LABORATORY PRODUCTION AND MAINTENANCE OF SPIROMETRA ERINACEI SPARGANA

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Abstract. The experiment was carried out to produce and maintain Spirometra erinacei spargana, the cause of human sparganosis, in the laboratory. It began with collection of eggs from gravid proglottids of adult worms obtained from naturally infected dogs. The eggs were incubated in distilled water at room temperature for embryonation. The fully developed embryos (coracidia) began to hatch 6 days after incubation. The coracidia were given to the first intermediate host, Mesocyclops aspericornis, where they developed into procercoids. After 12 days in Mesocyclops aspericornis, procercoids were infective to the second intermediate host, eg, tadpoles. After ingestion by tadpoles, the procercoid transformed to a plerocercoid or sparganum. As many as 64 spargana were found in a single tadpole. In addition, procercoids were able to grow and develop into spargana in mice, but the infection rate was very low (7%). Although mice were not very susceptible to procercoid infection, they were highly susceptible to spargana. By oral infection of mice, spargana survived in various organs without further development. Very high percentages of worm recoveries (60-100%) were obtained. The results show that mass production of spargana can be achieved by procercoid infection in tadpoles and the resulting spargana can be maintained in mice for specific experimental purposes.

INTRODUCTION

Spirometra erinacei is a pseudophyllidean tapeworm in dogs and cats. Its life cycle requires two different intermediate hosts, freshwater copepods as first intermediate host, and some vertebrates, such as amphibians, reptiles, and some mammals, as second intermediate hosts (Mueller, 1974; Lee et al., 1990). Its larval stage, the plerocercoid or sparganum, causes sparganosis in man. Humans can be infected with sparganum in 3 ways. First, ingestion of raw snakes, frogs or other animals containing spargana. The ingested spargana penetrate the intestinal wall, migrate to organs and stay alive in the tissue. Second, ingestion of infected copepods contaminating drinking water. Procercoids emerge from copepods, penetrate the intestinal wall to organs and develop into spargana in the tissue. The third is the application of the flesh of infected frogs to a wound or sore eyes, whereby the sparganum is transferred from the second intermediate host to human tissue (Tansurat, 1975).

Human sparganosis has been reported sporadically with worldwide distribution (Beaver et al., 1984; Sarma and Weilbaecher, 1986; Kron et al., 1991; Tsai et al., 1993; Jeong et al., 1998; Cummings et al., 2000). In Thailand, sparganosis has been reported in all parts of the country (Kittiponghansa et al., 1988; Tesjaroen, 1991; Chamadol et al., 1992; Ausayakhun et al., 1993; Jirawattanasomkul and Noppakun, 2000; Phunmanee et al., 2001). So far, very little is known about the S. erinacei in Thailand. Neither information about the infection of this parasite in dogs, nor the development of the larval stages have been reported in Thailand. Therefore, this study was designed to produce and maintain the spargana of S. erinacei in the laboratory for further study.

MATERIALS AND METHODS

Adult S. erinacei were collected from the small intestines of naturally infected dogs from Sakon Nakhon Province, northeast Thailand. They were washed in distilled water, and the gravid proglottids isolated for egg collection.

Egg collection and incubation

The uteri in the gravid proglottids were teased apart by fine needles under a stereomicroscope. The released eggs were then collected, rinsed with distilled water and incubated at room temperature. The process of embryonation of the eggs was observed through a light microscope. The hatched larvae, coracidia, were also observed microscopically before infecting the copepods.

Infection of copepods with coracidia

Batches of Mesocyclops aspericornis were exposed for 24 hours to newly-hatched coracidia in a beaker containing dechlorinated water. The infected Mesocyclops aspericornis were then pooled and kept in a container at room temperature and fed with Paramecium. In Mesocyclops aspericornis, the coracidia developed to procercoids in the body cavity.
and became infective to the tadpoles after 12 days’
infection.

**Infection of tadpoles with procercoids**

Three-week-old tadpoles (*Rana* sp), purchased
from “Frog Farm” in Khon Kaen Province, were mixed
in a beaker with *Mesocyclops aspericornis* containing
fully developed procercoids. Twenty-four hours later,
the infected tadpoles were pooled in an aquarium and
fed twice a day with commercial food pellets. In
tadpoles, the procercoids developed to plerocercid or
sparganum.

**Infection of mice with procercoids**

Eight male Swiss albino mice, 6-8 weeks old, were
each orally infected with 50 procercoids in *Mesocyclops aspericornis*
by stomach intubation under light anesthesia. After infection, they were housed in
groups of 4 per cage and fed with commercial food
pellets (C.P. Thailand) and water *ad libitum*.

**Infection of mice with spargana**

Nine male Swiss albino mice, 6-8 weeks old, were
each infected with 5 spargana recovered from infected
tadpoles in the same manner and were looked after in
the same way as those infected with procercoids.

