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▣ BRIEF COMMUNICATION

Molecular Diagnosis of *Taenia saginata* Tapeworms from Two Residents of Northern Cambodia

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Abstract: *Taenia saginata* infection has seldom been reported in Cambodia. In this study, we performed a survey of intestinal parasites in 1,156 residents of Preah Vihear and Stung Treng Provinces in 2018. The results revealed that 26 (2.4%) cases were positive for *Taenia* spp. eggs. In order to obtain the strobilae of the tapeworms, 2 patients in Preah Vihear were treated with praziquantel and purged with magnesium salts. The proglottids expelled after the medication were morphologically and molecularly analyzed to determine the species. The main uterine lateral braches in gravid proglottids were > 15 in number suggesting that they are either *T. saginata* or *Taenia asiatica*. The sequences of the mitochondrial cytochrome c oxidase subunit 1 (*cox*1) gene and 2 nuclear loci, elongation factor-1 alpha (*ef*1) and ezrin-radixin-moesin-like protein (*elp*), were identical to the sequences of *T. saginata* available in GenBank but distant from *Taenia solium*, *T. asiatica*, and *T. saginata*-*T. asiatica* hybrid. This is the first report of the presence of *T. saginata* in the northern part of Cambodia bordering Lao PDR based on a molecular confirmation.

Key words: *Taenia saginata*, *cox1*, *ef1*, *elp*, molecular diagnosis, Cambodia

Taenia saginata, *Taenia asiatica*, and *Taenia solium* are 3 tapeworm species that can cause zoonotic infections in humans. They have unique life cycles, with humans as the only definitive host [1]. Beef is the infection source for *T. saginata*, whereas *T. asiatica* and *T. solium* infections are contracted by pork. The distribution of human taeniases is worldwide, and *T. saginata* is the most prevalent species [2].

Little has been known about human *Taenia* tapeworm infections in Cambodia with the exception of 2 studies reported in 2011 and 2014 [3,4]. According to the reports, fecal examinations of residents in Koh Kong Province [3] and 18 nationwide provinces [4] revealed the egg positive rate of *Taenia* spp. to be 1.5% (43/2,824) and 0.4% (115/32,201), respectively. In Koh Kong Province, eggs of *Taenia* spp. from 21 individuals were molecularly analyzed, and *T. saginata* (n=19) and *T. solium* (n=2) were identified by *cox1* sequencing and multiplex PCR [3]. However, no other information is available regarding the species of *Taenia* in other localities of Cambodia. In this study, we molecularly confirmed *T. saginata* proglottids expelled from 2 residents after praziquantel treatment and purging in a northern part of Cambodia.

In May 2018, the Institute of Parasitic Diseases, Korea Association of Health Promotion (KAHP), in cooperation with the National Center for Parasitology, Entomology and Malaria Control, Cambodia, conducted a survey on intestinal parasitic infections in 2 northern provinces (5 villages each), Preah Vihear and Stung Treng (IRB no. 099NECHR, approved by National Ethics Committee for Health Research, Cambodia). A total of 1,156 fecal samples were collected from the residents and examined by the Kato-Katz thick smear method. The results revealed that the overall egg positive rate of *Taenia* spp. was 2.4% (26/1,156); 1.7% (6/359) in Preah Vihear and 2.5% (20/797) in Stung Treng (Table 1). A small village named Kampong Sangkae located in the northeastern part of Preah Vihear and bordered with Lao PDR showed a fairly high prevalence (3.4%, 3/89) of *Taenia* spp. eggs. This village was selected for further analysis of *Taenia* tapeworms in this study.

Two egg positive cases from this village were treated with 10-

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15 mg/kg praziquantel (Shinpoong Pharm. Co., Seoul, Korea) followed by purging with 30-40 g $MgSO_4$. The 2 tapeworm strobilae (1 was with scolex) (Fig. 1A, B) discharged from the 2 cases were stored in 70% ethanol until morphological and molecular analyses. The scolex revealed 4 suckers but no recognizable rostellum nor hooklets (Fig. 1B). Several proglottids from each of the 2 cases were fixed in 10% formalin and stained with Semichon's acetocarmine, and morphologically observed using a light microscope. They were identified as either *T. saginata* or *T. asiatica* since they had >15 main uterine lateral branches filled with eggs (Fig. 1C) [5].

Table 1. Infection status of tapeworms in 2 northern provinces of Cambodia

Province	Village	No. of people examined	No. of *Taenia* spp. egg positive cases (%)
Preah Vihear	Kampong Pou	52	0 (0.0)
	Kampong Chey	42	2 (4.8)
	Kampong Sangkae[a]	89	3 (3.4)
	Kampong Sralau	74	0 (0.0)
	Kampong Sami	50	1 (2.0)
	Subtotal	359	6 (2.0)
Stung Treng	O' Chay	125	6 (4.8)
	Kanhchanh Tuek	114	4 (3.5)
	Ti Team	93	0 (0.0)
	Srae Russei	204	4 (2.0)
	Peam Khes	261	6 (2.3)
	Subtotal	797	20 (2.5)
Total		1,156	26 (2.4)

[a]The village where residents were recruited for praziquantel treatment and purging to recover adult tapeworms.

