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Emerging Problems of Parasitic Diseases in Southern Kyushu, Japan

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Abstract

In order to comprehend the recent trends in parasitic diseases in southern part of Kyushu, Japan, we closely analysed the patients whose sera and/or body fluid were sent to the Department of Parasitology, Miyazaki Medical College, for immunodiagnosis in the last 10 years. The number of total samples received, as well as positive ones, have been increasing every year since 1986. Paragonimiasis has been still the major parasitic disease in Miyazaki and adjacent areas. In addition, substantial number of other cases like gnathostomiasis, ascariasis, fascioliasis, and strongyloidiasis also occurred in this area. In 1995, 45 out of 129 serum samples were positive for binding to parasite antigens. Patients were mostly middle-aged, 55.8% of them being male. Direct demonstration of eggs or worm bodies was possible only in 2 cases. Major clinical manifestations of the patients were eosinophilia occurring together with lung and/or liver disorders. About two thirds (30/44) of the patients having eosinophilia of >20% were diagnosed as having parasitic diseases. Eosinophilia was the only clinical manifestation in 7 patients. Of 41 cases that had respiratory symptoms, 56.1% had parasitic diseases. The most frequently encountered abdominal symptom was hepatic nodular lesion(s) in abdominal scan. Of 10 such cases, 8 had parasitic diseases. In total, among patients who had eosinophilia together with lung and/or liver symptoms, 70.0% of them were diagnosed as having some kind of food-borne helminthic diseases of zoonotic nature. Requirement for a screening kit which covers various parasitic diseases was suggested, with which primary physicians can test their patients' sera immediately when they find eosinophilia.

Key words: parasitic diseases; serological diagnosis; eosinophilia; lung lesion; liver lesion.

Introduction

It is a general belief in public that parasitic diseases have practically almost disappeared in Japan, because eradication programs were so successfully carried out against soil-transmitted parasitic diseases as well as some other famous ones such as filariasis and schistosomiasis. Thus, not only lay people but medical professionals also forget about parasites and seem to consider that they have become free from parasites. However, this is merely an illusion and various kinds of parasites are still surviving, or imported in from foreign countries everyday. According to Kojima (1993), current parasitic diseases in Japan can be classified into five categories based on clinical aspects: 1. food-borne parasitic diseases, 2. zoonoses mainly transmitted from companion animals, 3. imported tropical diseases, 4. sexually transmitted diseases (STD), and 5. opportunistic infections. Among these, most helminthic parasitoses belong to food-borne diseases and zoonoses. Miyazaki Prefecture is located in the southeastern part of Kyushu, where food-borne parasitic diseases are still endemic. Department of Parasitology, Miyazaki Medical College was established in 1976, and a standardized immunological assay system using Ouchterlony's double diffusion in agarose method has been employed since 1986. Then, multiple-dot enzyme-linked immunosorbent assay (dot-ELISA) was introduced for rapid screening in 1991, because of increasing demand for faster diagnosis. Since then the number of sera we received for examination has increased every year and exceeded over 100 samples in 1995. Among these, about 1/3 were diagnosed as parasitoses, most of which were food-borne zoonoses. Since Miyazaki

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and adjacent areas are representative of rural Japan, it would not be surprising if other rural areas are in a similar situation.

Materials and Methods

Samples

Most samples we received were sera and/or body fluids such as pleural effusion. In addition, we occasionally received various kinds of samples including faeces, sputum, and tissue specimens and/or sections obtained by biopsy or surgical operations and sometimes from autopsied bodies, though the numbers of these specimens were far less than that of sera. In this study, we focus on patients whose sera and/or body fluids were sent to us for immunodiagnosis, because most of patients were egg- or worm-negative and immunoserological test was the only way to reach diagnosis.

Serological assays

We used to use Ouchterlony's method alone until 1991 and then introduced dot-ELISA. Today we employ dot-ELISA for a primary screening and, when positive reactions are obtained, the sample is tested further in microplate ELISA and/or Ouchterlony's test to confirm the diagnosis. Most cross reactivities in dot-ELISA could be ruled out in Ouchterlony's method. Inhibition ELISA was used for the determination of causative species, when Ouchterlony's test did not give a definite result.

