Table 1 Patient characteristics and antimalarial drugs used.

	P. vivax malaria ($n = 75$)	*P. ovale* malaria ($n = 19$)
Age in years (IQR)	28.0 (23.0–39.0)	29.0 (25.0–35.0)
Male (%)[a]	55 (74.3)	15 (78.9)
Body weight in kg (IQR)[b]	60 (54.0–65.8)	61 (60.0–68.0)
Region of disease acquisition		
Africa	8[c]	19
Asia	43	0
Oceania	14	0
Latin America	10	0
Schizonticide (primary attack)		
Chloroquine	32	8
Mefloquine	23	9
Artemether-lumefantrine	5	1
Other antimalarials or combinations of the above drugs	14	1
Data not available	1	0
Total primaquine dose (primary attack)		
210 mg	34	14
420 mg	33	4
Others	6	0
Data not available	2	1

[a] Gender not known in 1 *P. vivax* case.
[b] Body weight not known in 13 *P. vivax* and 4 *P. ovale* cases.
[c] 3 cases contracted in Uganda, 2 cases in Rwanda, 1 case each in Ghana, Mali, and Liberia.

the Research Group members provided treatment advice. After dispensing primaquine, the physicians were asked to complete and submit the clinical record to the Research Group. Primaquine treatment success was defined as the absence of a malaria episode for at least 1 year after the most recent primaquine treatment. To evaluate drug adverse events, cases were analyzed only if their blood exam data were available 7 days or later after commencing primaquine intake.

2.3. Study patients, materials, and the statistics

Only Japanese patients with *P. vivax* or *P. ovale* malaria, who were treated with primaquine for radical cure, were enrolled in this study. One reason for this is that foreign nationals could easily be lost to follow-up for relapses since they may return to the home country, and another is to know whether primaquine is safe in Japanese travelers. Analysis was performed principally using the submitted clinical records; however, when ambiguities arose, a direct inquiry was made to the patient's physician in order to obtain the relevant information.

Data were input into Microsoft Excel 2007 and GraphPad Prism 5. Patient characteristics are shown as median values with the interquartile range (IQR). Mann–Whitney *U* test was used to determine statistical significance between groups, and a p value <0.05 was considered significantly different.

3. Results

3.1. Patient characteristics

Between 2003 and 2012, 75 *P. vivax* and 19 *P. ovale* cases in Japanese travelers were enrolled, and the patient characteristics including the suspected regions of disease acquisition are shown in Table 1. The above *P. vivax* cases included 3 children, defined as patients aged <15 years. Individually, they were a 7-year-old boy (weighing 24.1 kg) who contracted malaria in Brazil, an 11-year-old boy (32.5 kg) who contracted malaria in Pakistan, and a 13-year-old girl (60 kg) who contracted malaria in India. No children were included in the *P. ovale* cases.

3.2. Schizonticide and primaquine use at primary attacks

Schizonticides used for acute-stage therapy against *P. vivax* and *P. ovale* primary attacks are also shown in Table 1. Mefloquine and oral quinine, but not chloroquine, were the only licensed antimalarials approved for malaria treatment in Japan.

Following the acute-stage therapy, *P. vivax* and *P. ovale* primary attacks were treated with primaquine, in most cases with a total dose of 210 mg or 420 mg, many of which being 15 mg daily for 14 days or 30 mg daily for 14 days, respectively (Table 1). Among the pediatric *P. vivax* cases, the 7-year-old boy received 7.5 mg daily primaquine for 14 days, and the 13-year-old girl received 30 mg daily for 7 days. The dosage the 11-year-old boy received was unknown.

3.3. Primaquine failures

Five cases experienced at least one malaria relapse, all of which were *P. vivax* malaria (Table 2), thus leading to a 6.7% relapse rate among our *P. vivax* cases. None of the above pediatric patients had a relapse. One *P. vivax* case, contracted in Indonesia (46/M), showed radical cure with the second round of standard primaquine therapy. Another case contracted in Papua New Guinea (63/F) involved treatment with 15 mg primaquine daily at the primary attack, which was discontinued prematurely due to an adverse event (described later). The patient experienced a relapse and was treated with 15 mg primaquine daily for 24 days. Nevertheless, the second relapse occurred which was successfully treated with the same primaquine regimen. In the 3 other patients (53/M, 48/M, 25/M), increased second dosages were effective, the last case having been reported elsewhere but without discussing the total dose per body weight [17]. Body weight was not known in one of those patients.

A breakdown by country of disease contraction could not be assessed systematically, since various countries were visited by the travelers, leading to unsuitably small denominator figures. However, we note that while relapse occurred in 2 of 3 cases acquired in Papua New Guinea and treated with a total dose of ≤3.5 mg/kg, none of the 6 similar cases acquired in India relapsed.

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Table 2 Relapsed cases of *Plasmodium vivax* after primaquine therapy for radical cure.

Age/gender	Body weight (kg)	Country of disease acquisition	1st primaquine therapy			2nd primaquine therapy			3rd primaquine therapy		
			Dosage	Total dose per body weight	Relapse, interval	Dosage	Total dose per body weight	Relapse, interval	Dosage	Total dose per body weight	Relapse, interval
46/M	71	Indonesia	15 mg/d 14 d	3.0 mg/kg	+, 320 d	15 mg/d 14 d	3.0 mg/kg	−	/	/	/
63/F	58	Papua New Guinea	15 mg/d 10 d	2.6 mg/kg	+, 105 d	15 mg/d 24 d	6.2 mg/kg	+, 50 d	15 mg/d 24 d	6.2 mg/kg	−
53/M	ND	Myanmar	15 mg/d 14 d	NA	+, 85 d	30 mg/d 14 d	NA	−	/	/	/
48/M	82	Papua New Guinea	15 mg/d 14 d	2.6 mg/kg	+, 132 d	30 mg/d 14 d	5.1 mg/kg	−	/	/	/
25/M	64	Brazil	15 mg/d 14 d	3.3 mg/kg	+, 81 d	30 mg/d 14 d	6.6 mg/kg	−	/	/	/

ND, not described, NA, not assessed.

In Fig. 1, individual evaluable *P. vivax* and *P. ovale* episodes including 5 *P. vivax* relapses are plotted in relation to the total primaquine dose per body weight received. In some columns, plots are divided into 2 clusters, one corresponding to lower primaquine doses per body weight, typically standard therapy, and the other corresponding to higher doses. While successfully treated *P. vivax* episodes had received a wide range of total primaquine doses per body weight (2.6–10.5 mg/kg), most of those that later relapsed had received lower doses (2.6–3.3 mg/kg). One exception was a case contracted in Papua New Guinea who did relapse once with 6.2 mg/kg but did not relapse thereafter with the same dosage. The median values of the "success" group and "failure" group in the 1st primaquine treatment were 4.7 mg/kg (IQR, 3.5–7.0) and 2.8 mg/kg (IQR, 2.6–3.2), respectively, and were significantly different. All *P. ovale* patients were successfully treated with total primaquine doses of 2.8–7.0 mg/kg.

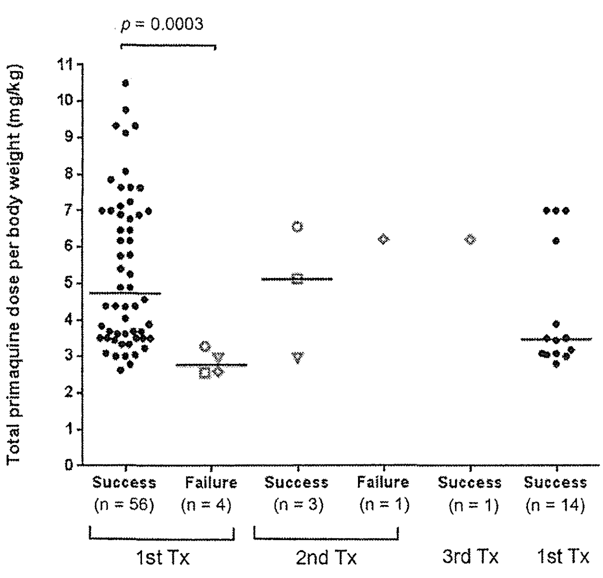

Figure 1 Treatment outcomes in relation to the total primaquine dose per body weight in radical cure of *P. vivax* and *P. ovale* malaria. Filled symbols represent a malaria case that was successfully treated with primaquine at the primary attack. Open symbols represent a malaria case that was not successfully treated with primaquine at the primary attack, and are shown individually by their own symbol in order for them to be followed up. Success = non-relapse after the primaquine, Failure = relapse after the primaquine therapy, Tx = treatment.

3.4. Drug adverse events

Among 60 evaluable adult patients, drug adverse events were reported in 3 patients (5.0%), 2 of whom exhibited liver function disturbance. There were no data reported concerning the drug's safety in the 3 children. One adult with hepatotoxicity (63/F) had underlying chronic hepatitis C and experienced 2 relapses, as described in Table 2. The patient had a pre-treatment aspartate transaminase (AST) level of 51 IU/L and pre-treatment alanine transaminase (ALT) level of 34 IU/L. On day 10 of the primaquine 15 mg daily treatment, AST and ALT levels rose to 244 and 222 IU/L, respectively, and medication was discontinued. Eight days after discontinuation, AST and ALT levels decreased to 101 and 138 IU/L, respectively. After the first relapse, the second primaquine treatment at 15 mg daily was initiated, and at day 10 of treatment, AST and ALT levels rose to 306 and 322 IU/L, respectively. Despite continuation of the medication this time, those levels began to fall (eventually reaching 50 and 104 IU/L, respectively), thus enabling the patient to complete the expected entire 24-day course. After the second relapse, the same treatment was administered, and at day 11 of treatment, no changes were noted in AST and ALT levels. In another patient (30/M), initial AST and ALT levels were 44 IU/L and 66 IU/L, respectively, and began to rise on day 5 of treatment, reaching 91 IU/L and 237 IU/L, respectively, on day 9 of treatment. Despite continuation of the primaquine treatment, AST and ALT levels returned to approximately half of the peak values. Abdominal pain was reported in one patient (28/M) receiving 30 mg daily, medication was discontinued on day

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5 of treatment due to this, and the patient was administered an H_2 blocker. After resuming 15 mg daily primaquine, no adverse events were noted, and the antimalarial was received for the full 14-day course.

