

# Two Human Cases of *Diphyllobothrium nihonkaiense* Infection in Korea

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**Abstract:** *Diphyllobothrium latum* and *Diphyllobothrium nihonkaiense* are the 2 reported main causes of human diphyllorbothriasis in the Republic of Korea. However, the differentiation of these 2 species based on morphologic features alone is difficult. The authors used nucleotide sequencing of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*) gene to diagnose *Diphyllobothrium* spp. Two patients visited the emergency room at Kyungpook National University Hospital on 3 April and 12 April 2013, respectively, with fragments of parasites found while defecating. The parasites were identified as *Diphyllobothrium* spp. based on morphologic characteristics, and subsequent *cox1* gene sequencing showed 99.9% similarity (1,478/1,480 bp) with *D. nihonkaiense*. Our findings support the hypothesis that *D. nihonkaiense* is a dominant species in Korea.

**Key words:** *Diphyllobothrium nihonkaiense*, human case, praziquantel, *cox1* gene

## INTRODUCTION

Diphyllobothriasis is caused by the adult tapeworm (*Diphyllobothrium* spp.), and is contracted by consuming raw or undercooked fish in Northwest Europe and East Asia. Many patients with diphyllorbothriasis are asymptomatic, while other patients develop gastro-intestinal discomfort, diarrhea, or abdominal pain [1]. Of the 18 *Diphyllobothrium* spp. known to cause human diphyllorbothriasis, *Diphyllobothrium latum* and *Diphyllobothrium nihonkaiense* are usually reported in Republic of Korea [2,3]. These 2 *Diphyllobothrium* species have similar morphologic features, and thus, clinicians are not able to differentiate the 2 parasites by naked eyes. As an alternative, genetic studies can be used to identify the species [4,5]. Recently, several nucleotide sequencing analysis studies revealed that previous identifications of *D. latum* based on morphologic characteristics were incorrect and that the parasite was in fact *D.*

*nihonkaiense* [5-7]. Although the chemotherapy required for both species is identical, the correct identification of *Diphyllobothrium* species is required from the perspectives of epidemiology and public health promotion, particularly by providing accurate information on intermediate hosts [8]. In this regard, we used nucleotide sequencing analysis of the mitochondrial cytochrome c oxidase subunit I (*cox1*) gene to identify *Diphyllobothrium* species in 2 Korean male patients.

## CASE RECORD

On April 3rd and 12th of 2013, 2 patients visited Kyungpook National University Hospital with a whitish yellow tapeworm segment. The patient that visited on April 3 was a healthy 50-year-old man (patient A), who visited the emergency room after finding a part of a tapeworm while defecating. He had not experienced any abdominal discomfort or pain, and had not visited any foreign country recently, and reported infrequent consumption of raw salmon and trout in side dishes. His blood test results were normal. The other patient was a healthy 20-year-old man (patient B). He also felt a foreign body while defecating, and visited the same emergency room. He stated that he seldom consumed raw fish. No abnormal clinical signs, such as, abdominal discomfort or pain,

