Molecular Detection of Diphyllobothrium nihonkaiense in Humans, China

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The cause of diphyllobothriosis in 5 persons in Harbin and Shanghai, China, during 2008–2011, initially attributed to the tapeworm Diphyllobothrium latum, was confirmed as D. nihonkaiense by using molecular analysis of expelled proglottids. The use of morphologic characteristics alone to identify this organism was inadequate and led to misidentification of the species.

Diphyllobothriosis is a fishborne cestodiasis caused by infection with adult tapeworms belonging to the genus Diphyllobothrium Cobbold, 1858 (1–5); the most frequent etiologic agents are D. latum and D. nihonkaiense. Humans are infected by ingesting raw or undercooked fish infected with larval plerocercoids. Adult tapeworms can grow to \(\approx 2–10\) m in length in the human small intestine (1–6). Despite the large size of the tapeworms, clinical symptoms can be absent or mild and include mild abdominal pain, watery diarrhea, and abdominal discomfort (3–7). D. latum infection can also cause vitamin \(\text{B}_{12}\)-deficiency anemia (5).

Diphyllobothriosis caused by D. nihonkaiense has been extensively reported in Japan (3,4), but it has also occurred autochthonously in South Korea (8) and the Far Eastern Federal District of Russia (originally reported as D. klebanovskii infection [9]). Sporadic cases have been reported in Europe (6), North America (10), and New Zealand (7) in recent years.

In mainland China, 15 cases of diphyllobothriosis among humans have been reported since the first report in 1927 through 2012; the etiologic species was identified as D. latum by morphologic characteristics (11–13; Table) and molecular markers (14,15). No cases of diphyllobothriosis had been reported in large cities such as Beijing and Shanghai during 1954–2007 (11). However, we confirm 4 cases of D. nihonkaiense infection in humans in Shanghai, previously identified as D. latum infection, during 2008–2011, as well as 1 case in the moderately populous city of Harbin in Heilongjiang Province.

The Study

We examined 5 recent infections of humans with Diphyllobothrium spp. (Table, cases 12, 16–19) that occurred in China. Each case had been originally reported as a D. latum infection on the basis of morphologic identification only. Case 12 was reported in Harbin City, Heilongjiang Province, in 2009 (13). The 4 cases reported in Shanghai were diagnosed at the National Institute for Parasitic Diseases, Shanghai, on the basis of morphologic features of passed strobila. Case-patient 16 lived in Japan, but it was suggested that he acquired the tapeworm in Shanghai where he had frequently eaten raw salmon. Case-patient 17 was a 10-year-old girl from Japan. Whether she became infected in Shanghai or Japan was unclear because of lack of information. Case-patients 18 and 19 acquired the infection in Shanghai because they had never been abroad.

Because all patients in Shanghai had eaten raw salmon, we decided to re-examine how the causative Diphyllobothrium spp. were identified. D. latum infection is associated with consumption of freshwater fish such as perch (Perca spp.), not Pacific salmon (Oncorhynchus keta, O. masou) and Atlantic salmon (Salmo salar) in the Northern Hemisphere (1–5). To expand diagnostic parameters and clarify the point of misidentification, we re-identified Diphyllobothrium spp. by examining the tapeworms’ morphologic features and using a molecular marker. In a sample from case-patient 12, only proglottids stained with acetic acid–carmine were available for testing by both methods (Figure 1, panel A). Proglottids obtained from 4 case-patients in Shanghai were preserved in either 10% formalin (case-patient 16) or 70% ethanol (case-patients 17–19) after collection (Table). Parts of the proglottids were embedded in paraffin, and sagittal sections were prepared for morphologic observation.

