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▣ CASE REPORT

Diphyllobothrium nihonkaiense Infections in a Family

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Abstract: *Diphyllobothrium latum* and *Diphyllobothrium nihonkaiense* are morphologically similar to each other, and only genetic method can differentiate clearly between the 2 species. A strobila of diphyllobothriid tapeworm discharged from a 7-year-old boy was analyzed to identify the species by mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene sequencing. He and his family (total 4 persons) ate slices of 3 kinds of raw fish 16 days before visiting our outpatient clinic. All family members complained of abdominal pain and watery diarrhea. They all expelled tapeworm strobilae in their stools. They were treated with a single oral dose of praziquantel and then complained of no more symptoms. The *cox1* gene sequencing of the strobila from the boy revealed 99.9% (687/688 bp) similarity with *D. nihonkaiense* and only 93.2% (641/688 bp) similarity with *D. latum*. Thus, we assigned this tapeworm as *D. nihonkaiense*. This is the first report of *D. nihonkaiense* infection in a family in Korea, and this report includes the 8th pediatric case in Korea. The current report is meaningful because *D. nihonkaiense* infection within a family is rare.

Key words: *Diphyllobothrium nihonkaiense*, *Diphyllobothrium latum*, raw fish, *cox1* gene, praziquantel, tapeworm

INTRODUCTION

Diphyllobothriasis is a fishborne tapeworm infection caused by the genus *Diphyllobothrium*. These tapeworms are creamy white in color, and can grow as long as 2-15 m in the human small intestine [1,2]. Generally, the incubation period of *Diphyllobothrium* plerocercoids until they develop into an adult tapeworm is 2-6 weeks [3]. It can cause gastrointestinal symptoms such as abdominal pain and diarrhea, but typically does not cause severe problems. Humans can be infected with diphyllobothriid species through consumption of raw or poorly cooked fish containing larval plerocercoids.

To date, at least 13 of about 50 species of genus *Diphyllobothrium* have been reported as human pathogens, and among them, *D. latum* had been reported to be the main pathogen in Korea [1,4]. In 1919, human *D. latum* infection was first reported in Korea based on recovery of eggs in the stool passages of 2 residents in Jinju by Kojima and Ko [5]. In 1971, *D. latum* infection was first documented on the basis of morphological identification of the expelled proglottids [6]. The first Korean child case of *D. latum* infection was reported in 1980 by Jeong et al. [7]. Since then, 6 pediatric cases of *D. latum* infection have reported on the basis of morphological identification of the expelled proglottids [4]. However, in 2009, by Jeon et al. [8], all the 62 cases previously diagnosed as *D. latum* were verified to be actually *D. nihonkaiense* infection based on DNA analysis [8].

D. latum and *D. nihonkaiense* are morphologically indistinguishable. Therefore, alternative genetic sequencing has been used as the most discriminative method for identifying each species. Recently, a cheap and rapid molecular test using a multiplex PCR with the *cox1* gene was developed for verifying the most common diphyllobothriid species infecting humans [9]. Although the treatment regimen for these 2 species is identical, the correct identification is necessary to acquire the epidemiologic information including intermediate hosts of this tapeworm so as to prevent further infections. We encountered a family (4 people) who experienced abdominal discomfort and diarrhea and discharged tapeoworm strobilae after eating raw fish in Korea. We could collect some segments discharged from a 7-year-old boy and genetically analyzed by *cox1* gene sequencing to determine the species.

CASE RECORD

A 7-year-old boy visited our outpatient clinic with a strobila of a tapeworm, about 1.2 m long. The patient was a resident