A panel of antigens we are currently using are phosphate buffered saline (PBS)-extract of *Paragonimus westermanii, P. miyazakii, Fasciola* sp., *Spirometra erinacei-europaei* (plerocercoid), *Gnathostoma doloresi, Trichinella spiralis* (muscle larvae), *Dirofilaria immitis, Toxocara canis, Ascaris suum, Anisakis simplex* (L_3), *Ancylostoma duodenale*, and *Strongyloides ratti* (L_3). *A. suum* and *S. ratti* were used instead of *A. lumbricoides* and *S. stercoralis*, respectively, because of the difficulties to obtain these materials. Antigen preparations of 1–3 mg protein/ml were aliquoted and stored at –30°C until use.

For Ouchterlony's double diffusion test in agarose, 15 μl of antigen solution (1–3 mg protein/ml) and patient's serum were added to each well and the gel plate was incubated at 27°C for 24–48 hr. The gel plate was washed extensively, dried between two pieces of cellophane membrane, and stained with Coomassie brilliant blue.

Dot-ELISA was performed as previously described by Itoh and Sato (1990) with slight modifications. In brief, 1 μl of each antigen solution was dotted on the strip of a nitrocellulose membrane (0.45 μm pore size with 3 × 3 mm printed grid: HAWG 304 FO, Millipore Intertec, Bedford, MA, USA) by a Hamilton type microsyringe fitted with a repeating dispenser. Antigen concentration was 0.2 mg/ml except for *Fasciola* sp., *A. duodenale* and *S. ratti*, which was 0.05 mg/ml, to minimize nonspecific binding to normal human serum. One μl of a 2000-fold diluted normal human serum was also spotted on the strip as a positive control for the peroxidase-labelled secondary antibody. The strips were air-dried, soaked in blocking buffer (1% casein in 20 mM Tris-HCl, pH 7.6) for 30 min at room temperature, then dried again and kept at 4°C until used. The strips were wetted by blocking buffer and then incubated with 1 ml each of diluted (1:200 with blocking buffer) patient's serum at 37°C for 1 hr. After washing with blocking buffer, they were incubated with horse-radish peroxidase-labelled rabbit anti-human IgG (gamma chain specific, DAKO, Glostrup, Denmark) diluted at 1:1,000 at 37°C for 1 hr. After washing three times with blocking buffer, they were incubated in substrate solution (0.04% 4-chloro-1-naphthol, 0.015% H_2O_2, and 16% ethanol in 1/15 M phosphate buffer, pH 7.4) at 37°C for 30 min. The strips were washed with distilled water and dried. Coloring of each spot was visually observed.

For binding and binding-inhibition ELISA assays, wells of the microtiter plates (Nunc, Roskilde, Denmark) were coated with 10 μg/ml of parasite antigens by incubation at 4°C overnight. Wells were washed with PBS containing 0.05% Tween 20, blocked with blocking buffer, and then incubated with serially diluted patients' sera at room temperature for 1 hr. After washing, peroxidase-labeled anti human IgG (DAKO) was added and incubated at room temperature for 1 hr. ABTS (2,2'-azino-di[3-ethyl-benzthiazoline sulfonate]) was used as substrate (Matsuda *et al.*, 1984) and optical densities were read in a ELISA reader (Multiskan Bichromatic: Labsystems Oy, Helsinki, Finland). For inhibition of binding of patient sera to a particular antigen,

patient's sera diluted at 1:2,000–10,000 (depending on the intensity of binding) were preincubated with various concentrations of inhibitor antigens at 4°C overnight and then added to wells coated with 10 μg/ml of antigens. Binding was detected as described above, and % inhibition was calculated relative to buffer control.

Results

Number of samples examined in the last 10 years

The number of serum samples we received for immunodiagnosis in the last 10 years was summarized in Fig. 1. Patients' sera received for follow-up studies are not included in numbers, though they were about a half of those from newly appeared patients. The number of sera we examined was 20 to 30 until 1991 and then suddenly increased to about 70 in 1992 probably due to the introduction of dot-ELISA in 1991 as a primary screening, which enabled us to respond faster than before to attending clinicians. The number almost doubled again from 67 in 1994 to 129 in 1995. The number of positive samples was 2 to 11 during 1986 to 1993 and increased suddenly to 32 in 1994 and 45 in 1995 (Fig. 1). Relative positivity was 47.8% in 1994 and 34.9% in 1995. These positive samples represent confirmed ones that were further tested in microplate ELISA and Ouchterlony's test. The drastic increase in number of samples examined in 1995 was obviously due to consultations from outside Miyazaki, which is clearly illustrated by Fig. 2.