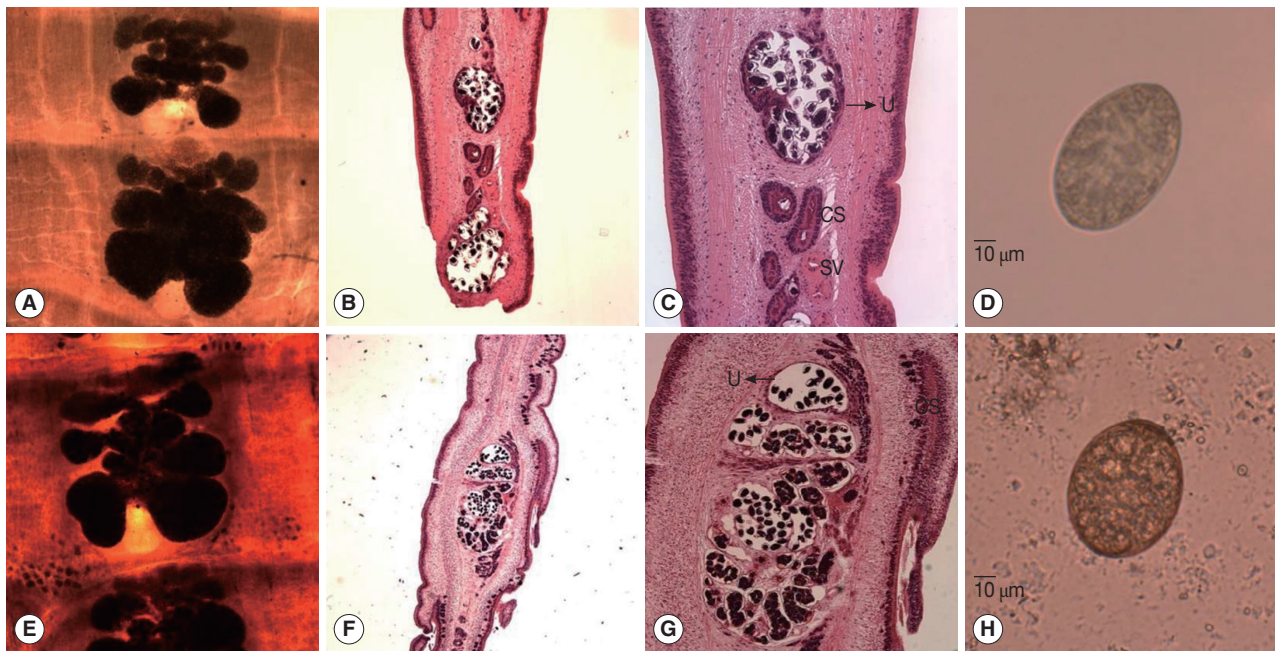
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**Fig. 1.** Gravid proglottids and eggs of *Diphyllbothrium nihonkaiense* from the 2 patients. (A, E) Whole-mounted specimens of a proglottid showing the uterus and cirrus sac ( $\times 40$  magnification). (B, C, F, G) Longitudinal sections of a mature proglottid showing the uterus (U), cirrus sac (CS), and seminal vesicles (SV) (H&E stain; B and F,  $\times 40$ ; C and G,  $\times 100$ ). (D, H) Eggs discharged from the gravid proglottid of *D. nihonkaiense* ( $\times 400$ ). (A-D, *D. nihonkaiense* from patient A; E-H, *D. nihonkaiense* from patient B).

were observed, and he has taken a vermicide during September 2012. His blood test results were also normal.

The specimens obtained from the 2 patients were forwarded to our laboratory with a request to identify the parasite species concerned. The parasite samples from the 2 patients consisted of a number of proglottids; gravid proglottids showed a rosette-shaped uterus as shown in Fig. 1A and E. Testes were follicular and ovaries were kidney-shaped in the posterior region. In longitudinal sections of gravid proglottids, uterine and genital pores were separated at midline, and the uterus was filled with eggs (B, C, F, and G in Fig. 1; H&E stain), which were oval and enclosed by a shell (D and H in Fig. 1). Based on these morphologic characteristics, both parasites were identified as *Diphyllbothrium* spp.

For genetic analysis, total genomic DNA was extracted from each specimen, and oligonucleotide primers were designed to amplify the 1,480-bp product of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene (forward primer, 5'-AT-GACTAATCTTAAAGTTTT-3'; reverse primer, 5'-TAAAGCCAA-CATACTATAATC-3'). PCR was performed as follows; initial denaturation at 96°C for 5 min, followed by 30 amplification cycles (96°C for 1 min, 58°C for 1 min, and 72°C for 1 min), and a final extension step at 72°C for 10 min. The purified PCR-

