พัฒนาการของระยะตัวอ่อนของพยาธิ *Spirometra erinacei* ซึ่งทำให้คนเป็นโรคสปาร์กาโนซิส ในสัตว์ทดลอง

Development of larval stages of Spirometra erinacei, the cause of human sparganosis, in experimental animals

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บทคัดย่อ

ศึกษาพัฒนาการของระยะตัวอ่อนของพยาธิ *S. erinacei* ในห้องทดลอง โดยเริ่มจากการรวบรวมระยะตัวเต็มวัยของพยาธิจากลำไส้ของสุนัขที่ซื้อมาจากจังหวัดสกลนคร จำนวน 834 ลำไส้ ซึ่งพบการติดเชื้อพยาธิ 68 ลำไส้ หรือ 8.15% ไข่พยาธิที่รวบรวมได้จากมดลูกในปล้องสุกของตัวเต็มวัย สามารถเจริญเติบโตเป็นตัวอ่อนระยะ coracidium และฟักตัวในน้ำกลั่นภายใน 6 วัน ระยะ coracidia เจริญเติบโตเป็นระยะ procercoid ในไรน้ำ (*Mesocyclops aspericornis*) ซึ่งเป็นโฮสต์ตัวกลางที่ 1 (1^{st} IH) และเป็นระยะติดต่อภายใน 12 วัน Procercoidsสามารถเข้าไปเจริญเติบโตในลูกอ๊อด (tadpoles- *Rana* sp. : 2^{nd} IH) จนได้ระยะ plerocercoid หรือ sparganum ในเนื้อเยื่อต่างๆ หลังจากถูกป้อนให้หนูแล้ว Spargana จากลูกอ๊อดสามารถเจริญเติบโตในหนู (mice) โดยไม่มีการเปลี่ยนแปลงรูปร่างในอวัยวะต่างๆ โดยเฉพาะอย่างยิ่ง ใต้ผิวหนัง ไขมัน ไต กล้ามเนื้อ และช่องท้อง หนู (mice) เป็นโฮสต์ที่ดีสำหรับ sparganum ของ *S. erinacei* อัตราการติดเชื้อในหนูบางตัวสูงถึง 100% (60%-100%).

Abstract

The development of larval stages of *Spirometra erinacei* was studied in the laboratory. The experiment began with the collection of adults of *S. erinacei* from intestines of dogs obtained from Sakon Nakhon Province. Sixty-eight out of 834 dogs examined (8.15%) were infected with *S. erinacei*. Eggs isolated from the uteri of gravid proglottids were cultured in distilled water at room temperature. The eggs became embryonated and began to hatch on day 6 after culture. After ingested by *Mesocyclops aspericornis* (first intermediate host: 1^{st} IH), the coracidia, developed into procercoids within 12 days and they were infective to tadpoles (*Rana* sp.: second intermediate host: 2^{nd} IH). In tadpoles, procercoids developed into plerocercoid or sparganum in various organs. After orally introduced to mice, the spargana

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survived and grew without further development in various organs especially s ubcutaneous tissue, adipose tissue, kidney, muscle and abdominal cavity. Mice were highly susceptible to spargana of *S. erinacei* with the infection rates of 60-100%.

คำสำคัญ : สไปโรเมตรา สปาร์กานัม

Keyword : Spirometra, Sparganum

Introduction

Spirometra erinacei is a pseudophyllidean cestode of dogs and cats (Mueller, 1974). Its life cycle requires two different intermediate hosts, the fresh water cyclops as first intermediate host (1^{st} IH) and some vertebrates such as amphibians and reptiles as second intermediate host (2^{nd} IH) (Mueller, 1974; Lee *et al.*, 1990). Its larval stages, the procercoid develops in cyclops, the plerocercoid or sparganum develops in frogs or snakes. The plerocercoid or sparganum causes sparganosis in man. Humans can be infected with sparganum by 3 possibilities. First, ingestion of raw snakes, frogs or other animals that harbour the spargana. The ingested spargana penetrate the intestinal wall, migrate to organs and stay alive in the tissue. Second, ingestion of infected cyclops contaminated in drinking water. Procercoids emerge from cyclops penetrate the intestinal wall to organs and develop to spargana in the tissue. The third possibility 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. In the tissue, the sparganum survives without further development.

