


CASE REPORT

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A co-infection case report of *Taenia saginata* in a patient with subclinical clonorchiasis confirmed by the combination of diagnostic tools

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Abstract

Background: Clonorchiasis is the common parasitic infection in the general population of the Republic of Korea, however, taeniasis is scarcely reported recently. Here, we describe a case of co-infection with the cestode *T. saginata* in a patient with subclinical clonorchiasis diagnosed by a combination of diagnostic tools in Korea.

Case presentation: A 56-year-old man visited the hospital having passed proglottids in his stool for the past two months and brought a stool sample with segments to our hospital. He had no abdominal symptoms, such as nausea, vomiting, abdominal pain, diarrhea, or constipation. He used to consume raw beef and fish frequently. We could not find evidence of gravid proglottids which contain fully developed uteri filled with ova or branched uterine structures, within the submitted sample. To identify the tapeworm species, we carried out molecular analyses on the proglottids. The *cox1* and *ef1a* sequences had a 100% match with those of *T. saginata* and differed from the sequences of the other *Taenia* species. Upon examination of stool samples fixed by formalin-ether concentration method, no *Taenia* species ova were observed in 10 slides. Instead, *C. sinensis* ova were observed, despite the level of IgG specific to *C. sinensis* being within the normal range. The patient was treated with praziquantel (25 mg/kg, three times a day) for 3 days, and subsequently *C. sinensis* ova were not found in his stool.

Conclusion: Our case indicates that a combination of morphological, serological, and molecular diagnostic tools should be used for the accurate diagnosis of subclinical parasitic infections.

Keywords: *Taenia saginata*, *Clonorchis sinensis*, Molecular diagnosis, Ova, ELISA

Background

Intestinal parasitic infections are still a major public health issue in worldwide [1]. Until the 1970s, many Koreans had intestinal parasitic infections, mostly from soil-transmitted helminthes [2]. Although overall helminth egg-positive rate was 84.3% in the 1st Nationwide Survey [2], a dramatic decline of the egg-positive rate has been shown to 2.6% in the 8th Nationwide Survey, 2013 [3]. Thus, at present, intestinal parasitic infections are not recognized as a critical problem in Korea. The public health focus has also shifted

from soil-transmitted helminthiases to food-borne parasitic infections. Of food-borne parasites, *Clonorchis sinensis* showed the highest prevalence in Korea showing 2.4% of egg-positive rate in general population [3]. The parasite is more prevalent in populations living by rivers, with an egg-positive rate of 11.4% [4], as humans usually become infected after ingesting raw or undercooked freshwater fish harboring *C. sinensis* metacercariae [5]. Human taeniasis, another food-borne parasitic infection, is a zoonotic disease, because it involves pigs (*Taenia solium* and *Taenia asiatica*) or cattle (*Taenia saginata*) as an intermediate host and humans as the definitive host [6, 7]. Studies dating back to 1924 reported *Taenia* prevalence in Korea at 7.2% or higher [8–10], but the national prevalence was 1.98% in 1971 [2].

