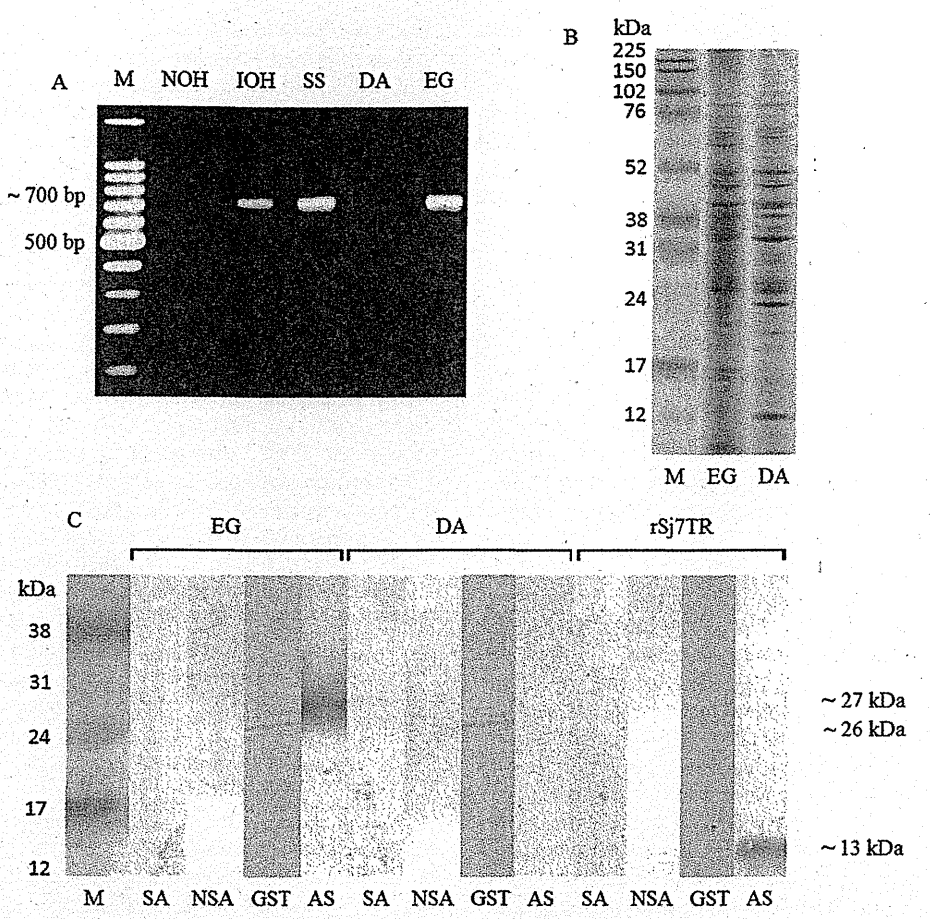

Fig. 2. Gel electrophoresis of reverse transcriptase-PCR products, polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis of Sjp_0110390 in different stages of *Schistosoma japonicum*. Gel electrophoresis (A) shows the presence of cDNA band at ~700 bp in the *S. japonicum*-infected *Oncomelania hupensis quadrasi* snail (lane IOH), schistosomula (lane SS) and schistosome eggs (lane EG) but none in the non-infected snail (lane NOH) and adult parasites (lane DA). SDS-PAGE (B) showed the fractionated proteins of the schistosome egg extract (lanes EG) and the adult parasite extract (lanes DA), M, marker. (C) These extracts and the recombinant Sj7TR (lanes rSj7TR) were transferred onto a PVDF membrane and reacted individually with anti-rSj7TR antisera (AS), anti-GST polyclonal antibody (GST), non-specific mouse IgG (NSA) and anti-mouse IgG (SA).

these stages while none on the adult parasite and the non-infected snail (Fig. 2A). Sequencing was then done to verify the identity of the cloned sequences using the ABI Prism 100 Genetic Analyzer (Applied Biosystems, Carlsbad, CA).

Western blotting using anti-rSj7TR antisera was performed to examine the expression levels of Sjp_0110390. Sodium dodecyl sulfate (SDS) extraction was done for both *S. japonicum* eggs and adults. The eggs and adults were homogenized in SDS buffer with a Potter-Elvehjem homogenizer. The homogenates were incubated at 100 °C for 5 min and centrifuged at 10,000 × *g* for 20 min at 4 °C. The SDS protein extract of both the eggs and adult were collected and fractionated on 15% SDS-polyacrylamide gel. The extracts were electrically transferred onto polyvinyliden difluoride (PVDF) membranes (Immobilon, Millipore, Billerica, MA). Blocking was done with 2% skim milk in T-PBS for 1 h, and then the membranes were incubated with anti-rSj7TR diluted 1:50 with the blocking buffer for 1 h. After washing with T-PBS, the membranes were incubated with the secondary antibody HRP-conjugated anti-mouse IgG (Southern Biotechnology Associates, Birmingham, Al) at 1:200 dilution for 1 h. Specific binding of anti-rSj7TR was detected using 3,3-diaminobenzidine (Merck, Darmstadt, Germany) as the substrate. PVDF membranes containing the adult and egg extract proteins incubated with the non-specific mouse IgG and the secondary antibody separately served as negative controls and with the mouse-derived polyclonal antibody against *S. japonicum* glutathione S-transferase (GST) as a positive control.

Immunoblotting result showed the presence of a band in the infected snail, schistosomula and egg protein extract (Fig. 2C) whose molecular sizes is found to be approximately 27 kDa. This result also coincides with the RT-PCR result showing a band at approximately 700 bp. According to the database, the expected size of the protein is 31 kDa [16] but based on the Western blot, the protein was expressed in nature lower than the expected molecular size. This was further confirmed by the sequencing done on the Sjp_0110390 cDNA showing that instead of having five repeats, it just possessed four repeats which made the protein smaller than expected (Supplementary Fig. 1). Tandem repeat analysis of this sequence revealed 4.2 copies of the 165 bp-long repeat unit [8] lower than that in the previous analysis of the sequence from the database. These differences might be due to strain differences of the parasites used (Yamanashi strain) against the one used in the whole genome sequence available online (Anhui strain). On the other hand, the recombinant Sj7TR showed a band with the molecular size of 13 kDa as expected. Furthermore, no bands were seen on the negative controls used while an approximately 26 kDa band was seen for the anti-GST positive control as expected on both the adult and egg lysates. The Western blot result is consistent with that of the IFAT and RT-PCR showing that Sjp_0110390 was not expressed in the adult stage of the parasite.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molbiopara.2012.12.002.

In conclusion, Sjp_0110390 was expressed mainly at the young stages of the parasite including the eggs, aquatic and intramolluscan stages and the schistosomula. However, it was not expressed on the adult forms of the parasite. Interestingly, the

significant antibody titer against Sj7TR found among *S. japonicum* infected individuals in the previous study [1] may be caused by the parasite's eggs or by continuous exposure to the cercaria but not from the adults. The titer value therefore might somehow be related to the number of eggs dislodged in the host's tissues eliciting immune response. It is known that the main inflammatory response seen on infected humans was caused by the schistosome egg deposition resulting to a strong egg antigen-specific cell-mediated granulomatous reaction [17].

It was also shown that the antigen was localized in the developing suckers of the parasite suggesting that it might be important in the feeding mechanism of the young schistosomes. However, the role and function of Sjp_0110390 in the young stages should be further studied through RNA interference techniques [18–21] to fully characterize the protein.

Acknowledgments

The authors thank Prof. Satoru Kawai and Dr. Naoko Kato-Hayashi of the Laboratory of Tropical Medicine and Parasitology, Dokkyo Medical University, Japan for giving some insights on the experiments done in this study, Asst. Prof. Junya Yamagishi of National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine for the additional bioinformatics analysis done on our gene of interest; Ms. Noriko Suzuki of Division of Clinical Support, Research Support Center, Dokkyo Medical University for preparing the paraffin sections of the parasite used in this study and Ms. Miho Usui of NRCPD, Obihiro University of Agriculture and Veterinary Medicine for helping in the animal experiments done in this study. This work was supported in part by a grant from Global COE Program from the Japanese Ministry of Education, Science, Sports, Culture and Technology, a Grant H23-Shinkosaiko-014 for research on emerging and re-emerging infectious diseases from the Ministry of Health, Labour and Welfare of Japan; and a Grant-in-Aid for Scientific Research (24405044) from the Japan Society for the Promotion of Science.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molbiopara.2012.12.002.

References

[1] Angeles JM, Goto Y, Kirinoki M, Leonardo L, Tongol-Rivera P, Villacorte E, et al. Human antibody response to thioredoxin peroxidase-1 and tandem repeat proteins as immunodiagnostic antigen candidates for *Schistosoma japonicum* infection. American Journal of Tropical Medicine and Hygiene 2011;85(4):674–9.
[2] Boguski MS, Lowe TM, Tolstoshev CM. dbEST—database for expressed sequence tags. Nature Genetics 1993;4(4):332–3.
[3] Howard RJ, Uni S, Aikawa M, Aley SB, Leech JH, Lew AM, et al. Secretion of a malarial histidine-rich protein (Pf HRP II) from *Plasmodium falciparum*-infected erythrocytes. Journal of Cell Biology 1986;103:1269–77.
[4] Taylor DW, Voller A. The development and validation of a simple antigen detection ELISA for *Plasmodium falciparum* malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene 1993;87:29–31.
[5] Beadle C, Long GW, Weiss WR, McElroy PD, Maret SM, Oloo AJ, et al. Diagnosis of malaria by detection of *Plasmodium falciparum* HRP-2 antigen with a rapid dipstick antigen-capture assay. Lancet 1994;343:564–8.
[6] Du W, Hu F, Yang Y, Hu D, Hu X, Yu X, et al. Molecular cloning, characterization, and immunolocalization of two lactate dehydrogenase homologous genes from *Taenia solium*. Parasitology Research 2011;109:567–74.
[7] Wellcome Trust Sanger Institute, Pathogen Sequencing Unit, GeneDB. http://www.genedb.org/ [accessed 13.07.10].
[8] Benson G. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Research 1999;27:573–80.
[9] Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, et al. Protein identification and analysis tools on the ExPASy server. In: Walker JM, editor. The proteomics protocols handbook. Humana Press; 2005. p. 571–607.
[10] Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nature Methods 2011;8:785–6.
[11] Rost B, Yachdav G, Liu J. The PredictProtein server. Nucleic Acids Research 2004;32:W321–6.
[12] Chou KC, Shen HB. Euk-mP.Loc: a fusion classifier for large-scale eukaryotic protein subcellular location prediction by incorporating multiple sites. Journal of Proteome Research 2007;6:1728–34.
[13] Angeles JM, Goto Y, Kirinoki M, Asada M, Leonardo L, Tongol-Rivera P, et al. Utilization of ELISA using thioredoxin peroxidase-1 and tandem repeat proteins for diagnosis of *Schistosoma japonicum* infection among water buffaloes. PLOS Neglected Tropical Diseases 2012;6(8):e1800.
[14] Sturrock RF, Karamsadkan SJ, Ouma J. Schistosoma infection rates in field snails. *Schistosoma mansoni* in *Biomphalaria pfeifferi* from Kenya. Annals of Tropical Medicine and Parasitology 1979;73:370–5.
[15] Kumagai T, Furushima-Shimogawara R, Ohmae H, Wang TP, Lu S, Chen R, et al. Detection of early and single infections of *Schistosoma japonicum* in the intermediate host snail, *Oncomelania hupensis*, by PCR and loop-mediated isothermal amplification (LAMP) assay. American Journal of Tropical Medicine and Hygiene 2010;83:542–8.
[16] Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Research 2011;39(database issue):D32–7.
[17] King CL. Initiation and regulation of disease in schistosomiasis. In: Mahmoud A, editor. Schistosomiasis. London: Imperial College Press; 2001. p. 218–38.
[18] Zhao ZR, Lei L, Liu M, Zhu SC, Ren CP, Wang XN, et al. *Schistosoma japonicum*: inhibition of Mago nashi gene expression by shRNA-mediated RNA interference. Experimental Parasitology 2008;119:379–84.
[19] Cheng GF, Lin JJ, Shi Y, Jin YX, Fu ZQ, Jin YM, et al. Dose-dependent inhibition of gynecophoral canal protein gene expression in vitro in the schistosome (*Schistosoma japonicum*) by RNA interference. Acta Biochimica et Biophysica Sinica 2005;37:386–90.
[20] Zou X, Jin YM, Liu PP, Wu QJ, Liu JM, Lin JJ. RNAi silencing of calcium-regulated heat stable protein of 24 kDa in *Schistosoma japonicum* affects parasite growth. Parasitology Research 2011;108:567–72.
[21] Kumagai T, Osada Y, Ohta N, Kanazawa T. Peroxiredoxin-1 from *Schistosoma japonicum* functions as a scavenger against hydrogen peroxide but not nitric oxide. Molecular and Biochemical Parasitology 2009;164:26–31.

ISSN (Print) 0023-4001
ISSN (Online) 1738-0006

Korean J Parasitol *Vol. 51, No. 1: 19-29, February 2013*
http://dx.doi.org/10.3347/kjp.2013.51.1.19

▣ MINI-REVIEW

Current Status and Perspectives of Cysticercosis and Taeniasis in Japan

Hiroshi Yamasaki*

Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan

Abstract: This mini-review describes recent epidemiological trends in cysticercosis and taeniasis in Japan. Some of the topics discussed herein were presented at the first symposium on "Current perspectives of *Taenia asiatica* researches", that was held in Osong in Chungbuk Province, South Korea, in October 2011 and organized by Prof. K. S. Eom, Chungbuk National University School of Medicine. To better understand the trends in the occurrence of cysticercosis and taeniasis in Japan, clinical cases reported in 2005 have been updated. In addition, the current status of *Taenia asiatica* infections successively occurring in Japan since 2010 is also discussed.

Key words: *Taenia solium*, *Taenia asiatica*, *Taenia saginata*, taeniasis, cysticercosis, Japan

INTRODUCTION

Cysticercosis, a parasitic disease caused by *Taenia solium* cysticercus, is one of the important parasitic diseases. Neurocysticercosis (NCC) is accepted to refer to cysts in the central nerve system, including the parenchyma and ventricles of the brain and the spinal cord. Subcutaneous cysticercosis (SCC) is used for the cysticercosis presenting the form of firm, mobile nodules, mainly in the soft tissues and muscles of on the trunk and extremities. NCC is clinically more serious than SCC because of the severity of the neurologic symptoms, such as epileptic seizures and paralysis that can result from infection. The disease constitutes a major public health problem in many parts of the world, including China, Southeast Asia, India, sub-Saharan Africa, and Latin America [1]. Cysticercosis has also become an important parasitic disease in developed countries, such as the United States, particularly in California and other states with a large immigrant population [2]. In Japan, although *T. solium* cysticercosis/taeniasis was endemic to the Okinawa region in southern Japan 50-60 years ago [3,4], the disease is no longer endemic in the area. Nonetheless, sporadic cases of cysticercosis have been reported in Japan, primarily among Japanese returning from abroad and foreigners coming to Japan (Table 1) [5].