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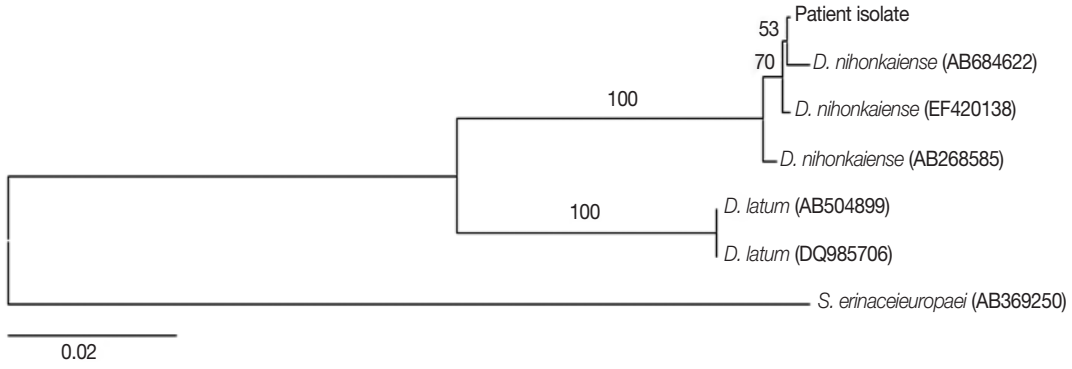

Fig. 1. Neighbor-joining tree of human *Diphyllobothrium* tapeworms based on nucleotide sequences of the *cox1* gene. Numbers above the branches detect the bootstrap values (1,000 replicates). The scale bar represents the estimated number of nucleotide substitutions per nucleotide site. The phylogenetic tree reveals that the *cox1* gene from our case was closer to *D. nihonkaiense* than *D. latum*.

of an urban area (Goyang, Korea) and was a first-grade student at an elementary school. His height was 123 cm (50-75th percentile) and weight was 24 kg (50-75th percentile). He had no previous medical history of note. He and his family members (4 persons), including his parents, had been on a trip to a coastal area (Mukho Harbor, Donghae, Korea) 16 days before they visited our hospital. There, they ate slices of 3 unknown kinds of raw fish and it was the first time when the child ate raw fish. Two weeks after the trip, 4 people who had been on the trip, including the boy, complained of lower abdominal pain and watery diarrhea. At day 16 after eating the fish, his mother found a noodle-like worm in his stool, and tried to pull out the tapeworm, but it snapped. His mother and the patient visited our outpatient clinic with some segment of the tapeworm. He had no other symptoms abide by the gastrointestinal problems. His bowel sounds were hyperactive, but other physical examinations were unremarkable, with no signs of anemia or neurological manifestations. We found no abnormal laboratory test results, including vitamin B_{12} level which was 1,033 *p*g/ml (normal range: 200-950 *p*g/ml). In stool examination, no parasite eggs or larvae were found. We treated the patient with a single oral dose of praziquantel. On the next day, he expelled the rest of the tapeworm, which was about 30 cm long and all of his symptoms disappeared. The other 3 members, i.e., his parents and a relative, also expelled strobilae in their stools (we could not collect the tapeworm specimens). They also took praziquantel and recovered from their illnesses.

The tapeworm specimen was fixed in 10% formalin, and sent to the Department of Parasitology and Tropical Medicine, Seoul National University College of Medicine, Seoul, Korea. Formalin was substituted with 1× PBS to remove the formalin for 24 hr. Before the molecular identification, the sample was identified as *Diphyllobothrium* sp., according to the microscopic examinations (×400 magnification) of eggs extracted from the uteri. Genomic DNA was then extracted from a single proglottid following a spin-column protocol using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The partial *cox1* gene was amplified by PCR using the forward primer (D1/nco1f1 5'-TAG CTG CTG CTA TAC AAT GTT GTT ATT-3') and the reverse primer (D1/nco1r1 5'-ACG ACG TGG TAA ACG GCA CAC ACC AAA-3'). PCR was carried out with 1 cycle of 94˚C for 5 min, 35 cycles of 94˚C for 1 min, 46˚C for 1 min, and 72˚C for 1 min, plus a final cycle at 72˚C for 5 min. DNA sequencing and construction of a neighbor-joining tree using our samples were performed according to the protocol described by Jeon et al. [8]. The reference data we used for a phylogenetic study were *D. nihonkaiense* (GenBank no. AB684622, EF420138, AB268585), *D. latum* (AB504899, DQ985706), and *S. erinaceieuropaei* (AB369250). The *cox1* sequences (688 bp) of the tapeworm showed 99.9% (687/688 bp) similarity with the reference sequence of *D. nihonkaiense* (EF420138), whereas the similarity with *D. latum* (DQ985706) was 93.2% (641/688 bp). Thus, the pathogen was identified as *D. nihonkaiense* (Fig. 1).

