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動物の日本住血吸虫感染症の診断と予防に関する最近の知見

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要　約

日本住血吸虫の宿主動物はヒトを含めて40種以上に及ぶが、ヒト以外の動物はヒト日本住血吸虫症の蔓延を招く重要な保虫宿主となる。しかしながら、これら動物の日本住血吸虫感染症の診断や予防に関する技術は、フィリピンを含めた流行国でさえ、十分に確立されていない。すなわち、ヒトの日本住血吸虫症の予防には多大な努力が注がれてきた一方で、保虫宿主である動物には十分な対策は行われてこなかった。この総説では、スイギュウ、ウシおよびイヌといった主要な保虫動物に対する日本住血吸虫の疫学と制圧に関する最新の知見を紹介する。

Keywords：日本住血吸虫症、保虫宿主、人獣共通感染症、疫学調査、感染症制圧

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■ MINI-REVIEW

Current Status and Perspectives of Cysticercosis and Taeniasis in Japan

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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-

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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-

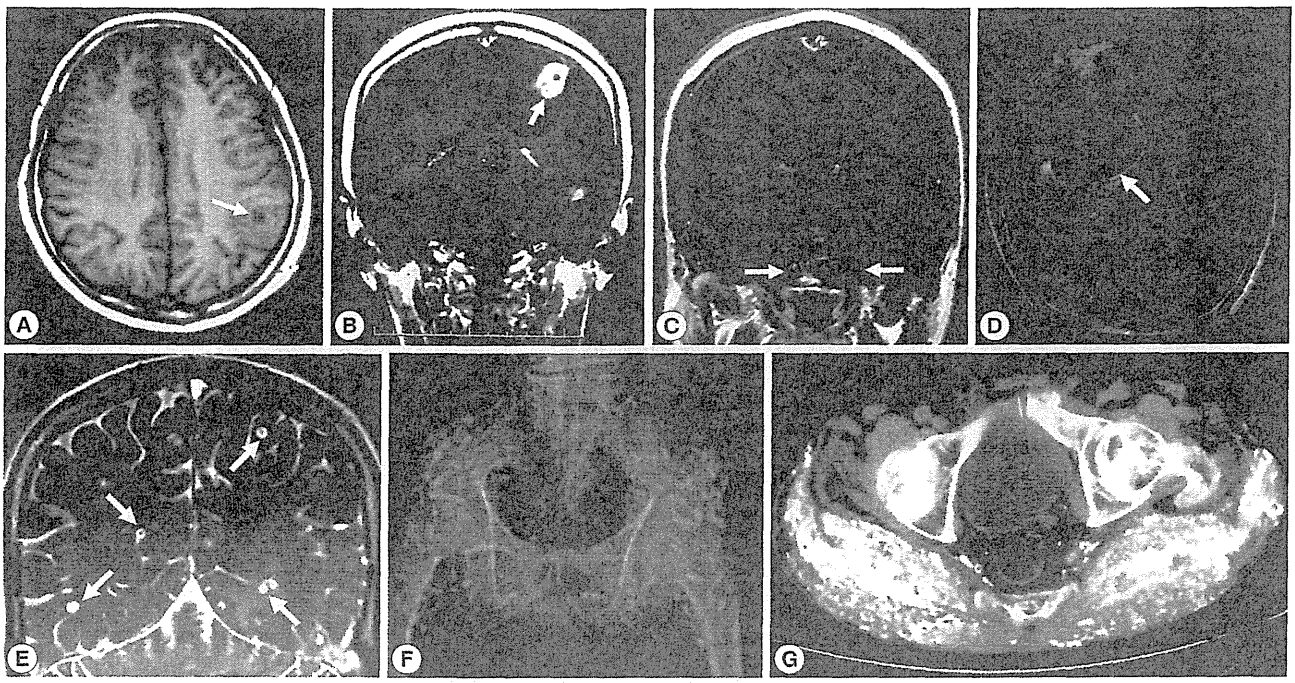

Fig. 1. Imaging findings of selected cysticercosis cases. (A) plain CT image showing a solitary lesion at the left occipitoparietal area (case 48 [32], courtesy of Prof. H. Matsuoka). (B) MRI showing one of multiple cystic lesions in the left frontal and temporal lobes (case 49 [33]). (C) MRI showing 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