Conversely, taeniasis, which is caused by infection with the adult tapeworm of *T. solium* or *Taenia saginata*, occurs worldwide, except in countries where people do not eat pork and beef for religious reasons [1]. Taeniasis caused by *Taenia asiatica* is restricted to countries in Asia, including South Korea, China, Taiwan, the Philippines, Vietnam, Thailand, Indonesia, and Japan [6]. In Japan, sporadic cases of taeniasis have been reported and most of them were caused by infection with *T. saginata* and were imported cases until *T. asiatica* infections were confirmed in 2010 (Table 2). Compared to cysticercosis, taeniasis is innocuous or asymptomatic, with most patients presenting with slight intestinal illness and mental discomfort due to persistent expulsion of the proglottids.

In Japan, the "Ordinance for Enforcement of the Food Sanitation Act" based on the Food Sanitation Law stipulates that food-borne parasitic diseases such as cysticercosis and taeniasis be treated as cases of food poisoning and that authorities be notified of their occurrence immediately. However, because parasitic diseases have never reported based on the law, it is not possible to accurately estimate the incidence of cysticercosis/taeniasis in Japan. Therefore, the author previously examined the epidemiological trends in cysticercosis and taeniasis based on clinical cases in Japan published in scientific journals [5]. Since then, new cases of cysticercosis and taeniasis have been reported and several cases of cysticercosis have been newly diagnosed in our department. The Department of Parasitol-

• Received 20 February 2012, revised 18 December 2012, accepted 18 December 2012.

* Corresponding author (hyamasak@nih.go.jp)

Table 1. Demographic and clinical data for cysticercosis cases reported in Japan (1990-2011)

Case No.	Year	Patient (Nationality/Age/Sex)	Type of cysticercosis	Diagnostic criteria	Presumed locality of infection	References
1	1990	Japanese/40/F	NCC (multiple)	CT/Pathology	Japan (Tokunoshima, Kagoshima)	55
2	1991	Korean/73/M	NCC (multiple)	CT/MRI/Pathology	Korea	86
3	1991	Japanese/33 /M	NCC (solitary)	CT/MRI/Serology	Honduras	87
4	1991	Japanese/29/F	SCC (solitary)	Pathology/Serology	Thailand	41
5	1991	Japanese/48/M	Intramedullary spinal (solitary)	CT/MRI/Pathology	Thailand	44
6	1992	Chinese/20/M	NCC (multiple)	CT/MRI/Pathology	China (Heilongjiang province)	88
7	1992	Japanese/41/F	NCC (multiple)	CT/MRI/Serology/Pathology	Hong Kong, Korea or Japan	89
8	1992	Japanese/30/M	NCC (multiple, racemose-type)	CT/MRI/Pathology	Japan (Ginowan, Okinawa)	50, 51
9	1992	Korean/42/M	NCC (multiple)	CT/MRI/Serology	Korea	90
10	1993	Japanese/44/F	NCC (solitary)	CT/Pathology	Japan	56
11	1993	Japanese/46/M	Ocular (solitary)	Funduscope/Pathology	Vietnam or Cambodia	45
12	1993	Japanese/41/F	NCC (solitary)	CT/MRI/Pathology	Japan	57
13	1993	Brazilian/26/F	NCC (multiple)	CT/Pathology	Brazil	91
14	1993	Japanese/49/F	NCC (multiple)	CT/MRI/Pathology	China	92
15	1993	Japanese/53/M	NCC (multiple, racemose-type ?)	CT/MRI/Pathology	Taiwan	55
16	1994	Korean/48/M	NCC (multiple) and SCC (systemic)	CT/MRI/X ray/Pathology	Korea	42
17	1994	Korean/43/F	NCC (multiple) and SCC (systemic)	CT/MRI/X ray/Pathology	Korea	42
18	1994	Japanese/72/F	NCC (racemose-type)	CT/MRI/Serology/Pathology	China	53
19	1994	Chinese/24/M	NCC (racemose-type)	CT/MRI/Pathology	China	54
20	1994	Japanese/44/M	NCC (solitary)	CT/MRI/Pathology	Japan	54
21	1994	Japanese/52/F	NCC (solitary)	CT/MRI/Serology	Japan	58
22	1995	Japanese/21/F	NCC (multiple)	MRI/PET	Japan	59
23	1996	Japanese/39/M	NCC (solitary)	MRI/Pathology	Japan	60
24	1996	Japanese/39/M	SCC (solitary)	Pathology	China	43
25	1996	Korean/70/F	NCC (multiple)	CT/MRI/Serology/Pathology	Korea	93
26	1997	Chinese/68/M	NCC (multiple) and SCC (multiple)	CT/MRI/X ray/Serology	China (Heilongjiang province)	7
27	1998	Chinese/48/M	NCC (multiple)	CT/MRI	China	8
28	1998	Japanese/37/M	SCC (solitary)	Pathology	Japan	9
29	1998	Japanese/34/M	NCC (multiple), SCC (multiple) and taeniasis	CT/MRI/Serology	China (Jiujiang, Jiangxi Province)	10
30	1998	Japanese/59/M	NCC (multiple)	CT/Pathology	China	11
31	1999	Japanese/19/F	NCC (solitary)	MRI/Pathology	India	12
32	1999	Chinese/55/M	NCC (multiple)	CT/MRI/Endoscopy/Pathology	China	13
33	1999	Japanese/46/M	NCC (solitary)	CT/MRI/Pathology	Indonesia, Nigeria, or Nepal	14, 15
34	2000	Japanese/45/F	SCC (multiple)	CT/Pathology	Thailand	16
35	2000	Cambodian/29/M	NCC (multiple) and SCC (multiple)	CT/MRI/X ray/Pathology	Cambodia	17
36	2001	Japanese/53/F	Ocular (solitary)	Funduscope/US	No information	18
37	2001	Japanese/43/F	NCC (solitary)	CT/MRI/Pathology	Thailand	19
38	2001	Unknown/73/M	NCC (multiple)	CT/MRI	No information	20
39	2001	Japanese/70/M	NCC (racemose type)	CT/MRI/Serology	Philippines	21
40	2002	Japanese/26/M	NCC (solitary)	CT/MRI/Pathology/Serology	Japan	22
41	2003	Japanese/22/F	NCC (solitary)	CT/MRI// US/Pathology	India	23
42	2004	Japanese/53/F	NCC (solitary)	CT/MRI/Pathology/DNA	India, Vietnam, Thailand or Myanmar	24, 25
43	2004	Chinese/50/M	Ocular (solitary) and NCC (solitary)	Funduscope/CT/Serology/Pathology	China (Heilongjiang province)	26
44	2004	Japanese/83/M	SCC (systemic)	CT/X ray/Pathology/DNA	China	27, 28
45	2005	Chinese/44/F	NCC (multiple) and SCC (multiple)	CT/MRI/X ray	China	29
46	2005	Chinese/21/F	NCC (solitary)	CT/MRI/PET/Serology	China (Harbin, Heilongjiang province)	30

(*Continued to the next page*)

Table 1. (*Continued from the previous page*) Demographic and clinical data for cysticercosis cases reported in Japan (1990-2011)

Case No.	Year	Patient (Nationality/Age/Sex)	Type of cysticercosis	Diagnostic criteria	Presumed locality of infection	References
47	2005	Filipino/9/F	NCC (solitary)	CT/MRI/Pathology/DNA	Philippines	31
48	2006	Japanese/24/F	NCC (solitary)	MRI/Pathology/DNA	Indonesia or Korea	32
49	2006	Indian/28/F	NCC (multiple)	CT/MRI/DNA	India	33
50	2006	Brazilian/42/F	NCC (racemose-type)	CT/MRI/Pathology/DNA	Brazil	34
51	2007	Japanese/38/F	NCC (solitary)	CT/MRI/Pathology/DNA	Nepal	35
52	2007	Japanese/84/M	SCC (systemic)	CT/X ray/DNA	Japan (Okinawa)	36
53	2007	Japanese/51/F	NCC (multiple)	CT/MRI	Japan (Okinawa)	This study
54	2007	Japanese/31/F	NCC (multiple)	CT/MRI	India	This study
55	2008	Indian/44 /F	NCC (multiple)	MRI/Serology/DNA	India	37
56	2008	Chinese/30/M	NCC (multiple) and SCC (multiple)	CT/MRI/X ray/Serology	China	This study
57	2008	Japanese/39/F	NCC (multiple)	CT/MRI/PET/Serology	Asian or African countries	This study
58	2009	Japanese/24/M	Ocular and taeniasis	Funduscope/US/Serology	Malawi	38
59	2009	Korean/38/M	NCC (multiple)	CT/MRI/SEM/Pathology	Korea	39
60	2009	Japanese/20/F	NCC (multiple) and taeniasis	CT/MRI/Serology/Capsule endoscopy/DNA	India	This study
61	2009	Japanese/61/M	NCC (multiple, racemose-type)	CT/MRI/US/Serology/Pathology/DNA	India, Thailand, China or Vietnam	This study
62	2010	Japanese/53/M	NCC (racemose-type)	CT/MRI/Pathology/Serology/DNA	Japan (Uruma, Okinawa)	40
63	2010	Japanese/58/F	SCC (multiple)	CT/MRI/X ray/DNA	Japan (Akita or Okinawa)	This study
64	2010	Chinese/46/F	NCC (multiple) and SCC (multiple)	CT/MRI/US/Serology	China (Harbin, Heilongjiang province)	This study
65	2010	Japanese/31/M	NCC (multiple), SCC(multiple) and taeniasis	CT/MRI/Serology/DNA	India	This study
66	2011	Nepalese/35/M	SCC (solitary)	CT/US/Serology/Pathology/DNA	Nepal	This study

ogy at the National Institute of Infectious Diseases, Tokyo routinely performs diagnostic tests requested for parasitic diseases from domestic and foreign medical institutions, and cysticercosis and taeniasis also are acceptable for diagnosis.

The purpose of this article is to overview the current status of cysticercosis/taeniasis in Japan and to update the data that was reported in 2005 [5] based on the cases cited in PubMed (National Library of Medicine) and Japana Centra Revuo Medicina as well as cases diagnosed in our department over the last 5 years (2007-2011).

CLINICAL CASES

Cysticercosis

According to Nishiyama and Araki [4], as many as 389 cases of cysticercosis were reported in Japan from 1908 to 1997. However, 24 cases reported between 1943 and 1979 were not included in the study. Furthermore, 41 cases, including 10 cases diagnosed by our department, have been newly confirmed between 1997 and 2011 (cases 26-66 in Table 1) [7-40]. Taken together, this gives a total of 454 cysticercosis cases that have been reported in Japan between 1908 and 2011. Table 1 shows 66 of the cysticercosis cases that have been reported over the last 22 years (1990-2011) along with cases confirmed by our department between 2007 and 2011.

Of these 66 cases, 54 (66.7%) were NCC; NCC with multiple cysts (28/54, 51.9%; Fig. 1E) was more frequent than NCC with a solitary cyst (13/54, 33.5%; Fig. 1A, B and Fig. 2A, B, E). Between 1990 and 2011, total 17 cases of SCC were reported as cases 4 [41], 16-17 [42], 24 [43], 26 [7], 28 [9], 29 [10], 34 [16], 35 [17], 44 [27,28], 45 [29], 52 [36], 56, 63, 64, 65, and 66. Two of them were systemic intramuscular cysticercosis with numerous calcified cysts; cases 44 [27,28] and 52 [36] (Fig. 1F, G; Fig. 2D, F). Very rarely, intramedullary cysticercosis in case 5 [44] and ocular cysticercosis in cases 11 [45], 36 [18], 43 [26], and 58 [38] have also been reported. Ten cases of NCC with either SCC or ocular cysticercosis were reported in cases 16-17 [42], 26 [7], 29 [10], 35 [17], 43 [26], 45 [29], 56 , 64, and 65 (Table 1). More interestingly, dual infection of cysticercosis and taeniasis was observed in 4 cases; 29 [17], 58 [51], 60, and 65 (Table 1). Furthermore, the adult tapeworm in case 41 was observed in the small intestine using capsule endoscopy to confirm the presence of the adult worm (Table 2).

Cysticercosis diagnosis is generally performed by imaging,

Table 2. Demographic and clinical data for taeniasis reported in Japan (1990-2011)

