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

*Korean J Parasitol* Vol. 51, No. 4: 471-474, August 2013
http://dx.doi.org/10.3347/kjp.2013.51.4.471

▣ CASE REPORT

# A Case of *Diphyllobothrium nihonkaiense* Infection as Confirmed by Mitochondrial *COX1* Gene Sequence Analysis

**Sang Hyun Park, Keesseon S. Eom, Min Sun Park, Oh Kyoung Kwon, Hyo Sun Kim and Jai Hoon Yoon***

*Department of Internal Medicine, Chuncheon Sacred Heart Hospital, Chuncheon 200-704, Korea; Department of Parasitology, School of Medicine, Chungbuk National University, Cheongju 361-763, Korea*

**Abstract:** *Diphyllobothrium nihonkaiense* has been reported in Korea as *Diphyllobothrium latum* because of their close morphologic resemblance. We have identified a human case of *D. nihonkaiense* infection using the mitochondrial cytochrome c oxidase subunit I (*cox1*) gene sequence analysis. On 18 February 2012, a patient who had consumed raw fish a month earlier visited our outpatient clinic with a long tapeworm parasite excreted in the feces. The body of the segmented worm was 2 m long and divided into the scolex (head) and proglottids. It was morphologically close to *D. nihonkaiense* and *D. latum*. The *cox1* gene analysis showed 99.4% (340/342 bp) homology with *D. nihonkaiense* but only 91.8% (314/342 bp) homology with *D. latum*. The present study suggested that the Diphyllobothrium spp. infection in Korea should be analyzed with specific DNA sequence for an accurate species identification.

Key words: *Diphyllobothrium nihonkaiense*, *Diphyllobothrium latum*, DNA, sequence, *cox1*, diagnosis

## INTRODUCTION

Most cases of diphyllobothriasis in Korea were reported to have been caused by *Diphyllobothrium latum* based on morphologic features of the specimens [1], which are closely similar to *Diphyllobothrium nihonkaiense*. However, genetic analysis can differentiate the 2 species [2,3]. In the Republic of Korea (=Korea), through a DNA analysis, 62 diphyllobothriasis cases previously reported as *D. latum* infection were verified to have been caused by *D. nihonkaiense* [3]. In addition, the 4 *D. latum* cases reported in 2012 [4], which were been verified based on DNA sequencing analysis, may also have been *D. nihonkaiense* cases.

It is difficult for clinicians to diagnose correctly the worms with naked eyes. Although the chemotherapy regimen to treat a variety of *Diphyllobothrium* spp. is the same, it is necessary to identify the species correctly to know the epidemiological characteristics and to prevent further infections by providing information on intermediate hosts. In the present study, a molecular diagnosis of *Diphyllobothrium* spp. was done using the mitochondrial cytochrome c oxidase subunit I (*cox1*) gene sequence of the tapeworm discharged from a Korean female patient.

## CASE RECORD

A female patient from Chuncheon, Gangwon-do, Korea who ate halibut, Matsubara's rock fish, trout, squid, sea squirts from Sokcho, Gangwon-do, and cherry salmon from Hwacheon, Gangwon-do, 12 months before visiting our clinic on 18 February 2012. She experienced abdominal discomfort for a month before presenting to our clinic [5]. Two days prior to the admission, she experienced abdominal discomfort again, and a part of the tapeworm was observed during the bowel movement. Her husband drew out of the 2 m long worm successfully, which was delivered to our clinic (Fig. 1).

The results of blood tests were within normal range, and no parasite eggs were observed in the feces during the outpatient follow-up. An abdominal examination did not reveal any atypical presentations. Also no atypical presentation was observed on gastroduodenal endoscopy and colonoscopy performed 2 days after the first visit. The worm specimen was fixed in 10% formalin and sent to the Department of Parasi-

• Received 8 May 2013, revised 6 July 2013, accepted 6 July 2013.
* Corresponding author (yoonjh@hallym.or.kr)

