〈Case Report〉

Diphyllobothrium nihonkaiense detected in a mother and daughter

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ABSTRACT *Diphyllobothrium nihonkaiense* and *Diphyllobothrium latum* are tapeworms that parasitize various mammals, including humans. These tapeworms can infect humans and cause a condition known as diphyllobothriasis. Both *Diphyllobothrium* species are morphologically similar, making differentiation difficult without parasitological expertise. In Japan, 80%-90% of cases of diphyllobothriasis are caused by *D. nihonkaiense*. However, owing to the globalization of food culture and habits, individuals have an increased opportunity to consume raw fish imported from abroad, including from Europe. To differentiate *Diphyllobothrium* species, molecular techniques such as DNA sequencing of the 18S rRNA gene have been introduced. DNA Sequencing of the mitochondrial cytochrome c oxidase subunit Ⅰ (*cox1*) gene is commonly used to identify *Diphyllobothrium* species.The *cox1* gene homology enables identifying the *Diphyllobothrium* species. Here, we diagnosed cases of diphyllobothriasis in a mother and daughter as sequential cases.

We report two cases of *D. nihonkaiense* infection in a mother and her daughter. The daughter (case 1), who noticed the passage of worms during defecation without experiencing abdominal symptoms, was the index case. She visited our hospital with her mother, bringing the tapeworm. Microscopic examination of the segmented proglottides morphologically identified the tapeworm as *D. nihonkaiense*. The daughter underwent no further examinations, including venipuncture or gastrointestinal examinations. The tapeworm was temporarily stored in saline solution and later preserved in 80% alcohol. The mother (case 2) complained of abdominal pain, diarrhea, and worm excretion. She had excreted tapeworms the previous week and had visited another hospital, where she was scheduled to be referred to our hospital. The mother was examined

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via Gastrografin® at the initial hospital, and her tapeworm samples were preserved in formalin. Molecular analysis of the mitochondrial *cox1* gene confirmed that both tapeworms were *D. nihonkaiense*. We considered this diphyllobothriasis to be coincidental in the same family, although no source of infection was identified. No scolex was found in the excreted samples from either patient. After follow-up of the family, no recurrence or new diphyllobothriasis were observed.

In both cases, two *D. nihonkaiense* tapeworms were found within the same period. The causative tapeworms were brought to the department of laboratory medicine in our institute, with one preserved in 80% alcohol and the other fixed in formalin. Before preservation or fixation, morphological examinations were performed, diagnosing the tapeworms as *D. nihonkaiense*. This diagnosis was tentative but reasonable given the high prevalence of *D. nihonkaiense* in Japan. Owing to shrinkage of the tapeworm sample from the mother, inspection was challenging. DNA typing is a confirmatory method for accurately diagnosing diphyllobothriasis. In our cases, molecular detection was effective in confirming differentiation of both worms. However, molecular methods require several days to confirm results, highlighting the need for laboratory technicians to develop expertise in parasitology.

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INTRODUCTION

The broad tapeworm, *Diphyllobothrium nihonkaiense*, is a large parasite common in Japan, consisting of over 3,000 proglottides, with a body length of several meters to a maximum of 10 meters and a body width of 10-20 mm[1]. In the 1980s, tapeworm disease in Japan was primarily considered by *Diphyllobothrium latum*, the same pathogen found in Europe[2]. However, the tapeworms found in Japan had different life cycles and histological structures. Although morphologically very similar to *Diphyllobothrium latum*, *D. nihonkaiense* has been reported as a novel species of *Diphyllobothrium*[2, 3]. Since then, adult tapeworms detected in Japan have been identified mainly via histopathology. DNA Sequencing of such as the 18S rRNA or mitochondrial *cox1* gene is commonly used to identify *Diphyllobothrium* species based on the homology of the target gene. A recent genetic analysis showed a 6.9%-15.2% difference in nucleotides between *D. latum* and *D. nihonkaiense* in the mitochondrial DNA[4]. Therefore, genetic diagnosis has been the mainstay for diagnosing tapeworms[5].