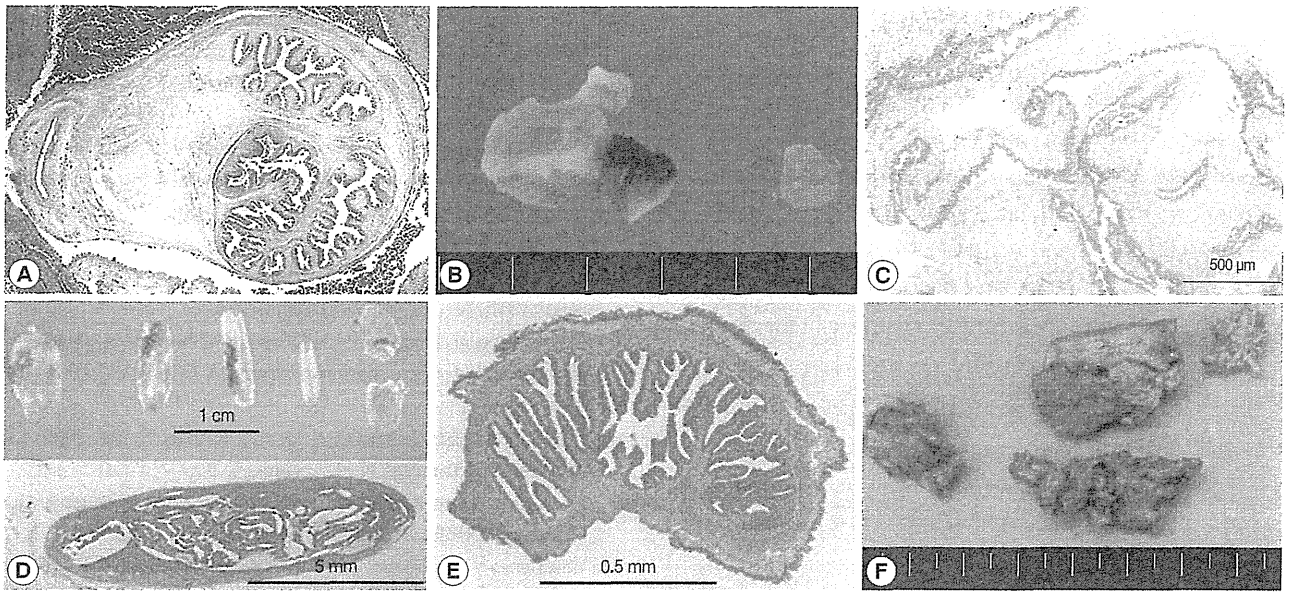

Fig. 2. Histopathologic findings of cystic lesions from cysticercosis patients. (A) A cellulose-type cysticercus characterized by rabyrinth-like structure (case 40 [22], courtesy of Dr. S. Matsunaga). (B) and (E) A resected lesion and a cellulose-type cysticercus (case 48 [32], courtesy of Prof. H. Matsuoka). (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.

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Application of Recombinant *Gnathostoma spinigerum* Matrix Metalloproteinase-Like Protein for Serodiagnosis of Human Gnathostomiasis by Immunoblotting

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Abstract. Matrix metalloproteinase (MMPs) is the extracellular zinc-dependent endopeptidase and is secreted for degrading extracellular matrix molecules of host tissues. A cDNA encoding MMP-like protein of *Gnathostoma spinigerum* larvae was amplified by reverse transcription-polymerase chain reaction, and was cloned into a prokaryotic expression vector, and expressed in *Escherichia coli*. Total immunoglobulin G class (total IgG) antibody responses to the recombinant MMP-like protein were analyzed by immunoblot diagnosis of human gnathostomiasis. Serum samples from proven and clinically suspected cases of gnathostomiasis, other parasitic diseases patients, and from healthy volunteers were tested. The immunoblotting gave high sensitivity (100%) and specificity (94.7%). Positive and negative predictive values were 85.4% and 100%, respectively. Recombinant MMP-like protein can be used as a diagnostic antigen and potentially replace native parasite antigens to develop a gnathostomiasis diagnostic kit.