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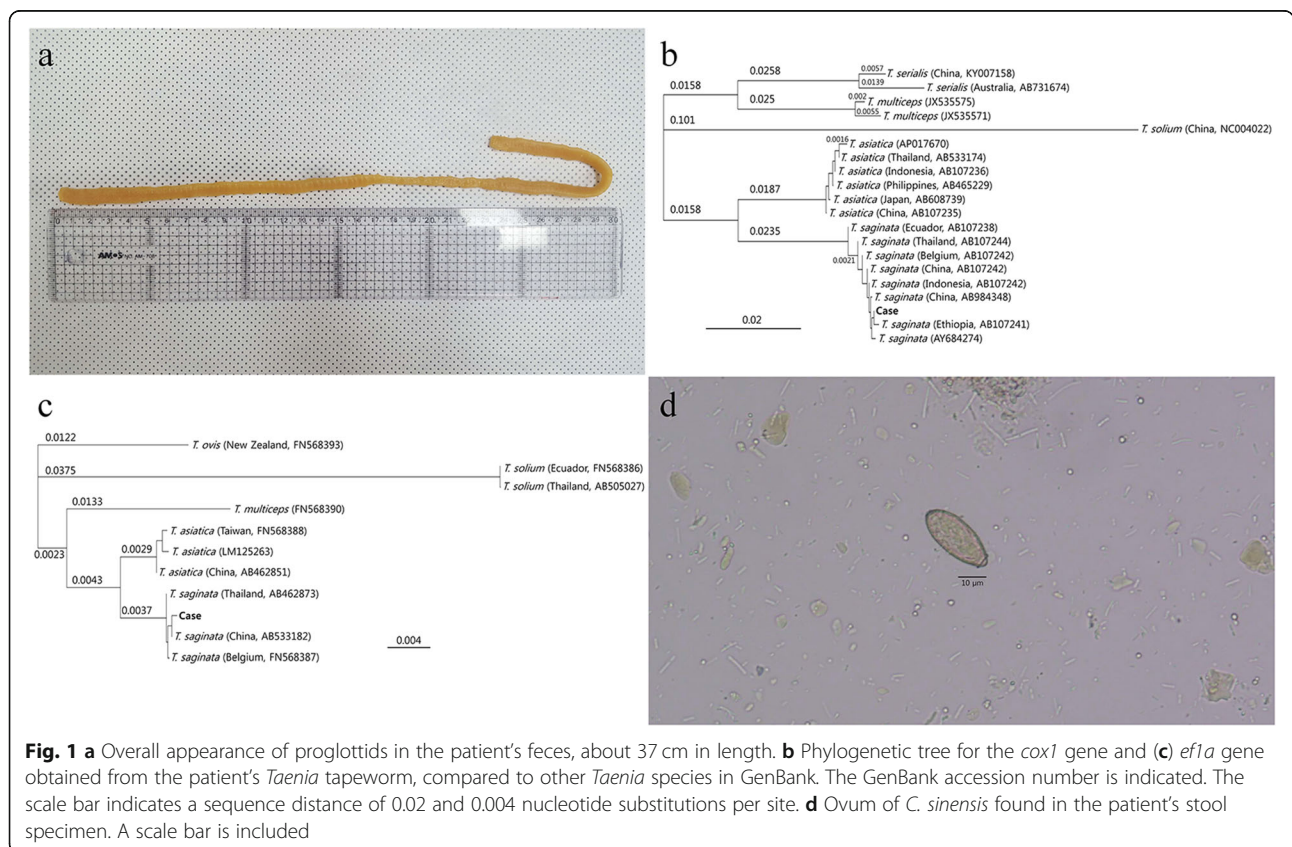


The national prevalence continued to decline, and was reported as 0.004% in 2013 [3]. Here, we describe a case of co-infection case with the cestode *T. saginata* and the trematode *C. sinensis* by using a combination of diagnostic tools in a patient with subclinical parasitic infection.

Case presentation

A 56-year-old man complained of passing proglottids in his stool intermittently over the last two months. No abdominal symptoms, such as nausea, vomiting, abdominal pain, diarrhea, or constipation were present. He reported frequent consumption of raw beef and fish (both marine and freshwater fish), and had no history of traveling abroad. He had previously obtained 400 mg of albendazole from the pharmacy and taken it once orally without clinical improvement. After that, he was prescribed 600 mg of praziquantel at a local clinic and had taken it once orally as well. He brought his stool sample, which included the passed segments to our hospital (Fig. 1a). The segments were pressed between two microscope slides and examined macroscopically without staining. We could not observe gravid proglottids, which contain fully developed uteri filled with ova, or branched uterine structures. To identify the tapeworm species, we conducted molecular analysis using the proglottid segments. Genomic DNA