For molecular identification of the Diphyllobothrium spp., genomic DNA samples were extracted from specimens by using a DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany). In specimens from case-patients 17–19, the mitochondrial cytochrome c oxidase subunit 1 gene (cox1, 1,566 bp) was amplified by PCR by using Ex Taq DNA polymerase (Takara Bio, Shiga, Japan) (7). In formalin-fixed samples of proglottids from case-patients 12

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DOI: http://dx.doi.org/10.3201/eid2002.121889

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and 16, DNA degradation caused by the fixative meant that only shorter \( cox1 \) fragments (249 bp, corresponding to sites 880–1128 of \( cox1 \)) could be amplified successfully by PCR by using KOD FX DNA polymerase (Toyobo, Osaka, Japan). DNA sequencing of amplicons was performed with a 3100-Advant (Applied Biosystems, Foster City, CA, USA). Phylogenetic analysis was performed by using the maximum likelihood method (MEGA 5.05, http://megasoftware.net/mega.php) and Bayesian inference (MrBayes version 3.1.2, http://mrbayes.sourceforge.net/). Clades were assessed by bootstrap resampling (1,000 replicates) and a posterior probability (10\(^6\) generations) for the maximum likelihood and Bayesian inference analyses, implying that the 3 isolates from persons in China (case-patients 16, 17, and 19) were identified as \( D. latum \) (Figure 1, panels B, C, and E) and the tapeworm found in case-patient 18 was identified as \( D. nihonkaiense \) (Figure 1, panel D).

Phylogenetic trees based on the complete \( cox1 \) nucleotide sequences showed the same topologies in maximum likelihood and Bayesian inference analyses, implying that the 3 isolates from persons in China (case-patients 16, 17, and 19) were identified as \( D. latum \) (Figure 1, panels B, C, and E) and the tapeworm found in case-patient 18 was identified as \( D. nihonkaiense \) (Figure 1, panel D).

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The 5 \( Diphyllobothrium \) spp. tapeworms examined in this study were previously identified as \( D. latum \) on the basis of morphologic characteristics, as were 3 of the 5 when we re-examined their morphologic characteristics. However, the 5 etiologic agents were confirmed as \( D. nihonkaiense \).
by molecular analysis. This discrepancy in the identity of these agents may be attributed to the morphologic similarities between the species and the century-long confusion between the parasite *D. latum* and the parasite that caused human diphyllobothriosis associated with the consumption of Pacific salmon in Japan (1–3). Diphyllobothriosis caused by *D. nihonkaiense* has also been reported in South Korea (8) and in the Far Eastern Federal District of Russia (9) and is considered to be autochthonous and linked to the consumption of wild Pacific salmon in these regions. Therefore, some cases of diphyllobothriosis reported in mainland China were probably caused by infections with *D. nihonkaiense*; case-patient 12 (13) in this study is considered to have had such a case. However, a recent report stating that the causative species of 2 diphyllobothriosis cases in northeastern China was *D. latum*, which suggests that *D. latum* is also indigenous to mainland China (14).

**Conclusions**

We confirmed human diphyllobothriosis caused by *D. nihonkaiense* in mainland China by using a mitochondrial DNA marker. Reassessment of a case in Harbin revealed that some, if not all, of the autochthonous diphyllobothriosis cases were likely initially misdiagnosed as *D. latum* infection because of morphologic similarities between *D. nihonkaiense* and *D. latum* tapeworms. Consequently, molecular analysis is indispensable not only for avoiding diagnostic confusion among *Diphyllobothrium* spp., but also for facilitating the acquisition of reliable epidemiologic and epizootic information and improving clinical relevance and preventive controls for diphyllobothriosis.

Information on diphyllobothriosis and warnings of the potential risks associated with infection by its local species should be disseminated to food handlers, restaurant owners, physicians, and consumers. Because...
we cannot determine with certainty whether previous diphyllobothriosis cases in mainland China were caused by *D. latum* or *D. nihonkaiense*, identification of *Diphyllobothrium* spp. should be performed with care. In addition, studies on the distribution and sources of infection of *D. latum* and *D. nihonkaiense* on mainland China should be undertaken.

Clinical studies were financially supported by the Parasitic Disease Control Program of the National Institute for Parasitic Diseases, the Chinese Center for Disease Control and Prevention, and the Department of Parasitology at Harbin Medical University, China. The histologic and molecular aspects of the study were supported in part by the Asian Laboratory Network Construction with the National Institute of Infectious Diseases, Tokyo, Japan of the Ministry of Health, Labour and Welfare, Japan, and a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science to HY (23406010).

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References


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