INTRODUCTION

Human gnathostomiasis is an important food-borne parasitic zoonosis caused by the spirurid nematode *Gnathostoma* spp. and the disease is endemic in Asia and the Americas,[1–4] and in returned travelers who had visited the endemic areas of this harmful parasite.[5,6] Humans acquire infection by consuming raw or undercooked meat, i.e., freshwater fish, frogs, chicken, etc., which harbor *Gnathostoma* advanced third-stage larvae (AL3). *Gnathostoma spinigerum* is a causative agent mainly in Asian countries, i.e., Thailand, Japan, Vietnam, etc.[2,7–9] The *Gnathostoma* AL3 migrates into the subcutaneous tissue and causes intermittent migratory swelling. Sometimes the worm migrates to vital organs, i.e., brain, eye, etc., producing severe pathologic signs and symptoms that can lead to harmful problems and death.[6,10–12] Definitive diagnosis for human gnathostomiasis can be made by detecting the migrating larvae from the human body. Because direct detection of the parasite is difficult and often unsuccessful, diagnosis of gnathostomiasis is practically made by relying upon clinical features, history of eating parasite-contaminated foods, blood eosinophilia, and serological outcomes, i.e., enzyme-linked immunosorbent assays[13–16] or immunoblotting using *Gnathostoma* AL3 extract, including an antigenic peptide with an approximate molecular mass of 24 kDa[17–19] and below 27–29 kDa.[20] Two-dimensional gel electrophoresis (2-DE) and immunoblotting revealed that *G. spinigerum* AL3 antigenic spots with an approximate molecular mass of 23–25 kDa and p*I* of 8.3–8.5 revealed a high potential for the serodiagnosis of human gnathostomiasis spinigerum.[21] The amino acid sequence of these antigenic spots was determined by liquid chromatography tandem mass spectrometry (LC/MS-MS) and the LC/MS-MS spectra[22] and one of the peptide sequences showed high similarity with a matrix metalloproteinase (MMP)-like protein of *G. spinigerum* database (GenBank accession no. AAF82802).[23]

Cloning and expression of *G. spinigerum* genes such as MMP-like protein,[23] cathepsin L-like cysteine protease,[24] and cyclophilin protein[25] have been reported. However, the diagnostic values of those recombinant proteins for human gnathostomiasis have not been validated. In this study, we produced a recombinant MMP-like protein of *G. spinigerum* and evaluated its sensitivity and specificity in immunodiagnosis for human gnathostomiasis. We selected MMP-like protein because its molecular mass and p*I* corresponded well with the 2-DE immunoreactive spots detected by the confirmed human gnathostomiasis sera.[22] The goal of this study is to setup a stable mass-production system for the standardized immunodiagnostic kit with recombinant *G. spinigerum* MMP-like protein antigen.

MATERIALS AND METHODS

Human sera. All serum samples were supplied by the serum bank of the Faculty of Medicine, Khon Kaen University. Serum samples consisted of three groups: 1) Negative control group, which included samples from healthy adult volunteers who were free from any intestinal parasitic infection and checked by stool examination by the formalin ethyl acetate concentration technique[26] at the time of blood collection. A pooled sera from all those healthy individuals was also used as negative control for each assay. 2) Gnathostomiasis group, which included samples from parasitologically confirmed gnathostomiasis patients and from patients showing clinical symptoms of suspected cutaneous and visceral gnathostomiasis,[2,27,28] with a history of eating food possibly contaminated with *Gnathostoma* larvae and were positive 24 kDa *G. spinigerum* antigen by immunoblotting.[29] 3) The third group ($N = 83$) consisted of serum samples from patients with other parasitic infections than gnathostomiasis. Their infections were confirmed by parasitological methods except that cysticercosis cases were diagnosed by a computerized tomography scan and found positive by the immunological method Table 1. Informed consent was obtained from all human adult participants and from parents or legal guardians of minors. The study protocol was approved by the Khon Kaen University Ethics Committee for Human Research (HE541293).

