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Nine Human Sparganosis Cases in Thailand with Molecular Identification of Causative Parasite Species

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Abstract. Human sparganosis is one of the neglected diseases but important food-borne parasitic zoonoses. The disease is caused by larvae (spargana) of diphyllobothriidean tapeworm. Here, we describe nine cases of human sparganosis, caused by *Spirometra erinaceieuropaei* in a hospital in Thailand during 2001–2012. Clinical characteristics, treatment, and outcome of cases were revealed. Diagnosis and identification of causative parasite species was made by histopathological investigations followed by a polymerase chain reaction-based molecular method using formalin-fixed paraffin embedded tissues. The DNA samples were extracted from tissues and a partial fragment of cytochrome *c* oxidase subunit 1 (*cox1*) gene was amplified for the detection of parasitic DNA. Infection could be prevented by increasing activities on health communication by responsible public health agencies.

INTRODUCTION

Humans are known to serve as the accidental host for several cestodes. Human sparganosis is one of the zoonotic diseases caused by plerocercoid type larvae called spargana of diphyllobothriidean tapeworm.[1–3] The disease can be classified into two types, non-proliferative sparganosis caused by an infection with canine and feline tapeworm,[4] genus *Spirometra*, and proliferative sparganosis caused by an infection with *Sparganum proliferum*.[5] Human non-proliferative sparganosis is endemic mainly in East and Southeast Asia, especially in China, Japan, Korea, Taiwan, and Thailand.[6–10] Genus *Spirometra* uses freshwater cycloploid copepods as the first intermediate host and a cluster of amphibians, reptiles, birds, and mammals as the second intermediate hosts/paratenic hosts.[5,11] Infections with the different species show no evident differences in clinical presentation, except for the disease caused by *S. proliferum*, which is a serious migratory form.[5,11] Human infection occurs by consuming raw or undercooked meat of the second intermediate hosts or paratenic hosts, including frogs and snakes. Infection also can occur by drinking water polluted with infected cycloploid copepods, or by the use of frog meat as a poultices.[5,11] In humans, spargana invade the brain, eyes, central nervous system (CNS), breast, and subcutaneous tissues, causing the disease in local tissue damage, blindness, paralysis, and even death, and are a major threat to human health.[6–10]

In Thailand, there were 55 cases of sparganosis (53 non-proliferative and 2 proliferative) in the period of 1943–2011.[10,12] This report describes nine cases of human sparganosis, caused by *Spirometra erinaceieuropaei* in a hospital in Thailand during 2001–2012. Diagnosis and identification of causative parasite species was made by histopathological investigations followed by molecular confirmation using formalin-fixed paraffin embedded (FFPE) tissues.

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MATERIALS AND METHODS

Patients and the specimens. Between March 2001 and January 2012, nine patients were diagnosed with human sparganosis by clinical characteristics and pathological findings (Tables 1 and 2) at Siriraj Hospital, Mahidol University, Thailand. All patients underwent surgery. Based on a retrospective review of medical records, clinical characteristics such as age, symptoms, duration and location of symptoms, past diet history, radiographic findings, and treatment were obtained. The definite diagnosis of sparganosis was made from the histological findings. Hematoxylin and eosin stained sections from parafilm blocks reveal parasites surrounded with tegument and several calcareous bodies were found in the parasitic stroma. This study protocol was approved by the Siriraj Institutional Review Board Certificate of Approval (COA no. *Si* 189/2012). Informed consent was obtained from all human adult participants and from parents or legal guardians of minors.

DNA extraction, polymerase chain reaction (PCR), DNA sequencing, and sequence analysis. For molecular identification of the causative parasite species, the parasites in the sections were confirmed by hematoxylin-eosin stain before preparing DNA. The DNA was extracted from 10 μm unstained serial sections (cut from the plerocercoid in the tissue of FFPE) attached to glass slides using a DEXPAT kit (TaKaRa Bio Inc., Shiga, Japan) as reported previously.[13,14] The resulting supernatants were used as the DNA template for PCR. Amplification of a partial mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene by PCR was performed as shown in Table 3. A fragment of *cox1* gene was amplified using the primers Se658-F and Se1124-R, which were designed from the *cox1* gene of *S. erinaceieuropaei* (GenBank accession no. AB369250). The PCR was carried out using a GeneAmp PCR System 9700 (Applied Biosystems, Singapore). Amplified product was run on a 1% agarose gel and a 467 base pair (bp) fragment was cut and sequenced using the Applied Biosystems 3730 × I DNA Analyzer and ABI big dye Version 3.1 (Foster City, CA). The partial *cox1* gene sequences of individual *S. erinaceieuropaei* from FFPE specimens of each patient were deposited in the GenBank Database with the accession nos. of KF539833–KF539841.