Human sparganosis has been reported sporadically worldwide in distribution. In Thailand, sparganosis has been reported from 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). At present, the knowledge about *S. erinacei* in Thailand is limited. No information about the infection of this parasite in dogs and little is known about the development of the larval stages in the intermediate hosts. Therefore, this study was designed to find out the infection rate in dogs and to observe the development of the larval stages of *S. erinacei* in experimental animals: the cyclops, tadpoles and mice.

Materials and Methods

Parasite collection

Adults of *S. erinacei* were collected from small intestines of dogs purchased from Sakon Nakhon Province, Northeast, Thailand. The worms were washed in distilled water and gravid proglottids were separated as the source of eggs.

Egg collection and incubation

The uteri in the gravid proglottids were teased apart by fine needles under a stereoscopic microscope. The released eggs (unembryonated) were 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 infection to the first intermediate host (cyclops).

Infection of cyclops by coracidia

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

Infection of tadpoles by procercoids

Three-week old tadpoles (*Rana* sp.), purchased from a frog farm in Khon Kaen Province, were mixed with cyclops containing fully developed procercoids in a beaker. Twenty four hours later, the infected tadpoles were pooled in an aquarium and fed twice a day with commercial food pellets. After 30 days in tadpoles, the procercoids developed to a new stage called plerocercoid or sparganum.

Infection of mice by spargana

Male Swiss albino mice, 6-8 weeks old, were used in this experiment. Each animal was infected with 5 spargana recovered from infected tadpoles by stomach intubation under light anesthesia. After infection, they were housed in group of 6 per cage, fed with commercial food pellets (C.P. Thailand) and water *ad libitum*.

Results

Natural infection of S. erinacei in dogs

It was found that 68 out of 834(8.15%) intestines examined harboured adult worms of *S. erinacei*. The number of worms recovered varied from dog to dog. Most of the infected dogs harboured only one worm while only a few dogs had high worm burden (Table 1).

Development of coracidia

After incubation of unembryonated egg (Fig. 1A) at room temperature for 2 days, the cloudy area appeared inside the egg shell (Fig. 1B). The development of the embryo gradually progressed and a complete embryo, the coracidium, appeared on day 5 of the incubation. The coracidia became mature (Fig. 1C) and began to hatch on day 6. Its entire surface was covered with cilia (Fig. 2). These coracidia moved actively, using their cilia, soon after hatching. They became exhausted and died within 2 days if they failed to infect fresh water cyclops (1st IH).

Development of procercoids

After coracidia were ingested by cyclops, they penetrated into the body cavity and moved freely. They were elongate in appearance at early stage. After 4 days in cyclops, procercoids (Fig. 3) were formed and became infective to the second intermediate host after 12 days in cyclops (Fig. 4).

Development of spargana

After eaten by tadpoles, procercoid larvae were released from the digested cyclops in the digestive tract and penetrated to the abdominal cavity. These procercoids migrated to various organs and developed to sparganum (Fig. 5). The number of spargana found in each infected tadpole varied depending on the number of procercoids ingested and the maximum was 64 spargana. They parasitized various organs especially the tail, abdominal cavity, muscle of the back and hind legs (Table 2).

Development and infectivity of spargana in mice

Table 3 shows that spargana from tadpoles were able to survive and grow in all mice infected. The sites of predilection of spargana in mice were

subcutaneous tissue (34.29%), kidneys (25.72%) and adipose tissue (17.14%). Spargana recovered from mice showed no changes in gross morphologies as their appearances were as the same as spargana obtained from tadpoles except the sizes of the worms. The spargana recovered from mice were much larger and longer than those recovered from tadpoles (Fig. 6A, 6B). The invaginated scolex and the tail were clearly visible.