Parasites, total RNA isolation, and synthesis of cDNA encoding MMP-like protein. The *G. spinigerum* AL3 were

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TABLE 1
Type of human sera and immunoblotting results against the purified fusion-tagged recombinant MMP-like protein*

Type of serum	Group	No. positive/no. total
Healthy control	1	0/30
Confirmed gnathostomiasis	2	13/13
Suspected gnathostomiasis	2	22/22
Cysticercosis	3	0/8
Taeniasis	3	0/5
Opisthorchiasis viverrini	3	2/8
Fascioliasis	3	3/10
Paragonimiasis	3	0/10
Angiostrongyliasis	3	0/9
Strongyloidiasis	3	0/5
Hookworm infection	3	0/5
Capillariasis	3	1/9
Ascariasis	3	0/5
Trichinosis	3	0/9

*MMP = matrix metalloproteinase.

collected from mice inoculated orally with early third-stage larvae recovered from copepod.[30] The worms (N = 40) were then placed into RNAlater (Promega, Madison, WI). The total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) and was finally dissolved in diethylpyrocarbonate-treated deionized water and stored at −70°C unit use. Based on the DNA sequence of a *G. spinigerum* MMP-like protein from the published data[23] (GenBank accession no. AF277294), we designed a primer pair to obtain the complete open reading frame of the MMP-like sequence. The primers used were as follows: GS-F1 5′-CAGTAAAG**ATG** AAACTACAGAGTGTG-3′ and GS-R1 5′-GACGT**TTA**C GGCATTGGAG-3′ (The start and stop codons are indicated in bold). A reverse transcription-polymerase chain reaction (RT-PCR) was performed using the RobusT II RT-PCR kit (Finnzymes, Espoo, Finland) according to the manufacturer's protocol. The PCR parameters were as follows: cDNA synthesis at 40°C for 60 minutes and at 94°C for 2 minutes; and then 35 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 1 minute at 72°C; and a final step at 72°C for 10 minutes. The PCR product obtained was checked by electrophoresis using 1% agarose gel, purified, and subcloned into pCR4-TOPO Vector using a TOPO TA Cloning kit (Invitrogen), and transformed into TOP10 competent cells (Invitrogen) for sequence confirmation.

DNA sequencing and analysis. The DNA sequencing was performed using the MegaBACE 1000 DNA analysis system (GE Healthcare, Piscataway, NJ), and the sequence obtained was analyzed using software programs including BLAST (www.ncbi.nlm.nih.gov), Multalin (http://bioinfo.genotoul.fr/multalin/multalin.html), BioEdit version 7.0.9 (www.mbio.ncsu.edu/BioEdit/BioEdit.html), and Compute pI/Mw tool (http://web.expasy.org/compute_pi/).

Expression, purification, and cleavage of the recombinant MMP-like protein. The primers carried restriction sites of *Eco*RI (GS-F2 5′- TGGCGTG**GAATTC**TATGAAAC TAC AGAGTGTG -3′) and *Hind* III (GS-R2 5′CGGAGGA **AA GCTT**TTACGGC ATTGGAG -3′) (Restriction sites are indicated in bold) were designed. The PCR parameters were as follows: initial heating at 94°C for 3 minutes; and then 35 cycles of 30 seconds at 94°C, 30 seconds at 69°C, and 1 minute at 72°C; and a final step at 72°C for 10 minutes. The PCR product was subcloned into a pET-43.1(+) expression vector (Novagen, Darmstadt, Germany). The recombinant plasmids were then transformed into *Escherichia coli* JM 109 and the accuracy of the nucleotide sequence harbored in the bacterial clones was verified by sequencing. The plasmid DNA presenting the correct codons was used to transform in *E. coli* Rosetta-gami 2(DE3) expression host (Novagen). Protein expression was induced by 1 mM isopropyl-β-D-thiogalactopyranoside. Suspension of bacterial cell in a lysis buffer (50 mM Tris–HCl, pH 8.0, 5% glycerol, 50 mM NaCl, 0.5 mg/mL lysozyme) was sonicated on ice, and the recombinant protein fused with N utilization substance A (NusA)-tagged and 6-Histidine (6-His)-tagged residues, and was purified using Ni–NTA His Bind Resin (Novagen). The recombinant MMP-like protein was separated from fusion-tagged proteins by cleaving with recombinant enterokinase (rEK) (Novagen) according to the manufacturer's instructions.