They were analyzed using BLAST-N search (National Center for Biotechnology Information, Bethesda, MD). Published *cox1*

TABLE 1
Clinical characteristics of 9 cases of sparganosis from year 2001 to 2012*

No.	Year	Age	Sex	Locations	Symptoms	Duration	Organ
1	2001	51	F	C	Firm mass at right lower eye lid	2 mo	Lymph node of right lower eye lid
2	2004	74	F	C	Firm mass at suprapubic area	N/A	Abdominal wall at pubic region
3	2004	26	F	NE	Firm mass at left scapular area	5 yr	Soft tissue of left scapular area
4	2005	45	F	S	Firm mass at right lower quadrant area of abdomen	6 mo	Abdominal wall at right lower quadrant area of abdomen
5	2007	26	F	C	Firm mass at left arm	2 mo	Soft tissue of left arm
6	2008	26	M	C	Complete ptosis and lateral rectus muscle palsy of left eye. Hearing loss of right ear. Hemiparesis of left leg.	2 yr	Right cerebellopontine angle and left interpeduncular region. Serpiginous-masslike lesion at L1–L5 region.
7	2008	51	M	C	Firm mass at left chest wall.	2 mo	Soft tissue of left chest wall.
8	2011	31	M	N	Left leg pain and numbness at left buttock	6 mo	Edematous conus medullaris lesion with an irregular tubular lesion in the caudal sac from L4–S1.
9	2012	46	F	C	Low back pain, paraparesis with sensory impairment.	2 mo	Intradural extramedullary serpiginous-cystic like lesion at C2–C5, C7, T1, T5–T10, L3–L5, S1.

*F = female; M = male; N = north; NE = northeast; C = central; S = south of Thailand; N/A = data not available.

sequences from *S. erinaceieuropaei* were aligned with our new sequences (alignment length 426 bp when trimmed to the length of the shortest sequence) using ClustalW[15] and a maximum likelihood tree constructed using MEGA 5.2.[16] The best-fit substitution model was determined in MEGA to be the Hasegawa-Kishino-Yano (HKY) model with uniform rates among sites, but assuming a proportion (0.65) of invariant sites. Sequences from *Spirometra mansonoides* (GenBank no. AF096239) were used as an out group.

RESULTS

According to this study ($N = 9$) there have been 10 cases of sparganosis in Siriraj Hospital since 2001 (including Boonyasiri and others).[12] Details of clinical characteristics of all patients were shown in Table 1. Subcutaneous sparganosis was six cases followed by CNS involvement (three cases). The CNS infections were all in spinal cord with one patient having both brain and spinal lesions. The mean age was 41.8 ± 16.2 years (range 26–74 years). There were six females and three males, a ratio of 2:1. Risk behaviors were not recorded in most cases. Only one patient had a history of eating uncooked snake meat (case no. 6, THA-Se6). Patients are mostly from the central part of the country (67%). The median duration before diagnosis was 4 months. Pathological findings, definite diagnosis, treatment, and outcome of cases were described in Table 2 and Figure 1. All patients underwent surgical excision or debridement for treatment. All of the cutaneous sparganosis patients were completely cured after excision and no recurrence after ~1 year of a followed period.

The partial *cox1* sequence of parasite DNA recovered from nine patients, which were deposited in the GenBank database under the accession nos. KF539833–KF539841 (Table 2), was almost identical (97–99%) with those of *S. erinaceieuropaei* from various geographical localities Figure 2. From these results and the previous report, the parasites obtained from nine patients were identified as *S. erinaceieuropaei*.