The genomic DNA of each tapeworm segment was extracted using the DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. PCR amplification and sequencing of mitochondrial cytochrome *c* oxidase (*cox1*, 1,620 bp), nuclear elongation factor-1α (*ef1*, 1,090 bp), and nuclear ezrin-radixin-moesin (ERM)-like protein (*elp*, 1,160 bp) genes were performed on each tapeworm sample with primers and conditions reported in a previous study [6]. A phylogenetic tree of *cox1* gene constructed from the representative selection of sequences available in GenBank, using the maximum-likelihood method available in MEGA and employing Tamura-nei model of nucleotide substitution with 1,000 bootstrap replications, showed that our samples were both identical with *T. saginata* (Fig. 2). The sequence homology (*cox1*) was 99.7% with *T. saginata* and 94.8-95.2% with *T. asiatica*. In addition, our samples contained the nuclear loci alleles *ef1C*/*ef1C* and *elpC*/*elpC*, which are homozygous for *T. saginata* based on the interpretation using Sanger chromatogram analysis (Table 2) [6]. This demonstrates that our samples are not a hybrid between *T. saginata* and *T. asiatica* but 'pure *T. saginata*'.

Human taeniases are known to be endemic in Southeast Asia, including Myanmar [7] and Lao PDR [8]. In Cambodia, a few previous surveys reported low-grade prevalence of *Taenia* spp. among the residents in several provinces [3,4]. Molecular studies on the species were reported in a paper in which the genom-

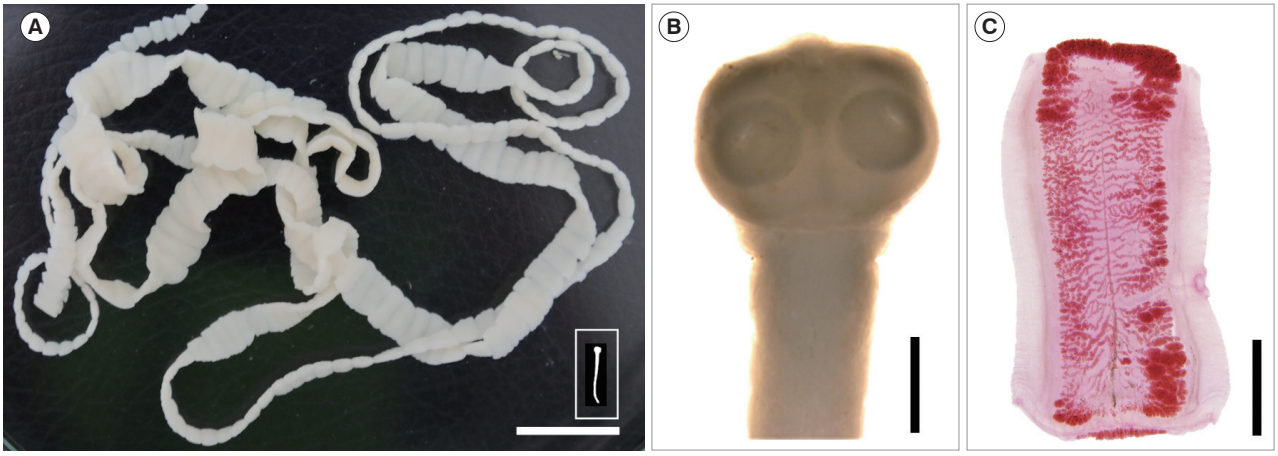

Fig. 1. (A) A complete tapeworm strobila, including the scolex (inset), of *Taenia saginata* discharged from a resident in Kampong Sangkae village, Preah Vihear Province, Cambodia. Scale bar=2 cm. (B, C) Enlarged views of the scolex and a gravid proglottid taken with a stereomicroscope (Leica, Wetzlar, Germany). Morphological characteristics of the unarmed scolex with no distinct rostellum (B) and the high number (>15) of main uterine lateral branches (C) designated the tapeworm as either *T. saginata* or *T. asiatica* and far from *T. solium*. Scale bar=1 mm in (B) and 3 mm in (C).