Antigenicity of the purified recombinant MMP-like protein by immunoblotting. The purified and rEK-cleaved MMP-like fusion-tagged proteins were electrophoresed on a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli,[31] and then transferred to a nitrocellulose membrane[32] that was cut into strips for immunoblotting. The purified fusion-tagged protein was detected by a reaction with an anti-NusA mouse monoclonal antibody (Novagen), according to the manufacturer's protocol. Briefly, after blocking the membrane strips with (3%) bovine serum albumin (BSA) in phosphate buffered saline (PBS, pH 7.5) incubating with an anti- NusA antibody, and then probing with horseradish peroxidase (HRP)-labeled goat anti-mouse IgG (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), the reaction was visualized with 3,3′-diaminobenzidine tetrahydrochloride (DAB) substrate. For demonstration of antigenicity of the cleaved recombinant MMP-like protein, the reaction was probed with a 1:100 diluted pooled sera of gnathostomiasis patients or pooled negative control sera (in 1% skimmed milk in PBS, pH 7.5) absorbed with *E. coli* lysate for 2 hours at room temperature. The membranes were washed with 1% skimmed milk in PBS, pH 7.5, containing 0.1% Tween-20 (PBST) (5 times), and incubated with goat anti-human IgG (H+L) HRP conjugate (Invitrogen) at a dilution of 1:4,000 (in 1% skimmed milk in PBST) for 2 hours at room temperature. After 5 washes with 1% skimmed milk in PBST, the strips were then developed with DAB substrate, and the reaction stopped with distilled water.

Evaluation of the purified fusion-tagged recombinant MMP-like protein as a diagnostic antigen for human gnathostomiasis. The purified fusion-tagged recombinant MMP-like protein was electrophoresed on 10% SDS-PAGE and then electrotransferred to a nitrocellulose membrane. After blocking nonspecific binding sites with 1% skimmed milk in PBST, pH 7.5 for 30 minutes, the membrane was cut into ~3 mm wide strips (9.8 μg protein/strip). Each strip was incubated with individual human serum samples absorbed with *E. coli* lysate and processed as described previously. The diagnostic parameters of sensitivity, specificity, and positive and negative predictive values were calculated as previously described.[33]

RESULTS

Synthesis of the complete cDNA encoding a MMP-like protein. We successfully amplified cDNA encoding a MMP-like protein of *G. spinigerum* (Figure 1A). The gene consisted

