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▣ CASE REPORT

Four Cases of *Taenia saginata* Infection with an Analysis of *COX1* Gene

Jaeeun Cho[1,†], Bong-Kwang Jung[1,†], Hyemi Lim[1], Min-Jae Kim[1,2], Thanapon Yooyen[3], Dongmin Lee[4], Keeseon S. Eom[4], Eun-Hee Shin[1,5], Jong-Yil Chai[1,*]

[1]*Department of Parasitology and Tropical Medicine, [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul 110-799, Korea; [3]Department of Biology, Faculty of Science, Thaksin University, Phatthalung 93110, Thailand; [4]Department of Parasitology, Medical Research Institute and Parasite Resource Bank, Chungbuk National University School of Medicine, Cheongju 361-763, Korea; [5]Seoul National University Bundang Hospital, Seongnam 463-707, Korea*

Abstract: Human taeniases had been not uncommon in the Republic of Korea (=Korea) until the 1980s. The prevalence decreased and a national survey in 2004 revealed no *Taenia* egg positive cases. However, a subsequent national survey in 2012 showed 0.04% (10 cases) prevalence of *Taenia* spp. eggs suggesting its resurgence in Korea. We recently encountered 4 cases of *Taenia saginata* infection who had symptoms of taeniasis that included discharge of proglottids. We obtained several proglottids from each case. Because the morphological features of *T. saginata* are almost indistinguishable from those of *Taenia asiatica*, molecular analyses using the PCR-RFLP and DNA sequencing of the cytochrome *c* oxidase subunit 1 (*cox1*) were performed to identify the species. The PCR-RFLP patterns of all of the 4 specimens were consistent with *T. saginata*, and the *cox1* gene sequence showed 99.8-100% identity with that of *T. saginata* reported previously from Korea, Japan, China, and Cambodia. All of the 4 patients had the history of travel abroad but its relation with contracting taeniasis was unclear. Our findings may suggest resurgence of *T. saginata* infection among people in Korea.

Key words: *Taenia saginata*, case report, molecular diagnosis, *cox1*, PCR-RFLP, sequence divergence

INTRODUCTION

Human taeniasis results from an intestinal infection with the parasitic tapeworm of the genus *Taenia*. The disease is caused by 3 species including *Taenia asiatica*, *Taenia saginata*, and *Taenia solium* [1]. Taeniasis is zoonotic because they involve pigs (*T. solium* and *T. asiatica*) or cattle (*T. saginata*) as the intermediate host and humans as the definitive host [2]. In case of *T. saginata*, when cattle digest the eggs, oncospheres hatch and then invade the intestinal mucosa and migrate via circulation to muscles where they develop into metacestodes (=cysticerci). Humans can become infected by ingesting raw or undercooked infected beef [2]. Usually in humans, a single *T. saginata* worm is infected, grows to become an adult in about 3 months. The adult tapeworm produces gravid proglottids, which crawl out of the anus of the infected humans or are diffused in the feces [2]. The main symptom is anal pruritis caused by outward migration of proglottids. Abdominal discomfort, mild diarrhea, and loss of body weight can also occur due to the presence of the tapeworm in the intestine [2].

Before 1993, when *T. asiatica* was first reported as a new species in the Republic of Korea (=Korea) [3], all *T. asiatica* had been considered as *T. saginata* because of the almost indistinguishable morphology of the 2 species [3]. Therefore, human *Taenia* tapeworms in Korea were diagnosed as either *T. solium* or *T. saginata*, or undetermined until 1993 [4]. In 1993, it was reported that *T. asiatica* could be distinguished from *T. saginata* by the existence of the rostellum on its scolex, presence of small posterior protuberances in gravid proglottids, higher numbers of uterine twigs, and wart-like formations on the larval bladder surface [3]. However, these distinctions are difficult to observe in each individual strobila, and both morphological and genetic analyses were required to clearly distinguish the 2 species [5].

Until the 1980s, in Korea, human taeniases had been one of the not uncommon parasitic infections [6]. An old report in 1924 documented that the prevalence of *Taenia* spp. eggs was

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* Corresponding author (cjy@snu.ac.kr)
† These authors contributed equally to this study as the first author.

