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CASE REPORT

Molecular Identification of *Diphyllobothrium latum* from a Pediatric Case in Taiwan

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Abstract: Human diphyllobothriasis is a parasitic disease caused by ingestion of larvae (plerocercoids) in raw or undercooked fish and commonly found in temperate areas. Rare cases were reported in tropical or subtropical areas especially in children. The first documented case of pediatric diphyllobothriasis in Taiwan had been reported 11 years ago. Here, we report another 8-year-old girl case who presented with a live noodle-like worm hanging down from her anus, with no other detectable symptoms. We pulled the worm out and found the strobila being 260 cm in length. Examination of gravid proglottids showed that they were wider than their lengths, containing an ovoid cirrus sac in the anterior side and the rosette-shaped uterus. Eggs extracted from the uterus were ovoid and operculated. *Diphyllobothrium latum* was confirmed by molecular analysis of the mitochondrial DNA cytochrome *c* oxidase subunit 1 (*cox1*) gene. The girl was treated with a single oral dose of praziquantel, and no eggs or proglottids were observed from her stool in the subsequent 3 months. The reemergence of human diphyllobothriasis in non-endemic countries is probably due to prevalent habit of eating imported raw fish from endemic areas. This pediatric case raised our concern that human diphyllobothriasis is likely underestimated because of unremarkable symptoms.

Key words: *Diphyllobothrium latum*, diphyllobothriasis, pediatric case, *cox1*

INTRODUCTION

Tapeworms of the genus *Diphyllobothrium*, known as "fish tapeworms" or "broad tapeworms", are worldwide in distribution and commonly found in temperate freshwater ecosystems. Infections of *Diphyllobothrium* spp. (diphyllobothriasis) are mostly reported in Western Europe, North America, South America, and the Far East, including Korea, Japan, and Russia [1,2]. Rare cases were reported in tropical or subtropical countries where these cases may be related to imported fish from endemic areas [2]. The most prevalent human diphyllobothriasis are caused by *D. latum*, *D. dendriticum*, *D. nihonkaiense*, and *D. pacificum*. The life cycles of these species are complex, comprising of 2 intermediate hosts (a copepod and a fish) and a definitive host (humans or other piscivorous mammals).

Human diphyllobothriasis takes place through the ingestion of larvae (plerocercoids) in raw or undercooked fish. In recent years, eating raw fish becomes popular in Taiwan, resulting in the demand for imported fish. This may also increase the risk of *Diphyllobothrium* infection due to eating imported fish. Since Taiwan is not in an endemic area, rare cases were previously reported, especially for pediatric cases. The first published pediatric case of *D. latum* infection in Taiwan had been reported 11 years ago [3]. Here, we present another rare case of *D. latum* infection in an 8-year-old girl, which was confrimed by molecular analysis of the mitochondrial DNA cytochrome *c* oxidase subunit 1 (*cox1*) gene.

CASE REPORT

An 8-year-old Taiwanese girl who was 127 cm tall and weighed 24 kg presented without discomfort, but a live noo-

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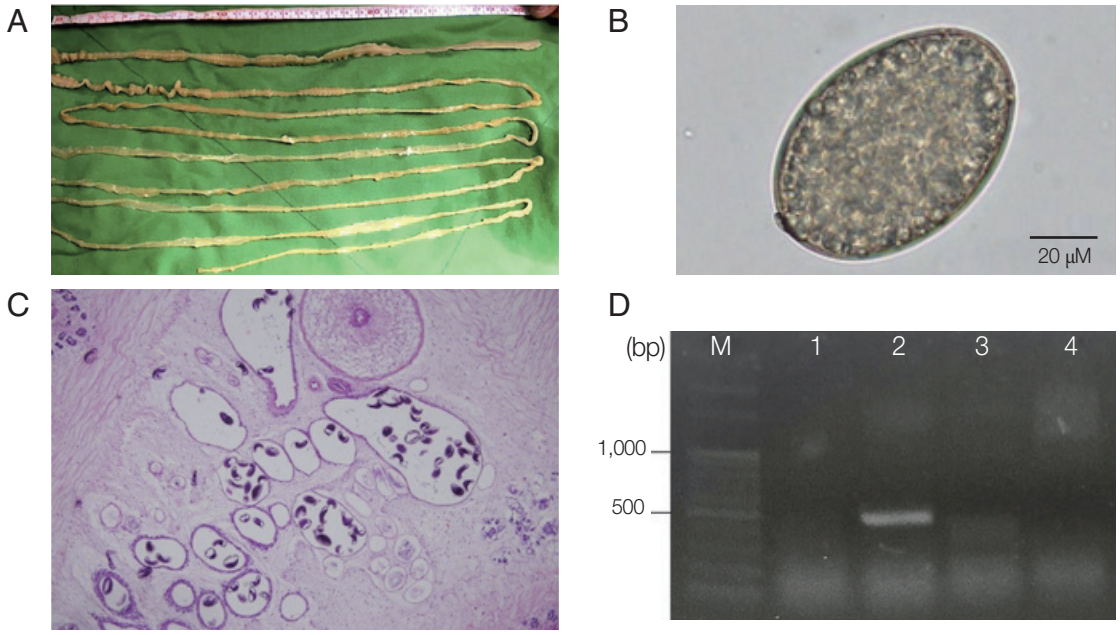