Table 1 Natural infection of *S. erinacei* in dogs from Sakon Nakhon Province.

Date of collection	No. of dogs examined	No. of infected dogs	% Infection	Worm burden	
				Mean ± SD	Range
23 January 2002	60	6	10.00	1.16 ± 0.40	1-2
4 May 2002	50	5	10.00	1.00 ± 0	1
6 June 2002	68	6	8.82	1.33 ± 0.81	1-3
6 July 2002	62	5	8.06	2.40 ± 2.60	1-7
3 August 2002	55	3	5.45	2.00 ± 1.00	1-3
18 August 2002	57	0	0	0	0
31 August 2002	63	3	4.76	1.33 ± 0.57	1-2
8 September 2002	60	5	8.33	1.80 ± 0.83	1-3
7 October 2002	57	3	5.26	1.0 ± 0	1
8 October 2002	72	9	12.50	2.00 ± 1.00	1-4
21 April 2003	40	1	2.50	1	1
26 April 2003	40	2	5.00	6.50 ± 7.77	1-12
24 May 2003	70	9	12.86	1.44 ± 0.88	1-3
5 June 2003	80	11	13.75	1.54 ± 1.21	1-4
Total	834	68	8.15		

Table 2 Site of infection of spargana in tadpoles (Total animals examined = 111)

Site of infection	Mean + SD (range)
Tail	3.52 ± 3.31 (0-18)
Abdominal cavity	6.23 ± 8.30 (0-40)
Back	3.11 ± 2.69 (0-11)
Hind legs	2.76 ± 2.23 (0-10)
Mouth area	2.34 ± 2.67 (0-13)
Abdominal wall	2.12 ± 1.58 (0-7)
Front legs	1.42 ± 0.53 (0-2)
Eyes	4.50 ± 3.53 (0-7)

