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.

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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 plerocercoid 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 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.

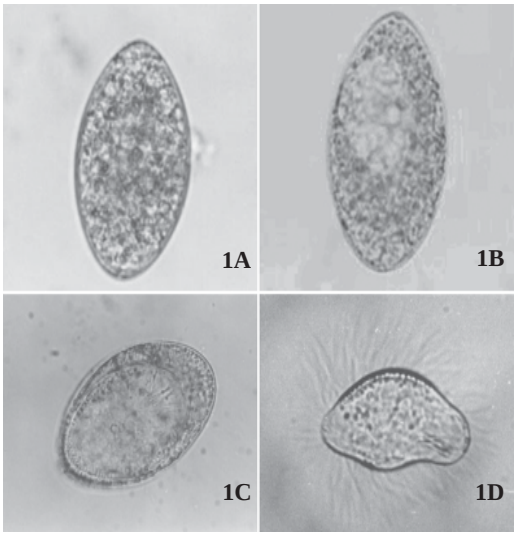

Fig 1- Embryonation of *S. erinacei* eggs in distilled water. 1A) Unembryonated egg from gravid proglottids. 1B) After 2 days of incubation. 1C) Fully developed coracidium 6 days after incubation. 1D) Coracidium after hatching.

Table 1
Infected organs and number of spargana in tadpoles infected with procercoids (Total tadpoles examined = 111).

Infected organs	No. of spargana
Tail	254
Abdominal cavity	187
Back	184
Hind legs	127
Mouth area	54
Abdominal wall	34
Front legs	10
Eyes	9
Total	859

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

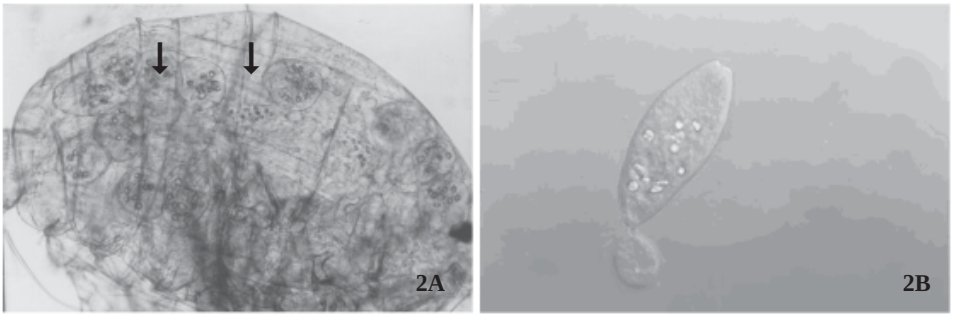

Fig 2- Morphology of the procercoid. 2A) Procercoids in the body cavity of *Mesocyclops aspericornis* (arrowed = procercoids). 2B) Procercoid released from *Mesocyclops aspericornis*.

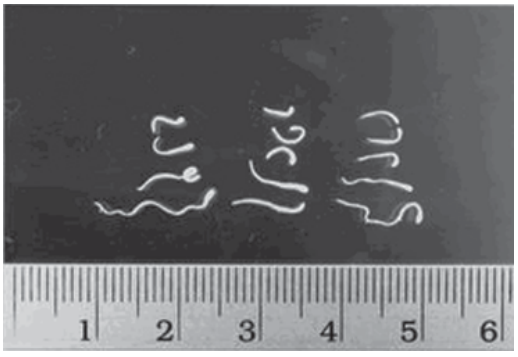

Fig 3- Spargana recovered from tadpoles after 30 days of infection.

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.

Table 2
Worm recoveries and parasitized organs in mice infected with 50 procercoids.

Mice no.	No. of spargana recovered (%)	Parasitized organs
1	6 (12)	Subcutaneous tissue, peritoneal cavity
2	6 (12)	Muscle, kidney
3	8 (16)	Liver, adipose tissue, peritoneal cavity
4	5 (10)	Adipose tissue, muscle
5	0 (0)	-
6	3 (6)	Muscle
7	0 (0)	-
8	0 (0)	-
Total	28 (7)	

ACKNOWLEDGEMENTS

This study was supported by a research grant from the Faculty of Medicine, Khon Kaen University. We thank Dr La-orsri Sanoamuang, Department of Biology, Faculty of Science, Khon Kaen University, for identification of *Mesocyclops aspericornis*.

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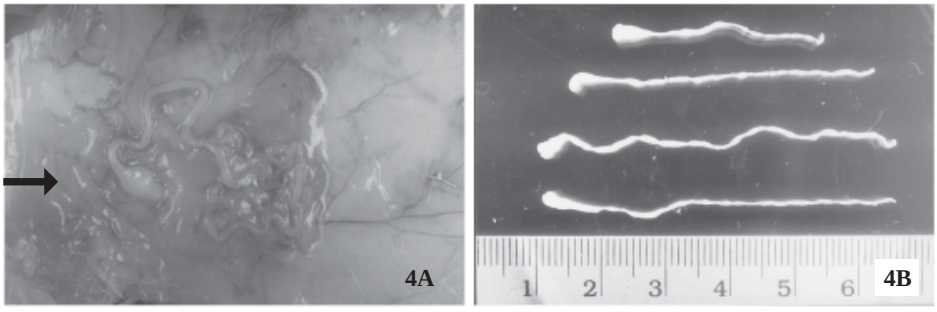

Fig 4- Spargana recovered from mice. 4A) A sparganum in subcutaneous tissue of a mouse (arrowed = scolex region). 4B) Spargana fixed in 10% formalin.

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

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

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