Diphyllobothriasis Nihonkaiense: Possibly Acquired in Switzerland from Imported Pacific Salmon

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Abstract

A 5-year-old Japanese boy passed tapeworm strobila while he was living in Switzerland. During a short visit to Japan, he was successfully treated with a single dose of praziquantel. DNA sequences of ITS1, cox1 and nd3 genes from the tapeworm were compatible with those of *Diphyllobothrium nihonkaiense* rather than *Diphyllobothrium latum*, which is prevalent in Europe. The patient consumed imported salmon in Switzerland. This case highlights the globalization of *D. nihonkaiense*, which was once restricted to the Far East, and reflects the worldwide demand for seafood.

Key words: *Diphyllobothrium nihonkaiense*, tapeworm, praziquantel, salmon

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Introduction

Since 1889 the broad tapeworm infections in Japan had been considered to be caused by *D. latum* (1), the same species that was and still is prevalent in Europe. However, Yamane et al (1986) revised the identification of the Japanese broad tapeworm and established the new species *D. nihonkaiense* (2). Although the morphological features of *D. latum* and *D. nihonkaiense* are similar, their life cycles exhibit notable differences: while *D. latum* employs freshwater fish such as perch, char and pike as its second intermediate host, *D. nihonkaiense* employs wild Pacific salmon such as *Oncorhynchus masou* (masou salmon), *O. gorbuscha* (pink salmon) and *O. keta* (chum salmon), which migrate in the Northern Pacific Ocean including the Sea of Okhotsk and the Bering Sea (1, 3, 4). In addition, recent studies revealed distinct differences in the DNA sequences of *D. nihonkaiense* and *D. latum* (5-8) that have rendered species diagnosis more reliable.

In European countries, a number of cases of tapeworm infection caused by *D. latum* are reported annually (9). Interestingly, however, molecular genetic analyses showed that a few diphyllobothriasis patients in Europe were in fact infected with *D. nihonkaiense*, and these patients were reported to have consumed imported Pacific salmon (5, 6).

We recently encountered a patient who passed tapeworm strobila while he was living in Switzerland. We initially suspected that this case was caused by *D. latum* because of the place of infection, but DNA sequence analyses identified *D. nihonkaiense* as the causative tapeworm. It is possible that this patient contracted *D. nihonkaiense* by consuming imported Pacific salmon, which are now more widely available in EU countries than before (10).

Case Report

The patient is a Japanese 5-year-old boy with an unremarkable past medical history and family history. He was living in Switzerland with his family for several years, and had not traveled outside Europe during the previous 12 months. In late September 2006, he passed a tapeworm strobila at his home in Switzerland. He presented with neither abdominal pain nor diarrhea or vomiting and did not consult a physician. Two weeks later, he made a short visit to Japan with his family. Shortly after their arrival in Japan, his mother once again noticed the boy was passing a tapeworm and she tried to draw it out, but it was torn off spontaneous...
ously at a length of 50 cm. Two days later, he and his mother consulted our clinic.

His body temperature was 37.1°C, the pulse was 102, and the respiration rate was 30. The boy was normally developed and well-nourished. The findings of the physical examination were unremarkable. Hematologic values were normal except for a mild increase in eosinophil count: the hematocrit was 40.0%; the white cell count was 5,900 with 55.8% neutrophils, 28.9% lymphocytes, 7.7% monocytes, 7.1% eosinophils and 0.5% basophils; the platelet count was 293,000; the red cell count was 4,880,000; Hb concentration was 13.9 g/dL; mean corpuscular volume (MCV) was 82.0 μm³; mean corpuscular Hb (MCH) was 28.5 pg/cell; and mean corpuscular Hb concentration (MCHC) was 34.8 g/L. Chemical values were unremarkable. Stool examination revealed operculate eggs that measured 57.5 to 65.0 by 40.0 to 42.5 μm, consistent with those of *Diphyllobothrium* spp. (Fig. 1).

The patient was given a single dose of praziquantel at 12 mg/kg, followed by magnesium citrate solution (Magcorol®). The next day, the patient passed a complete tapeworm strobila with scolex (Fig. 2).

### Species Diagnosis of the Isolate

The strobila was thin, 4.0 m long, and was widening posteriorly to maximum width of 10 mm (Fig. 2). Gravid uterus with 5 - 6 loops on each side was visible in the central part of each segment. The scolex was spatulate and 2.2 mm long by 0.6 mm in maximum width (Fig. 2). These and other features were compatible with those of *D. latum* and *D. nihonkaiense*.

DNA sequence analyses were carried out to make the species identification. The method was described elsewhere (11). Briefly, DNA was extracted from a mature segment using a QIAmp DNA Mini Kit (Qiagen GmbH, Germany), and the ribosomal DNA internal transcribed spacer (ITS), mitochondrial *cox1* gene for cytochrome oxidase subunit 1, and mitochondrial *nd3* gene for NADH dehydrogenase subunit 3 were targeted for PCR amplification. Primers used were: 5’-AACAAGTTTTCCGTAGGTGA-3’ and 5’-AGCACGTCTGGGATCATT-3’ for ITS, which yielded a 649-bp product including 18S rRNA and 5.8S rRNA; 5’-TTGATCGTAAATTTGGTTC-3’ and 5’-AAAGAACCTATTGAACAAAG-3’ for *cox1*, which yielded a 748-bp product; and 5’-AATTCTGTGTTCTAGTGAAT-3’ and 5’-GACAATAAGTTATTAGCAGT-3’ for *nd3*, which yielded a 475-bp product. The PCR amplification products were directly sequenced on both strands using the Big-Dye Terminator Cycle Sequencing Ready Reaction Kit version 3.1 (Applied Biosystems, Langen, Germany) and an Applied Biosystem 3730XL DNA sequencer.

