MAINTENANCE OF RADIX SNAILS AND ARTIFICIAL INFECTION WITH FASCIOLA GIGANTICA MIRACIDIUM IN LABORATORY CONDITION

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ABSTRACT

The snail intermediate hosts of Fasciola gigantica include Radix (Lymnaea) auricularia and Radix (Lymnaea) luteola are widely distributed in various regions of Tamil Nadu. These snails were found to be susceptible to experimental infection with Fasciola gigantica miracidium. A colony of adult R. auricularia and R. luteola was reared. Various developmental stages of lymnaeid snails from eggs to adult were studied under laboratory condition. The miracidia were developed from the eggs of the Fasciola gigantica flukes. The snails were exposed to the miracidia for infection for 30 days. It was found that snails were negative for any developmental stages after infection. The miracidium did not infect the snails. It showed that unsuccessful infection might be due to the variation between strains of F. gigantica and snails.

Key words: Fasciola gigantica, Infection, R. auricularia, R. luteola, Miracidium

INTRODUCTION

Tropical Fasciolosis caused by Fasciola gigantica is regarded as one of the most important single helminth infections of ruminants in Asia and Africa (Dalton, 1999). Prevalence of F. gigantica in ruminants was reported up to 80-100% in south east asian countries (Schillhorn van veen, 1980; Fabiyi, 1987). Kendall (1965) concluded that F. gigantica is transmitted worldwide by snails not readily distinguishable on morphological grounds or on grounds of their ecological requirements from the single super species Radix auricularia sensu lato. These snails are usually found in ponds and pools which have abundant aquatic vegetation and can be effectively maintained in the laboratory conditions.

However, reports on study of various developmental stages and infection with F. gigantica miracidia of Lymnaeid snails in India are scanty. The present study focuses on standardization of breeding and maintenance of lymnaeid snails and the infectivity of F. gigantica miracidia in laboratory conditions.

MATERIALS AND METHODS

Collection of snails

Field Radix spp. were located by prescreening snails from two different regions (Ooty and Vellore) in Tamil Nadu. They were collected in large plastic containers with care to avoid shell damage and transported to the laboratory.
Identification of snails

The snails collected were morphologically studied for identification and sent to Division of Parasitology, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh for confirmation.

Maintenance of snails in the laboratory conditions

The collected snails were divided into two groups and placed in 2x1 1/2 x1 feet glass aquaria. Each aquarium was provided approximately 10 lts of dechlorinated water with aeration. The aquaria were maintained on wooden shelves with natural light exposure. The water temperature and pH were monitored day after day. Radix spp snails were fed with lettuce leaves. Uncolored plastic sheets were set into the aquarium to collect eggs. The laid egg mass seen in the plastic sheets or sides and bottom of the tanks were transparent and jelly. The faeces were removed regularly by siphon method and the water was changed for once in two days (Prasad, 1989).

Maintenance of developmental stages of snails

The egg mass collected from snails was kept for development. The developmental stages from egg to adult were studied.

Collection of F. gigantica flukes and recovery of eggs

F. gigantica eggs were collected from the gall bladder washing of buffaloes slaughtered at Perambur slaughter house, Chennai, Tamil Nadu. The live flukes collected were washed thoroughly to remove the blood contaminants and immediately transported to the laboratory in normal saline. The flukes were incubated in normal saline at 37°C for the release of fertile eggs for nearly 3-4 hours. It was sieved and washed. The pellet containing the eggs was collected.

Development of miracidia for the infection in snails

Recovered eggs from F. gigantica flukes were transferred to petridishes containing distilled water and incubated at 26°C for 15 days for the development of miracidium. They were subsequently exposed to light to stimulate eclosion of miracidia.

Infection of snails

The snails from each species of mean length sizes (0.94 cm) (SD 0.08) and (0.7 cm) (SD 0.00 cm) of R. luteola and R. auricularia respectively, were placed in individual petridishes containing the released miracidium. 3-4 snails were introduced with 10-12 miracidium in a petridish. The preparation was maintained for 3-6 hours to ensure contact of the miracidium with the snail. After exposure, all snails were removed to the individual test tubes and the infection in these snails was confirmed after 4 weeks after exposure in sunlight by checking for the shedding of cercariae (Prasad, 1989).

