ISSN (Print) 0023-4001
ISSN (Online) 1738-0006

Korean J Parasitol Vol. 49, No. 2: 195-197, June 2011
DOI: 10.3347/kjp.2011.49.2.195

■ BRIEF COMMUNICATION

Molecular Identification of *Taenia* Tapeworms by *Cox1* Gene in Koh Kong, Cambodia

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Abstract: We collected fecal samples from 21 individuals infected with *Taenia* tapeworms in Koh Kong Province, Cambodia, and performed nucleotide sequencing of the *cox1* gene and multiplex PCR on the eggs for DNA differential diagnosis of human *Taenia* tapeworms. Genomic DNA was extracted from the eggs of a minimum number of 10 isolated from fecal samples. Using oligonucleotide primers Ta7126F, Ts7313F, Tso7466F, and Rev7915, the multiplex PCR assay proved useful for differentially diagnosing *Taenia solium*, *Taenia saginata*, and *Taenia asiatica* based on 706, 629, and 474 bp bands, respectively. All of the *Taenia* specimens from Kho Kong, Cambodia, were identified as either *T. saginata* (n=19) or *T. solium* (n=2) by *cox1* sequencing and multiplex PCR.

Key words: *Taenia saginata*, *Taenia solium*, molecular survey, egg DNA, multiplex PCR

Since 2006, intestinal parasite control has been performed among primary school children in Cambodia by the Korea Association of Health Promotion (KAHP) and the National Center for Parasitology, Entomology, and Malaria Control, Cambodia (CNM). These organizations carried out a parasitological survey of inhabitants and primary school children in Koh Kong, Kam Pot, and Kampong Som Provinces, and performed stool examinations using the Kato-Katz smear and scotch-tape anal swab methods. Since 1988 in Cambodia, parasitic helminth infections have revealed malaria, schistosomiasis, and other intestinal helminthic infections [1-4]. Previous studies reported that the intestinal helminth eggs detected were *Ascaris lumbricoides*, *Echinostoma* sp., hookworms, *Hymenolepis nana*, *Opisthorchis* sp., *Rhabditis* sp., and *Trichuris trichiura*, and protozoan cysts were *Entamoeba histolytica*, *Entamoeba coli*, *Giardia lamblia*, and *Iodamoeba buetschlii*. The overall infection rate of intestinal parasites was 54.2% in 2002 [5], 2-40% in 2003 [6], and 25.7% in 2004 [7]. However, there are few reports on taeniasis in Cambodia. Epidemiologically, *Taenia solium* and *Taenia saginata* are found worldwide and *Taenia asiatica* is found mostly in Asian countries, including Korea, China, Taiwan, Thailand, Indonesia, Vietnam, Japan, and the Philippines [8]. We have been monitoring the epidemiological status of *T. solium*, *T. saginata*, and *T. asiatica* infections in Cambodia, and now it is considered necessary to clarify the distribution of these tapeworms in Cambodia. To identify *Taenia* species, we analyzed nucleotides for sequence variations, and performed multiplex PCR using the copro-DNA from the eggs.

We collected a total of 2,824 fecal specimens from Koh Kong (n=904), Kam Pot (n=1,002), and Kampong Som (n=918). Of them, 2,052 specimens were collected from primary school children, and the remaining 772 specimens were from inhabitants. We collected *Taenia* sp. eggs from 21 patients in Koh Kong (Table 1). The eggs were detected by the Kato-Katz smear technique, in which the number of eggs counted in the entire field of 41.7 mg of stools is multiplied by 24 in order to obtain the number of eggs per gram of feces. The *Taenia* eggs were isolated from the stools and subjected to DNA sequencing and mul-

• Received 31 March 2011, revised 21 April 2011, accepted 22 April 2011.
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Table 1. Helminth egg positive rates in the feces of schoolchildren and residents in Koh Kong, Kam Pot, and Kampong Som, Cambodia in 2007

Province	No. of people examined	Total No. of positive (%)	No. of multiple positive	No. of positive (%)								
				Al	Hw	Tt	Ov	*Taenia* sp.	Hn	Ev	Ech. sp.	Others
Koh Kong	904	321 (35.5)	43 (4.8)	202 (22.3)	33 (3.7)	63 (6.7)	7 (0.8)	42 (4.6)	21 (2.3)	16 (1.8)	4 (0.4)	1 (0.1)
Kam Pot	1,002	293 (29.2)	48 (4.8)	65 (6.5)	183 (18.3)	79 (7.9)	0	0	0	20 (2.0)	0	1 (0.1)
Kampong Som	918	320 (34.9)	77 (8.4)	178 (19.4)	54 (5.9)	139 (15.1)	20 (2.2)	1 (0.1)	2 (0.2)	28 (3.1)	7 (0.8)	0
Total	2,824	934 (33.1)	168 (5.9)	445 (45.8)	270 (9.6)	281 (9.8)	27 (1.0)	43 (1.5)	23 (0.8)	64 (2.3)	11 (0.4)	2 (0.2)

Al, *Ascaris lumbricoides*; Hw, hookworms; Tt, *Trichuris trichiura*; Ov, *Opisthorchis viverrini*; Hn, *Hymenolepis nana*; Ev, *Enterobius vermicularis*; Ech, *Echinostoma* sp.; Others, unidentified parasite eggs.

tiplex PCR for differential diagnosis according to the protocols already established [9].