D. nihonkaiense, also called sanada-mushi, is named after Sanada fabrics, a type of ancient Japanese clothing (kimono), because of its morphology and is one of the most common parasitic diseases reported in Japan[1, 6]. The larvae, which live in the muscles of the intermediate hosts, salmon and cherry salmon, are transmitted orally from unheated or undercooked foods such as raw fish (sashimi)[5]. Consuming raw fish comes with risks, such as exposure to bacteria and parasites, including *D. nihonkaiense*. Hence, the fish must be fresh and properly handled to minimize these risks.

D. nihonkaiense is the predominant cause of tapeworm infections in Japan, with an 86% prevalence among all tapeworms[3]. *D. nihonkaiense* infections have also been reported outside of Japan.

However, in globalizing clinical infectious diseases, imported infectious diseases, such as *D. latum*, must be diagnosed and treated. Although *D. latum* is a minor and rare tapeworm in Japan, the risk of *D. latum* infection is more prevalent when consuming raw fish imported from abroad. In laboratory medicine, immediate testing of patient samples enables a better chance of diagnosing the disease. Clinical infectious diseases, including parasitosis, require laboratory technicians to handle stool samples that may contain parasite eggs or worm samples derived from patients. Here, we report two cases in which a daughter and her mother were simultaneously infected with a parasite.

CASE REPORTS

In this case series, the mother initially experienced abdominal pain and excreted parasitic worms. The mother planned to visit our hospital after consulting another clinic. During the waiting period, the daughter developed similar symptoms and visited our hospital directly before the mother's scheduled consultation.

Case 1

Indexed patient: 7-year-old girl (eldest daughter)

Chief complaint: excretion of worms from anus

Physical examination: nothing special

Life history: no raw food consumption except sushi

Travel history: No history of out-of-prefecture or out-of-country travel

Medical history: In April 20XX, she observed an appendage during defecation and attempted to pull it out, but the appendage was torn in the process. Because her mother was scheduled to visit our hospital for similar symptoms, she visited our emergency outpatient clinic for a thorough examination.

Clinical course: At the time of the visit, the patient brought the body of a worm that had been discharged in the morning, wrapped around disposable chopsticks and placed in a plastic bag. Upon arrival, the physician carefully unwrapped the body and suspended it in saline solution. Based on the worm's morphological characteristics, the physician decided that it was most likely the Japanese broad tapeworm *Diphyllobothrium nihonkaiense* and subsequently inspected the tapeworm in detail. The adult body of the worm was approximately 2 m long and 1 cm wide, with no observable scolex (Fig. 1). A single blackish uterine structure was observed in the center of each segment of the tapeworm body, suggesting that the segments

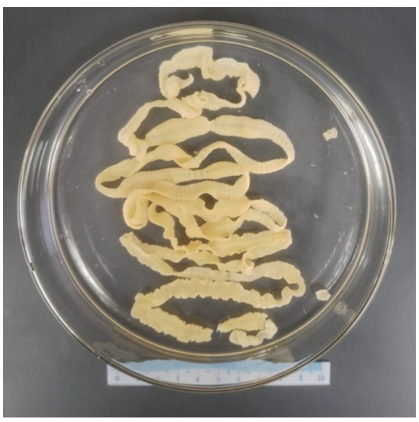

Fig. 1. Tapeworm from Case 1 suspended in saline solution
The total length was 2 m.

