Human *Diphyllobothrium nihonkaiense* Infection in Washington State

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Abstract. A patient in Washington State harbored a fish tapeworm most likely acquired from raw salmon. *Diphyllobothrium nihonkaiense* was identified by *cox1* sequence analysis. Although this is the first documented human *D. nihonkaiense* infection in the U.S., the parasite may have been present earlier but misidentified as *D. latum*.

*Diphyllobothrium* spp. are tapeworms acquired by the ingestion of raw or undercooked fish. Approximately 20 million people worldwide are believed to harbor fish tapeworms (1). Since the description of a *D. latum* infection in Minnesota in 1906 (2), diphyllobothriasis in the U.S. has been attributed to this species. The majority of reported cases since that time have followed the ingestion of freshwater fish such as perch or pike from the Great Lakes region.

However, more than 50 *Diphyllobothrium* species have been described, and at least 14 of these have been detected in humans (1). Species identification on the basis of morphology is unreliable, and molecular assays to distinguish individual species have been developed but are not widely available (3, 4). Of particular note, *D. nihonkaiense* is commonly found in Japan (5, 6) and has subsequently been reported in Korea (7), China (8) and in Europeans who ingested raw fish originating from Canada (9, 10).

The life cycle of *Diphyllobothrium* spp. is complex and involves copepods as first intermediate hosts, freshwater, anadromous or marine fish as second intermediate hosts, and fish-eating birds or mammals as definitive hosts (1). *D. nihonkaiense* has been found in Pacific salmon (*Onchorhynchus* spp.), including pink, chum and sockeye species. Brown bears are believed to be the natural definitive host (11).

We recently encountered a 20 year-old previously healthy resident of Washington State who passed a parasite that was identified as a section of a *Diphyllobothrium* sp. strobila on
morphological grounds. Due to the absence of risk factors for *D. latum* exposure such as ingestion of raw freshwater fish or travel to areas in which *D latum* is endemic, molecular analysis of DNA extracted from a proglottid was performed and identified the parasite as *D. nihonkaiense*. The patient was otherwise asymptomatic, was not taking any medications, and had not traveled outside the Pacific Northwest with the exception of a visit to Costa Rica at age 15. She admitted to eating sushi including, in particular, raw salmon. After the identification of *Diphyllobothrium* sp., she received a single dose of praziquantel.

The proglottids were approximately 2.5 mm long x 7.5 mm wide after fixation, with a rosette-shaped central uterus characteristic of *Diphyllobothrium* spp. (Fig. 1). Ova were expressed from a gravid proglottid. Thirty ova were measured, yielding a mean size of 44.9 (±2.4 SD) x 67.2 (±3.3 SD) µm.

A section of the parasite was fixed in formalin-acetic acid-alcohol (FAA) prior to microscopic examination, and an equivalent portion was submitted in 70% ethanol without fixation for molecular analysis. DNA was extracted from the unfixed sample using the Animal Tissue protocol for the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). A custom primer pair was designed to amplify a 349-bp region of the *Diphyllobothrium* cytochrome c oxidase subunit 1 (cox1) gene, based on publicly available sequences (forward primer 5`-TGCACTCTCTACACAGGTTT-3`; reverse primer 5`-ATGCCCCCACACACACTAC-3`). This specific region was selected because it contained residues that discriminate between *D. nihonkaiense* and *D. klebanovskii* (12). PCR was performed using the AccuPrime Pfx DNA polymerase kit (Invitrogen, Carlsbad, CA) using the following cycling conditions: 95°C for 2 minutes, followed by 35 cycles of 95°C for 15 seconds, 55°C for 30 seconds, and 68°C for 90 seconds and a final extension at 68°C for 2 minutes.
A DNA band of ~350 bp was isolated from a 1.5% agarose gel from the clinical sample.

A control PCR reaction performed on a known *Taenia solium* sample did not produce a PCR product under the same conditions. Sequencing of the isolate revealed multiple differences from the *D. latum* *cox1* gene and 100% homology to a known *D. nihonkaiense* sequence (Fig. 2A).

The phylogeny of this U.S. *D. nihonkaiense* isolate was consistent with that of other reported *D. nihonkaiense* isolates (Fig. 2B).