4. Discussion

Our current study of primaquine was not conducted as a formal clinical trial, and is therefore subject to several limitations. First, drug intake was mostly not supervised, and many patients may not have been hospitalized when primaquine was dispensed. Second, evaluation of drug adverse events may not have been conducted uniformly by the reporting physicians. Third, some relapses may have been missed due to study termination. However, it is also possible that the physicians have established close relationships with their patients due to the unique nature of this study, resulting in many late-occurring events, such as delayed relapses or drug adverse events, being reported after data was submitted to the Research Group. Specifically, possible effects of the preceding schizonticides on the primaquine efficacy results [10] could not be assessed due to inability to control other factors. Despite these limitations, the present data provide an invaluable foundation for evaluating the efficacy and safety of primaquine in Japanese travelers.

Numerous reports support the increased efficacy of higher total doses of primaquine for radical cure of *P. vivax*. Increases in dosage are usually performed with higher daily doses, but can also be achieved by prolonging drug administration [6,18]. Higher total dose regimens have proved effective treatments for *P. vivax* malaria contracted in countries such as Papua New Guinea [2,19], Somalia [9], and Thailand [20], where the plasmodial strains are regarded as tolerant, or even resistant, to primaquine. The total dose concept was generated in an experimental model of infection with *Plasmodium cynomolgi*, a simian malaria parasite morphologically identical to *P. vivax* that also forms hypnozoites in the liver, where treatments with the same total doses of 8-aminoquinoline drugs but of varying daily dose and duration of treatment resulted in the same radical cure effect [21]. This seems to hold true in humans treated for the *P. vivax* Chesson strain (common in Papua New Guinea), where 30 mg daily of primaquine for 14 days [22] was equally effective to a regimen of 60 mg daily for 7 days [19].

The other important aspect of primaquine efficacy is total dose per body weight of the patient, implying that the same doses in persons with a higher body weight could constitute suboptimal doses. Interestingly, over 35 years ago, a recommendation was made that patients who acquire *P. vivax* malaria in Papua New Guinea should receive a total primaquine dose of 6 mg/kg [19]. With the exception of one patient who contracted malaria in Papua New Guinea (receiving a total dose of 6.2 mg/kg), our data are consistent with those of 2 previous studies, which showed that a total dose of 3.5 mg/kg was required for successful radical cure of *P. vivax*. One study, conducted in Israeli travelers returning from Ethiopia found all of the 8 malaria episodes in 5 patients with a subsequent relapse had been treated with a total dose of $\leq$3.5 mg/kg [23]. In addition, a Brazilian study conducted on *P. vivax* cases revealed that 7 relapsed cases had primaquine doses ranging from 2.1 to 3.5 mg/kg [24]. The total dose of 3.5 mg/kg is achieved by those weighing 60 kg on standard primaquine therapy.

Conversely, *P. vivax* cases with a body weight <60 kg may well be effectively treated with standard therapy (resulting in a total dose >3.5 mg/kg), unless they are contracted in Papua New Guinea or other countries with primaquine resistance. This should minimize the risk of unrecognized and very rare drug adverse events at higher doses, even though these higher dose regimens are generally considered safe. However, we should also be keen to a possible exception to this rule, for example, in a French study, wherein 3 of 10 *P. vivax* cases, contracted in French Guiana, relapsed after 30 mg daily of primaquine for 14 days, equaling total doses of 4.0, 4.2, and 6.0 mg/kg, respectively [25].

In agreement with our results, *P. ovale* relapses after standard primaquine therapy are rare, compared to relapse in cases of *P. vivax*. In a Belgian referral center, 2 out of 34 *P. ovale* episodes relapsed after standard primaquine therapy, yielding a 6% relapse rate, while 8 (16%) out of 48 *P. vivax* cases relapsed with the same regimen [26,27].

Previously, primaquine has been regarded as a toxic and poorly tolerated drug. However, this opinion is being revisited in favor of the drug's safety, mainly based on study results examining its preventive use. In one study, Indonesian adults took 0.5 mg/kg daily for up to 1 year [28], and in another, Kenyan children received almost the same daily doses per body weight as above for 11 weeks [29]. Neither patient groups exhibited symptoms that might indicate a safety concern. Adverse events related to primaquine use in malaria cases are typically minor, including abdominal pain, nausea/vomiting, and diarrhea [30,31]. Although liver function disturbance has been documented in animals receiving lethal doses of primaquine [32], to the best of our knowledge, there is no literature revealing significant hepatotoxicity in humans. However, the possibility of hepatotoxicity in patients with a preexisting chronic liver disease should be treated with caution.

Three children received primaquine in our study, but unfortunately, without formal information about the drug's safety. A Japanese questionnaire study conducted in pediatric malaria cases between 1980 and 1999 showed that 17 malaria episodes, of which 8 were in Japanese children, had been treated with primaquine [33]. In this study, there seemed to be no particular safety concern when used by Japanese children (Mizuno Y, personal communication).

In conclusion, primaquine appears to be effective and safe for radical cure of malaria in Japanese travelers. To optimize treatment outcomes of *P. vivax*, the total primaquine dose per body weight should be considered, at least 3.5 mg/kg or even more if contracted in countries with significant drug resistance. However, for *P. ovale*, standard primaquine therapy is still recommended.

Conflict of interest

The authors declare no conflicts of interest.

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□ ORIGINAL ARTICLE □

Paragonimiasis in Japan: A Twelve-year Retrospective Case Review (2001-2012)

Eiji Nagayasu[1], Ayako Yoshida[1], Amy Hombu[1], Yoichiro Horii[2] and Haruhiko Maruyama[1]

Abstract

Objective Paragonimiasis, or lung fluke infection, is a food-borne parasitic disease caused by infection with trematodes belonging to the genus *Paragonimus*. Although paragonimiasis was once considered successfully controlled in the 1970s, new cases began to emerge in the late 1980s. To apprehend the current-day situation of the re-emergent cases of paragonimiasis in Japan, we conducted a retrospective review of 443 patients who were referred to our laboratory and diagnosed as having paragonimiasis during 2001-2012.

Methods Patients were diagnosed as having paragonimiasis based primarily on immunodiagnostic methods in addition to clinical, laboratory, and radiographic findings. Patient data were extracted from consultation sheets from attending physicians and were analyzed.

Results Majority of the patients were residents of Kyushu Island. However, a substantial number of cases were also from other parts of Japan. Immigrants (mostly from China, Thailand, and Korea) accounted for a quarter of the cases. Native Japanese contracted paragonimiasis by consuming wild boar meat or freshwater crabs, whereas immigrants contracted the infection almost exclusively by consumption of freshwater crabs. Eosinophilia and elevated serum IgE levels were found in around 80% of the patients. Parasite egg detection was documented only in 11.7% of the cases, showing the reliance on serological tests for diagnosing paragonimiasis in current clinical practice.

Conclusion Paragonimiasis remains a public health issue in Japan, and the situation should be closely monitored.

Key words: paragonimiasis, lung fluke, immunodiagnosis, eosinophilic pneumonia, immigrants, re-emergence

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Introduction

Paragonimiasis, or lung fluke infection, is a food-borne parasitic disease caused by infection with a number of trematode species belonging to the genus *Paragonimus*. In Japan, two species, *P. westermani* and *P. skrjabini miyazakii*, are pathogens causing human paragonimiasis, with the former being tremendously dominant (1-3). Human infections occur by consuming freshwater crustaceans (second intermediate host) or wild boar meat (paratenic host) (4). In Japan, *Eriocheir japonica* (Japanese mitten crab), *Geothelphusa dehaani* (Japanese freshwater crab) and *Sus scrofa leucomystax* (Japanese wild boar) are important sources of human infections (2).

Previously, paragonimiasis was endemic in most parts of Japan. In the late 1950's, it was estimated that more than 300,000 people were infected with the disease (5). However, intensive mass screening and treatment campaigns performed in endemic areas from the 1950's to 1960's successfully reduced the number of new cases, and paragonimiasis was considered to be a rare local disease of the past by the 1970's (6, 7).

However, new cases began to emerge in the late

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Table 1. Multiple Logistic Regression Analysis for White Blood Cell Count and Percentage of Eosinophil

	Dependent variables			
	WBC counts ≤10,000 (0), >10,000 (1)		Eosinophil (%) ≤7.0 (0), >7.0 (1)	
Independent variables	Odds ratio (95% CI)	p value	Odds ratio (95% CI)	p value
Age				
≤50 years old (0) >50 years old (1)	0.647 (0.327-1.281)	0.212	1.040 (0.519-2.085)	0.912
Sex				
male (0) female (1)	1.133 (0.559-2.298)	0.729	1.807 (0.795-4.112)	0.158
Ethnicity (Birth origin)				
native Japanese (0) immigrants (1)	1.679 (0.746-3.778)	0.195	0.931 (0.336-2.578)	0.890
Eating history				
wild boar meat (1) freshwater crab (0)	2.935 (1.366-6.308)	0.006*	1.694 (0.764-3.753)	0.194

1980's (8), and a gradual increase in the annual number of paragonimiasis cases was observed during the 1990's (1, 8). Detailed analyses of the clinical features of "re-emergent" cases were reported using clinical records collected between 1986 and 1998 (1) and 1999 and 2001 (9). Among these "re-emergent" cases, the majority of patients were middle- to old-aged men, and the major source of infection was the consumption of raw wild boar meat.

