

ABNORMAL *TAENIA SAGINATA* TAPEWORMS IN THAILAND

Wanna Maipanich¹, Megumi Sato², Somchit Pubampen¹, Surapol Sanguankiat¹, Teera Kusolsuk¹, Urusa Thaenkham¹ and Jitra Waikagul¹

¹Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; ²Laboratório de Parasitologia, Escola de Medicina Veterinária e Zootecnia, Universidade Federal do Tocantins, Araguaína, Tocantins, Brasil

Abstract. Sixty-eight residents of Ban Luang and Ban Pang Kae villages, in Nan Province, northern Thailand, visited our mobile field station in September 2006 and March 2007, seeking treatment for taeniasis. After treatment, 22 cases discharged tapeworm strobila in their fecal samples and 17 scolices were recovered. Among these, 3 were morphologically abnormal, with six suckers on the scolex. To confirm the species of these tapeworms, the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was used as a molecular marker. The partial COI sequences (800 bp) of the abnormal tapeworms were identical to the sequences of *Taenia saginata* deposited in Genbank.

Keywords: *Taenia saginata*, abnormal morphology, molecular marker, Thailand

INTRODUCTION

The Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, Thailand conducts education and research related to the medically important helminthes. To understand the problem of helminth infection in Thailand, our mobile team often travels to remote villages for epidemiological studies, and to provide medical services. In rural areas, people with worm infections visit our field station seeking treatment.

Taeniasis has a cosmopolitan distribution in countries where meat (especially

pork and beef) is eaten raw, or undercooked. In Thailand, many dishes contain raw or half-cooked meat, such as “*Larb*”, “*Lu*”, and “*Sa*”. The dishes are popular among people in northern Thailand. The ingredients are usually obtained from a small village market or shop-house. Meat for sale is brought to the rural area from the city and is parasite-free. Households in the community have pigsties where sows are kept. Many young piglets escape from their mothers after weaning and forage for food in the village. It is possible these piglets fed on human excreta. Stool examinations have shown 1.9% of the people in Nan Province have taenia infections (Muennoo *et al*, 2005). During festivals or other special occasions, piglet meat is cooked. In the kitchen, many men offer their help while the women prepare food for the guests. The men sometimes cooked their favorite dishes while drink-

Correspondence: Dr Jitra Waikagul, Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand.

Tel: 66 (0) 2643-5600

E-mail: tmjwk@mahidol.ac.th

ing alcohol, and do not notice cysticerci in the meat (personal observation). It has been noticed many cases of taeniasis occur among males after a party.

MATERIALS AND METHODS

Study sites

During 2006 and 2007, we visited northern Thailand to investigate the prevalence of opisthorchiasis (Ethics Committee approval number: MUTM 2006-040). The target groups were villagers in Ban Luang and Ban Pang Kae communities, Nan Province (Fig 1).

Parasitological examination

In the study areas, stool containers were distributed to villagers by village health volunteers. On the day of collection, stool samples were examined by modified cellophane thick smear technique (Katz *et al*, 1972). Villagers with and without parasitic infections are included for physical examination. Those with a history of expelling *Taenia* proglottids were also treated. They were asked to evacuate their bowels and eat a non-fiber meal the night before treatment. Early the next morning, they were given 4 tablets of niclosamide (0.5 g), 2 hours after a purgative (60 ml saturated magnesium sulfate solution) followed by large amounts of drinking water. After treatment, all the evacuated fecal material was collected 4-5 times per individual. The collected materials were repeatedly washed with water until clean, and tapeworm samples were collected from the sediment materials and processed for further study.