was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and subsequently used as a template for polymerase chain reaction (PCR). The mitochondrial cytochrome *c* oxidase subunit I (*cox1*) gene and partial sequences of elongation factor-1 alpha (*ef1a*) were targeted for PCR amplification. The sequences of the PCR primers used were: T1F (5'-ATATTTACT TTAGATCAT AAGCGG-3') and T1R (5'-ACGAGAAAATATATTAG TCATAAA-3') for *cox1*, and Tae_ef1/F4 (5'-TGTGGT GGAATCGATAAAAGG-3') and Tae_ef1/R4 (5'-TCGA TCTCATGTCACGAACG -3') for *ef1a* [11, 12]. PCR was carried out using a 30 µL reaction mixture containing 15 µL Smart 2 × PCR Pre-Mix (SolGent Co., Ltd., Daejeon, Korea), 2 µL template DNA, 10 µM of each primer, and 11 µL distilled water, as described in a previous study [13]. The amplification process comprised 35 cycles of denaturation (94 °C for 30 s), annealing (60 °C for 30 s), and extension (72 °C for 80–90 s). The PCR products were sent to Macrogen (Korea) for direct sequencing using the same PCR primers. The 480-bp *cox1* sequence had a 100% match with the *T. saginata* sequence and a 94.8% match with the *T. asiatica* sequence. The 1078-bp *ef1a* sequence also had a 100% match with the sequence of *T. saginata*, a 99.3% match with the sequence of *T. asiatica* and a 95.3% match with the sequence of *T. solium*. The three *Taenia*



species that infect humans had nucleotide differences at 73 and 57 polymorphic sites for the *cox1* and *ef1a* sequences, respectively (Additional file 1). Of these polymorphic sites, three sites (nucleotides 294, 336, 405) in *cox1* differentiated the three species. DNA sequences were aligned using the CLUSTAL W computer program [14]. Phylogenetic trees were constructed using the neighbor joining method [15] and genetic distances were computed using the Tamura-Nei method using the Geneious computer program (Fig. 1b and c). The neighbor joining tree used GenBank sequences derived from samples collected in Asia, and indicated that our specimen was in the same phylogenetic group as *T. saginata*, but not in the same group as *T. asiatica* or *T. solium*. In addition to molecular analysis, we also examined the patient's stool specimen using the formalin-ether concentration method. However, we could not observe any *Taenia* species ova on the 10 slides examined. Ova were not observed inside the proglottids either, indicating that these proglottids were immature. Instead, *C. sinensis* ova were observed on one slide (Fig. 1d). Conversely, the level of serum IgG specific to *C. sinensis* measured using Enzyme-Linked Immunosorbent Assay (ELISA) was within the normal range. He was treated with praziquantel at the recommended dose of 25 mg/kg three times daily for 3 days [16]. After treatment, no proglottids or ova of *C. sinensis* were found in his stool.

Discussion and conclusions

Human taeniasis is usually diagnosed by observing ova or gravid proglottids in the patient's stool. From diagnoses conducted after 1993, the *Taenia* tapeworms infecting humans in Korea were identified as *T. solium*, *T. saginata*, and *T. asiatica* [5, 17, 18]. However, additional differential modalities may be required to clearly distinguish among these species, as morphological characteristics, such as the presence of an unarmed rostellum on the scolex of adult, the large number of uterine twigs, and the presence of a posterior protuberance, can be difficult to observe in individual strobili [17, 18]. Direct sequencing of *cox1* sequences have been used to identify the species causing human taeniasis. Cho et al. successfully used *cox1* to distinguish between *T. saginata* and *T. asiatica* [11]. Recently, studies conducted in China and Lao People's Democratic Republic reported that hybridization between the three *Taenia* species can occur, based on sequencing nuclear and mitochondrial genes [19, 20]. In our case, as we did not observe uterine structures or ova in the proglottids in our patient's stool sample, these proglottids were likely immature. As such, we were unable to identify the tapeworm to the species-level using morphological characters, and thus sequenced both the nuclear gene, *ef1a*, and the mitochondrial gene, *cox1*, from the proglottid sample as well. Matches at several polymorphic sites

confirmed that the *Taenia* specimen in this case is most likely *T. saginata*, and closely related to *T. saginata*, specimens sampled previously in Korea, China, Indonesia, Thailand and other countries (Fig. 1b and c). Here, we diagnosed that this case of human taeniasis was caused by *T. saginata*. We confirmed that tapeworm species by sequencing nuclear and mitochondrial genes, which successfully differentiated *T. saginata* from other *Taenia* species. Notably, we discovered that co-existence of taeniasis in a patient with subclinical clonorchiasis using a combination of diagnostic tools. Even in the era of molecular tools, the expertise in microscopy is still essential to achieve a correct diagnosis. Further, subclinical clonorchiasis in this case was confirmed only by examining the stool sample using microscopy. Regarding the use of ELISA to diagnose the presence of *C. sinensis*, ELISA results should be interpreted with caution, as the result can be negative even with an egg-positive [21, 22].