DISCUSSION

In Thailand, there were 55 sparganosis cases (53 nonproliferative and 2 proliferative).[10,12,13] As was pointed out in their comprehensive review,[10] previously Tesjaroen[17] reviewed 34 cases of sparganosis in Thailand including 13 cases from Siriraj Hospital, the same hospital as this study. Because the overall sparganosis cases in Thailand is just over 60 cases including nine cases reported here, about one-third of the cases have been diagnosed and treated in the Siriraj Hospital, Mahidol University, Bangkok, Thailand. When Anantaphruti and others[10] reviewed sparganosis cases in Thailand, 18 of 52 cases were ocular sparganosis. However, the majority of them was recorded before 1990 and has become rare since 1991. In this study, six cases were subcutaneous sparganosis and three cases were CNS involvement, but we did not come across ocular cases. The chronological changes of the clinical features of sparganosis in Thailand indicate the reduced access to the traditional medicine and persistence of eating habits.

Previous study of sparganosis in Thailand, only three cases were identified as infection with *S. erinaceieuropaei* plerocercoids by PCR-based molecular identification of the worms preserved as FFPE tissues.[12,13] In this study, we added nine cases (one case was reported previously)[12] of human sparganosis in Thailand with molecular identification of the causative parasite species as *S. erinaceieuropaei*. In this study, we used partial mitochondrial *cox1* gene as a marker and found slight variation among the samples. Such an intraspecies variation is comparable with the intraspecies variation of *cox1* gene of *S. erinaceieuropaei* previously reported.[18] Although *S. erinaceieuropaei* plerocercoids is a causative agent of nonproliferative sparganosis in Asia/Oceania, *S. mansonoides* is proven as the causative agent of sparganosis in the Americas.[4] This report is the biggest sparganosis case series in Thailand.

Present results together with the previous review[10] clearly show that sparganosis remains an important food-borne zoonosis and infection can occur anywhere in Thailand. The infection is possibly caused by drinking water collected directly from ponds[19] or eating uncooked meat, including frogs and snakes.[10] In fact, in this study, case no. 6 (THA-Se6) had a history of eating raw snake meat. From the results described here, sparganosis is one of the neglected but important food-borne parasitic zoonoses in Thailand. Infection could be avoided by expanding focus on health communication regarding safe food and water by responsible agencies.

TABLE 2
Pathological finding, definite diagnosis, treatment, and outcome of nine cases of sparganosis from 2001 to 2012

Case no.	Gross finding	Histological finding	Diagnosis	Treatment	Recovery
1. [THA-Se1] KF539833	An irregular piece of rubbery well-circumscribed mass measuring 1.8 × 1 × 0.8 cm, the cut surface shows grayish white and light brown non-homogenous tissue.	Acute necrotizing lymphadenitis with calcified parasite, probable *Sparganum* spp. like.	Necrotizing lymphadenitis of right lower eye lid (Cutaneous sparganosis).	Excision	Completed
2. [THA-Se2] KF539834	An irregular piece of rubbery light brown tissue measuring 4.5 × 1 × 0.5 cm. The cut surface shows brown non-homogenous tissue.	Probable *Sparganum* spp. liked cyst.	Subcutaneous mass of abdominal wall at suprapubic area (Cutaneous sparganosis).	Excision	Completed
3. [THA-Se3] KF539835	An irregular piece of rubbery light brown tissue measuring 4.5 × 4 × 2 cm. The cut surface shows a 1.5 × 1.5 × 0.7 cm yellow material containing cyst in muscular tissue.	Intramuscular wall of abscess with infected *Sparganum* spp. like.	Intramuscular mass at left scapular area (Cutaneous sparganosis).	Excision	Completed
4. [THA-Se4] KF539836	Irregular pieces of soft fibrofatty tissue, varying from 1.5, 2.2, and 2.5 cm. The cut surface shows fatty yellow homogenous tissue.	Parasitic infection, morphology identical to *Sparganum* spp. like.	Subcutaneous mass of abdominal wall at right lower quadrant area (Cutaneous sparganosis).	Excision	Completed
5. [THA-Se5] KF539837	Irregular pieces of soft light brown tissue, measuring 0.4 × 0.5 × 0.4 cm and 0.9 × 1.2 × 0.5 cm.	Parasitic infestation morphologically compatible with *Sparganum* spp. like.	Subcutaneous mass of left arm (Cutaneous sparganosis).	Excision	Completed
6. [THA-Se6] KF539838	It consists of multiple pieces of small irregular soft light brown and grey white tissue, measuring 1.2 × 1.1 × 0.3 cm.	Compatible with parasite infection compatible with *Sparganum* spp. like.	Sparganosis of brain (right cerebellopontine angle and left interpeduncular region) and spinal cord (L1–L5) (Cerebral and spinal sparganosis).	Laminoplasty at L1–L5 and duraplasty with tumor removal, Right median pressure ventriculoperitoneal shunt.	Partial
7. [THA-Se7] KF539839	It consists of 3 pieces of irregular soft light brown and grey white tissue, measuring from 1.2–3 cm in greatest dimension.	Parasitic infestation associated with necrotic tracts in the subcutaneous tissue, morphologically compatible with *Sparganum* spp. like.	Subcutaneous mass of left chest wall (Cutaneous sparganosis).	Excision	Completed
8. [THA-Se8] KF539840	It consists of a piece of irregular soft grey white tissue, 0.3 × 0.3 × 0.2 cm.	Parasitic infestation, cestode. morphologically suggestive of with *Sparganum* spp. like.	Sparganosis of spinal cord (conus medullaris, L4–S1) (Spinal sparganosis/ involved conus medullaris).	Laminotomy at L1–L4 with laminectomy at L5–S1 and partial cystic mass removal.	Partial
9. [THA-Se9] KF539841	Multiple pieces of light brown, soft tissue measure 1 × 1 × 0.7 cm.	Specimen consists of membranous tissue fragments. They are foreign tissue and composed of three distinctive layers. Consistent with *Sparganum* spp. cyst like.	Sparganosis of spinal cord (C2–C5,C7,T1, T5–T10, L3–L5, S1) (spinal sparganosis).	Laminectomy at T9–T11, L1–L2 with multiple debridement, corticosteroid, praziquantel and ivermectin therapy.	No