RESULTS AND DISCUSSION

The collected snails from Tamil Nadu (Ooty and Vellore) have been morphologically measured (Table 1) and were identified as R. luteola and R. auricularia. Previous reports on the occurrence of these snails from Tamil Nadu
Several researchers have described Radix snail rearing techniques in the laboratory (Sanchez et al., 1995). Snail rearing techniques, maintenance of the aquaria and diet are extremely variable. Large water tanks were used for the maintenance of snails with suitable temperature and pH of water. The methodology used to rear and maintain Radix snails in the present study was found to be suitable in the laboratory (Souza and Magalhaes, 2000). The observation of the snail colonies was simplified since each colony is visible in the glass tank. Cleaning and feeding required approximately 10 minutes for each tanks. The natural environment is achieved through aerators for aeration stimulated snails as evidenced by their constant mobility position and egg mass production in aquatic environment. The water maintained between pH 6-7 with the temperature controlled at 26-27°C. Most materials employed are standard laboratory equipment which requires limited shelf space and not necessarily in an air conditioned room. The general description of normal developmental stages of fresh water pulmonate snails (Radix spp.) has been previously described elsewhere (Khangarot and Das, 2010). Four common developmental stages of snails were identified (Fig.1). The following normal embryonic stages were recorded.

i) The embryo (Morula stage) leaves the vitelline membrane and moves freely within the egg capsule by means of cilia (Fig. 1a).

ii) Larva (Trochophore-3-days old), showing the shell gland and prototroch. Embryo rotates within the egg capsule and it was more transparent. Early and mid trochophore (Fig. 1b & 1c).

iii) When larvae were 5 days old (Veliger stage) foot, reddish eyes, tentacles, and appearance of helical shell structure were noticed. Early and mid veliger stages (Fig. 1d & 1e).

iv) In Hipo or Veliconcha stage, the foot and viscera were well separated; embryo fully occupied the whole egg capsule (Fig. 1f). The larval structures degenerate gradually with following 2 days and then the young snail hatched at 10-15 days (Fig. 1g).

Boray (1963) described a standard procedure for culture of R.tomentosa, which has been widely adopted and successfully applied to R.truncatula, R.peregra, R. palustris and R. natalensis (Hildebrandt, 1968). The snail was successfully maintained under defined laboratory conditions (Sharma et al., 1989). Kendall (1954, 1965), regarded the race of main intermediate hosts in South, West and East Africa as R. auricularia natalensis and in India, Bangladesh and Pakistan as R. auricularia rufescens. The Molluscan intermediate host R. auricularia rufescens were found in Lucknow and Bareilly, Uttar Pradesh (Thapar and Tandon, 1952, and Prasad, 1989). R. auricularia (sensu stricto) were found in the Bareilly, Uttar Pradesh and in Kashmir (Sharma et al., 1989).

In the present study, the snails from the high altitude (Ooty) and plain north eastern (Vellore) zones in Tamil Nadu were collected and the methodology used to rear and maintain these snails was found to be suitable in our laboratory. Thus, it reveals the rearing of snail colonies were important and in the future, these snails will be subjected to experimental infection with Fasciola gigantica in order to obtain
cercaria, metacercariae and the adult
worms for vaccine production. The adult
F.gigantica flukes were collected from the local
abattoir and identified morphologically. The
length and breadth of the F.gigantica eggs in
the present study was 152.43±22.08 µm (n=10)
and 109.22±14.55 µm (n=10). The embryo and
miracidial developmental stages of eggs under
laboratory conditions were maintained and it
was observed microscopically (Fig.2 & 3). The
F.gigantica miracidia begin to swim immediately
after hatching. The epithelial cells contain cilia
arranged in regular longitudinal rows and the
anterior end of the miracidium contains stylet
for the penetration into snails (Fig 4).

The morphometrical measurement of
miracidium was presented in Table 2. The total
length and breadth of the body and stylet of the
miracidium was interpreted. Hatching of
F.gigantica eggs is achieved by muscular
contraction of the miracidium which when
stimulated by light. The miracidium is the first
free-living larval stage of F.gigantica and is a
non-feeding or 
ganism. The time required for the
development of miracidia in eggs of
F.gigantica varies with temperature. The
temperature maintained in our laboratory for the development
of miracidia was 26°C and 80% of the eggs were
developed successfully.