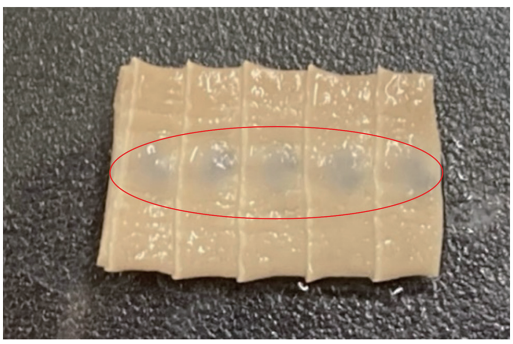

Fig. 2. Segments of the tapeworm from Case 1
Only one uterine structure was observed in each segment. The characteristics of the worm eggs in the observed segments are shown.

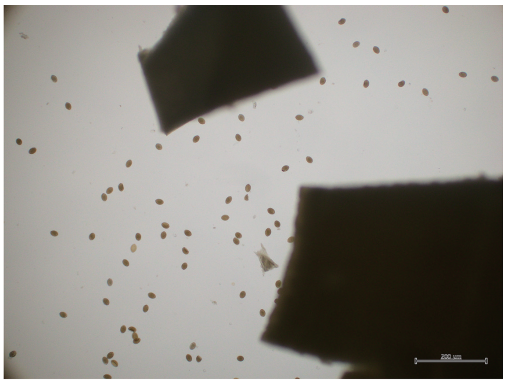

Fig. 3. Microscopic image of a fragmented section from Case 1
The segment contains many eggs (10×4).

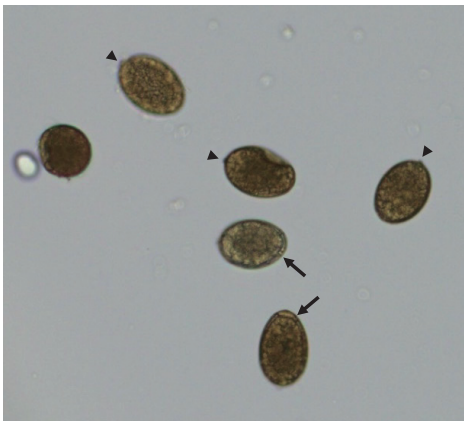

Fig. 4. Tapeworm eggs from Case 1
Ova with operculum (arrow) and knob (arrow head) were observed (10×40).

Table 1. Examination findings at presentation of Case 2

WBC	6.80 $\times 10^3/\mu$L	TP	7.6 g/dL	CRE	0.54 mg/dL
RBC	4.14 $\times 10^6/\mu$L	Alb	4.8 g/dL	UA	4.2 mg/dL
Hb	12.6 g/dL	A/G ratio	1.71	UN	20 mg/dL
Ht value	38.0 %	AST	18 U/L	Glu	89 mg/dL
PLT	266 $\times 10^3/\mu$L	ALT	23 U/L	Amy	51 U/L
Neutro	48.9	LD	162 U/L	T-Cho	210 mg/dL
Lympho	39.4	ALP	62 U/L	Na	144 mmol/L
Mono	4.1	γ-GT	10 U/L	K	5.2 mmol/L
Eosino	6.9	ChE	323 U/L	Cl	106 mmol/L
Baso	0.7			CRP	0.06 mg/dL

were mature or gravid (Fig. 2). Microscopic examination of the shredded segments revealed that many were still immature, but the segments were unicellular and contained eggs with operculum and knob (Fig. 3 and 4). We identified the tapeworm as *D. nihonkaiense* based on morphology. After inspection, the tapeworm body was temporarily stored in saline solution, then preserved in 80% alcohol the next day for permanent fixation.

Case 2

Patient: 30-year-old woman (mother of the patient in case 1)

Main complaints: abdominal pain, diarrhea, excretion of worms

Medical history: The patient had abdominal pain for several days in April 20XX, a week before her daughter visited our hospital. When she defecated, she discharged worms twice that were centimeters in length. She then consulted her primary physician and was referred to our hospital for deworming. At her first visit to our hospital, she brought the excreted worms, which were formalin-fixed by the nearby primary physician.