Case No.	Year	Patient (Nationality /Age/Sex)	Etiologic agent (diagnostic criteria)	Presumed locality of infection	References
1	1990	Japanese/72/M	*T. saginata* (/Morphology)	?	63
2	1990	Korean/52/M	*T. saginata* (Serology/Morphology)	?	64
3	1990	Japanese/34/M	*T. saginata* (Morphology)	Ethiopia	65
4	1990	Japanese/32/M	*T. saginata* (Morphology)	Japan	65
5	1990	Japanese/26/M	*T. saginata* (Morphology)	Ethiopia or Somalia	65
6	1992	Japanese/10/F	*T. saginata* (Morphology)	Japan	66
7	1994	Japanese	*T. saginata* (Morphology)	Iran	53
8	1994	Japanese	*T. saginata* (Morphology)	?	53
9	1994	Japanese	*T. saginata* (Morphology)	?	53
10	1994	Japanese	*T. saginata* (Morphology)	France or Germany	53
11	1994	Japanese	*T. saginata* (Morphology)	Germany	53
12	1996	Japanese/53/F	*T. saginata* (Morphology)	?	67
13	1996	Japanese/26/M	*T. saginata* (Morphology)	Bolivia	68
14	1996	Japanese/47/M	*T. saginata* (Morphology)	Cote D'Ivoire	69
15	1997	Japanese/23/F	*T. saginata* (Morphology)	Europe	70
16	1998	Brazilian/45/M	*T. saginata* (Colonoscope/Morphology)	Brazil	71
17	1998	Japanese/34/M	Probably *T. solium* with NCC	China (Jiujiang, Jiangxi Province)	10
18	2001	Filipino/32/F	*T. saginata* (Morphology)	Philippines	72
19	2001	Japanese/26/M	*T. saginata* (Morphology)	Japan or India	73
20	2001	Japanese/47/M	*T. saginata* (Morphology)	Indonesia	73
21	2001	Japanese/30/M	*T. saginata* (Morphology)	Ethiopia	73
22	2001	Japanese/60/M	*T. saginata* (Morphology)	Japan	73
23	2002	Japanese/30/M	*T. saginata* (Morphology)	Ethiopia	74
24	2002	Japanese/51/M	*T. saginata* (Morphology)	Thailand	74
25	2002	Japanese/46/M	*T. saginata* (Morphology)	Africa	75
26	2003	Japanese/24/F	*T. saginata* (Morphology)	Vietnam	77
27	2007	Japanese/45/M	*T. saginata* (DNA)	Thailand or Indonesia	76
28	2007	Cambodian/16/M	*T. saginata* (DNA)	Cambodia	94
29	2007	Japanese/58/M	*T. saginata* (DNA)	Korea	94
30	2007	Japanese/32/M	*T. saginata* (DNA)	Ethiopia	94
31	2007	Japanese/33/M	*T. saginata* (DNA)	Cambodia or Ethiopia	94
32	2007	Japanese/40/F	*T. saginata* (DNA)	China, Kenya, Monaco or Croatia	This study
33	2007	Japanese/25/M	*T. saginata* (Endoscope/Morphology)	Laos	78
34	2008	Japanese/26/F	*T. saginata* (DNA)	Nicaragua, Laos or Indonesia	This study
35	2008	Japanese/26/M	*T. saginata* (DNA)	Indonesia	This study
36	2008	Japanese/45/M	*T. saginata* (DNA)	Vietnam or China	This study
37	2009	Japanese/24/M	*Taenia* sp. (Morphology) with ocular type	Malawi	38
38	2009	Japanese/63/M	*T. saginata* (DNA)	Thailand	This study
39	2009	Japanese/57/M	*T. saginata* (DNA)	Thailand	This study
40	2009	Japanese/49/M	*T. saginata* (DNA)	Thailand	This study
41	2009	Japanese/20/F	*T. solium* (Capsule endoscope/DNA) with NCC	India	This study
42	2010	Japanese/58/M	*T. asiatica* (DNA)	Japan	81, 84
43	2010	Japanese/41/F	*T. asiatica* (DNA)	Japan	81, 84
44	2010	Japanese/55/M	*T. asiatica* (DNA)	Japan	81, 84
45	2010	Japanese/40/M	*T. asiatica* (DNA)	Japan	81, 84
46	2010	Japanese/31/M	*T. asiatica* (DNA)	Japan	82, 84
47	2010	Japanese/41/M	*T. asiatica* (DNA)	Japan	83
48	2010	Japanese/28/M	*T. asiatica* (DNA)	Japan	83
49	2010	Japanese/30/M	*T. asiatica* (DNA)	Japan	83, 84
50	2010	Japanese/60/M	*T. asiatica* (DNA)	Japan	83

(*Continued to the next page*)

Table 2. (*Continued from the previous page*) Demographic and clinical data for taeniasis reported in Japan (1990-2011)

Case No.	Year	Patient (Nationality /Age/Sex)	Etiologic agent (diagnostic criteria)	Presumed locality of infection	References
51	2010	Japanese/39/F	*T. asiatica* (DNA)	Japan	83, 84
52	2010	Japanese/24/F	*T. asiatica* (DNA)	Japan	83
53	2010	Japanese/31/M	*T. solium* (endoscopy/DNA) with NCC	India	This study
54	2010	Japanese/39/M	*T. asiatica* (DNA)	Japan	84
55	2010	Japanese/56/M	*T. saginata* (DNA)	Thailand	This study
56	2010	Japanese/26/F	*T. asiatica* (DNA)	Japan	84
57	2010	Japanese/43/F	*T. asiatica* (DNA)	Japan	84
58	2010	Filipino/31/F	*T. asiatica* (DNA)	Philippines	This study
59	2011	Japanese/46/M	*T. saginata* (DNA)	Malaysia	This study
60	2011	Japanese/35/M	*T. saginata* (DNA)	Mali	This study
61	2011	Japanese/52/M	*T. saginata* (DNA)	Thailand	This study
62	2011	Japanese/24/F	*T. saginata* (DNA)	Indonesia (Bali)	This study
63	2011	Japanese/41/M	*T. saginata* (DNA)	Senegal	This study
64	2011	Thai/21/M	*T. solium* (DNA)	Thailand	This study
65	2011	Japanese/33/M	*T. saginata* (DNA)	Sudan	This study
66	2011	Japanese/54/M	*T. asiatica* (DNA)	Japan	This study
67	2011	Japanese/38/M	*T. asiatica* (DNA)	Japan	This study
68	2011	Ethiopian/24/F	*T. saginata* (DNA)	Ethiopia	This study
69	2011	Japanese/12/M	*T. asiatica* (DNA)	Japan	This study
70	2011	Japanese/54/M	*T. asiatica* (DNA)	Japan	This study
71	2011	Japanese/42/F	*T. saginata* (DNA)	France	This study
72	2011	Ethiopian/26/F	*T. saginata* (DNA)	Ethiopia	This study
73	2011	Japanese/41/F	*T. asiatica* (DNA)	Japan	This study

serologic, and histopathologic examinations. In our department, molecular identification of the etiologic agents is routinely performed, if surgically removed materials are available [46-48]. Indeed, the usefulness of molecular methods for diagnosing the causative agents has successfully been demonstrated by the identification of 2 genotypes of *T. solium* cysticercus as well as confirmation of the agents in paraffin-embedded sections [24,25,28,31,33-35,37,40]. In addition, the localities where the patients were infected can also be inferred based on the DNA sequences of the causative agents [32,49].

In SCC, X-ray examinations have revealed the presence of rod-like, scattered, calcified lesions in the soft tissues of the extremities (Fig. 1F, G; Fig. 2D, F). These calcified cysts have histopathologically been confirmed to be *T. solium* in cases 16-17 [42], 26 [7], 52 [36], and 44 [27,28] (Fig. 2A, C, E).

Two types of *T. solium* cysticercus, cellulose- and racemose-types, are known to exist. The cellulose-type cysticercus is characterized by a single bladder measuring 3 to 18 mm in diameter with an invaginated scolex and primarily found in the cerebral parenchyma and musculature. The racemose-type presents as large multilobulated cystic lesions lacking a scolex and appears to prefer the cisternal and ventricular systems or subarachnoid space [2]. Indeed, the racemose-type cysticercus is frequently found in the subarachnoidal spaces as multilobulated lesions (Fig. 1C, D). Although cysticercosis due to racemose-type *T. solium* cysticercus is relatively rare, 8 cases have been documented in Japan in cases 8 [50,51], 15 [52], 18 [53], 19 [54], 39 [21], 50 [34], 61, and 62 [40] (Table 1; Fig. 1C, D; Fig. 2C). Of these, mitochondrial DNA analysis using histopathologic sections revealed that etiologic *T. solium* was the Asian genotype in 3 cases, 50 [32], 61, and 62 [40], and American/African genotype in case 50 [34] (Table 1). The racemose-type cysticercus is considered to be an aberrant, multilobular, non-viable *T. solium* cysticercus, possibly the degenerated form of a cysticercus in the basal subarachnoid space. Molecular analysis using formalin-fixed and paraffin-embedded histopathologic specimens has proved that the racemose-type cysticercus is *T. solium* in cases 50 [34], 61, and 62 [40].

Most of the cysticercosis cases in Japan are imported cases, meaning that the patients either lived in or visited countries where cysticercosis and taeniasis are still endemic, and where they are presumed to have been exposed to *T. solium* eggs. However, 13 cases have suggested that infection occurred within Japan (cases 1 [55], 8 [50,51], 10 [56], 12 [57], 20 [54], 21 [58], 22 [59], 23 [60], 28 [9], 40 [22], 52 [36], 53, and 62 [40]. NCC was diagnosed by imaging findings (Fig. 1), serology, histopa-

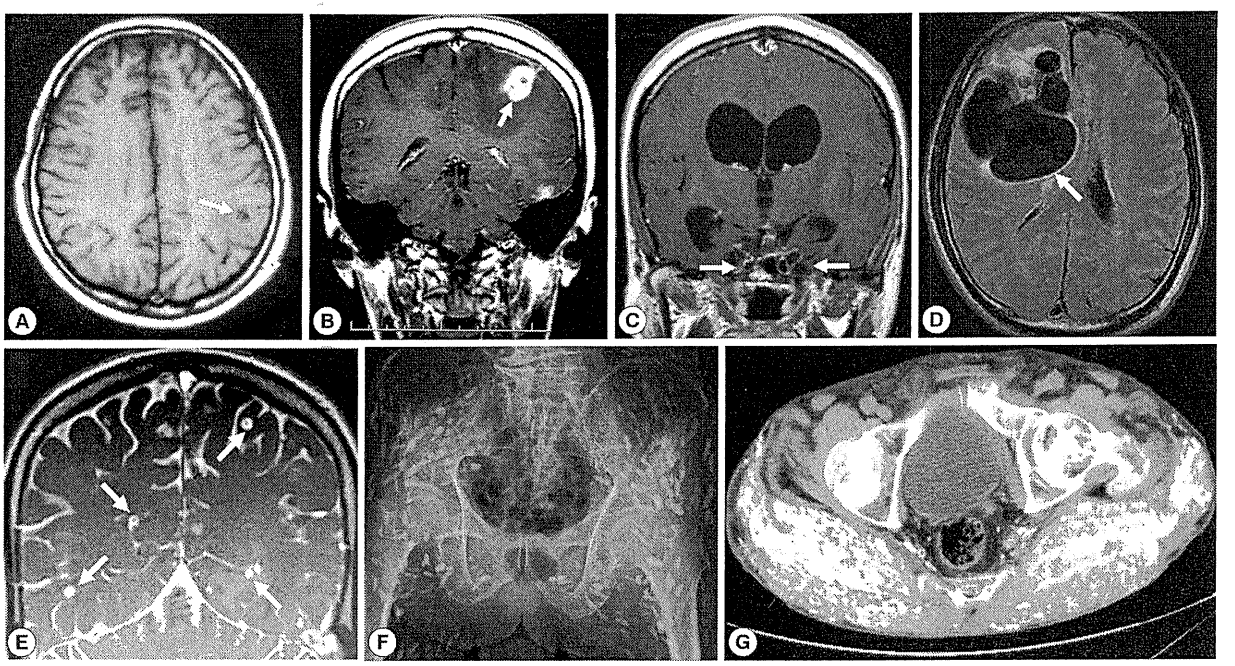

Fig. 1. Imaging findings of selected cysticercosis cases. (A) plain CT image showing a solitary lesion at the left occipitoparietal area (case 48 [32], courtesy of Prof. H. Matsuoka). (B) MRI showing one of multiple cystic lesions in the left frontal and temporal lobes (case 49 [33]). (C) MRI showing a rasemose-type lesion at the basal cistern (case 50 [34], courtesy of Dr. T. Oda). (D) MRI FLAIR findings showing a giant and multilobulated mass in the subarachnoidal spaces of the right frontal lobe (case 62 [40], courtesy of Dr. S. Shiiki). (E) Cisterography showing multiple cysts in the brain (case 60, courtesy of Prof. A. Chiba). (F) X-ray findings showing typical rice grain calcifications in the muscles of buttocks and lower extremity (case 44 [27, 28], courtesy of Dr. T. Nagase). (G) CT findings showing numerous calcified cysts in muscles of the of the buttocks (case 52 [36], courtesy of Dr. M. Tsuda).

thology (Fig. 2A, C, D, E), and molecular analysis.

Taeniasis

Table 2 shows 73 clinical taeniasis case reports that have been published in journals between 1990 and 2011 and diagnosed by our department between 2007 and 2011. In addition to these, 26 cases have been reported [61,62]. The most commonly encountered taeniasis cases were *T. saginata* infections and 48 cases (65.8%) have been confirmed to date (Table 2). Of these 48 cases, 45 were imported cases [63-78]. Although the route of infection is unknown, the possibility also exists that 4 of these cases may be attributable to domestic infections; cases 4 [65], 6 [66], 19 [73], and 22 [73]. *T. solium* taeniasis is extremely rare in Japan and only 1 case was reported in Okinawa in 1988 [79]. However, taeniasis solium cases with either NCC, SCC, or ocular cysticercosis have been confirmed, and all these were imported in cases 29 [10], 58 [38], 60, and 65 (Table 1) and cases 17 [10], 41, 53, and 64 (Table 2). Taeniasis caused by *T. asiatica* has been also recently successively confirmed in Japan and this will be discussed in the following chapter.

Taeniasis is usually diagnosed based on proglottid morphology. However, since *T. saginata*, *T. solium*, and *T. asiatica* are all morphologically similar, it is not always possible to accurately differentiate them. As a result, more reliable molecular diagnoses are currently employed to differentiate between taeniasis infections in our department [46-48]. Most recently, *T. solium* tapeworms have been observed in the small intestine using capsule endoscopy in cases 41 [23] and 53.

CURRENT STATUS OF *T. ASIATICA* INFECTION IN JAPAN

Although *T. asiatica* was not previously considered to occur in Japan [5], retrospective molecular analyses of proglottids revealed that 2 *T. asiatica* infections occurred in Tottori Prefecture on Honshu Island, Japan, in 1968 and 1996 [6]. Unfortunately, it is unknown whether the 2 Japanese cases were domestic infections or imported cases. As the number of Japanese travelers visiting Asian countries has increased, so too has the number of people from other Asian countries visiting Japan. This

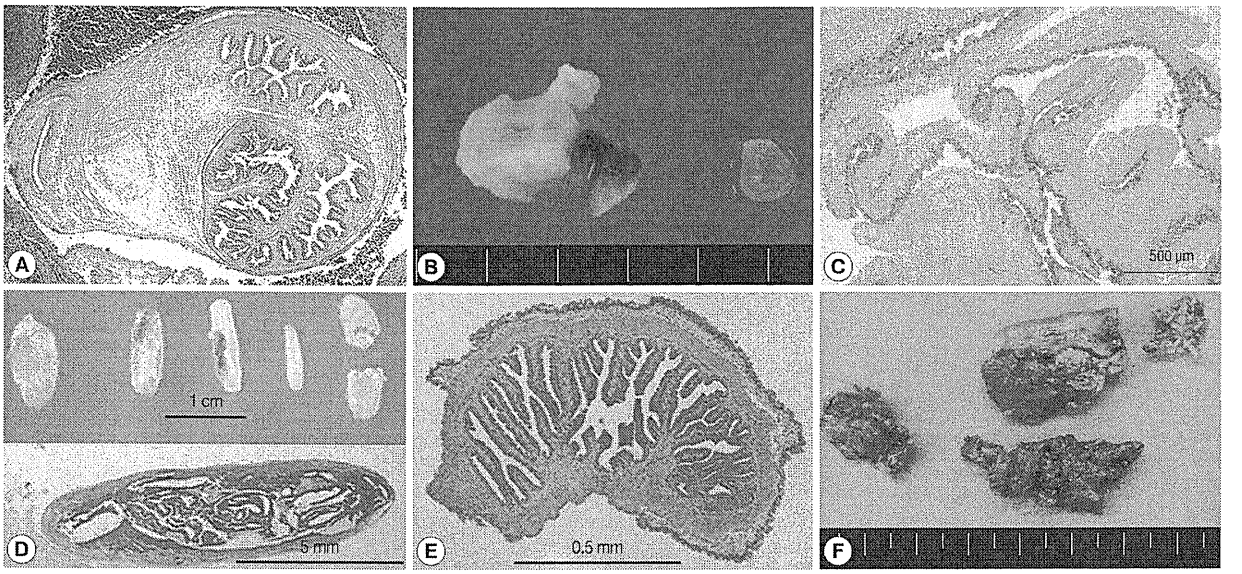

Fig. 2. Histopathologic findings of cystic lesions from cysticercosis patients. (A) A cellulose-type cysticercus characterized by rabyrinth-like structure (case 40 [22], courtesy of Dr. S. Matsunaga). (B) and (E) A resected lesion and a cellulose-type cysticercus (case 48 [32], courtesy of Prof. H. Matsuoka). (C) Racemose-type cysticercus characterized by complicated cystic walls (case 62 [40], courtesy of Dr. S. Shiiki). (D) SCC showing typical rice grain calcifications in the muscles of buttocks and lower extremity and the section of the calcified lesion (case 44 [27, 28], courtesy of Dr. T. Nagase). (F) Surgically removed calcified lesions (case 52 [36], courtesy of Dr. Tsuda). Sections (A, C , D, and E) were stained with hematoxylin and eosin.

may mean that the likelihood of encountering cases of imported *T. asiatica* is increasing. Surprisingly, from June 2010 to December 2011, an increasing number of human cases with taeniasis have been diagnosed in the Kanto region, including Tokyo and the neighboring 5 prefectures (Gumma, Tochigi, Saitama, Chiba, and Kanagawa) in central Honshu [80-84]. Of 31 taeniasis cases, 20 were attributed to *T. asiatica*. *Taenia asiatica* tapeworms were identified based on nucleotide sequence analysis of the mitochondrial cytochrome *c* oxidase subunit 1 gene [25] and allelic analysis of the 2 nuclear genes for elongation factor 1-α and ezrin-radixin-moesin-like protein genes [85].