12.8%, which was the highest value of *Taenia* prevalence ever recorded [7]. After 1927, the prevalence declined gradually until it became 0% in a national survey of 20,541 randomly selected Korean people in 2004 [1]. However, in a subsequent national survey performed in 2012 which targeted 23,956 people, 0.04% (10 egg-positive cases) prevalence of *Taenia* spp.was detected [8]. It was suggested that human taeniasis might be resurging recently.

In this article, we report 4 cases of *T. saginata* infection all of which were found during 6 months in 2013 (March to September), based on adult tapeworm recovery from the patients (neither *T. asiatica* nor *T. solium* cases during the same period). All the patients had the history of travel abroad to Asian countries, although no direct relationship was recognizable between the overseas travel and *Taenia* tapeworm infections. The specific identification of the worms was based on molecular analyses, including PCR-RFLP and partial sequencing of the cytochrome *c* oxidase subunit 1 (*cox1*) gene of the worms.

CASE RECORD

The 4 patients (cases A-D) found yellowish white tapeworm proglottids (4-5 in number in each patient) moving in their underwear or feces and consulted to the Department of Parasitology and Tropical Medicine, Seoul National University, Seoul, Korea. One of them complained of lower abdominal discomfort and anal itching; however, the other 3 had no special clinical complaints. All of them had experiences of visiting other countries including China, Cambodia, Japan, Dubai, or Turkey. Two of them tried flubendazole and albendazole based on wrong prescription, before prescribed with praziquantel in our department. All the 4 patients had the history of eating raw beef or rarely cooked beef steak recently (Table 1).

In case B (48-year-old male), an almost complete strobila (about 1.1 m long) without scolex was obtained after treatment with praziquantel (15 mg/kg in a single dose) and purging with 40 g magnesium sulfate ($MgSO_4$). The gravid proglottids from the 4 patients revealed 16-20 lateral uterine branches and grossly looked like either *T. saginata* or *T. asiatica*. It was needed to do molecular analysis to obtain a specific diagnosis [9-11].

MATERIALS AND METHODS

We performed PCR-RFLP and gene sequencing of the mitochondrial *cox1* for specific identification of the 4 *Taenia* tapeworms. In order to use as positive controls, we obtained 3 known samples from the Parasite Resource Bank in Chungbuk National University (Cheongju, Chungbuk Province, Korea); *T. asiatica* (cat. no. PRB081031800), *T. saginata* (cat. no. PRB080110080), and *T. solium* (cat. no. PRB071490001). The genomic DNA was extracted from a single segment by using the DNeasy® Blood & Tissue kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. The mitochondrial *cox1* gene was targeted in PCR amplification. The PCR primers used were T1F (5'-ATA TTT ACT TTA GAT CAT AAG CGG-3') and T1R (5'-ACG AGA AAA TAT ATT AGT CAT AAA-3') [9]. PCR was carried out in a 30 µl reaction mixture containing 15 µl of Smart 2x PCR Pre-Mix (SolGent Co., Ltd, Daejeon, Korea), 2 µl of template DNA, 10 µM of each primers, and 11 µl of distilled water. PCRs were progressed under 1 cycle of initial denaturation at 94℃ for 3 min followed by 40 cycles of denaturation (94℃ for 1 min), annealing (52℃ for 1 min), and extension (72℃ for 1 min), with a final extension at 72℃ for 10 min [12]. For PCR-RFLP, the PCR products (10 µl) were digested with the restriction endonuclease (1 µl of *Nco1*) in a final volume of 13 µl containing 2 µl of Ez-one 10x buffer for 1 hr at 37℃, and then

Table 1. Summary of 4 *Taenia saginata* cases

Case code	Age & sex	Clinical complaints	Suspected source of infection	Drug	Visiting date to our department	Countries traveled
A	27 M	Passage of proglottids	Raw beef	Praziquantel	Mar. 2013	China (2009) Cambodia (2010)
B	48 M	Passage of proglottids, abdominal discomfort, anal itching	Raw beef	Praziquantel	May 2013	China (2004-2008)
C	30 F	Passage of proglottids	Rare beef steak	Flubendazole Praziquantel	Sep. 2013	Japan (2012)
D	36 M	Passage of proglottids	Raw beef Rare beef sandwich	Flubendazole Albendazole Praziquantel	Oct. 2013	Dubai (Sep. 2013) Turkey (Sep. 2013)

analyzed by electrophoresis on 2% agarose gels.