A gradual increase in the number of immigrant cases attracted attention from 1998 to 2002 (10). Clinical features of paragonimiasis in immigrant patients were found to be different from those observed in native Japanese patients. For example, the mean age of the immigrant patients was lower, with the majority of affected individuals being women, than that of the Japanese patients. In addition, the major source of infection was freshwater crabs consumed either in Japan or their home countries.

In the present study, we analyzed patient records collected between 2001 and 2012. The number of patients diagnosed as having paragonimiasis in our laboratory totaled 443. We summarized the demographic and clinical characteristics of these patients. Our records likely represent a substantial portion of the paragonimiasis cases that occurred nationwide within the past 12 years and provide the opportunity to elucidate the clinical features of current cases of paragonimiasis.

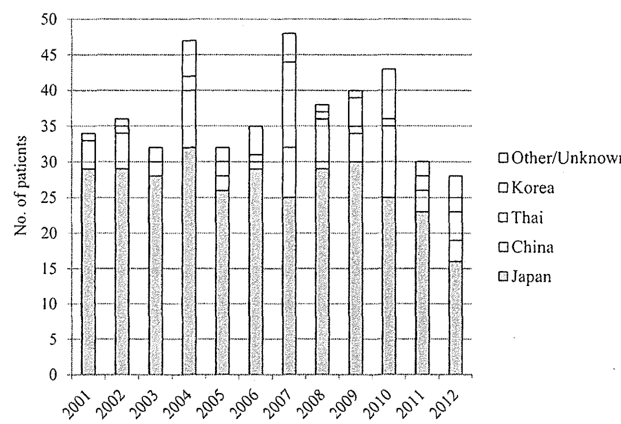

Figure 1. Annual number of paragonimiasis cases diagnosed in our laboratory (2001–2011) with a breakdown by country of birth.

Materials and Methods

Subjects and samples

A total of 443 of approximately 5,200 cases referred to our laboratory were diagnosed as paragonimiasis based on the patient's information, as provided by the attending physicians in the form of consultation sheets; this information included clinical symptoms, eating history, radiographic findings and laboratory data in addition to the results of immunodiagnostic tests performed in our laboratory. This study was approved by the Institutional Review Board of the Faculty of Medicine at the University of Miyazaki.

Immunodiagnostic methods

The details of the immunodiagnostic methods have been previously described (11). A multiple-dot enzyme-linked immunosorbent assay (ELISA) was used as a routine primary screening procedure.

Statistical analysis

The patients were categorized according to their age, sex, ethnicity (based on their birth place described on the consultation sheets) and eating history. Depending on the purpose of the analysis, the patients were also categorized based on

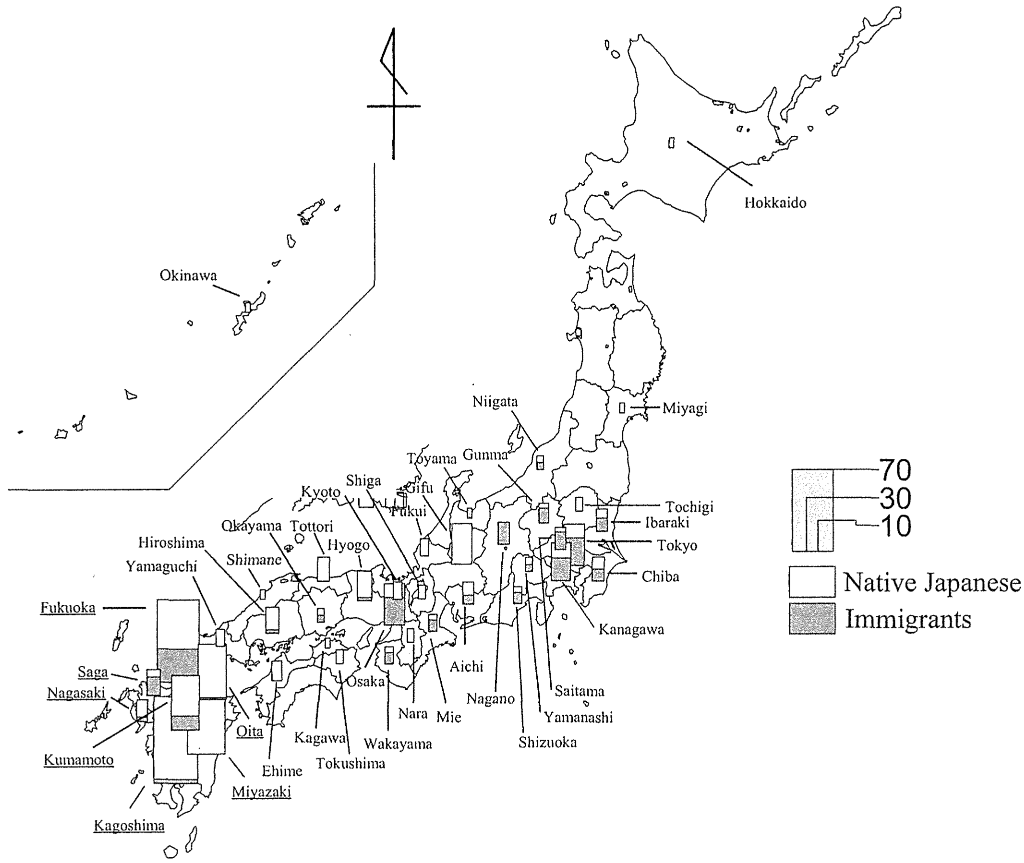

Figure 2. Geographical distribution of paragonimiasis cases diagnosed in our laboratory (2001–2012). The places of residence (prefecture) of the patients diagnosed in our laboratory during 2001–2012 are shown. The number of cases and proportion of native Japanese patients to immigrants are expressed in a variable width bar chart for each prefecture.

either the presence or absence of particular symptoms, abnormal chest radiograph findings or abnormal laboratory data.

Differences in the number of patients who exhibited various abnormalities were assessed using the chi-square test with Yates' correction or Fischer's exact test (when any of the expected cell frequencies were less than 5).

Multiple logistic regression analyses were conducted using the SPSS 22 software program (IBM Corp, Armonk, USA) to assess the factor(s) potentially affecting the frequency of a particular clinical symptom (chest/chest-back pain) or the absence of symptoms, as described in the Results section, and identify factor(s) potentially affecting the frequency of patients with a high white blood cell count (>10,000 cells/μL) or eosinophilia (>7.0% of white blood cells). For the analyses, all categorical variables were dummy-coded, as shown in Table 1 (male/female = 0/1, for example).

Results

Of approximately the 5,200 cases referred to our laboratory during the period of 2002-2012 for the serodiagnosis of various parasitic diseases, a total of 443 were diagnosed as paragonimiasis. The annual number of native Japanese patients with paragonimiasis was relatively consistent until 2009, ranging from 25 to 30, followed by a gradual decrease until 2012 (Fig. 1). The annual number of immigrant patients with the disease exhibited more marked fluctuation, ranging from 4 (in 2003) to 23 (in 2007) cases. Overall, immigrant cases represented 25.5% (113 of 443) of the total number of cases during the observation period. Among the immigrants, China (50 patients) was the most common country of origin, followed by Thailand (34 patients) and Korea (26 patients). These three countries accounted for 97% of all immigrant cases.

The geographical distribution of the cases is shown in Fig. 2. The majority (63.4%) of the patients were residents of Prefectures on Kyushu Island (Fukuoka, 71; Saga, 7; Nagasaki, 5; Kumamoto, 31; Oita, 32; Miyazaki, 56; and Kagoshima, 79), the most southwesterly of Japan's four main islands. Outside Kyushu Island, Tokyo (18 cases), Kanagawa (15), Gifu (17) and Osaka (18) each had a relatively large number of patients. Only a few cases were referred to us from Prefectures in northern Japan (Tohoku district and Hokkaido). More immigrant cases were seen outside Kyushu Island (65 of 162, 40.1%) compared with that observed on Kyushu Island (48 of 281, 17.0%). Prefectures with large

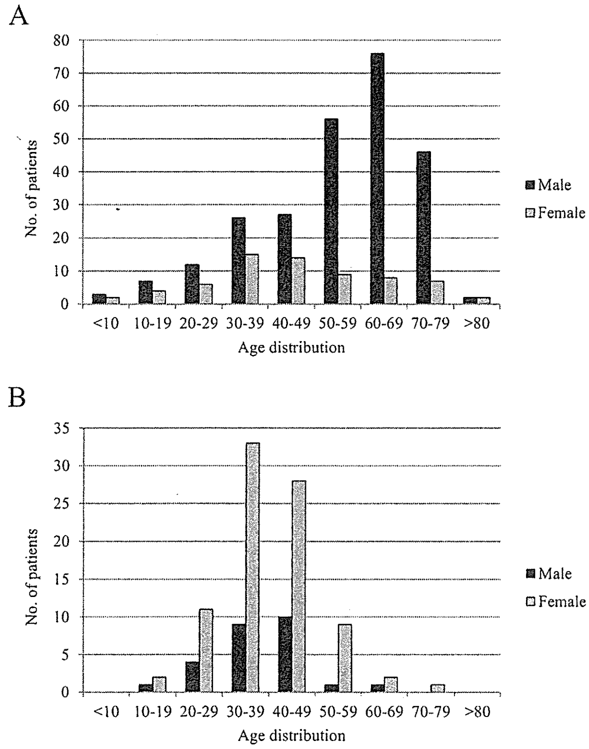

Figure 3. Age distribution of the paragonimiasis cases diagnosed in our laboratory (2001–2012). (A) Native Japanese patients and (B) immigrant patients

populations, such as Tokyo and Osaka, tended to have a high percentage of immigrant cases (12 of 18, 66.7%, for both).