amplified fragments obtained were cloned into pGEM<sup>®</sup>-T Easy Vector (Promega, Madison, Wisconsin, USA), and the inserted plasmids (*cox1* gene-pGEM<sup>®</sup>-T Easy Vector) were sequenced using a Big-Dye Terminator sequencing kit (Applied Biosystems, Foster City, California, USA) on an automated DNA sequencer (Applied Biosystems). Sequences were assembled and aligned using CLC Sequence Viewer 6.8.1. (CLC Bio, Aarhus, Denmark), and similarities in GenBank were examined using BLASTN. The *cox1* sequences (1,480 bp) of the 2 parasites showed similarities of 99.9% (1,478/1,480 bp), 95.1% (1,407/1,480 bp), 93.1% (1,378/1,480 bp), and 92.6% (1,371/1,480 bp) with *D. nihonkaiense* (GenBank no. AB781787), *D. ursi* (GenBank no. AB605762), *D. dendriticum* (GenBank no. AM412738), and *D. latum* (GenBank no. AM712906), respectively.

The patients were treated with a single oral dose of praziquantel (15 mg/kg) and no evidence of recurrence has been noticed.

## DISCUSSION

*D. nihonkaiense* was first identified by Yamane et al. [9] in 1986 by comparing its morphologic characteristics, such as

egg size, the pit shape of eggshells, and the angle between the long axis of the cirrus sac and seminal vesicle, with those of other *Diphyllobothrium* species [9]. *D. nihonkaiense* and *D. latum* had distinct morphologic characteristics. Specifically, the average size of *D. nihonkaiense* eggs was  $55.5 (\pm 1.0) \times 40.5 (\pm 1.5) \mu\text{m}$ , which is smaller than the average size of *D. latum* eggs (greater than  $60.0 \times 45.0 \mu\text{m}$ ). The eggshells of *D. nihonkaiense* had shallower pits distributed on the smooth surface than the eggshells of *D. latum* did. The cirrus sac is situated obliquely in *D. nihonkaiense*, and the angle between the long axis of the cirrus sac and seminal vesicle was sharper in *D. nihonkaiense* than in *D. latum*. In addition to these distinct morphologies, *D. nihonkaiense* and *D. latum* have different intermediate host specificities. The intermediate hosts of *D. nihonkaiense* are salmon, such as cherry, pink, chum, and sockeye salmon (*Onchorhynchus masou*, *O. gorbusha*, *O. keta*, and *O. nerka*, respectively), which are found in the northern Pacific, whereas the intermediate hosts of *D. latum* are perch, pike, burbot, and walleye [8]. Based on his history, patient A likely contracted parasites from these fish. On the other hand, patient B had no history of raw salmon consumption, and similarly, several other cases of *D. nihonkaiense* infection not associated with salmon consumption have been reported [1]. Accordingly, accumulating evidence indicates *D. nihonkaiense* has another intermediate host and that further research on the intermediate hosts of *D. nihonkaiense* in Korea is needed.

Previously, most cases of diphyllobothriasis in Korea were thought to be caused by *D. latum*. However, this paradigm shifted when Eom et al. [10] reported occurrence of *D. nihonkaiense* in Korea (35th annual meeting of The Korean Society for Parasitology, Kwangju, ROK). Owing to the publication of the complete mitochondrial genome of *D. nihonkaiense* [10], *D. nihonkaiense* can now be accurately identified by DNA sequencing of the mitochondrial *cox1* gene. In 2009, Jeon et al. [5] reported that 62 cases diagnosed as *D. latum* infection were actually caused by *D. nihonkaiense*, which were identified by genetic analysis. Moreover, another case of diphyllobothriasis was recently confirmed as *D. nihonkaiense* infection by *cox1* gene analysis [7]. These findings suggest that *D. nihonkaiense* has been misdiagnosed as *D. latum* and that *D. latum* might not exist in Korea. The present study, in which 2 Korean male patients were diagnosed as *D. nihonkaiense* by *cox1* gene se-

quencing, supports this suggestion. From the clinical point of view, this specific diagnosis might seem academic because diphyllobothriasis can be easily cured with praziquantel. Nevertheless, specific diagnosis at the species level is important in the contexts of epidemiology and public health, and would provide further insight into distribution of different taxa and improve our understanding of imported cases and outbreaks of diphyllobothriasis.

## CONFLICT OF INTEREST

We have no conflict of interest related to this study.

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