TABLE 3
The primers and polymerase chain reaction conditions

Primers	PCR master mixed (25 μL total volume)	PCR steps
Forward: Se658-F 5′-TTTGATCCTTTGGGTGGTGG-3′ Reverse: Se1124-R 5′-ACCACAAACCACGTGTCATG-3′	2.5 μL of 10× FastStart High Fidelity Reaction buffer with 18 mM $MgCl_2$ (Roche, Mannheim, Germany), 200 μM of dNTP, 0.2 μM of primer (Invitrogen, Carlsbad, CA), 0.625 units of FastStart High Fidelity Enzyme Blend (Roche).	Denatured 94°C 5 mins, 35 cycles at 95°C 30 sec, 59°C for 30 sec, 72°C 45 sec, a final extension at 72°C for 10 min.

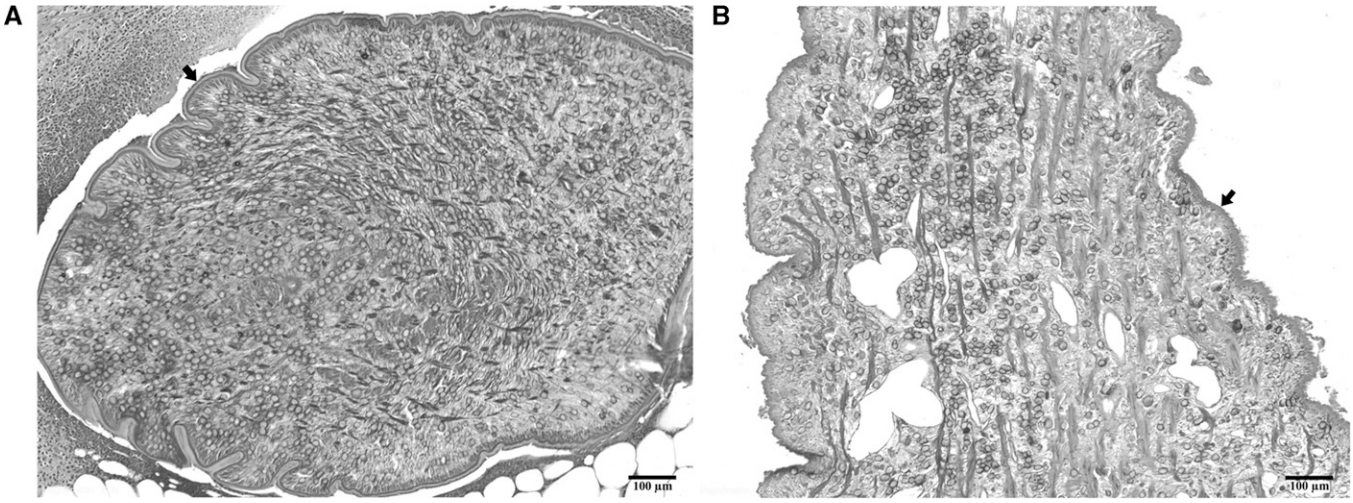

FIGURE 1. Representative plerocercoids detected in the paraffin-embedded sections used for molecular identification. (**A**) A plerocercoid (arrow) isolated from the subcutaneous nodule of the patient 4 (THA-Se4); (**B**) a