Among the native Japanese patients, there were more men than women in every age group, with the exception of patients over 80 years of age (Fig. 3A). Furthermore, there was a marked difference in the peak age of disease presentation between the sexes. In men, the peak was seen in the age group of 60-69 years (mean age: 55), compared to the age group of 30-39 years in women (mean age: 45). This difference with respect to gender was particularly marked in the age groups over 50 years. Regarding the immigrant patients, there were more women than men in every age group (Fig. 3B). The difference with respect to gender in the peak of disease presentation was not very clear, with men peaking at 40-49 years of age (mean age: 39) and women peaking at 30-39 years of age (mean age: 40).

In order to make comparisons between the "younger" and "older" age groups in terms of symptoms, chest radiographic findings and laboratory data, which will be presented later in this paper, we set an arbitrary cut-off of 50 years of age. As expected according to the age distribution graphs (Fig. 3), there were more patients over 50 years of age among the native Japanese than in the immigrant groups. In men, 175 (68.6%) patients were over 50 years of age among the native Japanese compared to two (7.4%) patients among the immigrants, a statistically significant difference ($p<0.0001$, χ^2 test). Similarly in women, more patients belonged to the older age group among the native Japanese (43 patients, 64.2%), compared to the only eight (9.4%) immigrants ($p=0.0002$, χ^2 test).

The patients' eating history of consuming freshwater crab and wild boar is summarized in Table 2. Approximately half (45.8%) and one-third (32.8%) of male and female native Japanese patients, respectively, had a history of consuming wild boar meat, although only a fraction of patients informed the physicians that they ate these foods raw. Among the immigrant cases, the majority of patients had a history of consuming freshwater crab, whereas wild boar meat was consumed by only three patients. Freshwater crab tended to be consumed raw more frequently by immigrants (34.6% for men and 32.5% for women) than native Japanese (6.2% and 11.9%, respectively). Twenty-four patients informed the attending physicians that they did not consume either freshwater or wild boar meat. Among these subjects, 19 had history of consuming raw deer meat.

Frequent symptoms documented on the consultation sheets are shown in Table 3. Coughing (28.9% of the patients), sputum, including hemosputum (27.3%), chest/chest-back pain (18.5%), fever (11.7%) and dyspnea (10.4%) were the most frequently observed symptoms. Other relatively common, but less frequent, symptoms included subcutaneous nodules (4.9%), rash/pruritus (3.1%), gastrointestinal (GI) symptoms (abdominal pain, vomiting and diarrhea, 6.5%) and back pain (2.3%).

Sixty-six (17.2%) of the patients were asymptomatic (Table 3). These patients were referred to us due to abnormal chest radiograph findings (50 patients), eosinophilia (seven patients) or both (one patient) at their annual check-up or upon visiting the physician for other medical reasons. Nine patients were referred to us in the absence of symptoms because their family members or close friends, who potentially had consumed the same food source of infection, were diagnosed with paragonimiasis.

The percentage of patients who exhibited each symptom was compared according to age groups (≤50 or >50 years old), sex (male or female), ethnic group (native Japanese or immigrant) and possible food source of infection (freshwater crab or wild boar meat). In these comparisons, we found the following differences with statistical significance: 1) chest/chest-back pain and GI symptoms appeared more frequently in the patients belonging to the younger age group; 2) chest/chest-back pain was more frequent in women than in men; 3) asymptomatic cases were more frequent in the older age group and native Japanese than in the younger patients and immigrants, respectively; 4) subcutaneous nodules were more frequent in the immigrants than in the native Japanese; 5) the potential food source of infection (freshwater crab or wild boar meat) did not affect the proportion of patients who presented with these symptoms.

Because these initial comparisons indicated that the presentation of chest/chest-back pain and "no symptoms" was affected by multiple factors, we performed multiple logistic regression analyses to identify factor(s) independently asso-

Table 2. Eating History of Patients

	Native Japanese		Immigrants	
	male	female	male	female
Number of patients	255	67	26	86
Freshwater crabs	63 (24.7%)	22 (32.8%)	17 (65.3%)	66 (76.7%)
Wild boar meat	117 (45.8%)	22 (32.8%)	0 (0%)	3 (3.4%)
Others	18 (7.0%)	5 (7.4%)	0 (0%)	1 (1.1%)
No information available	76 (29.8%)	24 (35.8%)	9 (34.6%)	19 (22.0%)

Note that the total number of patients is 434 instead of 443 because of 9 patients with unknown birth places.

The sums of each rows are more than the numbers of patients except for male immigrants, due to the patiens who consumed both freshwater crabs and wild boar meat (28 patients in total).

Cases were classified as others when the attending physitians described foods other than frashwater crabs or wild boar meat as potential sources of infection, which included raw deer meat (19 cases in total).

ciated with the presence or absence of symptoms. For the analysis of chest/chest-back pain, the age group and sex were used as independent variables. For the analysis of "no symptoms," the age group and ethnicity (native Japanese or immigrant) were used as independent variables. In these analyses, only the age group was found to be associated with the presentation of chest/chest-back pain and "no symptoms." Between the younger and older age groups, the odds ratio of having chest/chest-back pain was 3.23 [95% confidence interval (CI): 1.69-6.25], while that of having no symptoms was 0.31 (95% CI: 0.16-0.60).

We analyzed the chest radiographic findings documented on the consultation sheets. This information was available in 355 of 443 cases (80.1%). The most frequent observation was the presence of pleural effusion (in 208 or 47.0% of cases), followed by pneumothorax (75, 16.9%), nodular shadows (51, 11.5%), infiltrative shadows (39, 8.8%) and mass shadows (29, 6.5%). These findings were compared between the sexes, age groups, ethnic groups and possible food sources of infection. In these comparisons, we found that more patients 50 years of age or younger exhibited pleural fluid than the older patients (53.7% vs. 39.6%, p= 0.0042, χ^2 test). This younger age group also included more cases of pneumothorax than the older patients (22.1%, vs. 11.3%, p=0.0039, χ^2 test). The comparisons between the sexes, ethnic groups and possible food sources of infection did not yield any statistically significant differences.

It is known that peripheral blood eosinophilia and elevated levels of serum immunoglobulin E (IgE) are frequently observed in patients with paragonimiasis, as in other parasitic helminth infections (12, 13). On the other hand, it has been documented that the total while blood cell (WBC) count usually remains in the normal range or is moderately elevated (12, 13). In the present study, the total WBC, peripheral blood eosinophil and serum IgE levels were available in 422, 412 and 264 cases, respectively.

Overall, 29.9% of the patients showed an elevated WBC count, defined as being greater than 10,000 cells/μL. Likewise, 75.5% of the patients exhibited eosinophilia (>7% of the total white blood cell count) and 79.9% demonstrated an elevated (>170 IU/mL) serum IgE level.

These laboratory data were compared between the sexes, age groups, ethnic groups and possible food sources of infection. As shown in Table 4, more patients belonging to the younger age group (≤50 years old), women, immigrants and those who ate fresh water crab exhibited a high-WBC count (>10,000/μL) compared to that observed among the older patients (>50 years old), men and those who ate wild boar meat (χ^2 test). More women and patients who ate freshwater crab exhibited eosinophilia than did men and patients who ate wild boar meat (χ^2 test). As for the serum total IgE level, such comparisons did not yield any statistically significant differences.

In order to isolate factor(s) independently affecting these laboratory data, we performed a multiple logistic regression analysis with the age group, sex, ethnicity and food source of infection as independent variables and the presence or absence of a high WBC count (>10,000/μL) or eosinophilia (> 7.0% of the total WBC count) as dependent variables. As shown in Table 1, only the food source of infection was found to affect the presentation of a high WBC count with statistical significance, with freshwater crab consumption being associated with a 2.9-fold greater chance of a high WBC count. No single factor was proven to affect the incidence of eosinophilia in this analysis.

According to the clinical records, the detection of paragonimus eggs was documented in 52 of 443 cases (11.7%). Bronchoscopic techniques were most commonly used (in 18 cases), followed by examinations of sputum (17 cases), pathological inspection of surgically removed tissue (seven cases) and examinations of pleural fluid (three cases). Egg detection in feces was documented in only one case.