Laboratory findings at presentation: Table 1 shows the results of blood tests performed at the time of presentation. Neutrophils, eosinophils, and potassium were out of the reference range. The increase in eosinophils was likely due to the parasitic infection. Neutrophils were relatively decreased. The cause of the potassium elevation was unknown.

Clinical course: First, the patient visited the department of gastroenterology, and radiological

examination of the gastrointestinal tract with mono-contrast (Gastrografin®, amidotrizoic acid, Bayer, Japan) was scheduled to detect contents in the intestinal lumen. The worms were submitted to the department of microbiology, where they were visually inspected and identified as the tapeworm *D. nihonkaiense*. Thereafter, the gastrointestinal tract radiological examination was performed on another day, but no residual worms were found, and the patient was followed-up. No scolex was found in the excreted samples from either patient.

Morphological comparison and genetic analysis of the tapeworms derived from the two cases

The two patients in this study were a mother and her daughter. *D. nihonkaiense* was detected at the same time in both cases. In case 1, the specimen was collected during defecation at home. In case 2, a formalin-fixed specimen was brought to our hospital. We compared the morphologies of the two worms. Fig. 5 shows the tapeworm from case 1 fixed in 80% alcohol at our hospital; Fig. 6 shows the tapeworm from case 2 fixed in formalin. The two tapeworms appeared completely different, although they belonged to the same species. Alcohol is recommended for fixing parasitic worms[7]. The formalin-fixed tapeworm from case 2 was markedly shrunken in size due to the strong dehydrating effect of formalin. This case reaffirms that alcohol fixation should be used to preserve tapeworms after collection, considering the need for maintaining morphology and the possibility of later genetic analysis.

The National Institute of Infectious Diseases was commissioned to perform genetic analysis of the samples from both cases. PCR-restriction fragment length polymorphism testing targeting the partial length of the mitochondrial *cox1* gene[8] revealed that the specimens were digested by BspHI, but not by AfeI, AgeI, or BanI; therefore, they were identified as *D. nihonkaiense*.

DISCUSSION

In this case, a mother and her daughter were likely infected simultaneously from the same food source with *D. nihonkaiense*. The specific source of infection or hazardous food consumption within the family, including the mother and daughter, could not be identified. Given that ingested plerocercoids develop into mature adult worms within one month[7], it is estimated that the ingestion of food infected with plerocercoids occurred approximately one month prior (March 20XX). The reported infection rate of *D. nihonkaiense* in Japan is 51 cases per year[1]. Due to the rarity of this infection, it is reasonable to consider that the multiple infections of *D. nihonkaiense* within the same family were derived from the same source during a single intake

Fig. 5. Tapeworm from Case 1 fixed with 80% alcohol
The worm appeared intact.

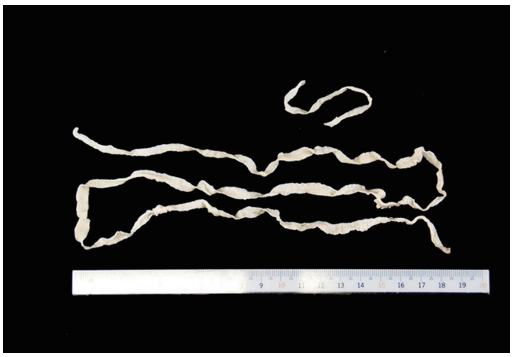

Fig. 6. Tapeworm from Case 2 fixed with formalin
The worm was shrunken in length and width.

episode. Among the second intermediate hosts, such as *Oncorhynchus* species, the infection rate of *D. nihonkaiense* varies[9]. In *Oncorhynchus keta* (chum salmon), the individual infection rate was 51.1% (24/47), while in *Oncorhynchus masou* (cherry salmon) and *Oncorhynchus gorbuscha* (pink salmon), the individual infection rates were 12.2% (10/82) and 18.5% (5/27), respectively. Therefore, it is understandable that even if family members consume the same food, some may become infected while others do not.