The sequences of *cox1* from *Taenia* tapeworm specimens were aligned using the Genieous program (version 6.1.4) (Biomatters Ltd., Auckland, New Zealand). The *Taenia* tapeworms were identified based on the similarity of nucleotide sequences and phylogenetic relationships with those of *T. asiatica* from Korea (GenBank no. AB465224), China (GenBank no. AB107235), and Japan (GenBank no. AB608742), *T. saginata* from Korea (GenBank no. AB465246), China (GenBank no. AB107239), Cambodia (GenBank no. AB275143), and Japan (GenBank no. AB644391), and *T. solium* from Korea (GenBank no. DQ089663). The neighbor-joining tree was constructed under the Kimura 2 parameter model by MEGA program version 5.2 to figure phylogenetic relationships [13].

RESULTS

When the PCR products were digested with *Nco1* restriction enzyme, all the cases (A, B, C, and D) revealed identical PCR-RFLP patterns which were consistent with the known *T. saginata* (Fig. 1). Only 1 band (491 bp) was observed for *T. saginata*, whereas 2 different bands (152 bp and 339 bp) were obtained for *T. solium* and *T. asiatica*.

Sequencing of the 491 bp *cox1* gene showed 99.8-100% identity with *T. saginata*; however, only 94.7-94.9% and 87.8-88.0% identity was seen with *T. asiatica* and *T. solium*, respectively (Table 2). Furthermore, the neighbor-joining tree revealed that our 4 specimens (cases A-D) were phylogenetically compatible to *T. saginata* but far from *T. asiatica* or *T. solium* reported from various Asian countries and deposited in GenBank (Fig. 2).

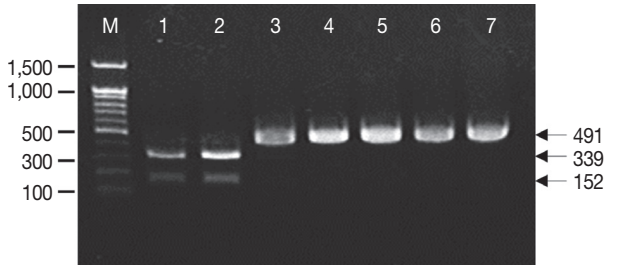

Fig. 1. Amplification of the *cox1* mitochondrial gene of Taenia tapeworms by PCR-RFLP analysis. The amplified products of *Taenia solium* and *Taenia asiatica* were digested with *Nco1* restriction endonuclease forming 2 different banding patterns (152 and 339 bp). Nonetheless, *Taenia saginata* and our 4 samples (491 bp) were not digested with the *Nco1* enzyme. Lanes 1 to 7 are *T. solium*, *T. asiatica*, *T. saginata* (positive samples), and cases A, B, C, and D (test samples), respectively. M: 1 kb size maker (bp).

DISCUSSION

We used *Nco1* restriction enzyme, which recognizes and digests C^CATGG sites, to analyze the mitochondrial *cox1* gene of our *Taenia* specimens. The *Nco1* was previously used for digestion of the *cox1* gene of *T. solium* which was divided into 2 fragments [14]. No other reports have been available which used this enzyme to digest *Taenia* spp. genes. In the present study, we observed for the first time that *T. asiatica* (from the Parasite Resource Bank, Chungbuk National University) was also divided into 2 fragments just like *T. solium* (from the Parasite Resource Bank) both having the same recognition sequenc-

Table 2. Nucleotide identity of our specimens with the known *Taenia* species in GenBank[a] for the mitochondrial cytochrome *c* oxidase 1 (*cox1*) gene

Our patients	*Taenia saginata*	*Taenia asiatica*	*Taenia solium*
Case A	491 (100.0)	465 (94.7)	431 (87.8)
Case B	491 (100.0)	465 (94.7)	431 (87.8)
Case C	491 (100.0)	465 (94.7)	431 (87.8)
Case D	490 (99.8)	466 (94.9)	432 (88.0)

The unit of each value is base pair (%).
[a]*T. asiatica* from Korea (GenBank no. AB465224), China (GenBank no. AB107235), and Japan (GenBank no. AB608742), *T. saginata* from Korea (GenBank no. AB465246), China (GenBank no. AB107239), Cambodia (GenBank no. AB275143), and Japan (GenBank no. AB644391), and *T. solium* from Korea (GenBank no. DQ089663).