Nineteen out of 20 patients infected with *T. asiatica* were Japanese nationals residing in the Kanto area and 1 was a Filipino woman living in same area (Tochigi). Fifteen patients stated that they frequently ate raw pig liver (*sashimi*). Sixteen had never been overseas or, if they had undertaken any international travel, they traveled to countries where *T. asiatica* is not endemic. The infection in the Filipino woman who has returned to the Philippines several times was also considered to have been occurred in Japan.

The occurrence of taeniasis due to *T. asiatica* infection is thus considered to have occurred within Japan by the following reasons: i) most of the patients had never been overseas or traveled to areas where *T. asiatica* is not endemic, ii) most patients had histories of eating raw pig liver, iii) based on interviews with patients and meat inspectors, pigs that had been produced and slaughtered in the Kanto region were strongly suspected to be possible sources of infection, iv) although Japan imports pork from Canada, Mexico, and Europe, no raw pig liver is imported from these countries. At present, the reasons why *T. asiatica* infections successively occurred in the Kanto region, a region within which the disease was not reported previously, have not yet been satisfactorily clarified. Considering that patients have occurred now, it is possible that the workers and pigs on farms in the Kanto region currently constitute the *T. asiatica* reservoirs responsible for these infections. We have been investigating the prevalence of *T. asiatica* metacestodes in pigs from these farms in collaboration with local meat inspection centers. In addition, we have also disseminated information describing precautions against *T. asiatica* infections in Infectious Agents Surveillance Reports (http://idsc.nih.go.jp/iasr/ 32/374/kj3741.html) published by the Infectious Diseases Information Center at the National Institute of Infectious Diseases [80-84].

CONCLUSIONS

It is expected that cysticercosis and taeniasis will primarily be detected as imported cases with the increasing numbers of Japanese travelers to foreign countries where these diseases are endemic or visitors from these areas increase. The occurrence of human infections due to *T. asiatica* is currently restricted to the Kanto region in Japan, and the origins of infection have not yet been clarified. Thus, further occurrence of the disease is likely to occur, medical practitioners should be aware of the importance accurately identifying the causative agent responsible for infection.

ACKNOWLEDGMENTS

The author thanks Prof. Keeseon S. Eom and Prof. Jong-Yil Chai for their initiation to submit a review paper. The author also thanks Prof. H. Matsuoka, Prof. A. Chiba, Drs. T. Oda, S. Shiiki, T. Nagase, M. Tsuda, and S. Matsunaga, for providing imaging pictures and pathology specimens. Drs. Y. Morishima and H. Sugiyama are thanked for their valuable discussions of clinical cases, and M. Muto is also acknowledged for her technical assistance with molecular and serologic examinations of cysticercosis and taeniasis cases. The study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare, Japan (H20-22-Shinko-Ippan-016 and H23-Shinko-Ippan-014) and from the Japan Society for the Promotion of Science (23650602).

REFERENCES

1. Garcia HH, Gonzalez AE, Evans CA, Gilman RH. Cysticercosis Working Group in Peru. *Taenia solium* cystericercosis. Lancet 2003; 362: 547-556.
2. White AC, Jr. Neurocysticercosis: Updates on epidemiology, pathogenesis, diagnosis, and management. Ann Rev Med 2000; 51: 187-206.
3. Araki T. Current trend of cerebral cysticercosis in Japan. Clin Parasitol 1994; 5: 12-24 (in Japanese).
4. Nishiyama T, Araki T. Cysticercosis cellulosae: Clinical features and epidemiology. In Otsuru M, Kamegai S, Hayashi S eds. Progress of Medical Parasitology in Japan. Tokyo, Japan. Meguro Parasitological Museum, 2003; 8: 281-292.
5. Yamasaki H, Sako Y, Nakao M, Nakaya K, Ito A. Research on cysticercosis and taeniasis in Japan. In Ito A, Wen H, Yamasaki H eds, Taeniasis/Cysticercosis and Echinococcosis in Asia, Asian Parasitology Vol. 2, p. 6-36, Federation of Asian Parasitologists, 2005.
6. Eom KS, Jeon HK, Rim HJ. Geographical distribution of *Taenia asiatica* and related species. Korean J Parasitol 2009; 47: S115-S124.
7. Okabe K, Tsunoda M, Irie H, Koike H, Yoshino Y, Tsuji M. A case of cerebral cysticercosis with facial spasm showing a change in MRI abnormalities in a short time course. J Kyorin Med Soc 1997; 28: 175-179 (in Japanese).
8. Niijima K. MRI of cerebral cysticercosis. Shinkei Naika (Neurol Med) 1998; 48: 202-203 (in Japanese).
9. Matsushima H, Hatamochi A, Shinkai H, Shimizu M, Hatsushika R. A case of subcutaneous cysticercosis. J Dermatol 1998; 25: 438-442.
10. Okada S, Kikuchi S, Takeda A, Ootani K, Saiga T, Kobayashi M, Niimura M, Yamanouchi N, Kodama K, Sato T. A case of neurocysticercosis successfully treated with praziquantel. Seishinka Chiryogaku 1998; 13: 345-351 (in Japanese).
11. Yamakawa Y, Tashima T. Intraventricular cysticercosis diagnosed by histology of small fragment floating in the shunt tube. No Shinkei Geka Sokuho 1998; 8: 833-837 (in Japanese).
12. Ohsaki Y, Matsumoto A, Miyamoto K, Kondoh N, Araki T, Ito A, Kikuchi K. Neurocysticercosis without detectable specific antibody. Intern Med (Tokyo) 1999; 38: 67-70.
13. Hatano T, Tsukahara T, Araki K, Kawakami O, Goto K, Okamoto E. A case of neurocysticercosis with multiple intraparenchymal and intraventricular cysts. Neurol Surg 1999, 27: 335-339 (in Japanese).
14. Ito A, Nakao M, Ito Y, Yuzawa I, Morishima H, Kawano N, Fujii K. Neurocysticercosis case with a single cyst in the brain showing dramatic drop in specific antibody titers within 1 year after curative surgical resection. Parasitol Int 1999; 48: 95-99.
15. Yuzawa I, Kawano N, Suzuki S, Fujii K, Ito Y. A case of solitary cerebral cysticercosis. Jpn J Neurosurg 2000; 9: 364-369 (in Japanese).
16. Miura H, Itoh Y, Kozuka T. A case of subcutaneous cysticercosis (*Cysticercus cellulosae cutis*). J Am Acad Dermatol 2000; 43: 538-540.
17. Sugiyama M, Okada T, Higuchi H, Yabe Y, Kobayashi N, Teramoto A. A case of neurocysticercosis presenting as focal seizure. Neurol Surg 2000; 28: 807-810 (in Japanese).
18. Kataoka H, Yamada T, Tsumura T, Kawamura H, Takenaka H, Maeno T, Mano T, Takahashi T. A case of presumed ocular cysticercosis. Ganka Rinsho Iho 2001; 95: 605-606 (in Japanese).
19. Matsuda M, Shimizu K, Shimizu Y, Hattori T, Tabata K. A case of neurocysticercosis with versive seizures as an initial symptom. Intern Med 2001; 87: 405-407 (in Japanese).
20. Miyagami M. Cerebral imaging diagnosis CT, MRI. Modern Physician,2001; 21: 1576-1581 (in Japanese).
21. Nakajima M, Tashima K, Hirano T, Nakamura-Uchiyama F, Nawa Y, Uchino M. A case of neurocysticercosis suggestive of a reinfection, 20 years after the initial onset. Clinica Neurol 2002; 42: 18-23 (in Japanese).
22. Matsunaga S, Asada H, Shuto T, Hamada K, Inomori S, Kawamu-

ra S, Hamada A, Okuzawa E. A case of solitary neurocysticercosis of unknown transmission route. Neurol Surg 2002; 30: 1223-1228 (in Japanese).
23. Mori Y, Kobayashi T, Kida Y. A case of neurocysticercosis removed successfully surgically. Jpn J Neurosurg (Tokyo) 2003; 12: 191-195 (in Japanese).
24. Yamasaki H, Ito A, Matsunaga S, Yamamura K, Chang CC, Kawamura S. A case of neurocysticercosis caused by *Taenia solium* Asian genotype confirmed by mitochondrial gene analysis of paraffin-embedded specimen. Clin Parasitol 2003; 14: 77-80 (in Japanese).
25. Yamasaki H, Matsunaga S, Yamamura K, Chang CC, Kawamura S, Sako Y, Nakao M, Nakaya K, Ito A. Solitary neurocysticercosis caused by Asian genotype of *Taenia solium* confirmed by mitochondrial DNA analysis. J Clin Microbiol 2004; 42: 3891-3893.
26. Harada Y, Naoi N, Nawa Y, Harada K. A case of zoonotic infection by *Cysticercus cellulosae*. Jpn J Clin Ophthalmol 2004; 58: 1985-1988 (in Japanese).
27. Nagase T, Kiyoshige Y, Suzuki M. A case of obsolete systemic cysticercosis cellulosae. Clin Parasitol 2004; 15: 24-26 (in Japanese).
28. Yamasaki H, Nagase T, Kiyoshige Y, Suzuki M, Nakaya K, Itoh Y, Sako Y, Nakao M, Ito A. A case of intramuscular cysticercosis diagnosed definitively by mitochondrial DNA analysis of extremely calcified cysts. Parasitol Int 2006; 55: 127-130.
29. Matsushita T, Tanaka C, Sakoh K, Mizobuchi M, Nihei A, Abe T, Seo Y, Murakami N. Neurocysticercosis - case report. Hokkaido Noshinkei Shikkan Inst Journal 2005; 15: 35-39 (in Japanese).
30. Matsumoto L, Ubano M, Uesaka Y, Kunimoto M, Kawanaka M. A case of neurocysticercosis diagnosed with positron emission tomography (PET). Shinkei Naika (Neurol Med) 2005; 63: 473-476 (in Japanese).
31. Yamasaki H, Nakao M, Sako Y, Nakaya K, Ito A. Molecular identification of *Taenia solium* cysticercus genotype in the histopathological specimens. Southeast Asian J Trop Med Public Health, 2005; 36(suppl 4): 131-134.
32. Matsuoka H, Gomi H, Kanai N, Gomi A, Kanda M, Yamasaki H, Sako Y, Ito A. A case of solitary neurocysticercosis lacking elevation of specific antibodies. Clin Parasitol 2006; 17: 102-106 (in Japanese).
33. Yamasaki H, Sako Y, Nakao M, Ito A, Nakaya K. *Taenia solium* cysticercosis cases diagnosed by mitochondrial DNA analysis of paraffin-embedded specimens. Clin Parasitol 2006; 17: 134-137 (in Japanese).
34. Oda T, Kikuchi B, Hoshino T, Koide A, Yoshimura J, Nishiyama K, Mori H. A case of hydrocephalus with stalactitic change of ventricular wall. Niigata Med J 2006; 120: 115 (in Japanese).
35. Ishikawa E, Komatsu Y, Kikuchi K, Yamasaki H, Kimura H, Osuka S, Tsurubuchi T, Ito A, Matsumura A. Neurocysticercosis as solitary parenchymal lesion confirmed by mitochondrial deoxyribonucleic acis sequence analysis - case report. Neurol Med Chir 2007; 47: 40-44.
36. Tsuda M, Mine R, Kiyuna M, Yamasaki H, Ito A. A case of subcutaneous cysticercosis with multiple calcified cysts in the gluteal region. J Jpn Plastic Reconst Surg 2007; 27: 381-385 (in Japanese).
37. Maeda T, Fujii T, Odawara T, Iwamoto A, Sako Y, Ito A, Yamasaki H. A reactivation case of neurocysticercosis with epithelial granuloma suspected by serology and confirmed by mitochondrial DNA analysis. Clin Parasitol 2008; 19: 150-152 (in Japanese).
38. Masuda T, Yamaji H, Shiragami C, Fukuda K, Ogaki S, Harada M, Kagei N, Shiraga F. A case of intraocular cysticercosis treated by vitreous surgery. Jpn J Clin Ophthalmol 2009; 63: 303-306 (in Japanese).
39. Komuro T, Okamoto S, Nitta T. A case of neurocysticercosis. No Shinkei Geka Sokuho 2009; 19: 1072-1076 (in Japanese).
40. Yamasaki H, Sugiyama H, Morishima Y, Ohmae H, Shiiki S, Okuyama K, Kunishima F. A case of neurocysticercosis caused by racemose-type *Taenia solium* cysticercus. Clin Parasitol 2010; 21: 29-32 (in Japanese).
41. Sato T, Kunishi K, Kameyama A, Takano T. Ota N. A case of cysticercosis cellulosae hominis. Inter Med 1991; 68: 190-192 (in Japanese).
42. Kuboyama K, Oku K, Seto T, Higasa S, Kimoto K, Akagi K, Iseki M. Radiological findings in two cases of cerebral cysticercosis. Clin Parasitol 1994; 5: 153-156 (in Japanese).
43. Hatsushika R, Umemura S, Ito J, Okino T. A case study of human infection with *Cysticercus cellulosae* (Cestoda: Taeniidae) found in Okinawa Prefecture, Japan. Kawasaki Med J 1996; 22: 81-87.
44. Hasegawa H, Bitoh S, Koshino K, Obashi J, Yamamoto H. Intramedullary spinal cysticercosis. A case report. Sekitsui Sekizui (Vertebra and Spinal cord) 1991; 4: 337-341 (in Japanese).
45. Kajiwara N, Muramatsu R, Goto H, Usui M. A case of intraocular cysticercosis. J Eye (Atarashii Ganka) 1993; 10: 119-122 (in Japanese).
46. Yamasaki H, Nakao M, Sako Y, Nakaya K, Sato MO, Mamuti W, Okamoto M, Ito A. DNA differential diagnosis of human taeniid cestodes by base excision sequence scanning thymine-base reader analysis with mitochondrial genes. J Clin Microbiol 2002; 40: 3818-3821.
47. Yamasaki H, Allan JC, Sato MO, Nakao M, Sako Y, Nakaya K, Qiu DC, Mamuti W, Craig PS, Ito A. DNA differential diagnosis of taeniasis/cysticercosis by multiplex PCR. J Clin Microbiol 2004; 42: 548-553.
48. Yamasaki H, Nakao M, Sako Y, Nakaya K, Sato MO, Ito A. Mitochondrial DNA diagnosis for taeniasis and cysticercosis. Parasitol Int 2006; 55(suppl): S81-S85.
49. Yanagida T, Yuzawa I, Joshi DD, Sako Y, Nakao M, Nakaya K, Kawano N, Oka H, Fujii K, Ito A. Neurocysticercosis: Assessing where the infection was acquired from. J Travel Med 2010; 17: 206-208.
50. Shimamoto Y, Sugiyama E, Inaba M, Shinoda J, Shimazaki K, Yamada F. Cerebral cysticercosis treated with praziquantel - a case report. Neurol Surg 1994; 46: 381-386 (in Japanese).
51. Sugiyama E, Shimamoto Y, Yamada F, Inaba M. A case of cerebral cysticercosis (cysticercosis racemosus). Clin Parasitol 1992; 3: 146-148 (in Japanese).