Although the daughter had no symptoms other than dropping of the worms during defecation, medical examination was performed without invasive testing such as venipuncture or colonoscopy. We advocate avoiding invasive diagnostic procedures and long medical examinations on pediatric patients. The pediatric patient's burden was reduced in this case because she brought the worm with her to the clinic. We identified the worms directly and gave appropriate instructions to the patient's family. However, because the infection source could not be determined, follow-up and observation of family members other than the mother and daughter were necessary, including periodic examinations for worms and eggs[10, 11].

In routine workups, our laboratory performs smears and egg collection tests to search for various worm eggs and protozoa. In the present case, the parasitic tapeworm itself was brought to our laboratory, not a stool specimen to search for worm eggs. This enabled immediately achieving a morphological diagnosis of *D. nihonkaiense*. In our institute, laboratory technicians with expertise in medical zoology or staff in the department of microbiology advise management of cases of clinical parasitic infections. We intend to maintain this response system at our institute[11].

For treating diphyllobothriasis, eradication of the tapeworms is standard, typically with praziquantel, an antiparasitic drug[10–12], or Gastrografin®, a gastrointestinal contrast medium[13]. Praziquantel is used as the first-line drug for treating tapeworms because its side effects are usually mild and do not require treatment. When combined with a laxative, it is easier to collect the worms and confirm detachment of the scolex to determine the effectiveness of the deworming[12]. Gastrografin® is not a first-line treatment because of the high level of patient pain caused by insertion of the duodenal sonde and the risk of radiation exposure[14]. Gastrografin® carries a risk of radiation exposure in cases requiring radiographical confirmation. In the mother's case, the mother was lactating, yet the Gastrografin® procedure was performed. Praziquantel was avoided because of its mutagenesis risk. Praziquantel is excreted during feeding; thus, breast milk should be discarded until 72 hours after praziquantel administration (according to the pharmaceutical information). In this case, no worm samples were obtained in our hospital; we identified the worms using samples brought in by the patients.

Formalin fixation destroys parasitic DNA over time[15]. When genetic analysis is to be performed later, freezing or alcohol fixation is considered the best preservation method[7, 10]. Here, we successfully identified the same tapeworm species in both cases, regardless of whether the samples were fixed in alcohol or formalin. In the present case, a genetic search requesting other institutions was not immediately performed owing to logistical restrictions during the coronavirus infection in Japan. Several years later, the request was accepted by the National Institute of Infectious Diseases, and the case was confirmed to be *D. nihonkaiense*.

According to the Information on the Detection of Pathogenic Microorganisms published by the National Institute of Infectious Diseases, most *Diphyllobothriidae* tapeworm cases diagnosed via DNA in Japan have been *D. nihonkaiense*[16, 17]. *D. latum* is the minority *Diphyllobothriidae* tapeworm in Japan[1]. Considering the frequency of

occurrences of other *Diphyllobothriidae* tapeworm, *D. nihonkaiense* is the most common tapeworm in Japan[1, 17]. Furthermore, because no *D. latum* has been reported as being detected in imported salmon or trout[1, 5], we believe *D. nihonkaiense* is the most common. However, both tapeworms, *D. nihonkaiense* and *D. latum*, have been diagnosed outside Japan[6], and both should be differentiated in clinics of clinical infectious disease, including imported infectious diseases. Clinical laboratory technicians must then appropriately advise physicians about patients concerning parasitic infections.

CONCLUSION

Although genetic identification of the tapeworms was delayed, we made a comprehensive diagnosis based on the tapeworm morphology and clinical findings of the patients. This contributed to clinical management for the family without wasting time waiting for the results of a molecular diagnosis.

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ETHICAL APPROVAL STATEMENT

This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Our institution does not require ethical approval for reporting individual cases or case series by institutional policy. Comprehensive informed consent was obtained from the patients at our institute.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article. Data are available on reasonable request due to privacy and ethical restrictions.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING STATEMENT

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