We could also find that the eggs were
not survived at the temperatures at 37°C or
higher than that. This is similar to that of
Grigoryan (1958) who reported the temperature of
24-26°C and pH of 6.5-7.0 were found to be
optimal and 70-80% of eggs would develop under
such conditions. He also found that eggs
did not survive at temperatures higher than 43-
44°C and that desiccation also was rapidly
fatal.

The infection of F.hepatica and
F.gigantica miracidium with their respective
snails were reported in many countries. Boray
(1966) obtained infection rates that varied from
0-100% in studies involving L.stagnalis,
L.palustris, L.peregra, L.truncatula,
L.auricularia, L.tomentosa and L.lessoni
originating from Germany, Austria, Kenya and
Australia and infected with miracidia of
compared the susceptibility of L.rufescens from
Pakistan with that of L.natalensis from Africa
to infection with miracidia of a West African
strain of F.gigantica. However infection with
F.gigantica miracidia to the snails collected
from Ooty and Vellore in Tamil Nadu in our
laboratory were unsuccessful and obtained no
cercariae from the suitable Radix snails. In
accordance with that of present study, Guralp
et al., (1964) who were unsuccessful in their
attempts to establish infection with F.gigantica
in L.truncatula obtained from Pakistan and
Turkey respectively. The study of non-
infectivity of F.gigantica miracidium from
Chennai strain with Lymnaeid snails collected
from Vellore and Ooty confirms the earlier
reports (Guralp et al., 1964). This may be due
to the disparity between strains of F.gigantica
and snails. Conflicting reports of this kind are
probably due to variations from place to place
between strains of F.gigantica and snails which
alter the host-parasite relationship in favour of
one or the other.

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Developing a validation portfolio to exploit key
virulence proteins in Fasciola species for
parasite control”
REFERENCES


Rao, C. M. P., (1966). On the comparative susceptibility of Lymnaea natalensis (Kraus) and L.rufescens (Gray) to infection with Fasciola gigantica (West African Strain) and the tissue responses in the snails. J. Helminth., 40: 131-140.


Table 1. Measurement of snails collected from Ooty and Vellore

<table>
<thead>
<tr>
<th>S.No</th>
<th>Area</th>
<th>Source</th>
<th>Stage of snails</th>
<th>Length of the shell (cm)</th>
<th>Breadth of the shell (cm)</th>
<th>No.of whorls</th>
<th>Aperture size (cm)</th>
</tr>
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<tbody>
<tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Length</td>
</tr>
<tr>
<td>1</td>
<td>Ooty</td>
<td>Ponds</td>
<td>Adult</td>
<td>0.94±0.06</td>
<td>0.52±0.01</td>
<td>3±0.00</td>
<td>0.62±0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Juvenile</td>
<td>0.7±0.00</td>
<td>0.4±0.00</td>
<td>3±0.00</td>
<td>0.5±0.00</td>
</tr>
<tr>
<td>2</td>
<td>Vellore</td>
<td>Ponds</td>
<td>Adult</td>
<td>0.90±0.07</td>
<td>0.50±0.03</td>
<td>3±0.00</td>
<td>0.55±0.08</td>
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</table>

Table 2. Morphometrical measurement of miracidium

<table>
<thead>
<tr>
<th>Length</th>
<th>Breadth</th>
<th>Length</th>
<th>Breadth</th>
<th>Length of the stylet</th>
</tr>
</thead>
<tbody>
<tr>
<td>With cilia (µm) n=10</td>
<td>Without cilia (µm) n=10</td>
<td>(µm) n=10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180.27±4.39</td>
<td>135.69±5.89</td>
<td>151.88±11.12</td>
<td>114.37±7.06</td>
<td>8.42±0.77</td>
</tr>
</tbody>
</table>
**Fig. 1** Normal developmental stages of *R. auricularia* at different days after egg laying until hatching. (a) Morula stage, (b) Early trocophore, (c) Mid trocophore, (d) Early and Mid veliger stages, (e) Late veliger stage, (f) Hipo stage, (g) Newly hatched snail.
Fig. 2 & 3. Embryo and miracidial developmental stages of *F. gigantica* egg

Fig. 4 Microscopical observation of *F. gigantica* miracidium released from eggs