Discussion

The re-emergence of paragonimiasis in Japan attracted attention in the late 1980's (1). Initially, cases diagnosed as paragonimiasis in our laboratory were almost restricted to residents of Kyushu Island. However, currently, a substantial number of patients (36.6% of total cases) living outside of

Table 3. Clinical Symptoms of Patients

Symptoms	Overall	Age			Sex			Ethnicity (birth place)			Eating history		
		≤50 y/o	>50 y/o	p	Male	Female	p	Native Japanese	Immigrants	p	Freshwater crabs	Wild boar meat	p
	(n=384)	(n=211)	(n=173)		(n=239)	(n=144)		(n=275)	(n=101)		(n=126)	(n=96)	
Cough	28.9	27.5	30.6	0.57	28.9	29.2	0.95	29.1	28.7	0.94	27.8	36.5	0.22
(Hemo)sputum	27.3	23.7	31.8	0.10	28.9	25.0	0.48	26.9	27.7	0.98	25.4	28.1	0.76
Chest, Chest-back pain	18.5	26.5	8.7	<0.0001*	13.8	25.0	0.01*	17.8	19.8	0.77	20.6	14.6	0.32
Dyspnea	10.4	9.5	11.6	0.62	10.5	10.4	0.99	10.5	10.9	0.92	10.3	15.6	0.33
Fever	11.7	14.7	8.1	0.07	11.3	12.5	0.85	13.5	7.9	0.20	11.1	8.3	0.65
Subcutaneous nodules	4.9	6.6	2.9	0.15	2.9	7.6	0.06	2.5	11.9	0.001*	8.7	2.1	0.07
Rash, pruritus	3.1	3.3	2.9	0.81	2.9	3.5	0.77	2.5	4.0	0.50	6.3	1.0	0.10
GI symptons	6.5	10.4	1.7	0.0012*	4.6	9.7	0.08	5.1	10.9	0.08	11.9	4.2	0.07
Back pain	2.3	2.8	1.7	0.52	2.5	2.1	1.00	2.2	3.0	0.71	3.2	1.0	0.39
None	17.2	9.0	27.2	<0.0001*	20.1	12.5	0.08	20.4	8.9	0.01*	11.1	18.8	0.16

Note that the total number is 384, instead of 443 because of exclusion of 59 cases that lack the appropriate descriptions of symptoms. Similarly, the sum of each categories such as male and female do not total 384 due to exclusions of the cases that lack the information necessary for the categorization. Patients who ate both fresh water crabs and wild boar meat were also excluded.

Gastrointestinal (GI) symptoms included abdominal pain, vomiting, and diarrhea.

p values were calculated using chi-square test with Yates' correction or Fisher's exact test when necessary.

Kyushu Island are also diagnosed as having paragonimiasis. One factor contributing to this phenomenon may be the sophistication of the distribution system of fishery products in Japan. Today, it is common to see live freshwater crab being sold at supermarkets in big cities. One study showed that 17% of freshwater crabs (*G. dehaani*) purchased in Tokyo contain *Paragonimus* metacercariae (14).

An age difference between native Japanese and immigrants has been noticed previously (immigrants are younger on average) (10) based on the clinical records collected in the period of 1986-1998 for native Japanese (1) and 1998-2002 for immigrants (10). In the present study, although the trend was the same, the mean age had increased from 48 to 53 for native Japanese and 36 to 40 for immigrants.

Among male native Japanese, paragonimiasis occurs predominantly in middle- and old-aged individuals who consume wild boar meat or freshwater crab. Among female native Japanese, paragonimiasis in the elderly is less common than that observed in men. Wild boar meat is also a less common suspected source of infection in female native Japanese. Regarding immigrants, paragonimiasis is diagnosed most frequently in middle-aged individuals. In contrast with native Japanese patients, more female patients were found among the immigrants in this study. The suspected food source of infection in this group is almost exclusively freshwater crab.

It is interesting to determine the source of infection in immigrants. In many cases, occasional visits to the immigrant's home country obscures the true source of infection. However, in a case of group infection among Thai patients presented to us in 2007, it was clear that the patients acquired the infection in Japan after eating the same dish containing raw freshwater crab (data not shown). In contrast, we also observed a case of a woman who developed symptoms two months after visiting Thailand. If she was truly infected in Thailand, the etiological agent should have been *P. heterotremus*, the only *Paragonimus* species affecting humans in Thailand (15). Although such a distinction does not directly affect treatment *per se*, it is expect to help construct a better public health strategy for controlling this disease.

We found that patients over 50 years of age were 3.18 times more likely to be asymptomatic compared to the patients belonging to the younger age group. The patients in this age group may have more occasions to receive health check-ups than those in the younger age group and therefore may have more opportunities to have paragonimus infection detected while symptoms are not apparent. Although a simple comparison showed that more Japanese patients than immigrants were asymptomatic (Table 3), this finding is most likely to reflect the fact that the Japanese group included more patients belonging to the older age group than did the immigrant group, based on the results of the multiple logistic regression analysis in which only age group, and not ethnicity, was found to be associated with the absence of symptoms.

Likewise, although the initial comparisons indicated that the frequency of patients presenting with chest/chest-back pain was affected by sex, with women more often exhibiting this symptom, this finding may be due to the fact that the female group included more patients belonging to the younger age group based on the results of the multiple lo-

Table 4. Laboratory Data of Patients

Classifications	WBC count			Eosinophil count (%)			Serum total IgE		
	n	>10,000/μL	p	n	>7.0%	p	n	>170IU	p
<=50 y/o	131	43.5	0.0003*	130	79.2	0.7493	89	76.4	0.96
>50 y/o	108	20.4		107	72.9		64	79.7	
male	149	24.8	0.0009*	147	71.4	0.033*	89	79.8	0.61
female	90	46.7		90	84.4		64	75.0	
Native Japanese	165	23.0	<0.0001*	164	73.2	0.1157	164	73.2	0.31
Immigrants	74	55.4		73	83.6		73	83.6	
Freshwater crab	130	47.7	<0.0001*	129	82.2	0.0321*	94	77.7	0.96
Wild boar meat	109	15.6		108	69.4		59	78.0	

The sum of each classifications are less than 443 due to exclusion of cases that lack the information necessary for the classification or laboratory data

Values are presented as percent.

p values were calculated using chi-square test with Yates' correction.

gistic regression analysis.

Significantly more cases of subcutaneous nodules were observed among the immigrants than among the native Japanese (Table 3). Subcutaneous nodules are one manifestation of extrapulmonary paragonimiasis, which is caused by the aberrant migration of juvenile worms. We suspect that high-density infections tend to occur more frequently in immigrants than in native Japanese, as suggested by Obara et al. (2004) (10), which may account for the higher occurrence of subcutaneous nodules, as it has been proposed that this manifestation is likely to occur more frequently in patients with heavy infections (16).

On the analysis of the chest radiograph findings, we observed pleural effusion and pneumothorax in the older age group more frequently than in the younger age group (Table 5). Because it has been reported that pleural manifestations are characteristic features of the early stage of paragonimiasis (17, 18), this finding again leads to the speculation that cases of paragonimiasis are detected earlier in older patients than in younger patients.

It is intriguing to find that more patients who ate freshwater crab exhibited a high WBC count than those who consumed wild boar meat (Table 1). The immune reactions elicited by paragonimus infection may differ quantitatively or qualitatively depending on the food source of infection. The biological mechanisms underlying this phenomenon remain to be investigated.

In the present study, eosinophilia and an elevated serum IgE level were found in a high percentage of patients (75.5% and 79.9%, respectively). Therefore, in patients presenting with typical symptoms, such as coughing and sputum production, and/or those who show abnormal chest X-ray findings, assessing the blood eosinophil count and serum IgE level is quite helpful when paragonimiasis is suspected, even when accurate data regarding the patient's eating history are not available.

Parasitic eggs (in the bronchoscopic fluid, sputum or pleural fluid in most cases) were detected in only 11.7% of cases, according to the available clinical information. The detection of eggs in feces was documented in only one case. We do not have information regarding the number of cases in which fecal examinations were performed. Only three records mentioned negative results on fecal examinations. It is most likely that fecal examinations were not performed in many of the remaining cases. We expect that the detection rate would be low on fecal examinations based on a past publication that reported the detection of paragonimus eggs in feces in only 1.9% of 365 individuals with a positive skin reaction against a paragonimus antigen (19).

The number of native Japanese patients with paragonimiasis has been decreasing over the past three years (since 2009). This may be due to a change in attitude toward eating raw meat, particularly after a widely broadcast food poisoning outbreak caused by eating raw beef tainted with enterohemorrhagic *Escherichia coli* (EHEC) in 2011 (20).

Traditionally, game meats, such as wild boar and venison, have been consumed locally by hunters and their families in Japan. However, in recent years, there have been efforts by some local governments to distribute such meats through consumer markets. In addition, several internet shopping sites are now selling game meats, propelled by the growing popularity of gibier dishes in Japan. The effects of the current easy access of general consumers to game meats on the pattern of paragonimiasis infection should be monitored closely. Because there is no nationwide surveillance system for this disease in Japan, we believe that the present study provides an important basis for such monitoring.

Although a treatment regimen with praziquantel (oral dose, 75 mg/kg body weight daily for three consecutive days) is recommended (21), we encountered at least 12 cases of underdosing. Therefore, there is still a need to increase awareness of this disease and its treatment among clinicians.

The authors state that they have no Conflict of Interest (COI).

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Table 5. Chest Radiograph Findings of Patients

Symptoms	Overall	Age			Sex			Ethnicity (birth place)			Eating history		
		≤50 y/o	>50 y/o	p	Male	Female	p	Native Japanese	Immigrants	p	Freshwater crabs	Wild boar meat	p
	(n=443)	(n=231)	(n=212)		(n=284)	(n=159)		(n=322)	(n=113)		(n=140)	(n=114)	
Pleural effusion	47.0	53.7	39.6	0.0042*	47.2	46.5	0.98	45.7	50.4	0.44	51.4	41.2	0.92
Pneuothorax	16.9	22.1	11.3	0.0039*	15.5	19.5	0.34	17.4	16.8	0.89	17.9	17.5	0.93
Nodular shadow	11.5	8.7	14.6	0.07	12.7	9.4	0.38	11.8	9.7	0.67	12.1	11.4	0.67
Infiltrative shadow	8.8	8.7	9.0	0.91	8.5	9.4	0.86	8.4	10.6	0.60	7.9	9.6	0.60
Mass shadow	6.5	4.3	9.0	0.08	8.1	3.8	0.12	8.1	2.7	0.08	3.6	11.4	0.73
No data available	19.9	17.7	22.2	0.30	18.0	23.3	0.22	12.7	23.9	0.0078*	22.9	15.8	1.00

Data are presented in percentage.
Note that the sum of each categories such as male and female do not total 443 due to exclusions of cases that lack the information necessary for the categorization.
p values were calculated using chi-square test with Yates' correction or Fisher's exact test when necessary.