DISCUSSION

In the Far East Asian countries, the main pathogen in almost all human diphyllobothriasis had been considered to be *D. latum*. However, in 1986, Yamane et al. [10] in Japan character-

ized *D. nihonkaiense* for the first time, describing morphological differences in eggs, adult worms, and plerocercoids of so-called *D. latum* between Japan and Finland [10]. *D. nihonkaiense* in Korea was first mentioned in 1990 on the basis of the morphological findings by Rim et al. [11].

In 2009, genetic analysis of *Diphyllobothrium* tapeworms collected from patients with diphyllobothriasis that had been reported as *D. latum* infection between 1982 and 2007 was conducted in Korea, and all of the 62 specimens analyzed were confirmed to be *D. nihonkaiense* [8]. Thereafter, additional 10 cases of diphyllobothriasis in Korea were confirmed to be *D. nihonkaiense* by *cox1* gene sequencing [12-15]. The current case (boy) is the 73rd case of *D. nihonkaiense* infection in Korea confirmed by genetic analysis and the 8th pediatric case of *D. nihonkaiense* infection in Korea. The current case is valuable in that there are rare pediatric cases of *D.nihonkaiense* infection reported in Korea, and *D. nihonkaiense* infection as a family unit rarely occurs. This is the first report on *D. nihonkainense* infection in a family unit.

The intermediate hosts of *D. nihonkaiense* are now known to be some kinds of Pacific salmon, which include *Oncorhynchus keta*, *O. masou*, *O. gorbuscha*, and *O.nerka* in Japan [3]. These kinds of salmon are also considered to be the main intermediate hosts of *D. nihonkaiense* in Korea because these fish migrate from Okhotsk, Bering, and the Pacific Ocean and return back to the East Sea, which Korea and Japan are sharing [8]. In Japan, a study investigating plerocercoids in wild Pacific salmon using PCR-based DNA sequence analysis targeting *cox1* and mitochondrial NADH dehydrogenase subunit 3 (*nad3*) genes was conducted; all plerocercoids obtained in salmon were identified as *D. nihonkaiense* [16]. However, there has been no reported trial analyzing genes of diphyllobothriid plerocercoids collected from intermediate host fish in Korea. Thus, there is a need for gene analysis of plerocercoids acquired from the fish that were ingested by patients to determine the source of infection in Korea.

The incidence of infection with *D. nihonkaiense* is increasing not only in the Far East Asian countries like Korea and Japan, but also in countries far from the East Asia, such as North America, New Zealand, and European countries due to ingestion of imported Pacific salmon [17-20]. For example, there were 9 cases of molecularly identified human diphyllobothriasis reported in Europe from 2005-2011. Among these, 6 cases were confirmed as *D. nihonkaiense* and most become infected with the pathogen by eating imported Pacific salmon [17,20-23].

Infection by diphyllobothriid species can be prevented by freezing and storing fish at -20℃ for 7 days or -35℃ for 15 hr before consumption [24]. Alternatively, larval plerocercoids can be destroyed by cooking the fish at 54-56℃ for 5 min [3]. However, people generally do not know about the risk of diphyllobothriasis when they eat raw or undercooked fish. Moreover, people tend to prefer never-frozen raw fish, like sushi or sashimi, to that prepared from frozen fish. Recently, consumption of raw fish or incompletely cooked fish, such as smoked salmon, has become popular globally. As a result, the chances of eating raw or undercooked fish have increased. This increased raw or insufficiently cooked fish consumption has resulted in increased risks for fishborne parasite infections such as diphyllobothriasis.

Because the treatment regimen of diphyllobothriasis is praziquantel, diagnosis through *cox1* gene sequencing can be considered too academic. Even though, accurate diagnosis for these species is necessary, regarding the epidemiology of these parasites and arrangement of protective methods. It led to a perception that dominant species is not *D. latum* but *D. nihonkaiense* in Korea. It would provide information whether the cause of diphyllobothriasis is imported cases or domestic species during its outbreak and lead to build protective methods for public health promotion.

CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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