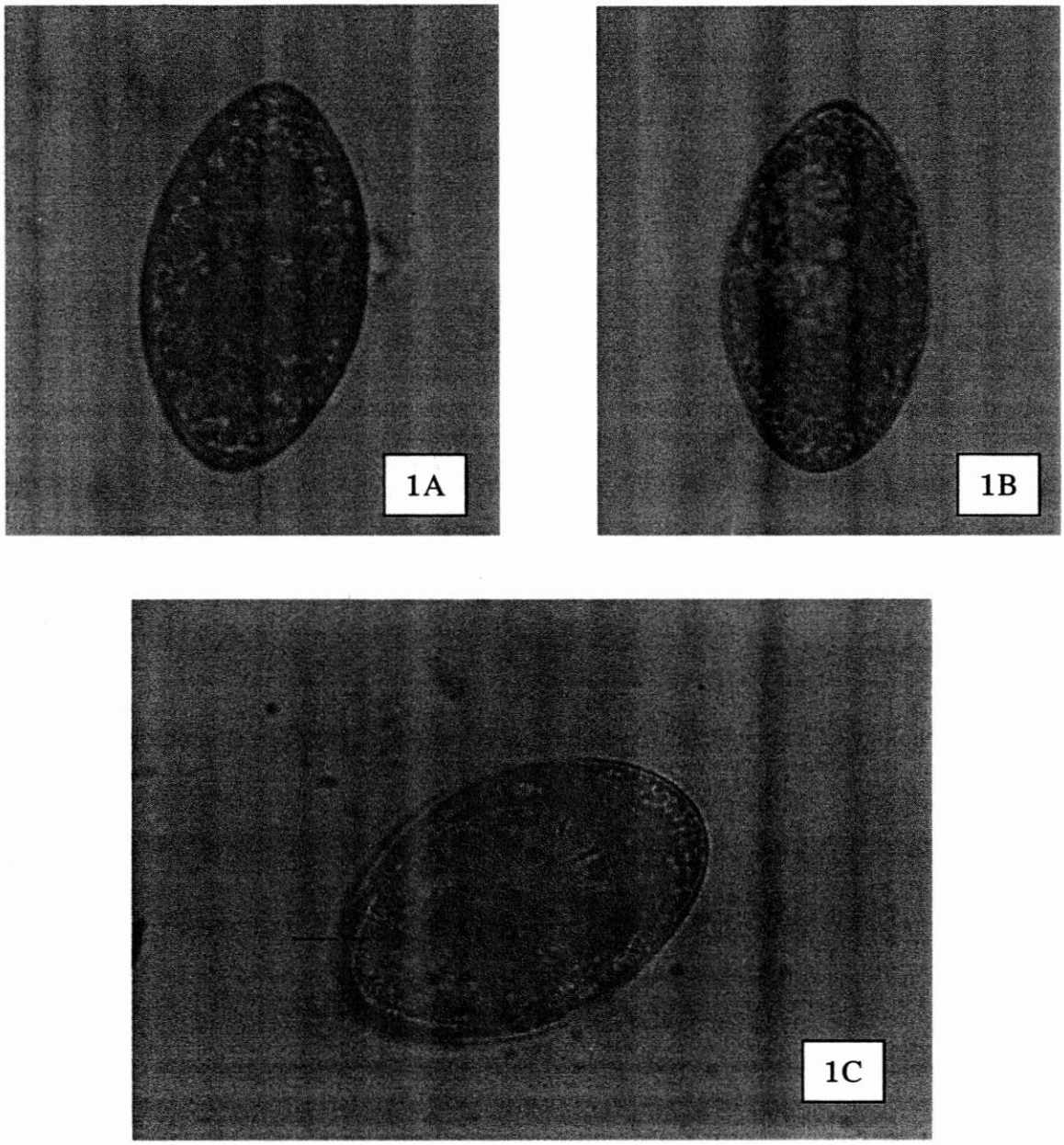

Figure 1 Embryonation of *S. erinacei* eggs in distilled water at different duration after incubation at room temperature.

1A : Unembryonated egg from gravid proglottids.

1B : After 2 days of incubation, the cloudy area appeared inside the egg shell.

1C : Fully developed coracidium (6 days after incubation). (arrowed = coracidium ; arrow head = hook)

