disease spreading into the new areas and food borne helminthozooses depends on the possible establishment of snail vectors (Narain et al., 1994; Chhabra and Singla, 2009).

The optimum temperature range for development of the snail is 15°C - 26°C. At this temperature rapid production of snail egg masses occurs. These eggs hatch within two weeks and the resulting snails mature a month later. Thus, one snail can produce several thousand descendants within a period of 10 - 12 weeks. No development and no reproductive activity takes place at temperatures below 10°C, however, snails may survive adverse conditions for months buried in the mud (Hansen and Perry, 1994). The overall metacercarial production is influenced by the temperature at which the snails are kept during infection. The snails at temperature ranges between 20°C and 27°C produced more metacercariae than those at temperature below 20°C (Lee et al., 1995).

High temperatures may cause a reduction in humidity, facilitating the dehydration of the snails and the desiccation of the soil, thus inhibiting the development of the green algae that is the main food source for these snails (Ranggel, 1999). High rainfall favour development and survival of both the intermediate host snail and the developmental stages of the parasite (Torgenson and Claxton, 1999). Optimal moisture for snail breeding and development of
larval stages within the snails is provided when rainfall exceeds transpiration and saturation is attained (Urquhart et al., 1996). In animals, the high prevalence of fluke infestation might be due to heavy contamination of snail habitats and ingestion of metacercariae as a result of high stocking density and local overcrowding around watering points (Keyyu et al., 2005; Kaur et al., 2013).

Numerous studies have been conducted regarding the investigation on the abundance and species of snails bearing trematodes larvae all over the world. However, in Myanmar, reports on the abundance of snails have not yet reported so far. Therefore, it is believed that the information on intermediate host snails in Taunggyi area could assist in the development of trematode infection control program in livestock production. Hence the present study was envisaged on the investigation on infection of trematodal larvae in snails in Taunggyi and Ayetharyar Areas, Myanmar.

MATERIALS AND METHODS

STUDY AREA AND STUDY PERIOD
Snail sample collections were carried out from April 2012 to July 2012 in Taunggyi and Ayetharyar Townships, Southern Shan State.

SAMPLE COLLECTION
Snail were collected from areas of snail habitat that were likely to be regularly contaminated with cattle faeces, for example, near paths emanating gateways, near watering points and favoured lying-up area (Figure 1). The snails were collected as per Gray and Parr (2000) through hand picking using the time-collection method that is, counting of total number of any snails collected during 30 – 40 min at collection site. Collected snails were placed alive in plastic containers and labeled separately by location and date.

The examination and identification of larval stages of parasites (miracidium, sporocyst, rediae and cercariae) was performed as per the method of Claxton et al. (1997). All the snails were taken to the laboratory and each was crushed in a motor by using a pestle and crushed pieces were transferred onto a glass slide. A few drops of normal saline was put onto the slide and covered with a cover slip and examined under a stereo microscope (×100) for the presence of developmental stages of larval trematodes.

SPECIES IDENTIFICATION OF SNAIL
Species identification of snail was based on morphology as described in wikepedia (https://en.wikipedia.org) and Mackie and Claudi (2010).

STATISTICAL ANALYSIS
ANOVA ‘F’ was used to test the significance of monthly abundance of infected snail among the study months. Independent sample ‘t’ test was used to compare the mean number of infected snail samples between summer and
Figure 3: Infected intermediate host snail species; A) Lymnaea truncatula; B) Lymnaea luteola; C) Viviparus species; D) Melanoides tuberculata; E) Biomphalaria glabrata

Figure 4: Trematodes' larvae observed in snails; A) Miracidium (×400); B) Redia (×400); C) Cercaria (×400)

rainy seasons and Simple Linear Regression analysis was done to find out the relationship between the meteorological data and abundance of infected snails. All the analyses were performed at the significance level of 0.05.

RESULTS

Based on the morphology of snail, ten different snail species could be identified. Among them, only 8 species were intermediate hosts of trematodes. Therefore, total number of collected intermediate host snails was 1,632 (Figure 2).

Among the observed intermediate host snail species, only 5 species (Figure 3), Lymnaea truncatula, L. luteola, Viviparus species, Melanoides tuberculata and Biomphalaria glabrata, were found to be infected with larval stages of trematodes (Figure 4). Physella acuta, Planorbis species and L. auriculata were free from any trematodal larval infection (Figure 5). Number of infected snails species in Taunggyi and Ayetharyar Townships was described in Figure 6.

The number of infected snails was significantly different among the months of study period. The number of infected snails was highest in July (98/612) and the followed by May (58/428), June (48/416) and lowest in April (12/176). There was not significant difference in infected snails between May and June, and between June and April (Figure 7). The percentage of infected snails was significantly higher in rainy season (146/1,028) than that of summer season (70/604) (Figure 8).