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Cutaneous paragonimiasis due to triploid *Paragonimus westermani* presenting as a non-migratory subcutaneous nodule: a case report

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Abstract

Introduction: Paragonimiasis is a food-borne infection caused by *Paragonimus* parasites. The lungs and pleura are the primary sites for the infection; however, ectopic infection can occur in other organs such as skin, liver and brain. It is difficult to make a diagnosis of ectopic paragonimiasis due to an ignorance of, and unfamiliarity with the disease. We report the case of a patient with subcutaneous paragonimiasis diagnosed by histopathological analysis and serological testing.

Case presentation: A 39-year-old Chinese immigrant woman presented with a subcutaneous nodule in her left lower back. The nodule was initially suspected of lipoma and she was followed up on without any treatment. However, it gradually indurated and the nodule was resected surgically. A magnetic resonance imaging scan revealed a polycystic lesion with inhomogeneous low or high intensity on T1- or T2-weighted images, respectively. The rim of the lesion was enhanced after contrast enhancement, but the inside did not show high-signal intensity. A histological analysis of the surgically resected specimen revealed variable-sized tubulo-cystic structures. The cyst wall showed a granulomatous change with scant eosinophilic infiltration. A number of parasite ova were observed in the necrotic tissue inside the cysts, and a parasite body with a presumed oral sucker and reproductive organ was also detected, suggesting a trematode infection. A subsequent serological examination showed a positive reaction of her serum to the *Paragonimus westermani* antigen. No abnormal findings were found on her chest computed tomography scan. The diagnosis of subcutaneous paragonimiasis caused by *Paragonimus westermani* was made.

Conclusions: We report a case presenting only as a non-migratory subcutaneous nodule without any pleuropulmonary lesion, which was initially suspected of lipoma but denied by magnetic resonance imaging scan results. The case was subsequently diagnosed as subcutaneous paragonimiasis from the results of histopathological analysis and serological testing.

Keywords: Subcutaneous nodule, *Paragonimus westermani*, Histopathology

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Introduction

Paragonimiasis is a food-borne infection caused by *Paragonimus* parasites, such as *Paragonimus westermani* and *Paragonimus skrjabini miyazakii*. Among the *Paragonimus* species, only *Paragonimus westermani* has a triploid variant, which can produce ova via parthenogenesis [1]. The primary organs for parasite infestation are the lungs and pleura, therefore most patients present with signs and symptoms involved in the lower respiratory tract and pleura such as cough, sputum, chest pain, dyspnea and pleural effusion. In some cases, ectopic infection occurs at unexpected sites such as skin, brain, liver and peritoneal cavity, due to erratic migration [1-3]. Ectopic paragonimiasis is difficult to confirm as a diagnosis because of its rarity and variable symptoms, which has caused an ignorance of, and unfamiliarity with of the disease. We report the case of a patient with subcutaneous paragonimiasis diagnosed using histopathological analysis and serological testing.

Case presentation

A 39-year-old Chinese immigrant woman had been aware of a subcutaneous nodule in her left lower back for a year and sought medical attention. In her past history, she had frequent opportunities to have been exposed to drunken crab (raw crab soaked in rice wine), especially before she emigrated in Japan seven years ago. Additionally, she had been a cigarette smoker in her twenties (5 cigarettes per day). No specific family history was addressed. The nodule was initially suspected of being soft tissue tumor, particularly lipoma, and followed up on for over a year without any treatment. However, the nodule gradually indurated and surgical resection was chosen as treatment. Her physical examination before the resection revealed neither fever nor abnormal pulmonary sounds. Her white blood cell (WBC) count was 6740/μL (reference range: 3500 to 8700/μL), and neither her eosinophil count nor serum immunoglobulin E (IgE) level was examined.

She was suspected of having lipoma and a magnetic resonance imaging (MRI) scan was performed. A cystic lesion was found in the subcutaneous tissue on her left lower back, suggesting a closely-aggregated tortuous and inflected tubular architecture. In the lesion, inhomogeneous low-signal intensity was observed on a T1-weighted image (T1WI) (Figure 1A), and inhomogeneous high-signal intensity was observed on a T2-weighted image (T2WI) (Figure 1B and C). A contrast-enhanced MRI scan revealed a high-signal intensity was not detected in the inside while the rim of the nodule was enhanced (Figure 1D). No infiltrative lesion to the muscle layer or retroperitoneum was detected. The signal intensity of the contents of the lesion was similar to that of water, suggesting serous or mucinous fluid rather than blood.

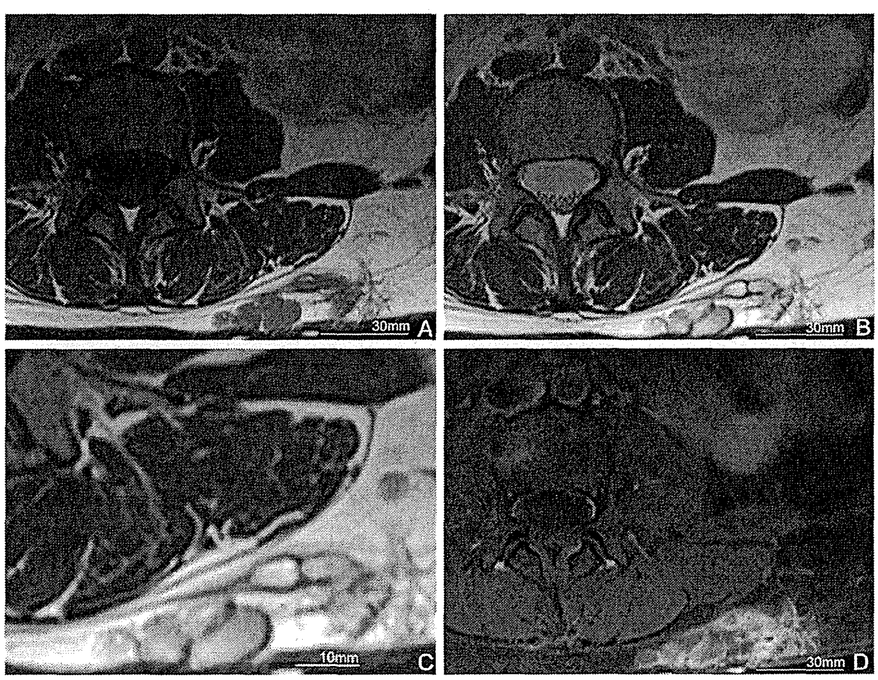

Figure 1 Magnetic resonance imaging (MRI) findings. A. Polycystic mass with inhomogeneous low intensity on T1-weighted image (T1WI). **B.** The mass with inhomogeneous high intensity on T2-weighted image (T2WI). **C.** The magnified image of the nodular lesion in **B. D.** Rim enhancement is seen but the inside of the mass shows no increment of density after contrast enhancement.

Based on these imaging findings, the preoperative diagnosis of the lesion was lymphatic vessel malformation or mucinous nodules. Subsequently, a surgical resection was performed.

Macroscopically, the resected specimen was an abscess-like nodular lesion beneath the skin. On the cut surface, a nodular lesion consisted of creamy yellowish material containing a small hematoma-like fragment and was surrounded by a subcutaneous adipose tissue. Microscopically, this lesion was composed of one large tubulo-cystic structure and several small cysts (Figure 2A). The cyst walls showed a granulomatous change with scant eosinophilic infiltration (Figure 2D). A number of parasite ova were observed in necrotic debris inside the cystic structures. The ovular shell thickness was uneven and some were distorted. There were several cells with round nuclei and eosinophilic cytoplasm in the ova, suggesting yolk cells (Figure 2B). Each ovum was approximately 80×50μm in size. They were also intensely birefringent under polarized light (Figure 2C) [4]. These findings were compatible with those of the ova of *Paragonimus*, particularly *P. westermani*, in terms of size. No apparent operculum was detected in the hematoxylin and eosin (HE) stained sections. Furthermore, histologic analysis of the hematoma-like fragment in the main cyst revealed a parasite body with a presumed oral sucker and reproductive organ (Figure 2E). These finding are compatible with the characteristics of adult trematodes, raising the possibility of subcutaneous *Paragonimus* infection. In addition, only a single worm was found in the lesion, suggesting that this worm was a triploid form of *P. westermani* producing ova via parthenogenesis, not a diploid form of the *Paragonimus* species.

On serological examination using multiple-dot enzyme-linked immunosorbent assay with antigens of *P. westermani* and *P. skrjabini miyazakii*, which are two major species causing human paragonimiasis in Japan [5], serum obtained from the patient was positive for *P. westermani* antigen rather than *P. skrjabini miyazakii*. However, no abnormal finding was found in both the lungs and pleura in her chest computed tomography (CT) scan. We made a diagnosis of subcutaneous paragonimiasis by *P. westermani*. One month after the resection, the WBC count in her peripheral blood was 6450/μL with an eosinophil count of 200/μL, and her serum IgE value was 51.7IU/mL, which were all within normal range. No further medication or treatment was assigned as she did not have any other symptoms and no lesion was detected in her chest CT scan.