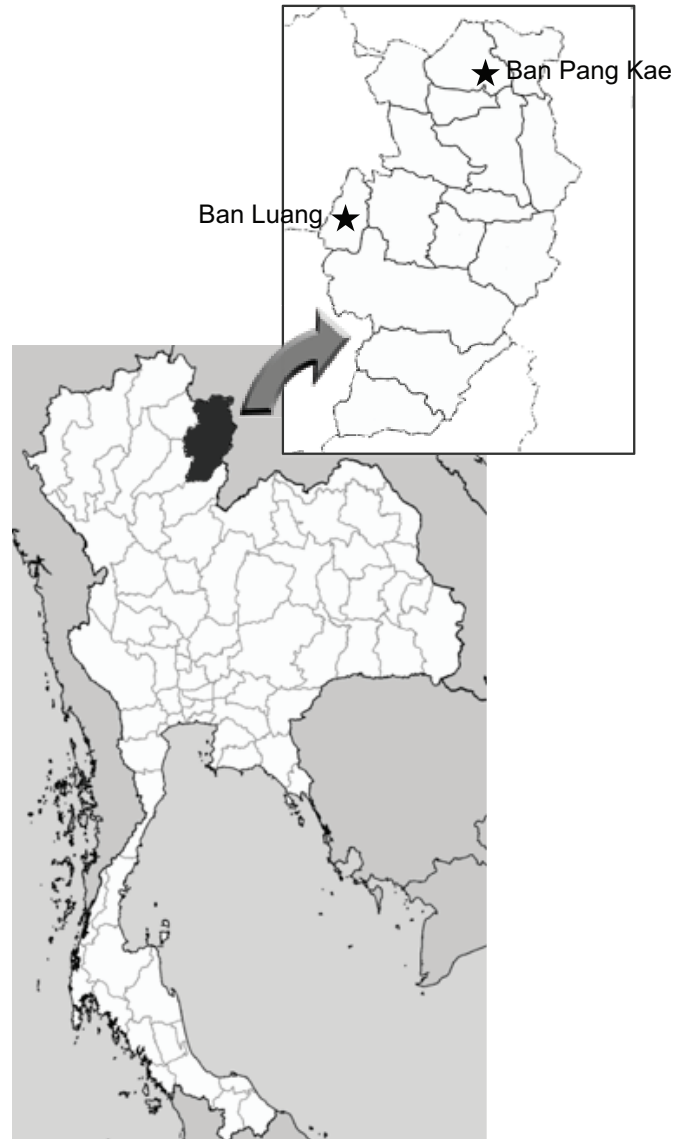


Fig 1–Map of study areas (★), Nan Province, Thailand.

The tapeworm scolices were fixed in 10% formalin and stained with acetic carmine for morphological study. The segments of each worm were fixed separately in 70% ethanol for molecular studies.

DNA analysis

Small fragments were cut from the strobila connected to each scolex of an

Table 1
Helminth infections among villagers in Ban Luang and Ban Pang Kae villages by Kato-Katz modified thick smear technique, 2006-2007.

Village	No. exam	Helm+ve (%)	Helminth eggs (%)					
			Minute int fluke	<i>Taenia</i>	<i>A.l</i>	Hw	<i>T.t</i>	<i>S.s</i>
Ban Luang	435	257 (59.1)	255 (58.6)	15 (3.5)	0 (0)	6 (1.4)	1 (0.2)	3 (0.7)
Ban Pang Kae	225	55 (24.4)	6 (2.7)	3 (1.3)	14 (6.2)	32 (14.2)	0 (0)	0 (0)

Minute int fluke, minute intestinal fluke; *A.l*, *Ascaris lumbricoides*; Hw, hookworm; *T.t*, *Trichuris trichiura*, *S.s*, *Strongyloides stercoralis*

Table 2
Taeniasis cases in Ban Luang and Ban Pang Kae villages.

	No. of cases in each category			
	Presence of eggs in stool	Recent discharging of segment	Expelled scolex during liver fluke treatment	Total
Ban Luang	15	8	2	25
Ban Pang Kae	3	40	0	43
Total	18	48	2	68

abnormal tapeworm. They were extracted individually using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany). The cytochrome *c* oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR) technique. The primers used were designed from the mitochondrial COI gene sequence of *Taenia saginata* (Accession no.: AY195858), the forward primer was Tae-F (5' GGG TTT GTG GTC AGG TTT TG3') and the reverse primer was Tae-R (5' CAT CTAACC CAA CCG TAA AC3'). About 10 ng of genomic DNA was used as a DNA template. The PCR mixture volume was 50 μ l, containing genomic DNA, 25 mmol/l of MgCl₂, 2.5 mmol/l of dNTPs, 40 μ mol of each primer, and 1 \times of *Taq* polymerase buffer and 1 unit of *Taq* polymerase. The

PCR products were sequenced directly by cycle sequencing reactions run on an ABI Prism 3100 automated sequencer. PCR primers were used as sequencing primers. DNA alignment analyses were conducted using CLUSTAL_X Program version 2 (Thompson *et al*, 1997).

RESULTS

Only a few taeniasis cases were detected on stool examination (Table 1). During our visits, 48 persons passed tapeworm segments and visited our clinic requesting treatment. We treated those who expelled segments within the past 2-3 months. Of the 68 subjects with *Taenia* infections (Table 2), worm(s) or parts of worms were collected from the

Table 3
Post-treatment discharge of tapeworms.