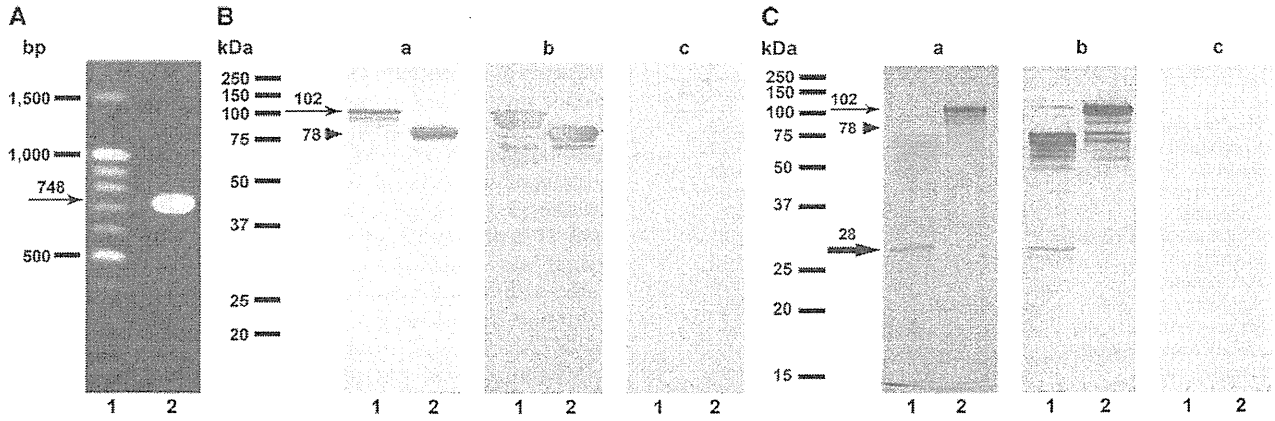

FIGURE 1. (**A**) Ethidium bromide stain patterns of the *Gnathostoma spinigerum* complete cDNA encoding a matrix metalloproteinase (MMP)-like protein on a 1.0% agarose gel. An arrow indicates band at approximate of 748 base pairs. Lane 1, DNA size markers (ZipEnzyme, Novosibirsk, Russia) and lane 2, the complete cDNA encoding a MMP-like protein amplified by reverse-transcriptase polymerase chain reaction (PCR) with gene-specific primers. (**B**) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the fusion-tagged protein with and without recombinant MMP-like protein expressed in *Escherichia coli*. Coomassie Brilliant Blue stained (a) and immunoblotting probed with (b) and without (c) an anti- NusA antibody. Lane 1, the fusion-tagged protein containing the recombinant MMP-like protein (102 kDa, arrow) and lane 2, the fusion-tagged protein alone (78 kDa, arrow head). (**C**) SDS-PAGE analysis of the recombinant MMP-like protein cleaved with recombinant enterokinase (rEK) and the recombinant fused-tagged MMP-like proteins. Coomassie Brilliant Blue stained (a) and immunoblotting probed with sera from pooled gnathostomiasis patients (b) and pooled healthy negative controls (c). Lane 1, the rEK-cleaved MMP-like protein and lane 2, the fusion-tagged MMP-like recombinant protein. A thin arrow indicates expression of the fusion-tagged MMP-like protein (102 kDa). An arrow head indicates 78 kDa band of the fusion-tagged protein alone. A thick arrow indicates band of the rEK-cleaved MMP-like protein (28 kDa).

of a single open reading frame of 735 basepairs encoding 244 amino acids with a predicted molecular mass of 28 kDa and a theoretical p*I* of 7.8. The sequence was identical to the nucleotide sequence of a *G. spinigerum* MMP-like gene (GenBank accession no. AF277294).

Expression, purification, cleavage, and immunocharacterization of the recombinant MMP-like protein. A complete cDNA encoding the MMP-like protein was cloned into an expression vector and the recombinant MMP-like fusion-tagged protein was expressed in an *E. coli* expression system. The purified recombinant protein gave a single band in SDS-PAGE (Figure 1B, panel a). By immunoblot analysis, the fusion-tagged MMP-like protein and fusion-tagged protein alone were visualized by anti-NusA antibody at approximate molecular masses of 102 and 78 kDa, respectively (Figure 1B, panel b). The rEK-cleaved MMP-like protein has a molecular mass of ~28 kDa (Figure 1C, panel a). The cleaved MMP-like protein showed strong positive reactivity with pooled gnathostomiasis patient serum (Figure 1C, panel b) but did not react with the pooled negative control serum (Figure 1C, panel c) by immunoblotting.

Evaluation of the diagnostic values of the purified fusion-tagged recombinant MMP-like protein. Immunoblot analysis employing the diagnostic values of the purified fusion-tagged recombinant MMP-like protein for human gnathostomiasis was evaluated using individual serum from healthy control, gnathostomiasis patients, and the patients with other parasitic diseases (Table 1; Figure 2A). All serum samples from the confirmed (N = 13) and suspected (N = 22) gnathostomiasis patients strongly reacted with the purified recombinant MMP-like fusion protein. In contrast, none of the 30 healthy control sera showed positive seroreactivity to this recombinant antigen. Some cross-reactivity was observed in serum samples of capillariasis (1 of 9), opisthorchiasis viverrini (2 of 8), and fascioliasis (3 of 10). The calculated diagnostic sensitivity, specificity, and positive and negative predictive values were 100%, 94.7%, 85.4%, and 100%, respectively. Non-reactive band was shown when all sera reacted with the fusion-tagged protein alone (78 kDa) (Figure 2B).

DISCUSSION

For the immunodiagnosis of gnathostomiasis, many attempts have been made[34] to establish a specific diagnostic test system for the disease caused by *Gnathostoma* spp., such as *Gnathostoma binucleatum*,[16,35] *Gnathostoma doloresi*,[36] and *G. spinigerum*.[17] However, the limitation is the small amount production of the specific antigen from native worm extracts. Maintenance of the lifecycle of *Gnathostoma* spp. in experimental animals in the laboratory is expensive and time consuming. The cDNA encoding MMP-like protein of *G. spinigerum* AL3 was cloned and the deduced amino acid sequence was correlated with that of the immunodominant spots[22] and this protein was considered as the potential antigen for detecting specific antibodies in infected patients. Thus, in this study, we intended to produce the recombinant *G. spinigerum* MMP-like protein for massive antigen supply for the serodiagnosis of human gnathostomiasis in Asian countries. The MMP-like gene was expressed in *E. coli* using pET 43.1(+), which contained the Nus A and 6-His tagged residues as fusion proteins. This expression system resulted in a high yield (~140 mg/one L of *E. coli* culture) and the fusion protein was obtained in the soluble fraction, as evaluated by Coomassie Brilliant Blue staining (shown in Figure 1B, panel a, lane 1). The fusion protein was seen as a molecular mass of ~102 kDa, which was somewhat greater than the theoretical molecular mass of 89 kDa (fusion tag, 61 kDa; MMP-like protein, 28 kDa). This could possibly be caused by the alteration of the constant charge: mass ratio in binding between the SDS and the protein carrying a large size of the fusion tag.