plerocercoid (arrow) detected in the spinal cord from the patient 8 (THA-Se8). The sections were stained with hematoxylin-eosin. Scale bar = 100 µm.

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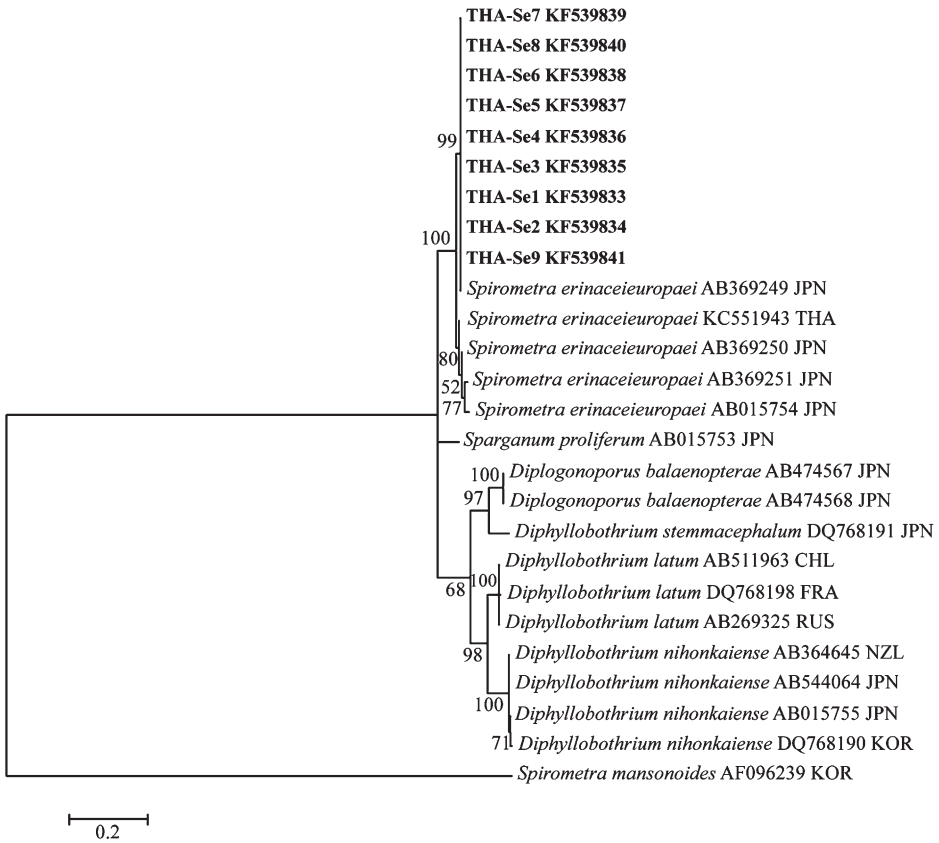

FIGURE 2. Maximum Likelihood tree based on partial cytochrome *c* oxidase subunit 1 (*cox1*) gene sequences. Sequences of *Spirometra* spp. and other tapeworm obtained from GenBank are indicated with accession number and country code (ISO 3166-1 alpha-3 codes). *Spirometra erinaceieuropaei* sequences of this study are presented in bold (KF539833-KF539841). The sequences were deposited in GenBank numbers as shown in Table 2. Numbers at the nodes indicate bootstrap *P* values (1,000 replicates).

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