Case no.	Age	Sex	Tapeworms discovered			
			Scolices	Strobila etc	Scolex anomaly	Identification
Ban Luang						
1	67	M	1	2 long strobila	No	<i>T. saginata</i>
2	35	M	ND	1 long strobila	-	-
3	59	M	2	Mature and gravid segments	No	<i>T. saginata</i>
4	34	F	1	Segments	No	<i>T. saginata</i>
5 ^a	45	M	2	2 long strobila	1 Yes, 1 Normal	<i>T. saginata</i>
6	54	F	ND	Long and short chains	-	-
7 ^a	48	M	1	Few immature segments	Yes	<i>T. saginata</i>
8	43	M	1	Segments	No	<i>T. saginata</i>
9	47	F	ND	Short chains	-	-
10 ^b	45	F	1	Segments	No	<i>T. saginata</i>
11 ^b	43	F	1	Segments	No	<i>T. saginata</i>
Ban Pang Kae						
12	40	M	1	Young segments	No	<i>T. saginata</i>
13	34	M	1	Long strobila, short chain, gravid segments	No	<i>T. saginata</i>
14	36	F	ND	Mature and gravid segments	-	<i>T. saginata</i>
15	49	F	1	Young segments	No	<i>T. saginata</i>
16	30	M	ND	Long strobila (neck to gravid)	-	<i>T. saginata</i>
17	54	M	ND	Short chain, proglottids	-	-
18	29	M	1	Long strobila, gravid segments	No	<i>T. saginata</i>
19 ^a	12	F	1	Long strobila, mature and gravid segments	Yes	<i>T. saginata</i>
20	23	M	1	Long strobila, short chains	No	<i>T. saginata</i>
21	32	M	1	Segments	No	<i>T. saginata</i>
22	27	M	ND	Long strobila, gravid segments	-	<i>T. saginata</i>

^aSubjects with 6-sucker tapeworms

^bSubjects in the liver-fluke treatment group who passed tapeworm in their fecal matter

ND, not discovered

bowel movements of 22 cases. Most of the worms were in short/long chains of immature, mature, and gravid proglottids; 17 scolices were found from 15 treated persons (Table 3).

Three scolices with 6 suckers were recovered from the feces of 3 subjects (Ban Luang Village: 2; Ban Pang Kae Village: 1). The stained gravid segments of the two

cases (cases 5 and 19) had characteristics of *Taenia saginata*, and their COI sequences indicated the 3 abnormal scolices (cases 5, 7, and 19) were *T. saginata*.

Case No. 5. A farmer in Ban Luang District enjoyed eating spicy raw meat dishes while drinking alcohol. He had frequently experienced "worms" crawling actively from his anus over the previous few months.

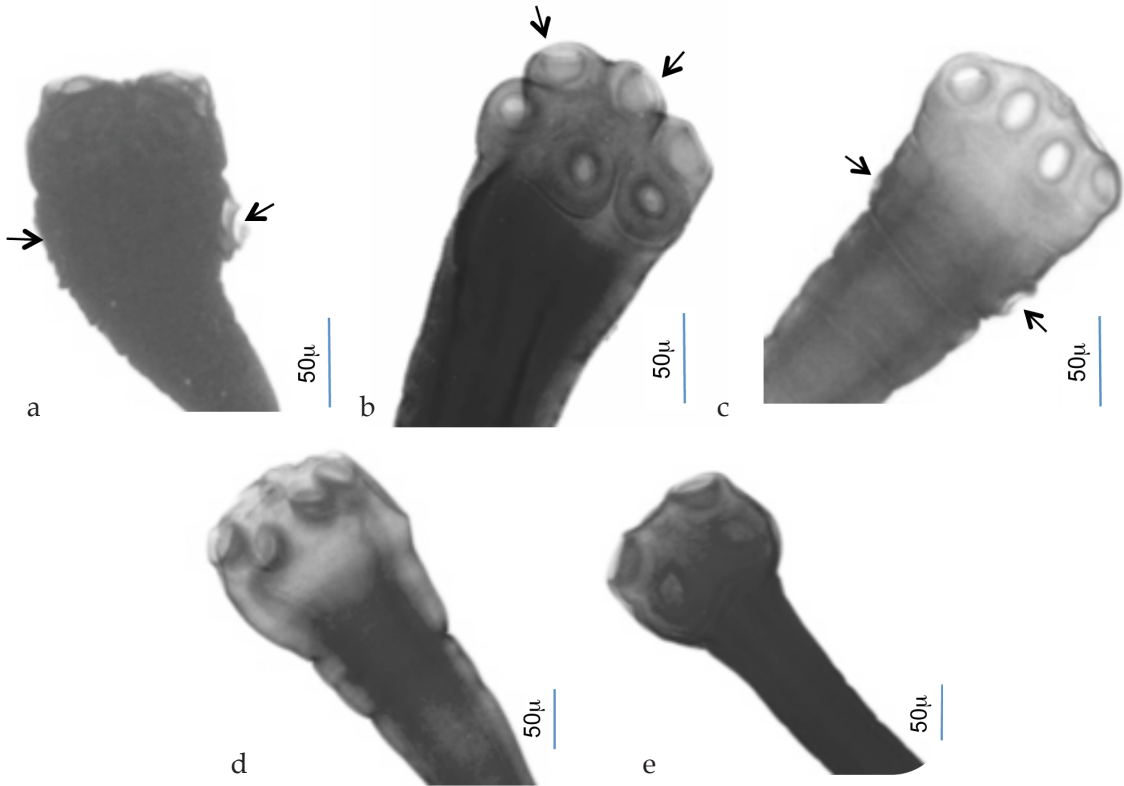


Fig 2—Abnormal scolex with 6 suckers extra suckers (arrows); case no. 5 (a), case no. 7 (b) and case no. 19 (c) and a normal scolex with 4 suckers (d-e).