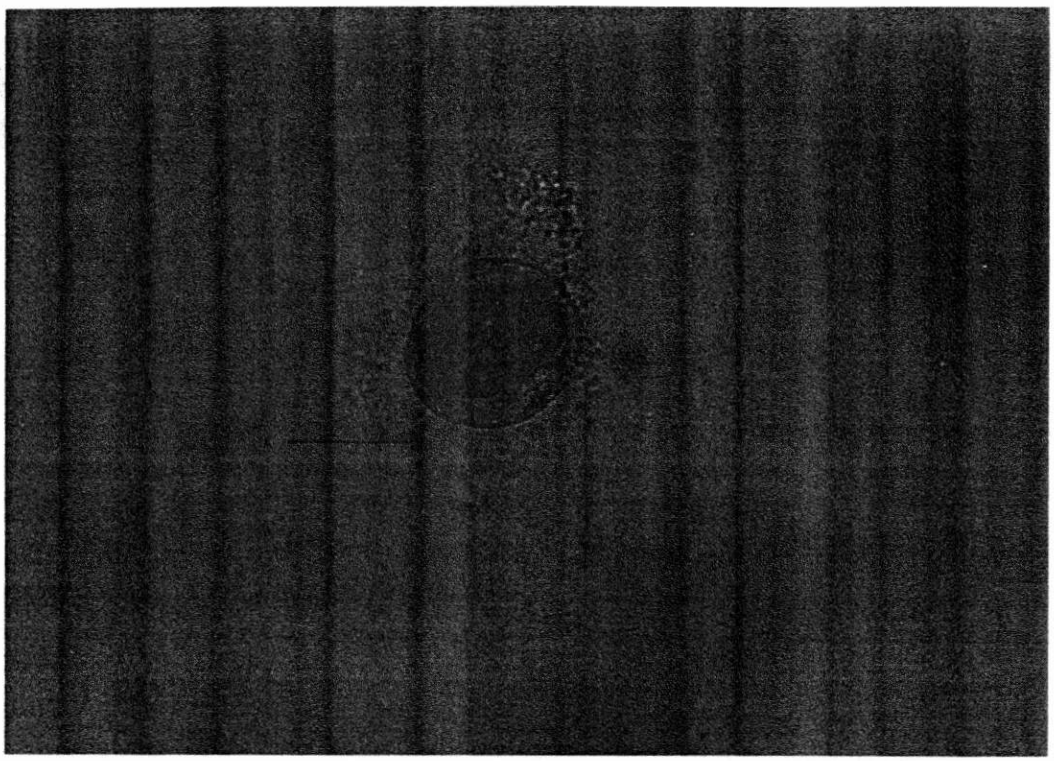

Figure 2 The morphology of a coracidium (formalin-fixed specimen). arrow = cilia on the entire body.

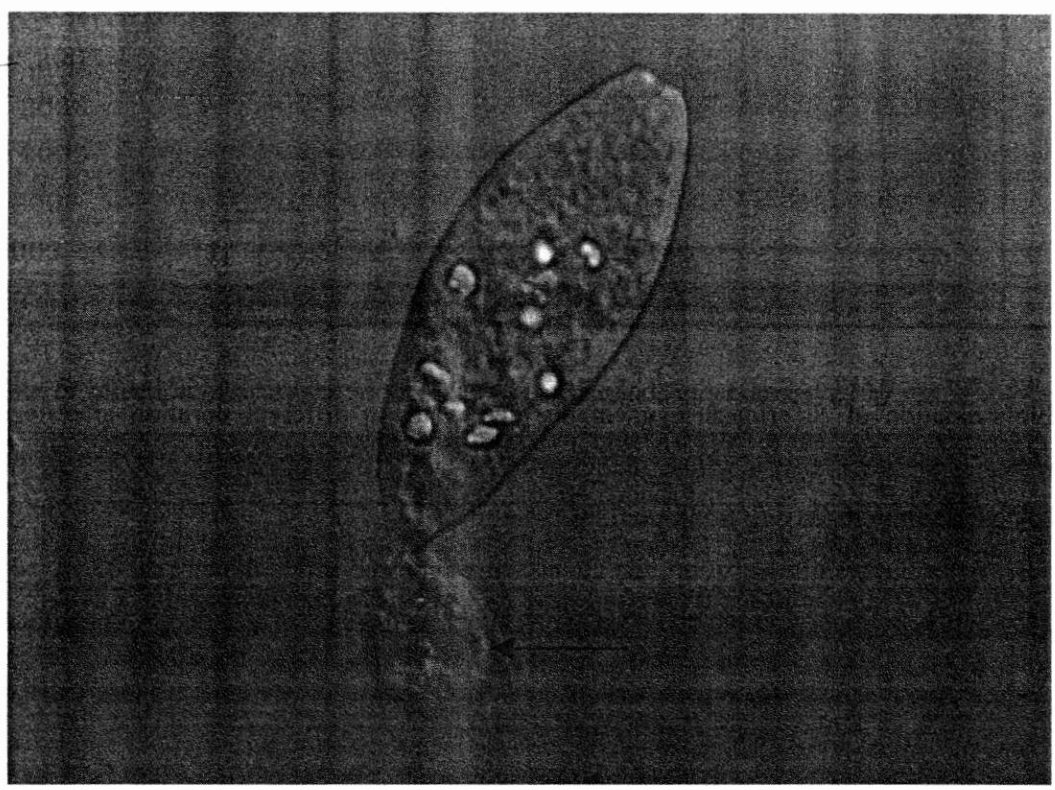

Figure 3 A 4-day old procercoid obtained from cyclop. (arrow = cercomer)