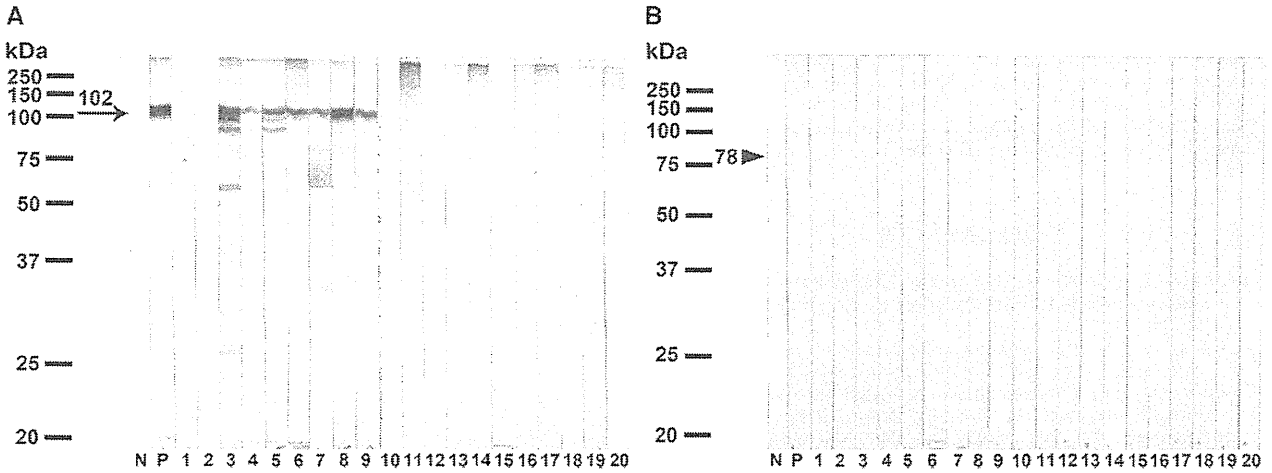

FIGURE 2. Representative immunoblotting patterns reacted with the purified fusion-tagged MMP-like protein (**A**) compared with the fusion-tagged protein alone (**B**) expressed in *Escherichia coli*. Antigens were probed with 1:100 diluted sera from pooled negative controls (N), pooled human gnathostomiasis patients (P), healthy control (1; 2), parasitologically confirmed gnathostomiasis (3–7), clinically suspected visceral gnathostomiasis (8) and cutaneous gnathostomiasis (9), cysticercosis (10), angiostrongyliasis (11), paragonimiasis (12), fascioliasis (13), trichinosis (14), ascariasis (15), opisthorchiasis viverrini (16), strongyloidiasis (17), taeniasis (18), capillariasis (19), and hookworm infection (20). The arrow indicates the specific immunoreactive band at ~102 kDa and arrow head indicates the fusion-tagged protein alone at ~78 kDa.

The immunodominant antigenicity of the recombinant fusion protein against human sera was shown by immunoblotting. The recombinant fusion protein specifically reacted with the gnathostomiasis patient sera, but not with sera from healthy control or from patients infected with other parasites. Only faint cross-reactivity was observed with the sera of capillariasis (1 of 9), opisthorchiasis viverrini (2 of 8), and fascioliasis (3 of 10) patients. These cross-reactions are possibly explained because these patients might have a previous history of subclinical infection with *G. spinigerum* and mixed infections with these parasites. Even when cross-reactions with fascioliasis, capillariasis, and opisthorchiasis sera were observed, it does not cause a real problem in the clinical setting because these parasitic infections usually present with clinical features different from those of gnathostomiasis. However, subclinical infection with *G. spinigerum* and mixed infections sera with other parasites need to be evaluated with more samples. In addition, we have also tested the immunoblotting patterns using various human sera as revealed in Table 1 and reacted with the recombinant MMP-like protein cleaved with rEK (28 kDa) (see Supplemental Figure 1), the diagnostic sensitivity and specificity were quite similar and revealed the results as presented when testing with the purified fusion-tagged MMP-like protein (102 kDa). These results ensure both types of antigen can be used for supportive diagnostic purpose.