Discussion

Paragonimiasis has been re-emerging in Japan due to the increase in immigrants and travelers from endemic areas, such as China and Thailand [5]. Nevertheless, paragonimiasis is still a rare disease in Japan, since annual new cases are around several dozen nationally [1]. Due to the rarity of the disease, it is significantly difficult to make a diagnosis of paragonimiasis and proper examinations are often not performed. For example, a case with bloody sputum, which is one of the typical clinical symptoms of pulmonary paragonimiasis, remained undiagnosed for three

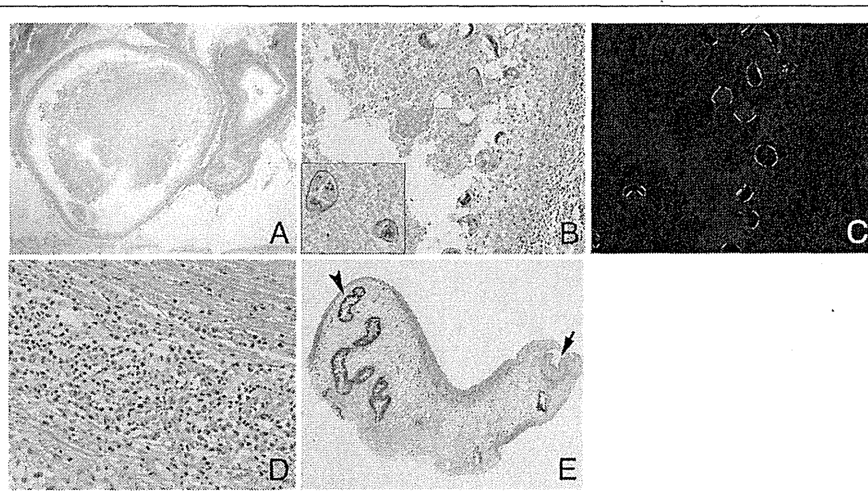

Figure 2 Histopathological findings. A. The lesion is composed of variable-sized tubulo-cystic structures embedded in subcutaneous tissue. Hematoxylin and eosin (HE) stain: 12.5×. **B.** A number of parasite ova are observed in the necrotic tissue inside the cystic structures. The shell thickness of ova is uneven and some are distorted. The ova contain several cells with round nuclei and eosinophilic cytoplasm, suggesting yolk cells (inset, 400×). HE stain: 200×. **C.** Egg shells of *Paragonimus westermani* are highlighted under polarized light in the same field as shown in **B**: 200×. **D.** The cyst wall with a granulomatous change and scant infiltration of eosinophils. HE stain: 400×. **E.** The section of the worm shows characteristics of trematode, the presumed oral sucker (arrow) and reproductive organ (arrow head). HE stain: 40×.

years [6]. A high eosinophil count in peripheral blood and an elevated serum IgE value are not specific findings; they are observed in around 80% of patients [5]. Even if a sputum test and biopsy are performed, the ova or body of *Paragonimus* parasites may not be detectable due to an insufficient amount of specimens [7].

Subcutaneous paragonimiasis is particularly rare compared to pleuropulmonary and the other ectopic paragonimiasis. According to a recent review of paragonimiasis cases in Japan, only 4.9% of the 384 cases presented with subcutaneous nodules [5]. In addition to the rarity, this case suffered from a lack of findings suggesting parasite infection: the subcutaneous mass was not migratory and an eosinophilic count in her peripheral blood and serum IgE value were unavailable. This caused difficulty in reaching the definitive diagnosis. In her histopathological examination the lesion was completely encapsulated by fibrosis, with a granulomatous change and scant eosinophilic infiltration. This may indicate a chronic phase of the infection, not an acute phase, which is in accordance with the long follow-up term before the resection. The resected tissue contained the parasite ova and body, which provided sufficient findings leading to the diagnosis of subcutaneous paragonimiasis. The following serological test confirmed an infection by *P. westermani.*

To the best of our knowledge, no report has been published regarding MRI findings of subcutaneous paragonimiasis in combination with histopathological examinations, although one case of subcutaneous paragonimiasis with a CT scan suggesting a localized abscess on the lower back has been reported [8]. On the other hand, MRI findings of liver and brain paragonimiasis have been reported, showing cystic lesions with low intensity signal in T1WI imaging and high intensity signal in T2WI imaging [9,10]. Subcutaneous nodules in this case presented similar MRI findings to those of the liver and brain cases, but additionally showed inhomogeneity of the content. A contrast-enhanced MRI scan revealed that the lesion was enhanced at the rim but not inside the nodules. Only a very few diseases, such as lymphatic vessel malformation, present similar MRI findings in subcutaneous tissue.

There are several infection sources of paragonimiasis. Freshwater crab (*Eriocheir japonica, Eriocheir sinensis* or *Geothelphusa dehaani)* is an intermediate host of *Paragonimus* and wild boar is a paratenic host. Humans become infected after eating these in a raw or undercooked state. Drunken crab and Japanese cuisine using freshwater crab or wild boar meat are thought to be the main infection sources in Japan [1,5,11]. A previous study suggested that the infection rate of freshwater crabs varies from 0 to 88%, according to the regions or seasons (the average infection rate is 17%) [12]. Paying careful attention to previous history related to freshwater crab and wild boar is important in making a diagnosis of paragonimiasis.

However, proper history-taking is often difficult, particularly in non-endemic areas of paragonimiasis. Since cutaneous and subcutaneous paragonimiasis lesions are relatively easy to reach, an excision biopsy for histopathological examination is useful. Even if a worm and/or ova are not observed in the lesion, findings such as an abscess with eosinophilic infiltration may be able to suggest parasite infection. In combination with serological testing, cutaneous and/or subcutaneous paragonimiasis can be diagnosed [13,14].

Conclusions

We report a case of 39-year-old Chinese immigrant woman presenting with a subcutaneous nodule on her left lower back. Her MRI findings showed cystic lesions with inhomogeneous low intensity signal in a T1WI and high intensity signal in a T2WI. Rim enhancement was shown on a contrast-enhanced MRI scan. A histopathological examination revealed a granulomatous lesion associated with necrosis, ova and a body of a parasite suggesting trematode. A serological examination confirmed an infection of *P. westermani.* Together with the absence of pleuropulmonary lesion, this case was diagnosed as subcutaneous paragonimiasis.

Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Abbreviations

CT: Computed tomography; HE: Hematoxylin and eosin; IgE: Immunoglobulin E; MRI: Magnetic resonance imaging; T1WI: T1-weighted image; T2WI: T2-weighted image; WBC: White blood cell.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MK and MA were involved in acquisition of data and drafting the manuscript, and equally contributed to this study. HT designed and organized this study. HM guided the diagnosis by parasitological, histopathological and serological examinations. EN helped in parasitological conception. TY performed dermatological aspects of this study. YA performed respirological aspects of this study. HK revised the manuscript critically for important intellectual content and supported financially. All authors read and approved the final manuscript.

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RESEARCH Open Access

New zoonotic cases of *Onchocerca dewittei japonica* (Nematoda: Onchocercidae) in Honshu, Japan

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Abstract

Background: Zoonotic infections with *Onchocerca* species are uncommon, and to date only 25 clinical cases have been reported worldwide. In Japan, five previous zoonotic infections were concentrated in Oita, Kyushu (the southern island), with one previous case in Hiroshima in the western part of Honshu (the main island). The causative agent in Japan was identified as *Onchocerca dewittei japonica* Uni, Bain & Takaoka, 2001 from Japanese wild boars (*Sus scrofa leucomystax* Temminck, 1842). Here we report two infections caused by a female and male *O. dewittei japonica*, respectively, among residents of Hiroshima and Shimane Prefectures in the western part of Honshu.

Methods: In both cases, nodules were surgically removed. The parasites in nodules were identified on the basis of their histopathological characteristics. Identification was confirmed by sequencing the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) gene from worms in the tissues used in the histological preparations.

Results: Case 1 was a 61-year-old woman from Hiroshima Prefecture who complained of a painful subcutaneous nodule on the back of her right hand. The causative agent was identified as a female *O. dewittei japonica* owing to transverse ridges on the cuticle and molecular analysis. Case 2 was a 78-year-old woman from Shimane Prefecture who had a painful nodule in the left temporal region. Histopathological characteristics and *cox*1 sequencing of the worm indicated that the causative agent was a male *O. dewittei japonica.*

Conclusions: For Cases 1 and 2, we diagnosed the causative agents as a female and male *O. dewittei japonica*, respectively. These findings indicate the spread of a zoonosis caused by *O. dewittei japonica* in the western part of Honshu, where wild boars have recently extended their habitats because of decreased annual snowfall, unused rice fields and a decline in the number of hunters in Japan. The *O. dewittei japonica* infection rate among wild boars was reported as 78% in Shimane Prefecture, in the western part of Honshu. Therefore, in the near future, zoonotic onchocercosis is likely to occur in Honshu as well as Kyushu, where wild boars, blackfly vectors and humans share the same habitat.

Keywords: Filarioid, Global warming, Japanese wild boar, *Onchocerca dewittei japonica*, Vector-borne disease, Zoonosis

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Background

Zoonotic filariosis is a human infection caused by animal filarioids, which are transmitted by blood-sucking vectors. The reported incidence of vector-borne parasitic zoonoses has recently increased throughout the world. The alterations in climate (particularly global warming), deforestation, urbanisation and human demographics have affected the transmission of parasites among vectors, host animals and humans. These factors have led to the occurrence of vector-borne parasitic zoonoses in areas where such infections have not been previously reported in humans [1-4].