After treatment, two scolices and strobila fragments were found among the fecal material. One scolex had six suckers, and another scolex had 4. The scolex with six suckers was small, 1.8 mm long and 0.7 mm wide. Two pairs of suckers were located closer in the group at the anterior end. Another pair of suckers was located posterior to the other suckers, located on both lateral sides, next to the neck (Fig 2a). The part of the anterior strobila that connected to the neck was 0.08 mm long and 0.5 mm wide, and comprised only very young segments. There were two worms in which the main part of the strobila was disconnected from the scolices, and mixed in the fecal material. The mature and gravid segments of

the worm could not be separated into individual parts; however, the stained gravid segments of the 2 strobilae had features of *T. saginata*.

Case No. 7. A male gardener aged 48 years, from Ban Luang District, sought treatment for taeniasis from the mobile team. He had a history of consuming improperly cooked meat, such as beef and pork, over several years. The first time he passed a tapeworm proglottid, he consulted a health personnel for treatment. After that, he repeated treatments with niclosamide when proglottids were seen. Two months before visiting the mobile team, he was admitted to the hospital due to botulism. He underwent gastric lavage and the strobila of a *Taenia*

worm was washed out. He believed he had a tapeworm infection and asked for treatment. After treatment, he passed a scolex and a few young proglottids. The scolex was fixed and examined under a stereomicroscope. An abnormal scolex was seen. After staining, the scolex was visibly large (2.2 mm long and 2.1 mm wide). Six suckers appeared in 3 pairs of equal size, 0.5mm x 0.5mm, and lay side by side in the anterior proximal region (Fig 2b). A rostellum and hooklet were absent. The neck was 1.7 mm long and 1.6 mm wide. The strobila was 0.1 mm long and 1.2 mm wide. Part of an immature segment was preserved for PCR analysis, and the results revealed *Taenia saginata*.

Case no. 19. A schoolgirl from Ban Pang Kae School submitted a stool sample for examination. The results revealed a tapeworm infection. She passed a complete *T. saginata* strobila after deworming. The worm had a scolex with 6 suckers; 4 in the normal position and extra suckers on the lateral side (Fig 2c). The scolex was 2.2 mm long and 2.1 mm wide, and the neck 1.7 mm long and 1.5 mm wide. The oval suckers were 0.7 mm long and 0.5 mm wide. The mature and gravid segments had the same characteristics as *T. saginata*. She told us that at home she regularly consumed improperly cooked meat, prepared by her mother. She had never been out of the area.

The partial COI sequences (800 bp) of the abnormal (6-sucker) worms from patient cases 5, 7, and 19 (Accession no. HQ606075-HQ606077) were aligned with the partial COI sequences of the normal (4-sucker) worms. The results show the nucleotide sequences of the abnormal and normal worms were identical.

DISCUSSION

Abnormalities among *Taenia* speci-

mens have been recorded since 1741 (McCulloch, 1913). These abnormalities comprise excess suckers, excess genital pores, and unusual segment formations. The specimens appeared as though *Taenia saginata* had been placed with their flat surfaces together and the two fused along one of their adjacent borders, as described by McCulloch (1913). Some scolices of the three-cornered taeniae possessed six suckers and polyradiate strobilae. Fourteen complete polyradiate specimens of *Taenia pisiformis* were reported from a "German police dog" (Dobrovolny and Dobrovolny, 1935). There were 2 theories about the abnormal specimens. One was it was a separate species, but no feeding experiments were conducted to support a suggestion that each egg would develop into a six-sucker cysticercus. The second theory was fusion of two worms and the malformation was the abnormality.

In Nan Province, the prevalence of taeniasis detected in stool examinations was 3.45% in Ban Luang and 1.33% in Ban Pang Kae. According to the infected persons involved in this study, 68 of 660 villagers had *Taenia* infection, so the prevalence of taeniasis in Ban Luang was raised to 5.74% and in Ban Pang Kae to 19.1%. The problem of taeniasis in northern Thailand is not significant due to the self-care habits of infected persons. However, if the habit of consuming raw or improperly cooked meat persists re-infections among the population may last a long time. The main *Taenia* infection among the sample population is taeniasis saginata. Although some abnormal worms were seen, these were proved to be *T. saginata*.

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