Fig. 1. (A) A strobila of *Diphyllobothrium latum* expelled by the patient (about 260 cm). (B) An egg extracted from the uterus of a gravid proglottid. The egg was ovoid and operculated. (C) Tissue section stained with hematoxylin and eosin showing the rosette-shaped uterus of a gravid proglottid. An ovoid cirrus sac is located in the anterior side of the gravid proglottid. Several eggs are observed in the loops of the uterus. (D) Molecular identification of *D. latum* by PCR. Lanes (1-4) represent the PCR products amplified by using different primers for the most common *Diphyllobothrium* species. Lane 1, *D. pacificum*; lane 2, *D. latum*; lane 3, *D. dendriticum*; lane 4, *D. nihonkaiense*. M: marker.

dle-like worm hanging down from her anus which was discovered after defecation. She was a healthy elementary school student, lived in a well-off family and loved to eat raw fish without any history of travel to a foreign country. Initially we pulled the worm out and found a strobila of about 260 cm in length (Fig. 1A). The worm was still alive and movable while we put it on the towel.

Physical examinations showed no specific signs, such as pale conjunctiva or abdominal tenderness. The laboratory data revealed the hemoglobin count to be 12.3 g/dl, the red blood cell count to be 4.51×10^6/μl, and the mean corpuscular volume (MCV) of 79.8 fl. Platelet count was 248×10^3/μl, and the white blood cell count was 5.07×10^3/μl (neutrophils 51.1%; lymphocytes 40.2%; eosinophils 3.2%).

Diphyllobothriasis was diagnosed by examination of the gravid proglottids and eggs. The segments are wider than their lengths, which contain an ovoid cirrus sac in the anterior side of each proglottid. The rosette-shaped gravid uterus has 5-6 loops on each side (Fig. 1B). Eggs extracted from the worm's uterus were found to be ovoid, yellow-brown, and operculated (Fig. 1C). The specific identification of *Diphyllobothrium* species is very difficult only by morphological observations. Hence, molecular analysis using PCR (Table. 1) is the only reliable approach to differentiate these parasites. Amplification of the mitochondrial DNA *cox1* gene was performed as previously described [4] (Fig. 1D). The expected product sizes were 437 bp for *D. latum*, 318 bp for *D. dendriticum*, 727 bp for *D. pacificum*, and 1,232 bp for *D. nihonkaiense*. DNA extracted from the tapeworm sample was successfully amplified for *D. latum*, yielding a specific product size close to the expected one, and no products were amplified for the other *Diphyllobothrium* species. The data confirmed that the species of *Diphyllobothrium* is *D. latum*.

Table 1. Primers used for PCR in this study

Specificity	Strand	Sequence (5'>3')
Common	Reverse	ATGATAAGGGAYAGGRGCYCA
Diphyllobothrium latum	Forward	GGGGTGTTACGGGTATTATACTC
D. dendriticum	Forward	GTGTTTTTCATTTGATGATGACCAGTC
D. pacificum	Forward	ACATGTGTGTAGTAACCTTGGC
D. nihonkaiense	Forward	CTTTGTTGTCTGGCCTTCCT

The girl was treated with a single oral dose of praziquantel (8.5 mg/kg/dose). After administration, no eggs or proglottids were observed from the stool in the subsequent 3 months. Ra-

diological examinations or gastrointestinal fiberoptic endoscopy were not performed due to her young age and no other remarkable symptoms.