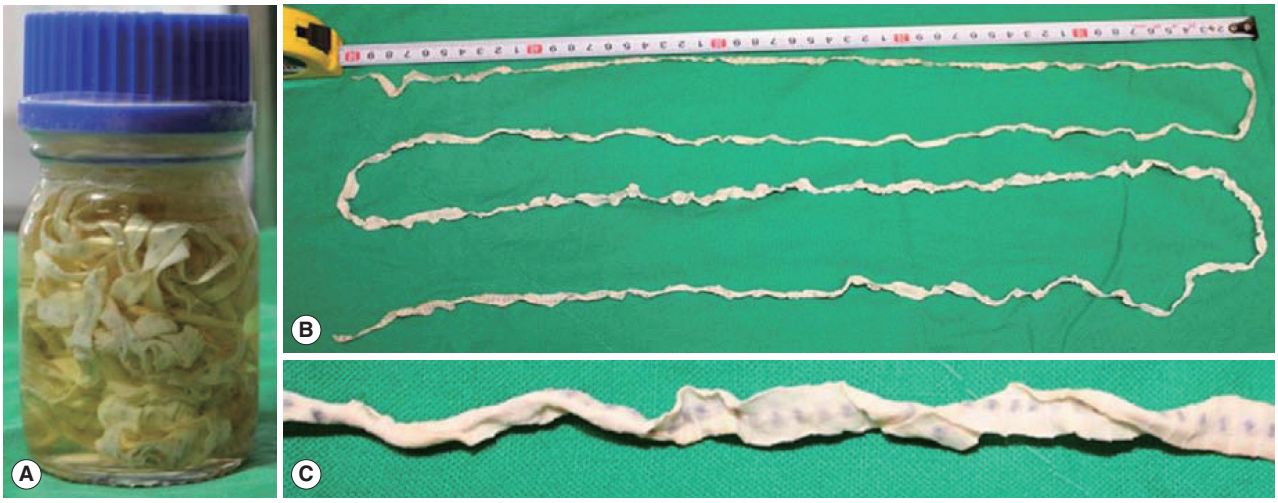


**Fig. 1.** Gross findings (A, B, C) of a spontaneously discharged strobila of D. nihonkaiense from this case. The whole length of this worm was about 2 m. (A, B) Whole discharged worm. (C) A close-up view of the segment.

tology, College of Medicine, Hallym University, Chuncheon, Korea. The proglottids of the worm showed a rosette-shaped uterus. The scolex was spoon-shaped with 2 sucking grooves. Therefore, it was identified as *Diphyllobothrium* spp. on the basis of the morphological finding. The specimen was dispatched to the Department of Parasitology, College of Medicine, Chungbuk National University to identify the species with molecular analysis. The purified PCR-amplified fragments of the *cox1* gene were directly sequenced. The primer walking method was employed to obtain direct sequences from each of the amplified fragments. Cyclic sequencing from both ends of the fragments was performed using a Big-Dye Terminator sequencing kit (Applied Biosystems, Foster City, California, USA) and the reaction products were electrophoresed on an automated DNA sequencer (model 3739KL, Applied Biosystems). The sequences were assembled and aligned using Geneous 6.1.5 (Biomatter, Auckland, New Zealand). The sequence regions were identified using BLAST searches and comparisons with sequences of *D. nihonkaiense* and *D. latum*, which had been deposited in the GenBank database. The PCR amplification and direct sequencing for the *cox1* target fragment (342 bp in length corresponding to the positions 781-1,122 bp of the *cox1* gene) were performed using the total genomic DNA extracted from this specimen. The *cox1* sequences (342 bp) of the worm showed 99.4% (340/342 bp) similarity to the reference sequence of the Japanese origin *D. nihonkaiense* (GenBank No. AB015755) and 91.8% (314/342 bp) similarity with the reference sequence of the Russian origin *D. latum* (GenBank No. DQ985706).

Praziquantel 10 mg/kg in a single dose was administered, and the patient developed headache after taking the drug but was relived soon. The patient's husband was also given the medication because he had eaten the same sea fish with the patient.

## DISCUSSION

*D. nihonkaiense* had been frequently misinterpreted as *D. latum* in Korea. In our case, the *cox1* gene sequencing analysis revealed it as *D. nihonkaiense*. The diphyllobothriasis reported after 2009 in Korea should be reconfirmed by DNA analysis, and if the same results as ours are obtained, then the *D. latum* cases should be revised as those caused by *D. nihonkaiense* [3]. The current case report is also expected to be a resource to aid in *D. nihonkaiense* epidemiology. Therefore, we suggest a possibility that *D. latum* might not exist in Korea. Although the known intermediate hosts of *D. nihonkaiense* are *Oncorhynchus keta* and *O. masou*, which thrive in the Pacific Ocean [6], our patient had no history of consuming these kinds of fish. Thus, our case report could prompt further research to discover other intermediate hosts.

## REFERENCES

1. Lee EB, Song JH, Park NS, Kang BK, Lee HS, Han YJ, Kim HJ, Shin EH, Chai JY. A case of *Diphyllobothrium latum* infection with a brief review of diphyllobothriasis in the Republic of Korea. Korean J Parasitol 2007; 45: 219-223.
2. Wicht B, de Marval F, Peduzzi R. *Diphyllobothrium nihonkaiense* (Yamane et al., 1986) in Switzerland: first molecular evidence

and case reports. Parasitol Int 2007; 56: 195-199.
3. Jeon HK, Kim KH, Huh S, Chai JY, Min DY, Rim HJ, Eom KS. Morphologic and genetic identification of *Diphyllobothrium nihonkaiense* in Korea. Korean J Parasitol 2009; 47: 369-375.
4. Choi HJ, Lee J, Yang HJ. Four human cases of *Diphyllobothrium latum* infection. Korean J Parasitol 2012; 50: 143-146.
5. Lee SH, Chai JY, Hong ST, Sohn WM, Huh S, Cheong EH, Kang SB. Seven cases of *Diphyllobothrium latum* infection. Korean J Parasitol 1989; 27: 213-216 (in Korean).
6. Scholz T, Garcia HH, Kuchta R, Wicht B. Update on the human broad tapeworm (genus *Diphyllobothrium*), including clinical relevance. Clin Microbiol Rev 2009; 22: 146-160.