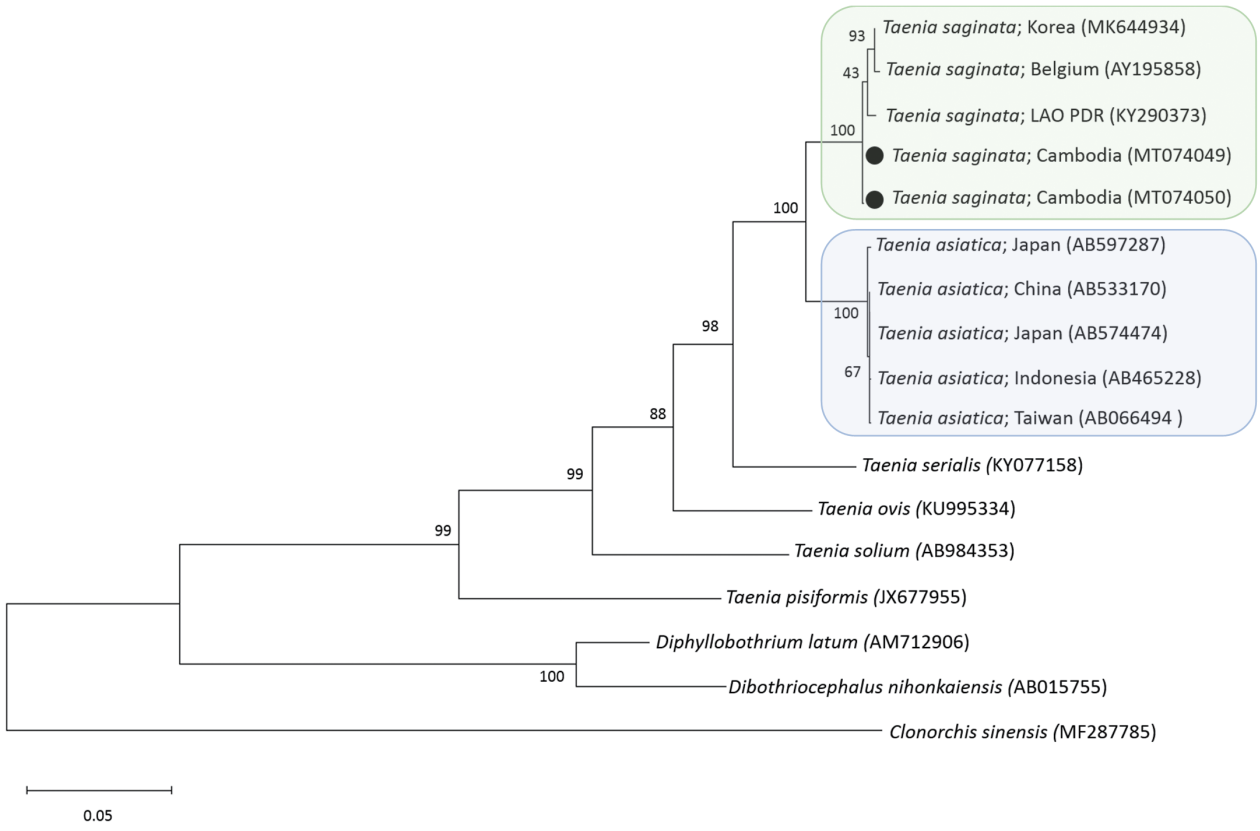

Fig. 2. A phylogenetic tree of 2 tapeworm samples obtained in this study in relation to tapeworm species drawn with *cox*1 DNA sequences in GenBank. Black dots indicate 2 samples identified in this study. *Clonorchis sinensis* was used as an outgroup. Scale bar indicates nucleotide substitutions per site.

Table 2. Genotype of tapeworm samples from 2 patients

Patient no.	mtDNA type (*cox*1)	Genotype[a] at *ef*1 locus	Genotype[a] at *elp* locus
1	*T. saginata*	*ef*1C/*ef*1C (*T. saginata*)	*elp*C/*elp*C (*T. saginata*)
2	*T. saginata*	*ef*1C/*ef*1C (*T. saginata*)	*elp*C/*elp*C (*T. saginata*)

[a]The allele types of these samples were analyzed following the previous study [6].

ic DNA was extracted from eggs collected from the feces [3]. In our study, we obtained gravid proglottids from 2 patients and observed that they had more than 15 main uterine lateral branches being consistent with *T. saginata* or *T. asiatica* but not with *T. solium*. To determine whether they are *T. saginata* or *T. asiatica*, it was necessary to perform molecular analyses [2].

Interestingly, hybrid descendants of *T. saginata* and *T. asiatica* were reported from humans in Thailand [6] and Lao PDR [8]. Therefore, we conducted molecular analyses of *cox*1, *ef*1, and *elp* genes to rule out this hybrid issue. The *cox*1 sequences revealed high homologies with *T. saginata* but low homologies with *T. asiatica*. The nuclear gene *ef*1 is known to have 3 alleles (*ef*1A, *ef*1B, and *ef*1C), and another nuclear gene *elp* has 4 alleles (*elp*A, *elp*B, *elp*C, and *elp*D); *T. saginata* has only 1 *ef*1 allele (*ef*1C) and 3 *elp* alleles (*elp*A, *elp*C, and *elp*D), whereas *T. asiatica* has 2 *ef*1 alleles (*ef*1A and *ef*1B) and 2 *elp* alleles (*elp*A and *elp*B) [6]. Our samples had *ef*1C and *elp*C alleles, and thus we could confirm them not a hybrid of the 2 species but pure *T. saginata*.

The geographical distribution of 3 human *Taenia* spp. is closely related to cultural characteristics of the people, which include the traditional food habit of consuming undercooked meat, including beef and pork, infected with viable metacestodes [8]. Our study suggests the necessity of continued surveillance of human *Taenia* tapeworm infections in Cambodia.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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