Previous immunoblotting reports have shown that the native 24 kDa *G. spinigerum* larval antigen reacting to total IgG antibody could contribute to the reliable diagnosis of human gnathostomiasis. The demonstrated sensitivity ranged from 83.3% to 100%, whereas the specificity ranged from 87.8% to 100%.[17,19,21] The native 21 kDa antigenic band of *G. spinigerum* AL3 reacting faintly to IgG4 antibody gave the 100% sensitivity and specificity,[18] whereas the 24 kDa *G. spinigerum* antigen revealed the sensitivity and specificity that ranged from 75% to 92.9% and 93.4% to 93.9%, respectively.[18,19] In another study, the antigenic bands below 27–29 kDa of *G. spinigerum* AL3 showed the 100% sensitivity and specificity.[20] This study showed high sensitivity and specificity of an immunoblot technique against the recombinant *G. spinigerum* MMP-like protein for detection of IgG antibody are quite similar with previous reports as described above.

In conclusion, we report the successful cloning of the *G. spinigerum* MMP-like gene and the fusion-tagged protein expressed as a soluble form in the *E. coli* cytoplasmic compartment. The recombinant *G. spinigerum* MMP-like protein has the potential for development of a serodiagnostic kit for human gnathostomiasis in endemic areas as a stable mass production system.

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VERSITA

Onchocerca lupi infection in Turkey: A unique case of a rare human parasite

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Abstract

Onchocerpa lupi was first isolated from a wolf in Russia. Since then, canine ocular onchocercosis has been increasingly reported, particularly in Europe and the United States. It is thought that blackflies and midges are the vectors of transmission, and it is possible that these vectors could transmit the parasite to humans. The first human case of *O. lupi* in Turkey was reported in 2011. In this report we present the third human case of *O. lupi* infection in Turkey. Our patient was a 28-year-old male who displayed a painless, immobile mass under the conjunctiva. The mass measured 10 x 12 mm in size. Pathological examination of the surgically excised tissue was suggestive of infection by a filarial nematode. Subsequently, the parasite was identified as *O. lupi* through molecular analysis. All of the previously reported cases of *O. lupi* in both humans and dogs were more symptomatic than in our patient, *Onchocerca* infection should not be ruled out during the differential diagnosis of the subconjunctival and orbital cystic mass in instances where there is little to no inflammation. It is important to consider biopsy and carry out molecular analysis to identify the parasite.

Keywords

Onchocerca lupi, orbita, Turkey, zoonotic infestation

Introduction

Ocular onchocercosis is a zoonotic parasitic disease caused by filarial nematodes of the genus *Onchocerca*. One of the species within this genus is *Onchocerca lupi*, which is known to parasitize dogs and other canids. *O. lupi* was first described from a sample taken from a wolf in Russia (Rodonaja 1967). Since then, canine ocular onchocercosis has increasingly been reported, particularly in Europe and the United States (Sréter and Széll 2008). Canine onchocercosis has been reported as both acute and chronic ocular and periocular infections, with either eye susceptible to infection. Additionally, a wide variety of ophthalmic manifestations have been reported, ranging from conjunctivitis to exophthalmos (Sréter and Széll 2008). It is thought that blackflies and midges are the vectors of transmission, and it is possible that these vectors could transmit the parasite to humans. The first two verified cases of *O. lupi* parasitization in humans occurred in the northwest of Turkey and were described by Otranto *et al.* (2011, 2012). In this report we describe the third confirmed human case of an *O. lupi* infection in Turkey.

Materials and Methods

A 28-year-old Turkish male with a tangible mass under the bulbar conjunctiva in the right eye was admitted to the Department of Ophthalmology at Dokuz Eylul University in Izmir, Turkey. The individual worked as a farmer in a village near Izmir (38°25′N, 27°09′E), a city on the Turkish Aegean coast. He had never traveled abroad nor in other regions of Turkey. Furthermore, there was no evidence of an insect bite nor was there a history of an animal attack.

Upon ophthalmologic examination, a painless, immobile mass measuring approximately 10 x 12 mm in size was detected in the patient's right eye. The patient's right eye was also slightly pseudoptotic (Fig. 1a). The anterior margin of the mass

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