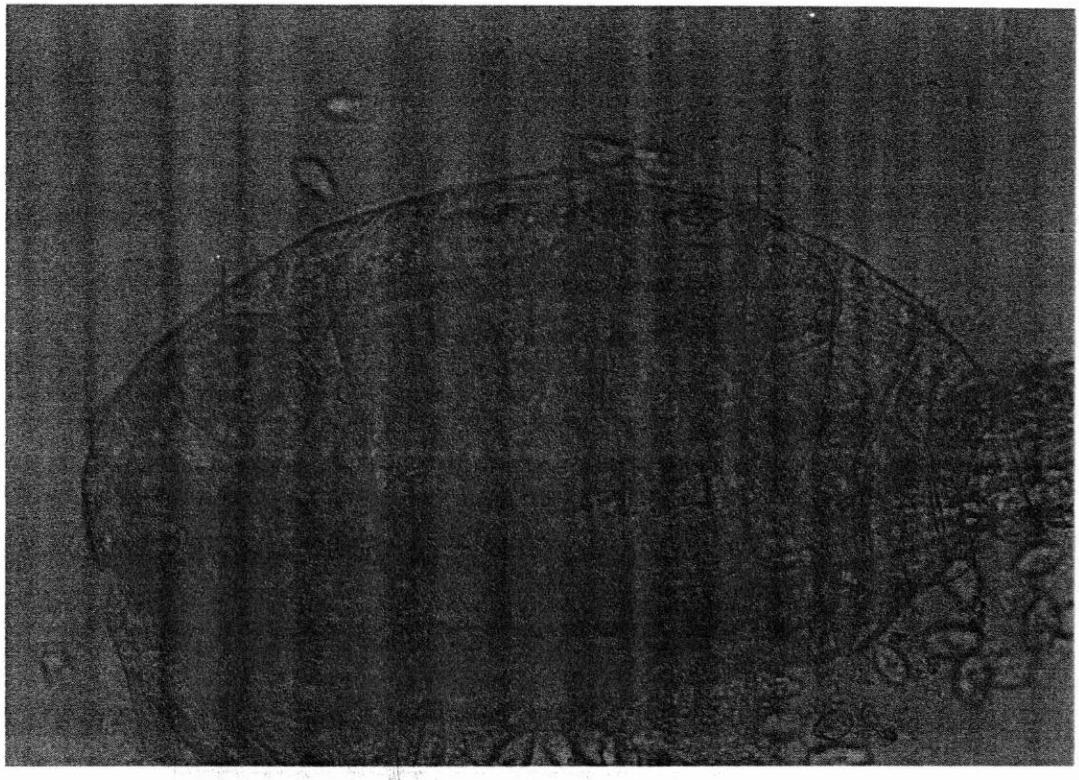

Figure 4 Procercoids (arrows) in the body cavity of cyclop.