52. Yawata Y, Kamata Y, Nagai C, Suzuki K, Shibata Y, Ajitsu S, Sano R, Takenaka K, Kamii H, Watanabe T. Diabetic come without preceeding thirst sensation in a case of cerebral cysticercosis. Yamagata Med J 1993; 11: 79-84 (in Japanese).
53. Yamasaki H, Araki K, Aoki T. Parasitic diseases examined during the past 16 years in the Department of Parasitology, Juntendo University School of Medicine. Juntendo Med J 1994; 40: 262-279 (in Japanese).
54. Takeshita I, Li HZ, Imamoto N, Cao YP, Gou CF, Liu DQ, Piao HZ, Fukui M. Unusual manifestation of cerebral cysticercosis. Fukuoka Med J 1994; 85: 29-34 (in Japanese).
55. Terada K, Seno K, Uetsuhara K, Asakura T. A case of cerebral cysticercosis: Cyst growth is confirmed by CT scan during 6 years of follow-up. Neurol Surg 1990; 18: 391-395 (in Japanese).
56. Ohnishi K, Murata M, Nakane M, Takemura N, Tsuchida T, Nakamura T. Cerebral cysticercosis. Int Med 1993; 32: 569-573.
57. Miyake H, Takahashi K, Tsuji M, Nagasawa S, Ohta T, Araki T. A surgical case of solitary cerebral cysticercosis. Neurol Surg 1993; 21: 561-565 (in Japanese).
58. Endo K, Hirayama K, Hida C, Tsukamoto T, Yamamoto T. Domestic infection of neurocysticercosis in a Japanese woman, who had not traveled overseas. Clin Neurol 1995; 35: 408-413 (in Japanese).
59. Nagayama M, Shinohara Y, Nagakura K, Izumi Y, Takagi S. Distinctive serial magnetic resonance changes in a young woman with rapidly evolved neurocysticercosis, with positron emission tomography results. J Neuroimag 1996; 6: 198-201.
60. Morioka T, Yamamoto T, Nishio S, Takeshita I, Imamoto N, Fukui M. Magnetoencephalographic features in neurocysticercosis. Surg Neurol 1995; 45: 176-182.
61. Shiota T, Yamada M, Uchikawa R, Tegoshi T, Yoshida Y, Arizono N. Epidemiological trend on diphyllobothriasis latum/nihonkaiense and taeniasis saginatus in Kyoto. Clin Parasitol 2003; 14: 81-83 (in Japanese).
62. Komura K, Hamada A, Okuzawa E. Parasitosis treated at Yokohama Rosai Hospital 2000-2008. Clin Parasitol 2008; 19: 103-105.
63. Okumura Y, Nakazawa S, Yoshino S, Yamao K, Inui K, Yamachika H, Arakawa A, Furuta T, Kishi K, Doai K, Toda M, Yamagishi S, Wakabayashi T, Suzui N, Watanabe K, Yamachika R, Asakura N, Okushima K, Nagase K. A case of taeniasis saginata detected by intestino-fiberscopical examination. Clin Parasitol 1990; 1: 112-114 (in Japanese).
64. Suzuki T, Haruma K, Shimamoto F, Tsuda T, Toyoshima H, Yoshihara M, Sumii K, Kajiyama G, Tsuji M, Sakamoto K, Kawamura M. A case of taeniasis saginata co-infected with clonorchiasis sinensis diagnosed by duodeno-fiberscope. Cho Shikkan no Rinsho 1990; 3: 30-34 (in Japanese).
65. Ohnishi K, Murata M. Gastrografin treatment for taeniasis saginata. Clin Parasitol 1990; 1: 115 (in Japanese).
66. Sekiya T, Kimura K, Sakuma M, Mishiku Y, Asakura Y, Kawamura I, Uchida A, Iwata T. A caseof taeniasis saginata. Shonika Rinsho 1992; 45: 585-588 (in Japanese).
67. Nakao A, Sakagami K, Hirohata T, Hara M, Obayashi N, Mitsuoka S, Uda M. A case of taeniasis saginata. Hiroshima Med J 1996; 49: 1369-1370 (in Japanese).
68. Tomizawa I, Takizawa Y, Sakamoto Y. A case of *Taenia saginata* entered our hospital. Sapporo City Hosp J 1996; 56: 45-47 (in Japanese).
69. Nihashi J, Shibata Y, Kajimura M, Ito G, Hanai H, Kaneko E, Terada M. A case of gastrografin-resistant tapeworm infection. Clin Parasitol 1996; 7: 131-134 (in Japanese).
70. Ohgami K, Ozasa S, Takahashi K. A case of taeniasis saginata. Teishin Igaku 1997; 49: 654-655 (in Japanese).
71. Nakayasu S, Kawabata M, Ishihara O, Matsuda Y, Ishii A, Terada M. A case of taeniasis saginata detected by mass-screening of the colon cancer, and treated with gastrografin method and praziquantel. Clin Parasitol 1998; 9: 16-18 (in Japanese).
72. Shiono Y, Hata K, Aosai F, Norose K, Yano A, Kawashima K, Yamada M. A case of taeniasis saginata diagnosed by spontaneously expulsed segments at labor. Clin Parasitol 2001; 12: 43-44 (in Japanese).
73. Ishida T, Araki T, Nishiyama T, Hirata I, Katsu K. Clinical study of diphyllobothriasis nihonkaiense and taeniasis saginata in Shinsei Hospital - special reference to treatment. Clin Parasitol 2001; 12: 48-53 (in Japanese).
74. Yoshimura R, Harada N, Sadamoto Y, Takahashi M, Kubokawa K, Ito K, Tanaka M, Toyoda T, Yamaguchi Y, Ohkumi A, Miyahara M, Nawada S. Two cases of taeniasis saginata. Rinsho to Kenkyu 2002; 79: 111-114 (in Japanese).
75. Yamamoto K, Kawahara K, Shimidzu N, Tanioka K, Takatoo T, Saida Y. A report of a case with taeniasis saginata. Iwata City Sogo Hosp J 2002; 4: 8-11 (in Japanese).
76. Nishimura Y, Yamaguchi S, Sahara T, Nakazaki Y, Tsuruta S, Fujimori I, Kino H, Yamasaki H, Nakao M, Ito A, Kuramochi T. Two cases of intestinal cestode infections diagnosed by genetic analysis. Clin Parasitol 2007; 18: 46-48 (in Japanese).
77. Miyamoto S, Komatsu N, Asai Y, Kurihara R, Asaoka A, Kawamura H, Arakawa Y, Takahashi K. A case of taeniasis saginata treated using gastrografin. Nippon Univ Med J 2003; 62: 229-231 (in Japanese).
78. Oyama N, Shiozaki H, Tahara T, Takada K. A case of taeniasis saginata treated by endoscopy using gastrografin. Progr Digest Endosc 2007; 70: 102-103 (in Japanese).
79. Arakaki T, Hasegawa H, Morishima A, Ikema M,Terukina S, Higashionna A, Kinjyo F, Saito A, Asato R, Toma S. Treatment of *Taenia solium* and *T. saginata* infections with gastrografin. Jpn J Trop Med Hyg 1988; 16: 293-299 (in Japanese).
80. Yamasaki H, Morishima Y, Sugiyama H, Muto M. *Taenia asiatica* infections as an emerging parasitic disease occurring in Kanto district since 2010. IASR 2011, 32: 106-107 (in Japanese).
81. Nakamura-Uchiyama F, Kobayashi K, Iwabuchi S, Ohnishi K. Four cases of taeniasis asiatica infected by eating raw pig liver or cattle liver. IASR 2011; 32: 107-108 (in Japanese).
82. Haruki K, Tamano M, Miyoshi Y, Araki J. A case of *Taenia asiatica* infection. IASR 2011; 32: 108 (in Japanese).

83. Kawai S, Kirinoki M, Chigusa Y, Matsuoka H, Suzuki T. Human cases due to *Taenia asiatica* occurring in Ryomo district of Gumma and Tochigi prefectures. IASR 2011; 32: 109-111 (in Japanese).
84. Yamasaki H, Muto M, Morishima Y, Sugiyama H, Kawanaka M, Nakamura-Uchiyama F, Ohgame M, Kobayashi K, Ohnishi K, Kawai S, Okuyama T, Saito K, Miyahira Y, Yanai H, Matsuoka H, Haruki K, Miyoshi Y, Akao N, Akiyama J, Araki J. Human cases infected with *Taenia asiatica* occurring in Kanto district, 2010. Clin Parasitol 2011; 22: 75-78 (in Japanese).
85. Okamoto M, Nakao M, Blair D, Anantaphruti MT, Waikagul J, Ito A. Evidence of hybridization between *Taenia saginata* and *Taenia asiatica*. Parasitol Int 2010; 59: 70-74.
86. Miyagami M, Satoh K. Diagnosis and treatment for an aged neurocysticercosis patient. Roka to Shikkan 1991; 4: 436-441 (in Japanese).
87. Uyama E, Cho I, Araki T, Osuga K. A case of neurocysticercosis diagnosed with severe headache during staying in Honduras. Zutu Kenkyu Kaishi 1991; 18: 64-66 (in Japanese).
88. Miyashita T, Miyaji A, Nishitani H, Mano K, Kunii O, Miyashita H, Shibuya T, Chen TH. A case of neurocysticercosis with cystic lesions in the lung. Clin Parasitol 1992; 3: 140-142 (in Japanese).
89. Kakizaki T, Kawai S, Takemura K, Tanaka M, Monobe T, Kim E Nagareda T, Kotoh R, Nishiyama T, Araki T. An operated case of neurocysticercosis. Clin Parasitol 1992; 3: 143-145 (in Japanese).
90. Kadotani H, Takatsuka K, Tanaka H, Yoshikawa N, Komatsu T. Cysticercosis. A case report with special reference to CT and MRI findings. Neuol Med (Tokyo) 1992; 36: 399-402 (in Japanese).
91. Kimura M, Sakatani K, Maekura S, Satou T, Furukawa T, Miyazato T, Hashimoto S. A case of cerebral cysticercosis cellulosae hominis. Acta Med Kinki Univ 1993; 18: 155-161.
92. Higashi K, Yamagami T, Satoh G, Shinnou M, Tanaka T, Handa H, Furuta M. Cerebral cysticercosis: a case report. Surg Neurol 1993; 39: 474-478.
93. Oka Y, Fukui K, Shoda D, Abe T, Kumon Y, Sakai S, Torii M. Cerebral cysticercosis manifestating as hydrocephalus - case report. Neurol Med Chir (Tokyo) 1996; 36: 654-658.
94. Yamasaki H, Nakaya K, Nakao M, Sako Y, Ito A. Significance of molecular diagnosis using histopathological specimens in cestode zoonoses. Trop Med Health 2007; 35: 307-321.

J. Parasitol., 98(6), 2012, pp. 1243–1247

VALIDITY OF THE BEAR TAPEWORM *DIPHYLLOBOTHRIUM URSI* (CESTODA: DIPHYLLOBOTHRIIDAE) BASED ON MORPHOLOGICAL AND MOLECULAR MARKERS

Hiroshi Yamasaki, Maki Muto, Minoru Yamada*, Naoki Arizono*, and Robert L. Rausch†

Department of Parasitology, National Institute of Infectious Diseases, Tokyo 162-8640, Japan. *e-mail: hyamasak@nih.go.jp*

ABSTRACT: The bear tapeworm *Diphyllobothrium ursi* is described based upon the morphology of adult tapeworms recovered from the brown bear (*Ursus arctos middendorffi*) and larval plerocercoids found in sockeye salmon (*Oncorhynchus nerka*) from Kodiak Island in Alaska in 1952. However, in 1987 *D. ursi* was synonymized with *Diphyllobothrium dendriticum*, and the taxonomic relationship between both species has not subsequently been revised. In this study mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) sequences of holotype and paratype *D. ursi* specimens that had been preserved in a formalin-acetic acid-alcohol solution since the time the species was initially described approximately 60 yr ago were analyzed. Molecular and phylogenetic analysis of the *cox1* sequences revealed that *D. ursi* is more closely related to *D. dendriticum* than it is to *Diphyllobothrium nihonkaiense* and *Diphyllobothrium latum*. In addition to molecular evidence, differences in the life cycle and ecology of the larval plerocercoids between *D. ursi* and *D. dendriticum* also suggest that *D. ursi* is a distinct species, separate from *D. dendriticum* and *D. nihonkaiense*, and also possibly from *D. latum*.