According to Simple Linear Regression analysis, abundance
Figure 5: Non-infected intermediate host snail species; A) Physella acuta; B) Planorbis species; C) Lymnaea auricularia

Figure 6: Number of infected snail species in Taunggyi and Ayertharyar Townships

Figure 7: Monthly abundance of infected snails

Table 1: Meteorological data and abundance of infected snails

<table>
<thead>
<tr>
<th>No.</th>
<th>Month</th>
<th>Temp. (°C)</th>
<th>Rainfall (mm)</th>
<th>% of infected snails</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>April</td>
<td>22.8</td>
<td>83.1</td>
<td>6.8</td>
</tr>
<tr>
<td>2</td>
<td>May</td>
<td>22.6</td>
<td>249.9</td>
<td>13.6</td>
</tr>
<tr>
<td>3</td>
<td>June</td>
<td>21.5</td>
<td>96.0</td>
<td>11.5</td>
</tr>
<tr>
<td>4</td>
<td>July</td>
<td>20.5</td>
<td>258.8</td>
<td>16</td>
</tr>
</tbody>
</table>

(Source: Taunggyi meteorological station, 2012)

Figure 8: Seasonal abundance of infected snails

Figure 9: Monthly temperature and monthly abundance of infected snails

of infected snails was significantly associated with monthly temperature and monthly rainfall. Meteorological data and abundance of infected snails during the study period from April 2012 to July 2012 are shown in Table 1, Figure 9 and 10.

DISCUSSION

In this first investigation on the prevalence of snails harbouring trematodes larvae in Myanmar out of eight species of intermediate host snails collected only five species (13.2%) were found carrying trematodal larvae.
In this study, 10.8% of *L. luteola* carried trematode infection. *Lymnaea luteola* is the first intermediate host for the trematodes such as *Schistosomes*, *F. gigantica*, *F. hepatica*, *Clonorchis sinensis*, *Paragonimus westermani*, *Metagonimus*, *Dicrochitrema formosanum*, *Opisthorchis sinensis*, *Haplorchis species*, *Centrocestus species* and *Diorchitrema formosanum*. Therefore, it is also required to have attention in human for the occurrence of clonorchiasis, metagonimiasis and opisthorchiasis because *Melanoides* species is the first intermediate host for fish borne trematodes.

The prevalence of infected *Melanoides tuberculata* was 17.9%. *Melanoides tuberculata* serve as first intermediate host for *Clonorchis sinensis*, *Paragonimus westermani*, *Metagonimus*, *Dicrochitrema formosanum*, *Opisthorchis sinensis*, *Philophthalmus* species, *Haplorchis species* and *Centrocestus formosanus*. Therefore, it is also required to have attention in human for the occurrence of clonorchiasis, metagonimiasis and opisthorchiasis because *Melanoides* species is the first intermediate host for fish borne trematodes.

In this study, 10.8% of *L. luteola* carried trematode infection. *Lymnaea luteola* is the first intermediate host for the trematodes such as *Schistosomes*, *F. gigantica*, *F. hepatica*, *Clonorchis gigantica*, *Echinostoma revolutum*, *Orientobilharzia dattae* and *Echinoparyphium bugulai*. Therefore, the presence of *L. luteola* species in this study area favours the occurrence of fasciolasis in cattle.

*Biomphalaria glabrata* and *Viviparus* species were observed harbouring trematodes’ larval infection as 10.5% and 13%, respectively. *Biomphalaria glabrata* is a vector of schistosomiasis. *Viviparus* species is host of many parasites in its native including cercaria, metacercaria, ciliated protozoans, annelids and chironomid larvae. Thus, it should be considered in the context of other concurrent trematode infections although trematodes of medical and veterinary importance, like the mammalian schistosomes do not occur in this study.

According to the findings of this study, the prevalence of infected snails was lower in the summer season (11.6%) than that of rainy season (14.1%). It was comparable with the finding of Rondelaud (1994) who stated that the number of released cercariae is low or none during summer; however, after rains, cercarial shedding immediately increase. As suggested by Rangel (1999) high temperature may cause a reduction in humidity, facilitating the dehydration of the snails and the desiccation of the green algae that are the main food source for the snails. The duration of the snails’ viability is directly related to relative humidity and inversely to temperature and exposure to sunlight (Spithill et al., 1999).

In the present study, the abundance of infected snails had negative relationship with monthly temperature positive relationship with monthly rainfall. The high rainfall favours the development and survival of both the intermediate host snail and the developmental stages of the parasites (Torgerson and Claxton, 1999). The abundance of infected snails was higher in Taunggyi Township (74.4%) than that of Ayetharyar Township (25.6%). It can be explained that different abundance of infected snails in two townships may be due to locating at different altitudes. Taunggyi Township has more availability of suitable habitat for the snail vectors due to different altitude and rainfall. Therefore, it is clear that trematode infection in snail is spreading in this study area. The specific strains of trematodes larvae found in snails should be investigated by advanced molecular techniques for further studies.

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CONFLICT OF INTEREST

No conflict of interest.

AUTHOR’S CONTRIBUTION

May June Thu, Lat Lat Htun and Saw Bawm carried out experimental works, data analysis, written manuscript and did all the correction and revision. Soe Soe Wai and Tin Tin Myaing advised study design.
REFERENCES

• Mackie GL, Claudi R (2010). Monitoring and control of macrofouling mollusks in fresh water systems (2nd ed) CRC press Taylor and Francis Group, LLC.