The nucleotide sequences of ITS, *cox1* and *nd3* determined in this study were deposited in DNA databases with the accession numbers AB288370, AB288373 and AB288376, respectively. The ITS sequence of the present clinical isolate was 100% identical with that of *D. nihonkaiense* isolated from Japanese and Korean patients, but was significantly different from *D. latum* found in French patients or *D. ditremum*, another diphyllobothriids species occasionally found in humans (Table 1). *Cox1* and *nd3* sequences also demonstrated a high level of similarity to those of *D. nihonkaiense*, but were significantly different from

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**Figure 1.** *Diphyllobothrium* eggs detected in the feces of the patient.

**Figure 2.** The *Diphyllobothrium* specimen discharged from the patient after administration of praziquantel. Left: the posterior portion of the strobila with gravid uterus in the central region of each segment. Right: scolex.
Table 1. Genetic Distances (Kimura parameter 2) of ITS1, cox1 and nd3 Sequences of the Present Clinical Isolate from Those of Diphyllobothrium nihonkaiense, D. latum and D. dendriticum

<table>
<thead>
<tr>
<th>Diphyllobothrium species</th>
<th>ITS1</th>
<th>cox1</th>
<th>nd3</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. nihonkaiense</td>
<td>0.000</td>
<td>0.000</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>(AB288368, DQ768182)</td>
<td>(AB288372)</td>
<td>(AB288374)</td>
</tr>
<tr>
<td>D. latum</td>
<td>0.020</td>
<td>0.078</td>
<td>0.111</td>
</tr>
<tr>
<td></td>
<td>(DQ768167)</td>
<td>(DQ768200)</td>
<td>(AY973606)</td>
</tr>
<tr>
<td>D. dendriticum</td>
<td>0.016</td>
<td>0.065</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td>(DQ768177)</td>
<td>(AB374223)</td>
<td>(AB374224)</td>
</tr>
</tbody>
</table>

Accession numbers are referenced in parentheses

those of D. latum or D. ditremum (Table 1).

Discussion

In Europe, D. latum is contracted by consuming raw or undercooked freshwater fish such as perch, char and pike. Many cases are reported annually across Europe, especially in the French and Italian speaking areas of subalpine lakes (9). In Switzerland, 8 to 12% of perch fillets from Lake Leeman are infested with plerocercoids of D. latum (9). Since Diphyllobothriasis latum is indigenous to Europe, we first suspected that the present case was caused by D. latum. However, the results of the molecular genetic analyses clearly demonstrated that the parasite was not D. latum but rather D. nihonkaiense, which is prevalent in the Far East. It is unlikely that the patient had contracted the tapeworm in Japan. First, he had been living in Switzerland with his family for several years and did not travel outside Europe during the previous 12 months, when his mother had noticed a tapeworm strobila passing from the boy for the first time. Secondly, the treatment with praziquantel was carried out within 2 weeks after his arrival in Japan, and the tapeworm obtained was mature with a length of 4 m. Considering that the development of the tapeworm requires at least 5 to 6 weeks for eggs to be produced and normally requires still more time for spontaneous passing of strobila (12), it is almost certain that the patient contracted the tapeworm in Switzerland. A detailed interview of the patient’s mother revealed that the patient and his family never consumed perch, char or pike; instead, the mother admitted that the family ate salmon purchased from nearby markets in Switzerland.

Although it has been reported that rainbow trout (Oncorhynchus mykiss) and brown trout (Salmo trutta) in Europe harbor D. dendriticum, which occasionally infect humans (13, 14), there have been no reports of D. nihonkaiense in these trout or in Atlantic salmon (Salmo salar). D. nihonkaiense larvae have only been isolated from Pacific salmon species including masou, pink and chum. In recent years, exports of wild Pacific salmon, including frozen or fresh chum salmon, to EU countries from the United States, mostly from Alaska, have markedly increased, reflecting an increased worldwide demand for seafood (10). Interestingly, 3 cases of Diphyllobothriasis nihonkaiense have been reported just recently, one from France and two others from Switzerland (5, 6). In the French case, the patient had eaten wild salmon carpaccio that was traced back to chum salmon imported from Canada (Gulf of Alaska) (5). In Switzerland, molecular genetic analyses of diphyllobothriid tapeworms obtained from 23 cases between 2004 and 2006 identified that 2 cases were caused by D. nihonkaiense and 21 cases by D. latum (6). At least one of the two diphyllobothriasis nihonkaiense cases in Switzerland was also attributed to chum salmon bought in a nearby supermarket in France.

Collectively, the present case is the fourth such case of D. nihonkaiense infection in Europe, probably through consumption of imported wild Pacific salmon. In Kyoto, Japan, 4-8 indigenous cases of diphyllobothriasis nihonkaiense are encountered every year, indicating that this tapeworm disease is still prevalent in Japan. The present case, together with the previous cases in France and Switzerland, clearly shows that diphyllobothriasis nihonkaiense is no more a local disease of the Far East.

Wild Pacific salmon are also reported to harbor the larvae of D. klebanovskii and D. ursi, which infect humans in Far East Russia and Alaska, respectively (15, 16). People should be aware of the risk of tapeworm infection when they eat raw or undercooked wild salmon. Infection can be prevented by cooking the fish at a temperature of 54°C to 56°C for 5 minutes. Alternatively, the plerocercoids can be destroyed by blast-freezing the fish at -35°C for 15 hours or by regular freezing at -20°C for 7 days before consumption (17).

References