The overall helminth egg positive rate was 33.1% (934/2,824) and 1.5% (43/2,824) for the *Taenia* sp. eggs. Other helminth eggs detected were *Ascaris lumbricoides* 15.8% (445/2,824), hookworms 9.6% (270/2,824), *Trichuris trichiura* 1.0% (27/2,824), *Enterobius vermicularis* 2.3% (64/2,824), and echinostomes 0.4% (11/2,824). Multiple parasite infections were found in 5.9% (168/2,824) of the samples. *Taenia* eggs had a positive rate of 4.6% (42/904) inhabitants in Koh Kong Province (Table 1).

The PCR amplification and direct sequencing of the *cox1* target fragment (456 bp in length corresponding to the positions 67-522 bp of the *cox1* gene) were performed using the total genomic DNA extracted from the eggs of parasites found in patients. The *cox1* sequences (456 bp) of the code number 97 and 166 showed 99% similarity with the reference sequences of *T. solium* (GenBank AB086256), and the other code numbers 70, 72, 76, 77, 83, 86, 89, 128, 129, 135, 139, 167, 180, 186, 190, 194, 196, 197, and 198 showed 99% similarity with *T. saginata* (GenBank AY684274). The diagnostic quality of the results obtained using multiplex PCR and species-specific primers were equal to that based on the nucleotide sequencing of the *cox1* gene. All of the 21 *Taenia* specimens from Koh Kong were identified by *cox1* sequencing and multiplex PCR as either *T. saginata* (n=19) or *T. solium* (n=2) (Table 2).

The most prevalent parasitic helminths in Cambodia were soil-transmitted nematodes, such as *Ascaris*, hookworms, and *Trichuris*. In addition, *Opisthorchis viverrini*, intestinal trematodes, and schistosomes are also found along the Mekong River [4]. Cestodes dominantly known were *Hymenolepis nana*; however, *Taenia* tapeworm species were not well recognized until 2006.

Table 2. Sample specimens of Koh Kong inhabitants analyzed in this study

Patient code	Sex/age	Stool exam.[a]	Cox1 sequence/ multiplex PCR
70	M/10	*Taenia*	*T. saginata*
72	F/12	*Taenia*	*T. saginata*
76	M/19	*Taenia*	*T. saginata*
77	M/27	*Taenia*	*T. saginata*
83	F/17	*Taenia*	*T. saginata*
86	F/54	*Taenia*	*T. saginata*
89	M/19	Al, *Taenia*	*T. saginata*
97	F/16	*Taenia*	*T. solium*
128	M/40	Al, *Taenia*	*T. saginata*
129	F/44	*Taenia*	*T. saginata*
135	M/50	*Taenia*	*T. saginata*
139	M/52	*Taenia*	*T. saginata*
166	F/36	*Taenia*	*T. solium*
167	F/24	*Taenia*	*T. saginata*
180	F/35	*Taenia*	*T. saginata*
186	M/28	*Taenia*	*T. saginata*
190	F/59	*Taenia*	*T. saginata*
194	M/48	*Taenia*	*T. saginata*
196	F/28	Al, *Taenia*	*T. saginata*
197	F/42	*Taenia*	*T. saginata*
198	F/38	*Taenia*	*T. saginata*

[a]Kato-Katz method; Al, *Ascaris lumbricoides*.

In our study, *Taenia* eggs were detected in schoolchildren and other inhabitants in Koh Kong and Kampong Som provinces and were genetically analyzed, but adult tapeworms were not collected. In Cambodia, according to a KAHP report (unpublished), the overall prevalence rate of *Taenia* tapeworms ranged from 0.3% to 1.5% between 2006 and 2009. In the present study, a higher prevalence of *Taenia* sp. was found in Koh Kong inhabitants who habitually eat raw beef and pork.

The diagnosis of *Taenia* tapeworm infections is usually based on microscopic detection of eggs or discharged proglottids in the stool. Coprological examination based on the morphology of tapeworm proglottids has a low sensitivity, and the eggs are morphologically indistinguishable. Molecular diagnostic methods, including the use of sequence specific DNA probes, PCR coupled to restriction fragment length polymorphism, and multiplex PCR, are highly useful for differential diagnosis of *Taenia* tapeworm eggs in stools. However, these methods may not be always suitable unless eggs and particles of proglottids are released into the stool. In our study, we collected 42 stool samples but 21 cases lacked the fecal volume for DNA extraction. The DNA recovery yield increased using ethanol precipitation of eggs in stools, and PCR was usually performed secondarily or thirdly for DNA sequencing. PCR products derived from fecal samples were detectable at a minimum number of 10 eggs.

There are countries showing sympatric distributions of 3 human *Taenia* tapeworms in South and East Asian regions. However, in this study, only *T. saginata* (n=19) and *T. solium* (n=2) were identified by *cox1* sequencing and multiplex PCR. The distribution of *T. asiatica* in Cambodia is yet to be cleared.

ACKNOWLEDGEMENTS

This work was supported by a research grant from Chungbuk National University in 2011. Parasite materials used in this study were provided by the Parasite Resource Bank of Korea, National Research Center (R21-2005-000-10007-0), Republic of Korea.

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