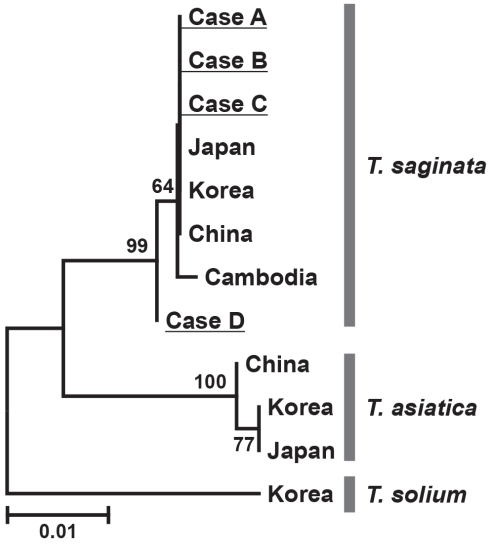

Fig. 2. Neighbor-joining tree of human *Taenia* tapeworms based on nucleotide sequences of the *cox1* gene. Numbers above the branches are bootstrap values. The scale bar represents the estimated number of nucleotide substitutions per nucleotide site. The phylogenetic tree reveals that the *cox1* gene from our 4 patients (case A-D) was closer to *T. saginata* than *T. asiatica* or *T. solium*.

es by *Nco1*. However, in case of *T. saginata* (our specimens), there was no site for enzyme recognition by *Nco1*, and thus the PCR products were preserved retaining only 1 band. Therefore, the *Nco1* enzyme appears to be useful to clarify the distinction between *T. saginata* and *T. asiatica* which are morphologically difficult to distinguish.

Korea had been one of the endemic areas for *Taenia* spp. until the 1980s [6]. However, several documents announced that the *Taenia* egg positive rate became as low as 0.02% nationwidely in 1997 and finally dropped to 0% in 2004 [15-17]. Thereafter, only 5 sporadic cases were diagnosed during 2006-2011 [1], and 10 additional cases were detected in 2012 in a national survey of 23,956 randomly selected people [8]. With regard to the occurrence of *T. saginata*, Jeon et al. [6] reported 14 specimens of *T. saginata* out of 68 *Taenia* spp. tapeworm specimens collected during 1935-2005 in Korea based on morphological and genetic analyses [6]. After 2008, 2 cases of *T. saginata* have been distinguished in Chungbuk National University [1]. However, case reports with species identification of *T. saginata*, including molecular analysis, have not been published since 2008. In this study, we reported 4 patients of *T. saginata* infection who were referrred to our department during March-September 2013. All the 4 patients had the history of travel abroad but no evidence for contracting taeniasis abroad could be identified. No matter what the source of infection is, our findings may suggest resurgence of *T. saginata* infection among the people in Korea.

Human *T. saginata* infection is caused by ingesting raw or undercooked infected beef [2]. However, in Korea, the infection status of cattle with *T. saginata* metacestodes has seldom been documented except in a few old articles which described 17.8-21.3% prevalence in 1924 among cattle in Seoul and Gongju [18], up to 37.6% in 1926 among cattle from different localities [19], and 30.9% (1936) and 5.1% (1943) among cattle in Jeju-do [20]. Particularly, after 1945, not a single official document has been published on the prevalence of *T. saginata* metacestodes among the Korean cattle. Only one episode concerning a measled cattle (=infected with *T. saginata* metacestodes) was available from a rural village of Gangwon-do in 1975 based on a personal communication [4].

This study is significant to collect 4 cases of *T. saginata* infection within a short time period of 6 months in a university parasitology department in 2013. Neither *T. asiatica* nor *T. solium* cases were detected during the same period in the department. Considering that only a limited number of *T. saginata* cases occurred previously in Korea compared to *T. asiatica* which occurred 4 times more frequently than *T. saginata* [6], there might be a resurgence of *T. saginata* infection recently in Korea. Reactivation of the domestic life cycle of *T. saginata*, if it was remained, and increased overseas travel of people, as well as import of beef from foreign countries, may be the possible responsible factors. Public attention should be paid to prevent human taeniasis avoiding intake of raw beef in Korea as well as in Asian countries endemic for taeniasis.

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CONFLICT OF INTEREST

We have no conflict of interest related with this study.

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