Although *Diphyllobothrium* Cobbold, 1858, contains at least 38 species (Kamo, 1999; Rausch, 2005), the taxonomic status of several of these species is currently unclear and in need of revision. *Diphyllobothrium ursi* Rausch, 1954 (Diphyllobothriidea; Diphyllobothriidae) was described in 1952 based on adult tapeworms collected from brown bear (*Ursus arctos middendorffi*) and larval plerocercoids obtained from sockeye salmon (*Oncorhynchus nerka*) from Kodiak Island in Alaska (Rausch, 1954). Adult *D. ursi* tapeworms were then found in black bears (*Ursus americanus*) from Alaska (Rausch and Hilliard, 1970) and Quebec, Canada (Frechette, 1978; Juniper, 1978), as well as in humans from British Columbia, Canada (Margolis et al., 1973; Ching, 1984). Subsequently *D. ursi* was synonymized with *Diphyllobothrium dendriticum* (Nitzsch, 1824) (Andersen et al., 1987), but no studies have attempted to resolve the taxonomic and phylogenetic relationships between these species at the DNA level since then.

Congeneric species of *Diphyllobothrium* in North America and the countries bordering the northern Pacific Ocean include *Diphyllobothrium alascense* Rausch et Williamson, 1958 (Rausch and Williamson, 1958), *Diphyllobothrium cordatum* (Leuckart, 1863) (Markowski, 1952), *Diphyllobothrium dalliae* Rausch, 1956 (Rausch, 1956), *D. dendriticum* (Andersen et al., 1987), *Diphyllobothrium ditremum* (Creplin, 1825) (Andersen et al., 1987), *Diphyllobothrium klebanovskii* Muratov and Posokhov, 1988 (Muratov and Posokhov, 1988), *Diphyllobothrium lanceolatum* (Krabbe, 1865) (Markowski, 1952), *Diphyllobothrium latum* (Linnaeus, 1758) (Andersen et al., 1987), *Diphyllobothrium nihonkaiense* Yamane et al., 1986 (Yamane et al., 1986; Wicht et al., 2008), and *D. ursi* (Rausch, 1954). Although *D. klebanovskii* has recently been synonymized with *D. nihonkaiense* based on molecular evidence (Arizono et al., 2009), the phylogenetic relationships among *Diphyllobothrium* species, including *D. alascense*, *D. dalliae*, and *D. ursi*, have not yet been clarified.

Received 7 December 2011; revised 17 May 2012, 31 May 2012; accepted 4 June 2012.

* Department of Medical Zoology, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan.

† Department of Comparative Medicine, University of Washington, Seattle, Washington 98195-7190.

DOI: 10.1645/GE-3063.1

The purpose of the present study was to determine whether *D. ursi* is a valid species or not based on morphological characters and phylogenetic analysis of mitochondrial DNA sequence data from *D. ursi* type specimens. The morphological characteristics of *D. ursi* were also redescribed because micrographs of the proglottids had not been published since the initial description of the species.

MATERIALS AND METHODS

Diphyllobothrium ursi type specimens examined in this study

The holotype and paratype specimens of *D. ursi* that were used in this study were collected from a brown bear in 1952. These specimens had been preserved in formalin-acetic acid-alcohol solution (FAA) over 60 yr, since the time the species was initially described and deposited at the Helminthological Collection of the U.S. National Museum (USNM; currently the U.S. National Parasite Collection, USNPC, slide no. 49355). Parts of holotype and paratype specimens preserved in FAA were sent to Department of Medical Zoology, Kyoto Prefectural University for morphological characterization and molecular analysis (Fig. 1).

Molecular analysis

For molecular analysis, 2 type specimens of *D. ursi* preserved in FAA were embedded in paraffin and sectioned to facilitate extraction of the DNA from the fixed proglottids (Yamasaki et al., 2007). Genomic DNA was then extracted from the 10-μm thick, unstained paraffin-embedded sections using a DEXPAT kit (Takara Bio Inc., Otsu, Japan) according to the manufacturer's instructions. Compared to *Ex Taq*–based DNA polymerase, preliminary results revealed that KOD-FX DNA polymerase (Toyobo, Osaka, Japan) showed higher fidelity, amplification efficiency, and elongation capability. The target DNA fragments were, therefore, amplified using KOD FX DNA polymerase in a reaction volume of 25 μl.

Because DNA was considered to have been degraded by long-term fixation, short overlapping DNA fragments (< 300 bp) of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) were amplified using primer pairs designed based on the *cox1* genes of *D. nihonkaiense* (AB268585) and *D. latum* (AB269325). The PCR protocol consisted of an initial denaturation step of 94 C for 15 min, followed by 35 cycles of 94 C for 30 sec, 58 C for 30 sec, 72 C for 1 min, and a final extension step of 72 C for 5 min. The short fragments amplified by PCR were then tentatively sequenced. The overlapping fragments of *cox1* were re-amplified using 27 primer pairs designed based on the tentative *cox1* sequences of *D. ursi* (Table I). Twenty-seven overlapping fragments were successfully amplified (data not shown), and all of the sequences were then subsequently joined. Samples for DNA sequencing were prepared using an ABI PRISM BigDye Terminator Cycle Sequencing Reaction kit (ver. 3.1, Applied Biosystems Inc., Foster City, California), and the resultant sequence ladders were read with a 3100 Genetic Analyzer and/or 3730 xl DNA Analyzer (Applied Biosystems Inc.).

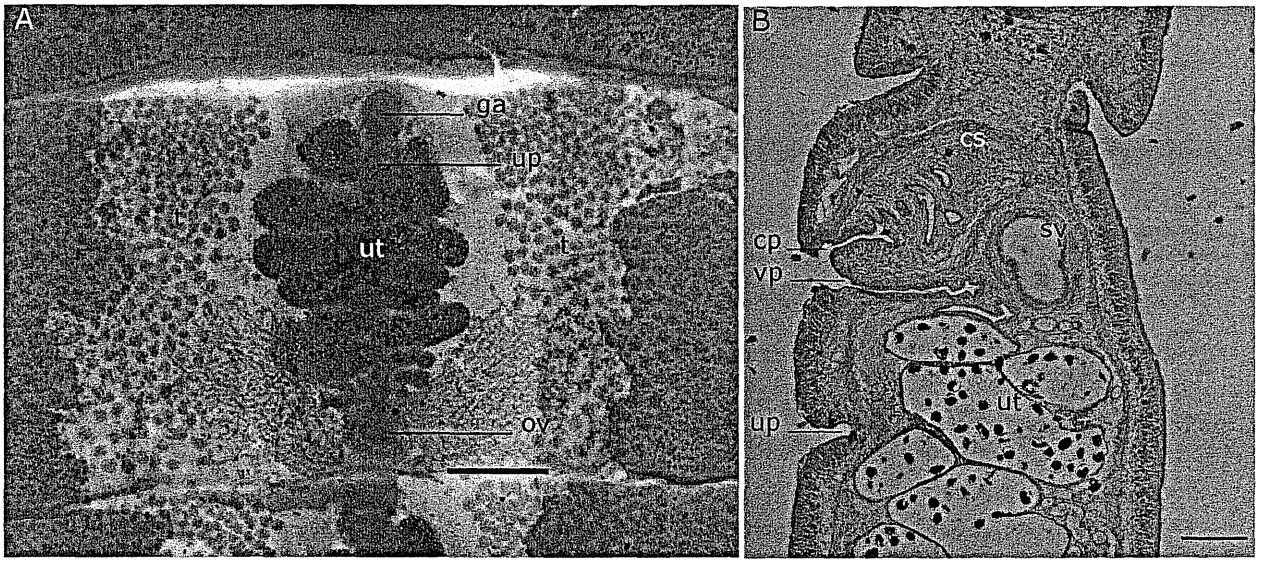

FIGURE 1 The holotype specimen of *Diphyllobothrium ursi* used for morphological characterization. (A) Gravid proglottid stained with acetic acid-carmine. ga, genital atrium; ov, ovary; t, testis; up, uterine pore; ut, uterus. (B) Medial sagittal section of a segment stained with hematoxylin and eosin. Cirrus sac is positioned obliquely to the anterior-posterior axis, and a seminal vesicle is located immediately posterior to the cirrus sac. cp, cirrus pore; cs, cirrus sac; sv, seminal vesicle; up, uterine pore; ut, uteri containing eggs; vp, vaginal pore. Scale bars = 1 mm (A) and 200 μm (B).

Phylogenetic analysis

Phylogenetic analyses were performed using Bayesian inference (BI) and the neighbor-joining (NJ) methods. Phylogenetic trees were constructed using the complete *cox1* sequences (1,566 bp) of 7 taxa of Diphyllobothriidae: *D. ursi* (AB605762, AB605763), *D. dendriticum* (AM412738, AB573182), *D. nihonkaiense* (AB364645, AB268585, AM412599, AM412560, AB544064, AB684623), *D. latum* (AB269325, AB504899, FM209181), *D. ditremum* (FM209182), *Diplogonoporus balaenopterae* (AB355623, AB355628), and *Spirometra erinaceieuropaei* (AB369249). The DNA sequence data for the BI and NJ analyses were converted into NEXUS and FASTA formats using the MEGA program (ver. 5.05). The BI analysis was performed using MrBayes (ver. 3.1.2), and the General Time Reversible model was selected (Ronquist and Huelsenbeck, 2003). Kimura's 2-parameter model (K2P) was selected for the NJ analysis (Kimura, 1980). Phylogenetic trees were rooted with *S. erinaceieuropaei* as an out-group. Clades were assessed by posterior probability and bootstrap resampling (1,000 replicates) of the BI and NJ trees, respectively. Pairwise genetic distances based on the complete *cox1* sequences (1,566 bp) were evaluated by the NJ method with the K2P substitution model (gamma parameter = 1), which is considered suitable for estimating genetic distances when using mitochondrial DNA (Brown et al., 1982).

REDESCRIPTION

Diphyllobothrium ursi

(Fig. 1)

Diagnosis

Genital atrium in anterior half of segment, near anterior margin; uterine pore on mid-line, immediately posterior to genital atrium; posterior portion of uterus markedly coiled, consisting of 5 to 8 loops each side of mid-line; anteriormost loops frequently extending considerably beyond genital pore. Ovary at posterior margin of segment, with 2 distinct lobes connected by narrow reticulated isthmus near center. Testes abundant in each proglottid and disposed in disconnected lateral fields or extending across mid-line, anterior to genital pore. Cirrus pore and vaginal pore in anterior portion in medial sagittal section of gravid proglottid (Fig. 1B). Cirrus pore and vaginal pore surrounded by elevated area of papilla-like protrusions. Uterine pore posterior to genital atrium. Cirrus sac pyriform and relatively large. Seminal vesicles spherical with thick wall, situated immediately posterior to distal end of cirrus sac.

Molecular-phylogenetic relationships between *D. ursi* and related *Diphyllobothrium* species

The nucleotide sequence data of *cox1* for *D. ursi* were deposited in the DDBJ/GenBank database under accession numbers AB605762 (paratype) and AB605763 (holotype). Phylogenetic trees were constructed using the complete *cox1* sequences (1,566 bp) of 7 taxa belonging to the Diphyllobothriidae produced trees with similar topologies. *Diphyllobothrium ursi* formed a monophyletic cluster and was more closely related to *D. dendriticum* than to *D. nihonkaiense* or *D. latum*, even though the posterior probability (0.76) and bootstrap value (63) were relatively low (Fig. 2). The genetic distance between the 2 *D. ursi* individuals was 0.0165, which is considered typical for intraspecific variation. Table II shows the pairwise genetic distances between *D. ursi* and related *Diphyllobothrium* species. The genetic distance between *D. ursi* and *D. dendriticum* ranged from 0.0442 to 0.0570 (mean = 0.0502). The genetic distance between *D. ursi* and *D. nihonkaiense*, and between *D. ursi* and *D. latum*, ranged from 0.0546 to 0.0617 (mean = 0.0580) and from 0.0735 to 0.0887 (mean = 0.0809), respectively.

DISCUSSION

Despite initially being described as a new species in 1954 (Rausch, 1954), *D. ursi* was synonymized with *D. dendriticum* for the following reasons (Andersen et al., 1987). The proglottids of adult *D. ursi* appear to exhibit considerable morphological variation in a variety of hosts; specimens from the brown bear deposited in the USNPC had large, broad segments and were more muscular than those of *D. dendriticum*. Even so, the specimens deposited at the USNPC by Andersen et al. (1987) conform to the description of *D. dendriticum*. However, *D. ursi* can be separated from *D. dendriticum* and *D. ditremum* by several characters, including relatively larger eggs (average 65 × 43 μm), larger number of testis (≤ 1,650), and a strobila exceeding 11 m (Rausch, 1954). As compared with adult *D. ursi*, *D. alascense*, *D. cordatum*, and *D. dalliae* that are distributed in North America

TABLE I. Oligonucleotide primer pairs used to amplify the *cox1* gene of *Diphyllobothrium ursi*.