DISCUSSION

There are several species of *Diphyllobothrium* infecting humans. Among these, *D. latum*, *D. dendriticum*, *D. pacificum*, and *D. nihonkaiense* have been shown to be the main species. *D. latum* was commonly reported all over the world, including Europe, North America (Alaska, Great Lakes), and Asia [2,5]. *D. pacificum* was found in the Pacific Coast of South America and Japan. *D. nihonkaiense* is prevalent in Japan and Korea, but rare in Europe or America. In Taiwan, only rare cases of diphyllobothriasis were reported in the past caused by *D. latum* and presumably related to imported salmon [3,6]. Recent taxonomic studies indicated that the majority of human diphyllobothriasis was caused by *D. nihonkaiense*. Many cases of *D. latum* infection reported in Korea were re-identified as *D. nihonkaiense* based on molecular analysis of the mitochondrial *cox1* gene [7]. Therefore, it was necessary to perform molecular analysis in order to identify the species of *Diphyllobothrium*. In our study, we successfully identified the broad tapeworm to the species level by PCR analysis of *cox1* gene. We used formalin- and ethanol-preserved samples for extraction of DNA and subsequent PCR analysis. However, DNA can be extracted only from ethanol-preserved samples but only with difficulty from formalin-preserved ones. This observation is consistent with the results of previous studies, indicating that formalin irreversibly affects the quality of DNA.

Since *D. latum* was identified, we sought to investigate the possible source of this parasitic infection. In previous studies, including rare cases reported in subtropical and tropical Asia [1,2,5], *D. latum* is worldwide in distribution but *D. nihonkaiense* seems to dominate in the northern Pacific region. The second intermediate hosts of *D. latum*, including freshwater, anadromous, and marine fish had been reported, and the pacific salmon was the major host which contributed to *D. nihonkaiense* infection [2,7]. More species of second intermediate hosts infected with *D. latum* possibly result in wider distribution, especially in non-endemic areas, due to imported fish. However, the reliable cause of *D. latum* infection in Taiwan instead of *D. nihonkaiense* or other *Diphyllobothrium* spp. infection was still unclear. The different environmental adaptability of *Diphyllobothrium* spp. is worthy of investigation. In our case, the girl had never ingested salmon. Thus, her diphyllobothriasis was presumably caused by ingestion of other species of imported fish, although it is hard to trace the specific source.

D. latum infections are mostly asymptomatic, but some patients may present with transient abdominal discomfort, abdominal pain, diarrhea, fatigue, and less commonly intestinal obstruction. Severe cases of *D. latum* infection may lead to megaloblastic anemia because vitamin B_{12} is absorbed by the worm. Lee et al. [8] showed 5 cases of *D. latum* infection among children in Korea, and 3 of them presented with abdominal pain, 1 with anemia, and 1 with passage of proglottids [8]. The first pediatric case in Taiwan, a 8-year-old boy, also presented with passage of proglottids [3]. In our case, the tapeworm was initially misidentified as a part of intestine and finally recognized as *D. latum*. Due to the unremarkable symptoms and chief complaints of our case, we propose that *D. latum* infection may be underestimated in Taiwan.

Praziquantel is the first choice of treatment for diphyllobothriasis. Oral administration of a single dose from 10 to 25 mg/kg has been recommended to treat *D. latum* infection. Almost all reported cases have been effectively cured with rare side effects. In our case, the proglottids were expelled before a single dose of praziquantel treatment (8.5 mg/kg/dose), and no eggs or proglottids were observed in the stool during follow-up. Intraduodenal gastrografin has been reported to be an effective treatment as an alternative therapy for diphyllobothriasis. However, this method is limited for choice because insertion of duodenal tube is painful, which is not suitable for children.

We examined the level of hemoglobin and the stool in the subsequent 3 months for follow-up. All of them showed negative results. We did not check the level of vitamin B_{12} because the levels of hemoglobin and MCV were normal, and no evidence of anemia was found. In previous reports, gastrointestinal fiberoptic endoscopy and abdominal magnetic resonance imaging were used for diagnosis and removal of the residual scolex [2,8]. However, these examinations are costly and difficult to be accepted for children.

Since eating raw fish imported from the endemic area becomes more popular, prevention of diphyllobothriasis should be taken seriously. Deep-frozen fish (at -10°C for 24-48 hr) or brine-treated fish (12% NaCl) is safe for consumption [6]. Additionally, plerocercoid larvae could be killed at a temperature of 55°C in 5 min [5]. Hence, it is not recommended to eat raw or smoked fish and consumption of well-cooked fish is the

better way to prevent the reemergence of diphyllobothriasis.

In conclusion, we should be aware of reemergence of human diphyllobothriasis in non-endemic countries due to prevalent habit of eating raw fish that may be imported from endemic areas. Additionally, it is noteworthy that human diphyllobothriasis is likely underestimated because of unremarkable symptoms.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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