**RESULTS**

**Embryonation of eggs**

Fig 1 shows that after incubation of an
unembryonated egg at room temperature for 2 days, the

![Fig 1- Embryonation of *S. erinacei* eggs in distilled water. 1A) Unembryonated egg from gravid proglottids. 1B) After 2 days of incubation. 1C) Fully developed coracidium 6 days after incubation. 1D) Coracidium after hatching.](image)

**Table 1**

<table>
<thead>
<tr>
<th>Infected organs</th>
<th>No. of spargana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tail</td>
<td>254</td>
</tr>
<tr>
<td>Abdominal cavity</td>
<td>187</td>
</tr>
<tr>
<td>Back</td>
<td>184</td>
</tr>
<tr>
<td>Hind legs</td>
<td>127</td>
</tr>
<tr>
<td>Mouth area</td>
<td>54</td>
</tr>
<tr>
<td>Abdominal wall</td>
<td>34</td>
</tr>
<tr>
<td>Front legs</td>
<td>10</td>
</tr>
<tr>
<td>Eyes</td>
<td>9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>859</strong></td>
</tr>
</tbody>
</table>

embryonation began to develop. Complete development
occurred within 5 days. The fully developed embryos
(coracidia) began to hatch 6 days after incubation. The
entire surface of the coracidium is covered with
numerous cilia, which are useful for swimming.

**Production of procercoids**

After coracidia were ingested by *Mesocyclops aspericornis*, they penetrated into the body cavity and
moved freely. They transformed to procercoids by day
4 and became infective to the second intermediate host
(tadpole) after 12 days (Fig 2).

**Production of spargana**

**In tadpole.** It was found that tadpoles were
susceptible to infection with procercoids. Procercoids
developed into spargana in various organs (Fig 3 and
Table 1). Most of tadpoles were infected with 1-6
spargana. However, as many as 64 spargana were
recovered from a single tadpole.

**In mice.** After the mice were orally infected with
50 procercoids in *Mesocyclops aspericornis*, only some
of them developed into spargana. Low percentages of
worm recoveries after 30 days were obtained (Table 2).

**Infection of mice with spargana**

Although mice were not very susceptible to infection
with procercoids (Table 2), they were highly susceptible
to infection with spargana. Table 3 shows high
percentages of worm recoveries (60-100%) in mice
infected with 5 spargana. All spargana survived and
increased in size without further development (Fig 4).

**DISCUSSION**

The *Spirometra* studied in the present experiment
Fig 2- Morphology of the procercoid. 2A) Procercoids in the body cavity of *Mesocyclops aspericornis* (arrowed = procercoids). 2B) Procercoid released from *Mesocyclops aspericornis*.

Fig 3- Spargana recovered from tadpoles after 30 days of infection.

was morphologically similar to *Spirometra erinacei* studied by Lee et al (1990) in Korea. The prevalence of *S. erinacei* in dogs in Sakon Nakhon Province, northeast Thailand was 8.15% (68 out of 834 dogs investigated). The first intermediate host in Khon Kaen was *Mesocyclops aspericornis* while *Mesocyclops leuckarti* and *Eucyclops serrulatus* were the first intermediate hosts in Korea (Lee et al, 1990). It has been known that frogs and snakes are 2nd intermediate hosts of *S. erinacei* (Miyazaki, 1991) but no information about the infection of spargana in these kinds of animals was available in Thailand. Therefore, it was not convenient to perform experiments using spargana. Production of spargana in the laboratory, as presented in this paper, can solve this problem. Tadpoles were suitable hosts for the production of spargana in the laboratory. They can be used for studying any specific purpose concerning spargana, eg, drug testing, host responses to sparganosis, and so on. However, keeping tadpoles or frogs in the laboratory is not convenient. Mice were the host of choice for maintenance of these larvae. They were susceptible to spargana infection and easily looked after in the laboratory. They allowed spargana to survive for a long time or until used.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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Table 3
Worm recoveries and parasitized organs in mice infected with 5 spargana of S. erinacei (30 days after infection).

<table>
<thead>
<tr>
<th>Mice no.</th>
<th>No. of spargana recovered (%)</th>
<th>Parasitized organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 (60)</td>
<td>Adipose tissue, kidney, subcutaneous tissue</td>
</tr>
<tr>
<td>2</td>
<td>3 (60)</td>
<td>Adipose tissue, kidney, subcutaneous tissue</td>
</tr>
<tr>
<td>3</td>
<td>5 (100)</td>
<td>Peritoneal cavity, subcutaneous tissue, hind legs</td>
</tr>
<tr>
<td>4</td>
<td>5 (100)</td>
<td>Adipose tissue, kidney, hind legs, subcutaneous tissue</td>
</tr>
<tr>
<td>5</td>
<td>4 (80)</td>
<td>Adipose tissue, kidney, subcutaneous tissue</td>
</tr>
<tr>
<td>6</td>
<td>3 (60)</td>
<td>Hind legs, kidney, subcutaneous tissue</td>
</tr>
<tr>
<td>7</td>
<td>4 (80)</td>
<td>Kidney, testes, peritoneal cavity, subcutaneous tissue</td>
</tr>
<tr>
<td>8</td>
<td>3 (60)</td>
<td>Kidney, subcutaneous tissue</td>
</tr>
<tr>
<td>9</td>
<td>5 (100)</td>
<td>Kidney, testes, hind legs, subcutaneous tissue</td>
</tr>
<tr>
<td>Total</td>
<td>35 (77.7)</td>
<td></td>
</tr>
</tbody>
</table>