trnW/F*:	5′-AAGGTCATTTGATGAATACAGA-3′
R185–160:	5′-GTGATTAAAAATTTATAACAGTCTGA-3′
F1–25:	5′-ATGACTAATYTTAAAGTTTTTAGTT-3′
R130–106:	5′-AATTAATCCGTATCATAACACTAAG-3′
F110–135:	5′-GTGTTATGATACGGATTAATTTTGTT-3′
R275–250:	5′-AGGCCAGACAGCAAAGGTATTAAATA-3′
F121–145:	5′-CGGATTAATTTTGTTGAGCCTTATT-3′
R265–241:	5′-ACAAAGGTATTAAATAGTTACCAAA-3′
F160–185:	5′-TCAGACTGTTATAAATTTTTAATCAC-3′
R344–320:	5′-AAAAACAAAATAGAAGGAAATAATA-3′
F310–335:	5′-AGTGCGTGATTATTATTTCCTTCTAT-3′
R475–453:	5′-CCCTAGATAAACCAGCTAAATGT-3′
F415–438:	5′-AGGGATAGTAAGGGTGTTGATTTT-3′
R640–616:	5′-TACGATCAAATAACAACATTGTAAT-3′
F575–600:	5′-CAATTCTTTTGCTTTTAACTATTCCT-3′
R745–721:	5′-GTAAAATTAAAACATATACTTCAGG-3′
F500–524:	5′-GTACCCTTTACACAGCCTTTGTTGA-3′
R640–620:	5′-TACGATCAAATAACAGCATTG-3′
F446–470:	5′-TTTCTTTACATTTAGCTGGTTTATC-3′
R640–620:	5′-TACGATCAAATAACAGCATTG-3′
F600–624:	5′-TGTATTAGCTGCTGCTATTACAATG-3′
R745–721:	5′-GTAAAATTAAGACATACACCTCAGG-3′
F634–655:	5′- GATCGTAAATTTGGTTCAGCTT-3′
R755–735:	5′-CCAAAACCAGGTAAAATTAAA-3′
F720–745:	5′-CCCTGAAGTATATGTTTTAATTTTAC-3′
R905–881:	5′-GCCGTCTTTACATCTAAACCCACTG-3′
F875–899:	5′-TGTTTACAGTGGGTTTAGATGTAAA-3′
R1180–1156:	5′-AAAACACTATTATACTTATATAAGA-3′
F880–905:	5′-ACAGTGGGTTTAGATGTAAAGACGGC-3′
R1060–1035:	5′-CACCCCCGATAGTGAATAACACAATA-3′
F900–925:	5′-GACGGCTGTTTTCTTTAGTTCAGTTA-3′
R1100–1076:	5′-ATATTATCAAGTACACAAGCAGAAA-3′
F1030–1055:	5′-TCATTTATTGTGTTATTCACTATCGG-3′
R1200–1175:	5′-AACTGGCCACCACCAAACAAAAAACA-3′
F1035–1060:	5′-TATTGTGTTATTCACTATCGGGGGTG-3′
R1205–1180:	5′-GTAATAACTGGCCACCACCAAACAAA-3′
F1040–1064:	5′-TGTTATTCACTATCGGGGGTGTTAC-3′
R1160–1135:	5′-TAAGAACCTAGTGACATTACATAATG-3′
F1050–1075:	5′-TATCGGGGGTGTTACGGGTATTATTC-3′
R1250–1225:	5′-ACTATACAATGACATTGTAGTAAATA-3′
F1095–1120:	5′-TAATATTTTGCATGATACTTGGTTTG-3′
R1325–1301:	5′-CACACACGACGRGGTAAACCGCACA-3′
F1180–1205:	5′-TTTGTTTGGTGGTGGCCAGTTATTAC-3′
R1360–1335:	5′-GAATATTAATTCAAGCATAACCTGAC-3′
F1180–1204:	5′-TTTGTTTGGTGGTGGCCAGTTATTA-3′
R1379–1354:	5′-AAGGAACCTATTGACAAAGAATATT-3′
F1220–1244:	5′-ATAAGTATTTACTACAATGCCATTG-3′
R1369–1345:	5′-TTGAGCAAAGAATATTAATTCAAGC-3′
F1305–1328:	5′-CGGTTTACCACGTCGTGTGTGTGT-3′
R1474–1449:	5′-TTGCTGAACTACCATAATAACCTAAA-3′
F1330–1354:	5′-TATGAGTCAGGTTATGCTTGAATTA-3′
R1566–1543:	5′-CTATAAAGCCAACATACTATAATC-3′
F1460–1485:	5′-ATGGTAGTTCAGCAACTTTATTAAAT-3′
trnT/R†:	5′-ATTTACAAAACCAGTATTCTAATTAAA-3′

* Based on the gene for *trnW* of *D. nihonkaiense*.
† Based on the gene for *trnT* of *D. nihonkaiense*.

are morphologically smaller and distinct from *D. ursi* (Rausch and Williamson, 1958; Markowski, 1952; Rausch, 1956).

Rausch and Hilliard (1970) reported that *D. ursi* may be a junior synonym of *Diphyllobothrium gondo* Yamaguti, 1942,

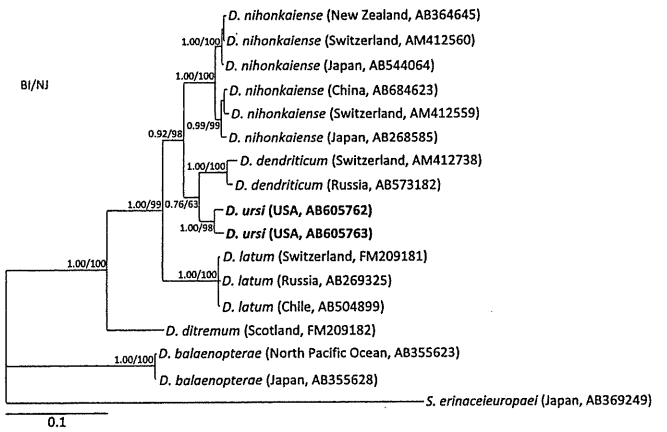

FIGURE 2. Phylogenetic tree inferred using complete *cox1* sequence data (1,566 bp) from *D. ursi* and related *Diphyllobothrium* species by Bayesian inference (BI) and neighbor-joining (NJ) methods. Numbers at nodes indicate posterior probabilities and bootstrap values (1,000 replicates) for the BI and NJ analyses, respectively. Posterior probabilities were estimated based on 1 million generations. Scale bar indicates the number of base substitutions/site. Nucleotide sequence data were obtained from the following accession numbers: *D. ursi* (AB605762, AB605763), *D. dendriticum* (AM412738, AB573182), *D. nihonkaiense* (AB364645, AB268585, AM412599, AM412560, AB544064, AB684623), *D. latum* (AB269325, AB504899, FM209181), *D. ditremum* (FM209182) and *Diplogonoporus balaenopterae* (AB355623, AB355628). *Spirometra erinaceieuropaei* (AB369249) was used as an out-group.

which was collected from the small intestine of a short-finned pilot whale *Globicephala scammoni* (currently *Globicephala macrorhynchus* Gray, 1846, Delphinidae, Cetacea) (Yamaguti, 1942). However, the scolex of adult *D. ursi* is relatively small and muscular, measuring 1.0–1.5 mm in length and 0.75 mm in width; it also has a variable shape and is always spatulate. Conversely, the scolex of adult *D. gondo* (syntype: MPM Coll. No. 22304) deposited at the Meguro Parasitological Museum (MPM) in Tokyo, Japan, is small and wrinkled or funnel-shaped, measuring 0.5–0.6 mm long by 0.3–0.45 mm wide (Yamaguti, 1942; Kamo et al., 1982). Given these marked differences in the adult morphology of *D. ursi* and *D. gondo*, the 2 species are not considered to be synonymous. Adult *D. nihonkaiense* and *D. latum* have spatulated scolices, measuring 2.4–2.8 mm long by 1.2–1.5 mm wide and 1.5–2.5 mm long by 0.8–1.6 mm wide, respectively. Collectively, these morphological features indicate that *D. ursi* is distinct from *D. nihonkaiense* and *D. latum*.

It is difficult to estimate how much genetic distance constitutes inter- or intra-specific variation, particularly since genetic distances vary depending on the target genes and the length of the nucleotide sequences examined (Nakao et al., 2007; Wicht et al., 2010). The genetic distance between *D. latum* and *D. nihonkaiense* calculated using data from 12 mitochondrial protein-coding gene sequences ranged from 0.0438 (cytochrome *b* gene, 1,070 bp) (Wicht et al., 2010) to 0.152 (ATPase subunit 6 gene, 510 bp) (Nakao et al., 2007). The genetic distance between *D. nihonkaiense* and *D. latum* measured using the *cox1* gene sequence data (1,566 bp) was 0.0804 ± 0.0035 (Table II), and the genetic distances between *D. ursi* and *D. dendriticum* was 0.0502. Arizono et al. (2009) reported that the genetic distances of *D. nihonkaiense* vs. *D. dendriticum* and *D. nihonkaiense* vs. *D. latum* estimated based on partial *cox1* sequences (711 bp) were 0.062–

TABLE II. Pairwise genetic distance between *D. ursi* and the related *Diphyllobothrium* species including *Diplogonoporus balaenopterae* and *Spirometra erinaceieuropaei*.

Species	*D. ursi*	*D. dendriticum*	*D. nihonkaiense*	*D. latum*	*D. ditremun*	*D. balaenopterae*	*S. erinaceieuropaei*
D. ursi							
D. dendriticum	0.0502 ± 0.0054						
D. nihonkaiense	0.0580 ± 0.0031	0.0749 ± 0.0036					
D. latum	0.0809 ± 0.0078	0.0767 ± 0.0006	0.0804 ± 0.0035				
D. ditremum	0.1130 ± 0.0001	0.1021 ± 0.0023	0.1118 ± 0.0016	0.1020 ± 0.0000			
D. balaenopterae	0.1718 ± 0.0062	0.1688 ± 0.0026	0.1709 ± 0.0030	0.1473 ± 0.0011	0.1557 ± 0.0013		
S. erinaceieuropaei	0.2310 ± 0.0041	0.2343 ± 0.0016	0.2333 ± 0.0032	0.2316 ± 0.0000	0.2163	0.2156 ± 0.0015	

* Genetic distance (mean ± SD) calculated by the neighbor-joining method using Kimura's 2-parameter model based on 1,566 bp-complete *cox1* sequences.

0.070 and 0.075–0.078, respectively. Although the genetic distances estimated using both partial and complete *cox1* sequences were slightly different, a genetic distance of 0.050 to 0.060 could be considered sufficient for distinguishing between *Diphyllobothrium* species. In this way, *D. ursi* can be separated from *D. dendriticum* and *D. nihonkaiense*, and probably from *D. latum*, at the molecular level. Given that the posterior probabilities and bootstrap values were relatively low, further studies are necessary to examine the relationships between *D. ursi* and *D. dendriticum* that are distributed in North and South America.

The number of *Diphyllobothrium* species that have been described since *Taenia lata* (=synonym of *D. latum*) was described has contributed toward the current confusion regarding the taxonomy, distribution, and host preference of species in this genus (Dick et al., 2001; Scholz et al., 2009). However, *Diphyllobothrium* species can be divided into 3 groups based on their life cycles: (1) freshwater (life cycle is completed in freshwater), (2) anadromous (associated with salmonids migrating from freshwater to the sea), and (3) marine (the entire life cycle occurs in the sea) (Scholz et al., 2009). Within the context of these different life cycles, several interesting differences have been observed in *D. ursi*, *D. dendriticum*, and *D. nihonkaiense*. For example, the plerocercoids of *D. ursi* form cysts on the stomach serosa of sockeye salmon, and the adult tapeworms parasitize bears (Carnivora, Ursidae) and, occasionally, humans as definitive hosts (Rausch, 1954; Hilliard, 1960; Rausch and Hilliard, 1970). Like *D. nihonkaiense*, *D. ursi* belongs to the anadromous group. Conversely, the plerocercoids of *D. dendriticum* are typically found in the viscera and on walls of the body cavity in Arctic chaar (*Salvelinus alpinus*), brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), Dolly varden (*Salvelinus malma*), rainbow trout (*Oncorhynchus mykiss*), and smolts and adult migrants of sockeye salmon in North America (Andersen et al., 1987). The adult tapeworms infect piscivorous birds, especially gulls and pelicans, mammals such as red foxes and bears (Andersen et al., 1987; Rausch and Hilliard, 1970), and, occasionally, humans (Wicht et al., 2007). The plerocercoids of *D. nihonkaiense* have been found in the muscle tissue of masu salmon (*Oncorhynchus masou*), chum salmon (*Oncorhynchus keta*), and pink salmon (*Oncorhynchus gorbuscha*) (Yamane et al., 1986; Ando et al., 2001), with adult tapeworms infecting brown bears and humans (Kamo, 1999).

In addition to the molecular evidence reported here, the differences in the life cycles, and the site tropism of the plerocercoids support the assertion that *D. ursi* is a valid species, which can be distinguished from *D. dendriticum*, *D. nihonkaiense*, and *D. latum* using molecular and morphological markers.

ACKNOWLEDGMENTS

The authors thank Dr. Jun Araki, Meguro Parasitological Museum, Tokyo, Japan, for the opportunity to examine syntype specimens of *D. gondo* and *Diphyllobothrium subtile*. Yuka Mori is acknowledged for technical assistance. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare, Japan (H20-H22-Shinko-Ippan-016) and from the Japan Society for the Promotion of Science (23406010) to H.Y.

LITERATURE CITED

ANDERSEN, K., H. L. CHING, AND R. VIK. 1987. A review of freshwater species of *Diphyllobothrium* with redescriptions and the distribution of *D. dendriticum* (Nitzsch, 1824) and *D. ditremum* (Creplin 1825) from North America. Canadian Journal of Zoology **65:** 2216–2228.

ANDO, K., K. ISHIKURA, T. NAKAKUGI, Y. SHIMONO, T. TAMAI, M. SUGAWA, W. LIMVIROJ, AND Y. CHINZEI. 2001. Five cases of *Diphyllobothrium nihonkaiense* infection with discovery of plerocercoids from an infective source, *Oncorhynchus masou ishikawae*. Journal of Parasitology **87:** 96–100.

ARIZONO, N., M. SHEDKO, M. YAMADA, R. UCHIKAWA, T. TEGOSHI, K. TAKEDA, AND K. HASHIMOTO. 2009. Mitochondrial DNA divergence in populations of the tapeworm *Diphyllobothrium nihonkaiense* and its phylogenetic relationships with *Diphyllobothrium klebanovskii*. Parasitology International **58:** 22–28.

BROWN, W. M., E. M. PRAGER, A. WANG, AND A. C. WILSON. 1982. Mitochondrial DNA sequences of primates: Tempo and mode of evolution. Journal of Molecular Evolution **18:** 225–239.

CHING, H. L. 1984. Fish tapeworm infections (Diphyllobothriasis) in Canada, particularly in British Columbia. Canadian Medical Association Journal **130:** 1125–1127.

DICK, T. A., P. A. NELSON, AND A. CHOUDHURY. 2001. Diphyllobothriasis: Update on human cases, foci, patterns and sources of human infections and future consideration. Southeast Asian Journal of Tropical Medicine and Public Health **32** (Suppl. 2): 59–76.

FRECHETTE, J. L. 1978. Seasonal changes in the prevalence of ova of *Diphyllobothrium ursi* and *Baylisascaris transfuga* in the feces of the black bear (*Ursus americanus*). Journal of Wildlife Diseases **14:** 342–344.

HILLIARD, D. K. 1960. Studies on the helminth fauna of Alaska. XXXVIII. Taxonomic significance of eggs and coracidia of some diphyllobothriid cestodes. Journal of Parasitology **46:** 703–715.

JUNIPER, I. 1978. Morphology, diet, and parasitism in Quebec black bears. Canadian Field Naturalist **92:** 186–189.

KAMO, H. 1999. Genus *Diphyllobothrium* Cobbold, 1858. *In* Guide to identification of diphyllobothriid cestodes, K. Hirai (ed.). Gendai Kikaku, Tokyo, Japan, p. 26–146.

———, J. MAEJIMA, S. YAZAKI, AND S. FUKUMOTO. 1982. Notes on morphology and taxonomy of the genus *Diphyllobothrium* found

from some marine mammals in Japanese environs. Yonago Acta Medica **33:** 261–270.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution **16:** 111–120.

MARGOLIS, L., R. L. RAUSCH, AND E. ROBERTSON. 1973. *Diphyllobothrium ursi* from man in British Columbia—First report of this tapeworm in Canada. Canadian Journal of Public Health **64:** 588–589.