Early documentation of human taeniasis in Korea reported that the prevalence of *Taenia* was ranging from 7.2 to 12.0% [8–10]. More recently (2004–2008), the prevalence had declined to low levels of 0–0.01% [3, 23]. Since 2008, there has only been one study that documented four cases of *T. saginata*, which were diagnosed by sequencing of *cox1* [11]. Some researchers have suggested that human taeniasis is now close to absent in Korea, but cases of infection may be hidden, especially in areas where taeniasis was previously ubiquitous. In our case, the patient was from Jeollanam-do, the province with the second highest prevalence of taeniasis after Jeju-do (Island) according to the 1st Nationwide Survey [2]. Co-infection with other parasites can frequently occur in areas with high parasite prevalence, even though the helminthes do not share the same intermediate host [16]. Moreover, people who enjoy eating both raw beef and freshwater fish, as with our patient, have a higher chance of co-infection. The habit of eating raw fish or beef is deeply rooted in traditional customs among residents of rural areas of Korea [4]. In general, educating the public about not eating raw beef and freshwater fish is important for reducing the incidence of food-borne parasitic infections in Korea.

Initially, the patient took albendazole obtained from a pharmacy instead of visiting a hospital, although the medication was not effective. This may be due in part to the National Deworming Campaign in Korea over the past years that focused on soil-transmitted helminthiasis and highlighted repeated administration of albendazole to control parasitic infections. An initial 600-mg tablet of praziquantel (10 mg/kg) may be sufficient for treating taeniasis in this case. The patient also received additional praziquantel at a dosage of 75 mg/kg/day for 3 days to treat clonorchiasis. Although we could not find scolex of *Taenia* species in the submitted specimens, the treatment may have been effective in controlling both taeniasis and clonorchiasis.

In this study, we described an unusual case of co-infection of *T. saginata* in a patient with subclinical clonorchiasis in Korea. Our results indicate that diagnosis through molecular methods may be helpful in cases with ambiguous morphological characters such as immature proglottids. Overall, a combination of diagnostic tools should be used for the accurate diagnosis of subclinical parasitic infections.

Additional file

Additional file 1: Polymorphic sites of *cox1* and *ef1a* sequences of the submitted proglottids compared with those of human *Taenia* tapeworms. Numbers indicate the positions from 5' end of the *cox1* or *ef1a* gene, and dots indicate sequence matches with *T. saginata*. *Three sites (nucleotide 294, 336, 405) were distinguishable substitutions in all of three human *Taenia* tapeworms. (XLSX 16 kb)

Abbreviations

cox1: Cytochrome c oxidase subunit I; *ef1a*: Elongation factor-1 alpha; ELISA: Enzyme-linked immunosorbent assay; PCR: Polymerase chain reaction

Acknowledgements

Not applicable.

Funding

No funding was required for the writing of this case report.

Availability of data and materials

Not applicable (no datasets were analyzed or generated during this study).

Authors' contributions

KHP participated in the clinical care of the patient. JSJ performed experiments. KIN, SHK and JHS1 advised about and supervised the experiments and interpretations. JHS2 and EJW wrote the manuscript. EJW was responsible for the concept of and critical contribution and revision to this manuscript. JHS1 corresponding to JHS and JHS2 corresponding to JHS. All authors critically reviewed the manuscript for publication. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The collection of fecal samples including tapeworm conducted in accordance with the guidelines and approval of the Institutional Review Board of Chonnam National University Hospital (IRB CNUH-2015-052) and all data analyzed were anonymized. No information was used that could lead to patient identification.

Consent for publication

Written consent to publish this case report was obtained from the patient (Consent No. 2018-P02).

Competing interest

The authors declare that they have no competing interests.

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Received: 2 September 2018 Accepted: 29 January 2019

Published online: 06 February 2019

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