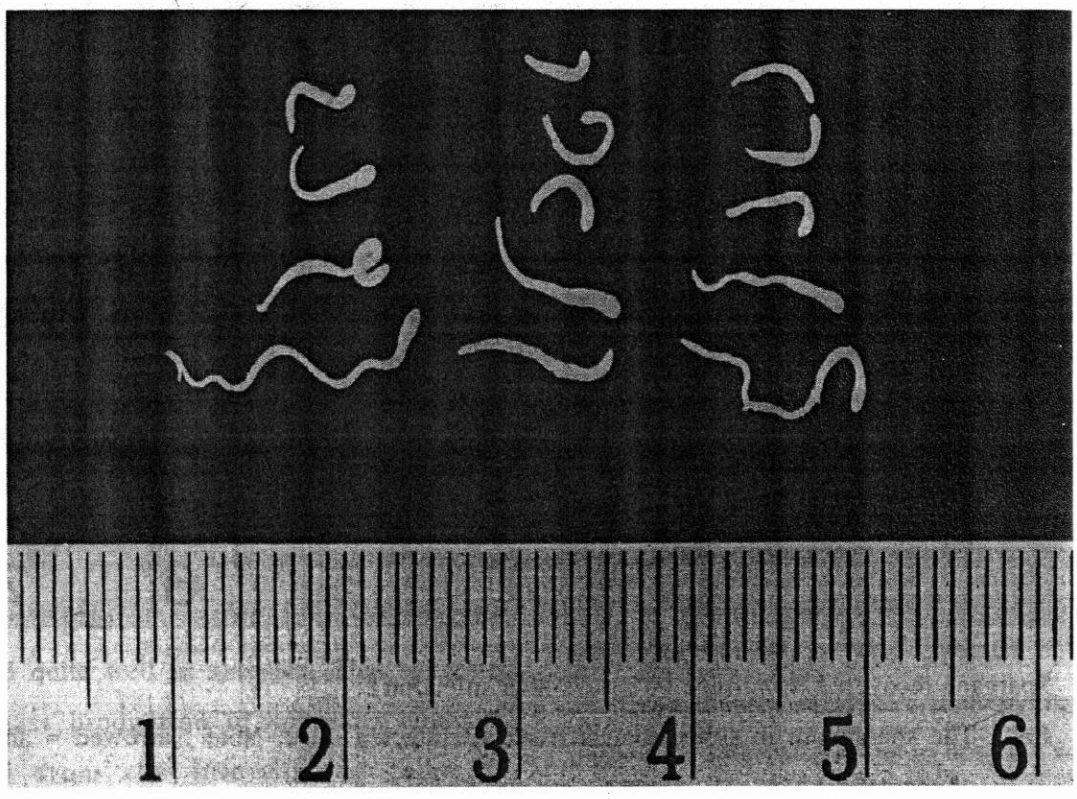

Figure 5 Spargana recovered from tadpoles after 30 days of infection.