Twenty-five clinical cases caused by *Onchocerca* spp. transmitted from animals have been reported worldwide: eight in North America, six in Japan, five in Europe, three in Turkey, one in Kuwait, one in Tunisia and one in Iran. Among these, five ocular infections and one cervical spinal mass caused by *Onchocerca lupi* Rodonaja, 1967 were recently reported in Turkey, Tunisia, USA and Iran [5-14]. Five suspected or identified causative agents were *O. gutturosa* Neumann, 1910 from cattle, *O. cervicalis* Railliet & Henry, 1910 from horses, *O. dewittei japonica* from Japanese wild boars, *O. jakutensis* (Gubanov, 1964) from European deer and *O. lupi* from carnivores (e.g. dogs) [7,15-18].

In Japan, seven *Onchocerca* species (*O. gutturosa*, *O. lienalis* Stiles, 1892, *O. cervicalis*, *O. suzukii* Yagi, Bain & Shoho, 1994, *O. dewittei japonica*, *O. skrjabini* Rukhlyadev, 1964 and *O. eberhardi* Uni & Bain, 2007) have been identified in domestic and wild animals [4,19-21]. Two unnamed *Onchocerca* species (*Onchocerca* sp. from wild boars and *Onchocerca* sp. Type A from blackflies) were recently differentiated from other congeneric species by molecular analyses [22-24]. Six clinical cases of zoonotic onchocercosis have been reported from Japan, where five infections were concentrated in Oita, Kyushu (the southern island), and another case occurred in Hiroshima in the western part of Honshu (the main island) [5]. The blackfly *Simulium bidentatum* (Shiraki, 1935), anthropophilic and zoophilic, was verified as a natural vector of *O. dewittei japonica* in Oita, Kyushu [22-24] and the blackfly vectors have been found in Honshu [4].

In the current study, we present two cases of *O. dewittei japonica* infections in the western part of Honshu that were identified on the basis of their histopathological and molecular characteristics. These findings indicate that zoonotic infections caused by *O. dewittei japonica* have occurred in the western part of Honshu, owing to the increase in numbers and the habitat expansion of wild boars in Honshu as well as Kyushu [25]. The nomenclature of parasitic diseases follows the guideline proposed by the Executive Committee of the World Association for the Advancement of Veterinary Parasitology [26].

Methods

Clinical history

Case 1 was a 61-year-old woman from the Higashihiroshima City, Hiroshima Prefecture, Japan. The patient was a housewife who lived in a rural area near mountains inhabited by wild boars. She often observed blackflies and had a dog as a pet. She had not travelled outside Japan during the previous 10 years. In November 2010, she developed a painful nodule on the back of her right hand. On 25 November, the nodule was surgically removed at a hospital in Hiroshima. After the surgery, she was examined for the parasitic infections at Hiroshima University Hospital until February 2011 and no signs were found.

Case 2 was a 78-year-old woman from Izumo City, Shimane Prefecture, Japan. The patient lived in a rural area near mountains inhabited by wild boars. She occasionally worked outside as a farmer. She reported that she had been bitten by blackflies and mosquitoes. She had never travelled outside Japan and had not visited Kyushu for 10 years. She had a cat as a pet. In January 2011, she developed a nodule in the left temporal region of her head. She reported that the nodule caused pain for 2 weeks and was increasing in size. On 1 April 2011, the nodule was surgically removed at Shimane Medical University Hospital. After this, she was treated with antibiotics and analgesics for 3 days. For 4 months after the surgery, her head, thoracic, abdominal and pelvic areas were continuously examined by computed tomography scan for any parasitic infections. The results showed that there were no lesions. Eosinophilia in the patient decreased from 10.2% after the surgery on 19 April 2011 to 4.2% (the normal value) on 29 June 2011. Laboratory examinations detected no immunological deficiencies.

Histopathological and molecular analyses

For Case 1, the excised mass (1 × 3 cm, Figure 1A) was fixed in 10% buffered formalin for several hours and embedded in paraffin. Sections (3 μm thick) were stained with haematoxylin and eosin (HE). Microscopic examinations revealed five longitudinal sections, two oblique sections and five transverse sections of a worm. Five histological sections stained with HE were used for the molecular analysis. These sections were immersed in xylene for 2-3 days to remove the cover glass. The worm tissues (0.6 mm^2) were scraped off with a sterile scalpel under a stereomicroscope for use in the molecular analysis. The tissues were used to determine the nucleotide sequence of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) gene. The tissues were incubated with 0.5 mL of DEXPAT (Takara Bio Inc., Otsu, Japan) for 10 min at 100°C and centrifuged for 10 min at 12,000 rpm at 4°C. The supernatants containing the extracted DNA were mixed and concentrated by ethanol precipitation. Approximately 30 ng

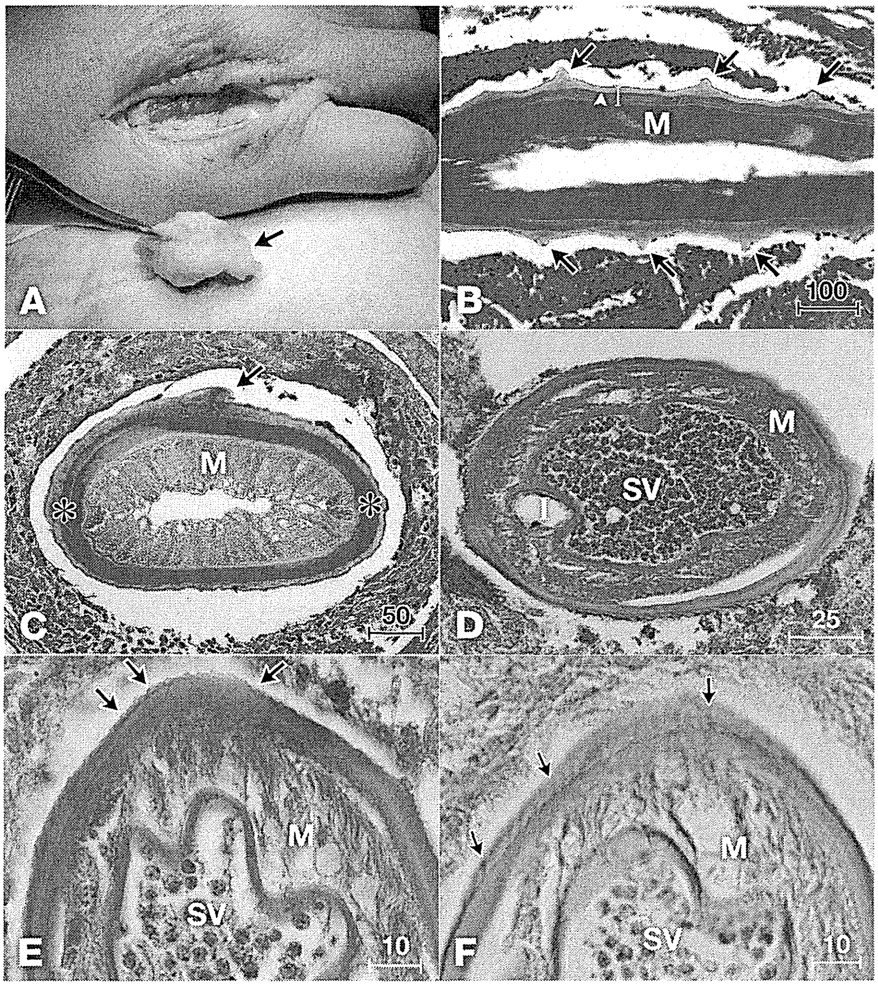

Figure 1 Histopathological characteristics of *Onchocerca dewittei japonica* in nodules excised from Cases 1 in Hiroshima (A–C) and 2 in Shimane (D–F). A) Mass (arrow, 1 × 3 cm) excised from the back of the right hand. **B)** Longitudinal section of the female *O. dewittei japonica*. Arrows, triangular transverse ridges; arrowhead, middle layer of the cuticle without the inner striae; white vertical line, cuticle; M, muscle layer. HE staining. **C)** Slightly oblique transverse section of the female worm showing the thick cuticle. Arrow, elevation of the cuticle, indicating the transverse ridge; asterisks, lateral chords; M, muscle layer. HE staining. **D)** Transverse section of the male *O. dewittei japonica*. I, intestine; M, muscle layer; SV, seminal vesicle with spermatozoids. HE staining. **E)** Enlarged transverse section. Arrows, small ridges on the cuticle; M, muscle layer; SV, seminal vesicle. HE staining. **F)** Slightly oblique transverse section. Arrows, small longitudinal ridges; M, muscle layer; SV, seminal vesicle. PAS staining. Unit of bars, μm.

of DNA was used as a template for PCR. PCR amplification was performed using the primers CO1fF-CO1fR (expected size: 239 bp) as described previously [6]. However, the primers failed to amplify *cox*1 from our specimens; therefore, we designed a new reverse primer called CO1f1R (5'- AAAATAATAACATAAACCTCAGG ATG-3') and used a new primer set CO1fF-CO1f1R for amplification. The position of the primer in the complete mitochondrial genome of *O. volvulus* [GenBank: AF015193] is 3013–3038. The thermal conditions were as follows: initial denaturation at 94°C for 2 min, followed by 40 cycles at 98°C for 10 s, 47°C for 30 s and 68°C for 30 s. The PCR product of the expected size (155 bp) was excised from the agarose gel, purified with a QIAEX II Gel Extraction Kit (Qiagen, Hilden, Germany) and cloned into the *Hinc*II site of the pUC118 plasmid vector with a Mighty Cloning Reagent Set < Blunt End > (Takara Bio Inc.). The inserted fragments from six colonies were sequenced using M13F (-20) and M13R primers, a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems).

The nucleotide sequence was aligned with the published sequences of 11 *Onchocerca* species using CLUSTAL W with the default settings in BioEdit ver. 7.0.5.3. [27,28]. Two unnamed species (*Onchocerca* sp. wild boar and