Although this represents the first documented *D. nihonkaiense* infection in the U.S., it is possible that this species has been present in the country for a long time without being recognized as distinct from *D. latum*. A cluster of 52 cases of diphyllobothriasis were investigated in association with fresh salmon ingestion in California, Hawaii, Oregon and Washington in 1980-1981 (13), but the parasites were not identified to the species level, and *D. nihonkaiense* had not yet been described as a species at that time (6). Morphological discrimination of *Diphyllobothrium* spp. can be difficult, and the diagnosis of diphyllobothriasis is usually made on morphological grounds. The ova of *D. nihonkaiense* have been reported to be somewhat smaller (mean size 45 x 57 µm) than those of *D. latum* (mean size 57 x 72 µm) (14), and it is noteworthy that the usual size criteria for identification of *D. latum* ova essentially encompass both species (40-50 x 58-75 µm) (15). The ova from our specimen averaged 45 x 67 µm.

Human *Diphyllobothrium* infections may be asymptomatic, as in the present case, or may be associated with vague gastrointestinal symptoms. Vitamin B12 deficiency has been described as a complication of longstanding *D. latum* infection but has not been reported with *D. nihonkaiense*. Mild anemia or eosinophilia may occasionally occur (11), but a causal relationship has not been established. Patients harboring *D. nihonkaiense* may repeatedly shed
large segments over extended periods of time, resulting in substantial psychological distress for both the patient and family members (1, 16).

Diphyllobothriasis has not been a reportable condition in the U.S. since 1982. The parasite appears to be re-emerging in Russia, Korea, Japan, South America and alpine regions of Europe (1). Treatment with praziquantel appears to be effective (5). U.S. Food and Drug Administration regulations (Code of Federal Regulations Part 123, Title 21) require that ready-to-eat raw, raw-marinated or partially cooked fish other than molluscan shellfish and tuna must be frozen at -20°C for 7 days or -35°C for 15 hours prior to service or sale. Thus, salmon sushi served in commercial establishments should be safe to eat. However it is notable that Diphyllobothrium spp. are not reliably killed by smoking (17), so that cold-smoked salmon represent a potential source. This report documents the presence of D. nihonkaiense in the United States and underscores the ability of molecular methods to reveal unappreciated diversity in human pathogens.
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Figure Legends

Figure 1. *Diphyllobothrium nihonkaiense*. A. Proglottids. B. Ovum. Scale bar = 10 microns (400x).

Figure 2. Phylogenetic relationship of U.S. *D. nihonkaiense* isolate and related isolates.

Phylogenetic tree of U.S. isolate in relation to other tapeworms. UPGMA tree on amplified 349bp fragment of *cox1* gene. Kimura 80 parameters. Bootstrap values for 10,000 replicates shown. Sample AB375003.1 was determined by the Hashimoto group to be *D. klebanovskii* (12).
References


2. Nickerson WS. 1906. The broad tapeworm in Minnesota, with the report of a case of infection acquired in the state. JAMA. 46:711–713.


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Table. *cox1* sequence alignment of U.S. isolate compared to other *Diphyllobothrium* spp. sequences by CLUSTAL-W.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Position in <em>cox1</em> gene</th>
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<tbody>
<tr>
<td></td>
<td>663 669 678 684 717 720</td>
</tr>
<tr>
<td>U.S. Isolate</td>
<td>C A A C C C A C A T T G T C T</td>
</tr>
<tr>
<td><em>D. nihonkaiense</em> AB684623.1</td>
<td>·· ·· ·· ·· ·· ·· ·· ·· ·· ··</td>
</tr>
<tr>
<td><em>D. klebanovskii</em> AB375661.1</td>
<td>·· ·· T T ·· ·· ·· ·· ·· C ··</td>
</tr>
<tr>
<td><em>D. nihonkaiense</em> Dn8 AB375003.1</td>
<td>·· ·· T T ·· ·· ·· C ·· ·· ··</td>
</tr>
<tr>
<td><em>D. latum</em> AB504899.1</td>
<td>T G G T T T G G T C A T A</td>
</tr>
</tbody>
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Dots indicate homology with USA Isolate sequence. Sample *D. nihonkaiense* Dn8 AB375003.1 was determined by the Hashimoto group to be of a distinct lineage closely related to *D. klebanovskii*. *D. latum* sequence included for reference.