MARKOWSKI, S. 1952. The cestodes of pinnipeds in the Arctic and other regions. Journal of Helminthology **26:** 171–214.

MURATOV, I. V., AND P. S. POSOKHOV. 1988. Causative agent of human diphyllobothriasis—*Diphyllobothrium klebanovskii* sp. n. Parazitologiiya **22:** 165–170.

NAKAO, M., D. ABMED, H. YAMASAKI, AND A. ITO. 2007. Mitochondrial genomes of the human broad tapeworms *Diphyllobothrium latum* and *Diphyllobothrium nihonkaiense* (Cestoda: Diphyllobothriidae). Parasitology Research **101:** 233–236.

RAUSCH, R. 1954. Studies on the helminth fauna of Alaska. XXI. Taxonomy, morphological variation, and ecology of *Diphyllobothrium ursi* n. sp. provis. on Kodiak Island. Journal of Parasitology **40:** 540–563.

———. 1956. Studies on the helminth fauna of Alaska. XXVIII. The description and occurrence of *Diphyllobothrium dalliae* n. sp. (Cestoda). Transactions of the American Microscopical Society **75:** 180–187.

———. 2005. *Diphyllobothrium fayi* n. sp. (Cestoda: Diphyllobothriidae) from the Pacific walrus, *Odobenus rosmarus divergens*. Comparative Parasitology **72:** 129–135.

———, and D. K. Hilliard. 1970. Studies on the helminth fauna of Alaska. XLIX. The occurrence of *Diphyllobothrium latum* (Linnaeus, 1758) (Cestoda: Diphyllobothriidae) in Alaska, with notes on other species. Canadian Journal of Zoology **48:** 1201–1209.

———, and F. S. Williamson. 1958. Studies on the helminth fauna of Alaska, XXXIII. The description and occurrence of *Diphyllobothrium alascense* n. sp. (Cestoda). Zeitschrift für Parasitenknde **1:** 64–72.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:** 1572–1574.

SCHOLZ, T., H. H. GARCIA, R. KUCHTA, AND B. WICHT. 2009. Update on the human broad tapeworm (genus *Diphyllobothrium*), including clinical relevance. Clinical Microbiology Reviews **22:** 146–160.

WICHT, B., F. DE MARVAL, B. GOTTSTEIN, AND R. PEDUZZI. 2007. Imported diphyllobothriasis in Switzerland: Molecular evidence of *Diphyllobothrium dendriticum* (Nitzsch, 1824). Parasitology Research **102:** 201–204.

———, N. RUGGERI-BERNARDI, T. YANAGIDA, M. NAKAO, R. PEDUZZI, AND A. ITO. 2010. Inter- and intra-specific characterization of tapeworms of the genus *Diphyllobothrium* (Cestoda: Diphyllobothriidea) from Switzerland, using nuclear and mitochondrial DNA targets. Parasitology International **59:** 35–39.

———, T. SCHOLZ, R. PEDUZZI, AND R. KUCHTA. 2008. First record of human infection with the tapeworm *Diphyllobothrium nihonkaiense* in North America. American Journal of Tropical Medicine and Hygiene **78:** 235–238.

YAMAGUTI, S. 1942. Studies on the helminth fauna of Japan. Part 42. Cestodes of mammals, II. 1–18.

YAMANE, Y., H. KAMO, G. BYLUND, AND B. J. WIKGREN. 1986. *Diphyllobothrium nihonkaiense* sp. nov. (Cestoda: Diphyllobothriidae)—Revised identification of Japanese broad tapeworm. Shimane Journal of Medical Science **10:** 29–48.

YAMASAKI, H., K. NAKAYA, M. NAKAO, Y. SAKO, AND A. ITO. 2007. Significance of molecular diagnosis using histopathological specimens in cestode zoonoses. Tropical Medicine and Health **35:** 307–321.

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Pediatrics International (2012) **54**, 163–165 doi: 10.1111/j.1442-200X.2011.03529.x

Rapid Communication

Two pediatric cases of *Diphyllobothrium nihonkaiense* infection in summer (July–August) 2010

Takahiro Ikeda,[1] Daisuke Tamura,[1] Yuko Sato,[1] Ko Ichihashi,[1] Hiroyuki Matsuoka[2] and Hiroshi Yamasaki[3]

[1]*Department of Pediatrics, Saitama Medical Center, Jichi Medical University, Saitama,* [2]*Department of Infection and Immunity, Division of Medical Zoology, Jichi Medical University, Tochigi and* [3]*Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan*

Key words child, *Diphyllobothrium nihonkaiense*, mitochondrial DNA analysis, praziquantel.

Diphyllobothrium nihonkaiense is a major cestode parasite causing diphyllobothriasis in Japan. This parasite was once endemic to coastal provinces of central and northern Japan, where salmon fisheries thrived. During the past several decades, however, the pattern of infection has changed because raw fish are now being transported around the country, meaning that the infection has been spread among urban people who eat sushi and sashimi. Although the numbers of clinical cases of infection in large cities has fluctuated over the past 20 years, the incidence was particularly high in 2008.[1] In addition, reports of pediatric cases have recently increased. We report the increasing tendency of *D. nihonkaiense* infection and two pediatric cases during a short period in summer 2010 (July–August).

Case report

Case 1

A healthy 6-year-old Japanese boy visited Saitama Medical Center Jichi Medical University in July 2010. He had never been overseas. His chief complaint was discharge of a white band like a thin cord from his anus without symptoms. He had regularly eaten raw fish as sushi, including salmon, several times per month. We considered this to be a tapeworm segment in morphology. We used a single dose of praziquantel (22.5 mg/kg) and a laxative for therapy. Approximately 2 h later, he expelled a tapeworm with a scolex (Fig. 1). We considered the antihelmintic treatment to be successful in this case. Five months after treatment, the patient has expelled no parasites.

Case 2

An 8-year-old Japanese boy visited Saitama Medical Center Jichi Medical University in August 2010 because of discharge of a white band from his anus without symptoms. He had not been in contact with patient 1. His past medical history included stable bronchial asthma. He had regularly eaten fresh raw fish several times per month, as with patient 1. He was given praziquantel (19 mg/kg) and a laxative. Approximately 6 h later, he expelled a tapeworm in his stool. The scolex was not identified in the segment. Four months after treatment, no parasites had been expelled and we ended treatment and follow up.

In both cases, the cestode parasites obtained from two patients were fixed in 70% ethanol, and molecular identification was performed at the Department of Parasitology, National Institute of Infectious Diseases, Tokyo, Japan. Briefly, genomic DNA was prepared using a DNeasy Blood and Tissue kit (Qiagen, Helden, Germany) and mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) was amplified by polymerase chain reaction (PCR), and the nucleotide sequences were analyzed according to the method.[2] The causative cestodes were identified as *D. nihonkaiense* on nucleotide sequence analysis and phylogenetic analysis (Fig. 2).

Discussion

With improvements in public health, the frequency and prevalence rates of infection by parasites, especially soil-transmitted parasites, have been decreasing in Japan.[3–5] *D. nihonkaiense* infection was endemic to coastal areas of central and northern Japan, where raw food culture, as typified by salmon, had been widely accepted. The prevalence of *D. nihonkaiense*, however, spread throughout Japan from the 1970s, and it is no longer endemic.[6] This is because developments in transport techniques have provided people living inland with increased opportunities to eat raw fish. Although the number of clinical cases of infection in large cities has fluctuated over the past 20 years, the incidence rate was particularly high in 2008.[1] Most patients were 20–59 years of age, which probably reflects more frequent consumption of sushi and sashimi (raw fish food) by those in this age group than in other age groups. In another report, the peak age of infection was the 40s and 50s, and this was because such people have more occasions to eat raw fish, which used to be a luxury and was eaten only in specialized restaurants.[7] It is now, however,

Correspondence: Takahiro Ikeda, MD, Department of Pediatrics, Saitama Medical Center, Jichi Medical University. Amanuma-cho, Omiya-ku, Saitama-city, Saitama 330-0834, Japan. Email: r0704ti@jichi.ac.jp

Nucleotide sequence data reported in the present paper are deposited at the DDBJ/EMBL/GenBank databases under accession numbers AB597273 and AB597274 for cases 1 and 2, respectively.

Received 9 September 2011; accepted 21 November 2011.

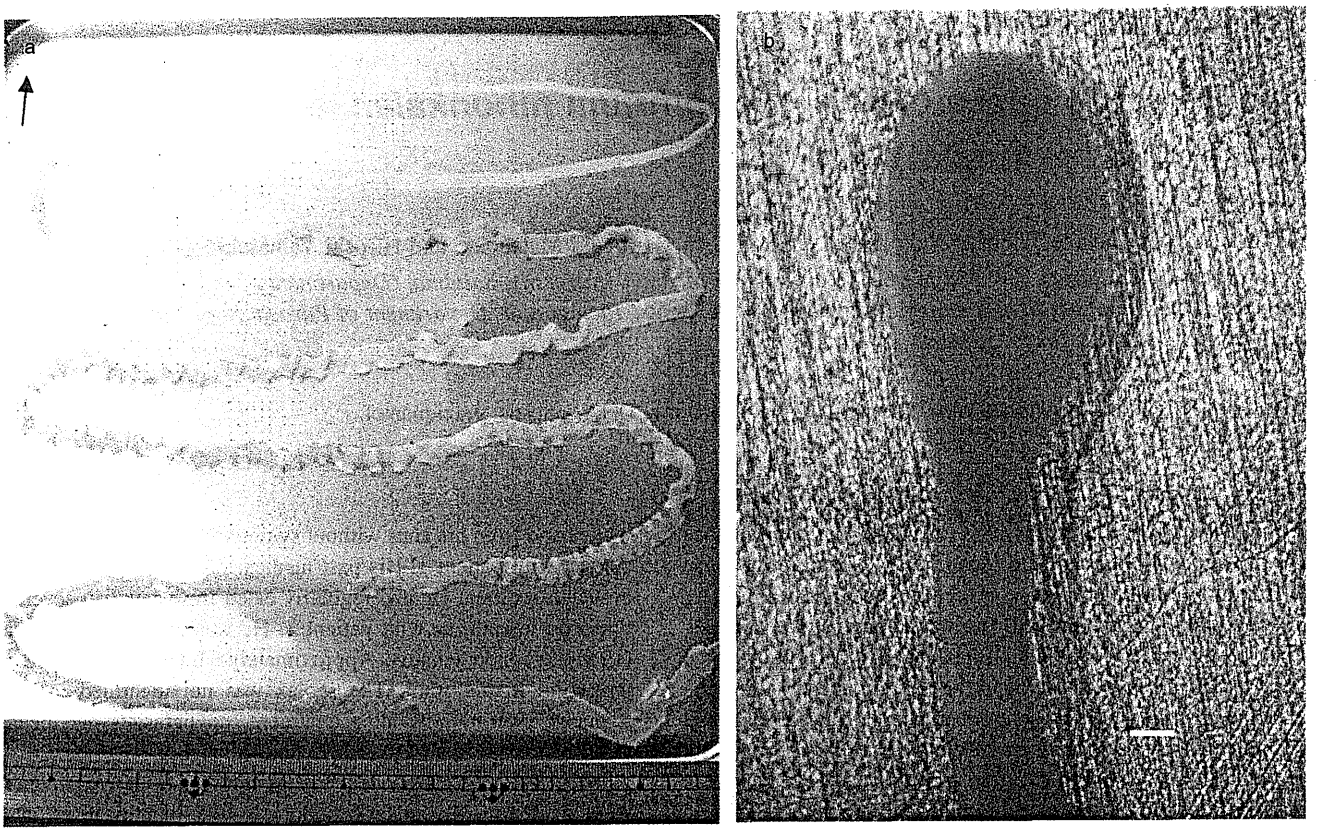

Fig. 1 (a) *Diphyllobothrium nihonkaiense* segments expelled from patient 1. The tapeworm was approximately 1.8 m in length. The scolex of the tapeworm was confirmed (arrow). (b) Magnification of the scolex characterized by club shape. Bar, 100 μm.

commonly eaten by ordinary families, including children.[8] Reports of pediatric cases of *D. nihonkaiense* in Japan have increased. Based on PubMed (for reports in English) and Ichushi (for reports in Japanese), the average number of patients was 1.3 per year from 1993 to 2007. In contrast, the average was 4.7, including the present cases, from 2008 to 2010. This trend suggests that the number of patients with *D. nihonkaiense* infection

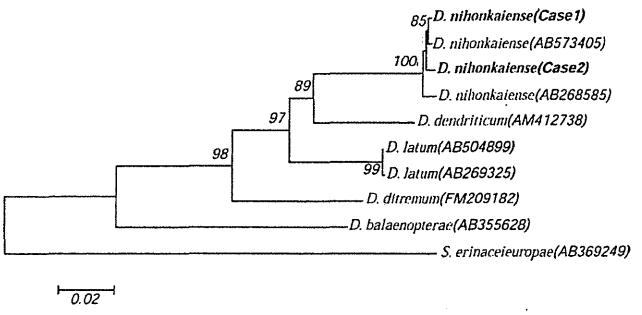

Fig. 2 Phylogenetic tree inferred from cox1 1566 bp nucleotide sequences using the neighbor-joining method. The numbers at branches indicate bootstrap values for 1000 replicates and the scale bar represents the genetic distance based on Kimura's two-parameter model. The tapeworms of patients 1 and 2 were included in the cluster of *Diphyllobothrium. nihonkaiense*.

may increase rapidly, even among children. *D. nihonkaiense* infections in humans have also been reported in Europe, where the parasite is non-indigenous.[9–11] Global demand for seafood as part of a healthy diet, and development of worldwide transport systems for raw fish may affect the prevalence of diphyllobothriasis, other than in Japan, as an emerging infection. The globalization of the salmon market indicates the possible emergence of diphyllobothriasis in non-endemic areas. Some types of *Diphyllobothrium* species are morphologically similar to each other, therefore it is very difficult to differentiate them from one another macroscopically. Genome analysis is necessary to confirm the diagnosis of the causative species. To date, pediatric cases of *D. latum* have not been reported in Japan.[12] In future, cases of *D. latum* or other non-native *Diphyllobothrium* species may appear in Japan, as *D. nihonkaiense* is no longer an endemic disease in Japan. Identification of causative species provides the epidemiology, which in turn facilitates planning of prevention measures. The present incidence of *D. nihonkaiense* has changed drastically from rural to urban areas, and not only adults but also children have been infected because of the rapid expansion of the transport system for fresh and frozen fish to meet the demands for seafood. The public is generally underinformed regarding the risks of diphyllobothriasis from eating salmon. Consumers and retailers should be made aware of the risk for tapeworm infection posed by eating raw salmon. In particular, a strong warning