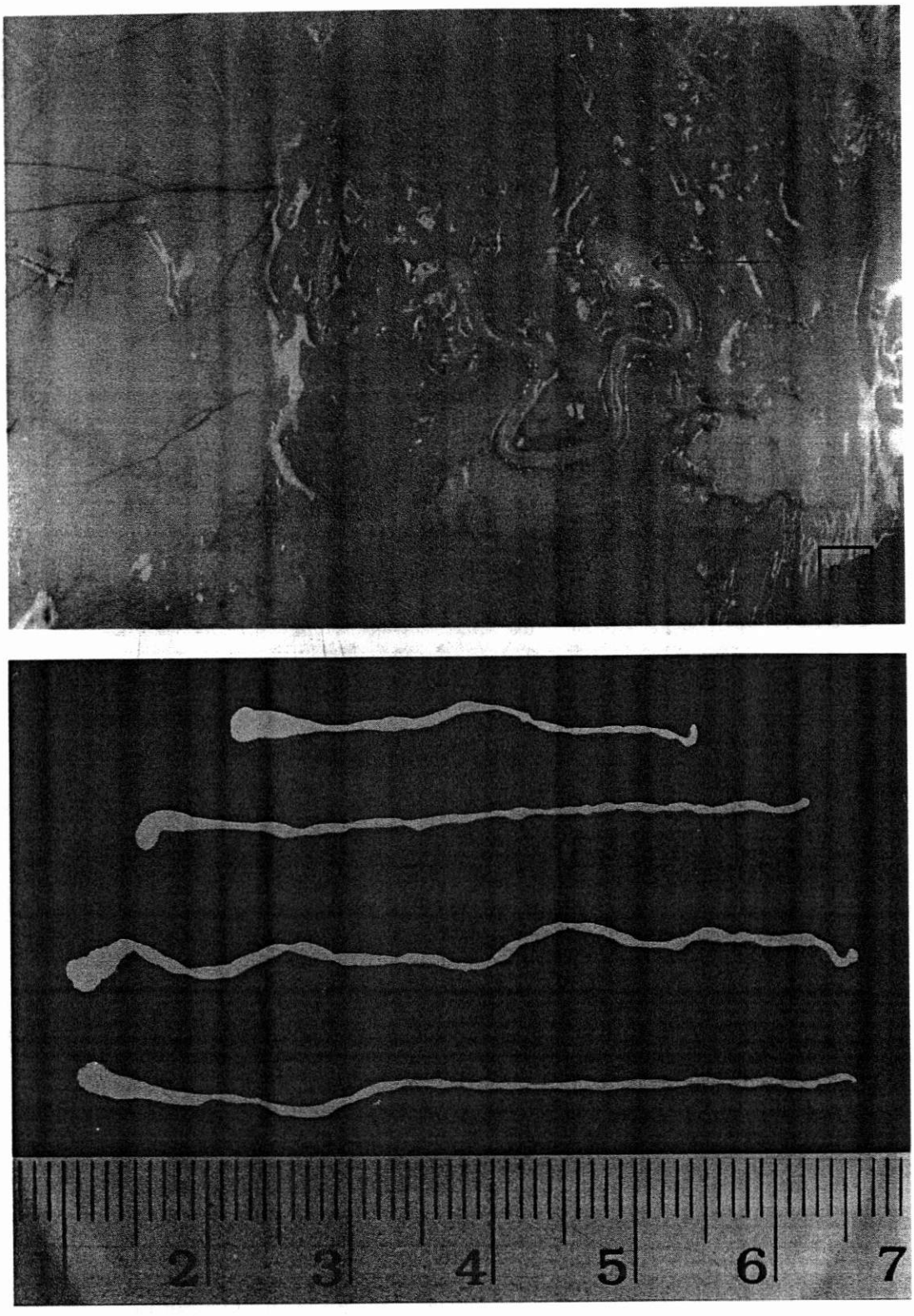

Figure 6 Spargana recovered from mice (30 days after infection).

6A : The sparganum in subcutaneous tissue of infected mice. Note : arrowed = anterior part of spargana.

6B : Formalin-fixed specimens.

H = head (anterior part), T = tail (posterior part)

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

Parasitized organs	No. of spargana recovered (%)
Subcutaneous tissue	12 (34.29)
Kidney	9 (25.72)
Adipose tissue	6 (17.14)
Hind legs	4 (11.43)
Peritoneal cavity	2 (5.71)
Testes	2 (5.71)
Total	35 (100)

Discussion and Suggestion

The present studies demonstrated that the larval stages of *S. erinacei* could develop in fresh water cyclops, tadpoles and mice. These findings agreed with the study in Korea (Lee *et al.*, 1990). They reported that *Mesocyclops leuckarti* and *Eucyclops serrulatus* were the first intermediate host in Korea. In the present study, *Mesocyclops aspericornis* is also susceptible to coracidia of *S. erinacei*. This indicated that several species of cyclops can be the host of this parasite. The ability to develop to procercoids of coracidia of *S. erinacei* was relatively the same. It took about 12 days for coracidia to develop to mature procercoids. Procercoids, in fact, can develop in mice but the infection rate was very low (Lee *et al.*, 1990; Sriswangwong *et al.*, 1996). In contrast, they developed quite well in tadpoles. This suggested that a mass production of spargana could be obtained from the infection of tadpoles. Unfortunately, it was not convenient to keep tadpoles or frogs in the laboratory for a long time. In addition, tadpoles are available only in rainy season. Therefore, other suitable experimental animals are needed to maintain spargana for research works.

Mice were the animals of choice to be used to maintain the spargana in the laboratory. They allowed the spargana to live and grow for a long time after spargana from tadpoles were orally introduced to them. Up to a hundred percent of spargana survived and increased in size. By this method, the spargana may be maintained in the laboratory for a long time or as long as readed.

It was clearly shown from this study and from previous reports (Lee *et al.*, 1990; Sriswangwong *et al.*, 1996) that the spargana, not procercoids, were succesfully survived in mice. This observation suggests that mammals, including human, become infected by receiving spargana themselves rather than by ingestion of procercoid infected cyclops in drinking water.

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