Causative species of parasitic diseases

In 1986 our major focus in immunodiagnosis was on paragonimiasis, since Miyazaki and surrounding area were, and still are, endemic for paragonimiasis. In fact, during 1986 to 1995, we experienced 52 paragonimiasis patients, most of which were infection with *P. westermanii*. Some cases with peculiar clinical features (Ichiki *et al.*, 1989;

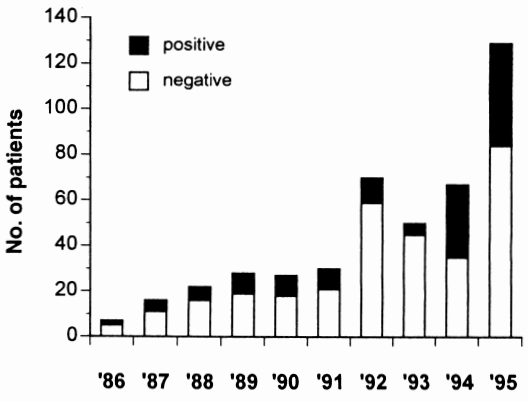

Fig. 1 Number of patients whose serum samples were sent to the Department of Parasitology, Miyazaki Medical College, for immunodiagnosis during 1986 to 1995. Closed columns represent number of patients diagnosed as having parasitic diseases.

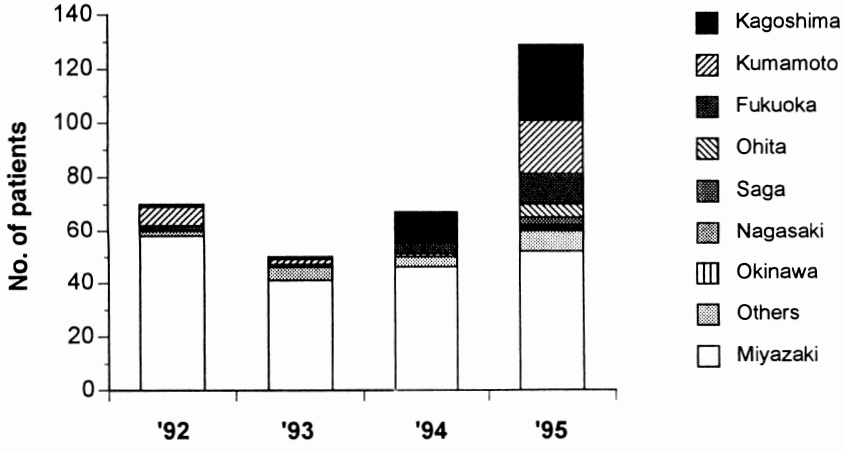

Fig. 2 Geographical distribution of institutions that sent serum samples to the Department of Parasitology, Miyazaki Medical College, for immunodiagnosis during 1986 to 1995.

Kan *et al.*, 1995; Ishikawa *et al.*, 1995), or extrapulmonary infections (Ogata *et al.*, 1990; Nabeshima *et al.*, 1991; Shimao *et al.*, 1994) were already reported. In 1991, we encountered the first case of paragonimiasis miyazakii in Miyazaki (Ono *et al.*, 1992). During 1986 to 1991, before the introduction of dot-ELISA, we already experienced various parasitic diseases other than paragonimiasis, some of which were entirely unexpected. The cases included intestinal capillariasis (Nawa *et al.*, 1988), gnathostomiasis doloresi (Ogata *et al.*, 1988; Nawa *et al.*, 1989), pulmonary dirofilariasis (Suzumiya and Nawa, 1990), severe metagonimiasis (Ichiki *et al.*, 1990), vomited *Philometroides* sp. (Kuroda *et al.*, 1991), and intestinal obstruction due to massive infection with *A. lumbricoides* (Oshikawa *et al.*, 1992). These results imply that paragonimiasis was merely the tip of the iceberg of an array of parasitic diseases in Miyazaki. As shown in Fig. 3, although paragonimiasis has been still the major parasitic disease in Miyazaki, considerable number of other cases like gnathostomiasis, ascariasis, and strongyloidiasis (Hidaka *et al.*, 1995; Tanaka *et al.*, in press) occurred. When patients from outside

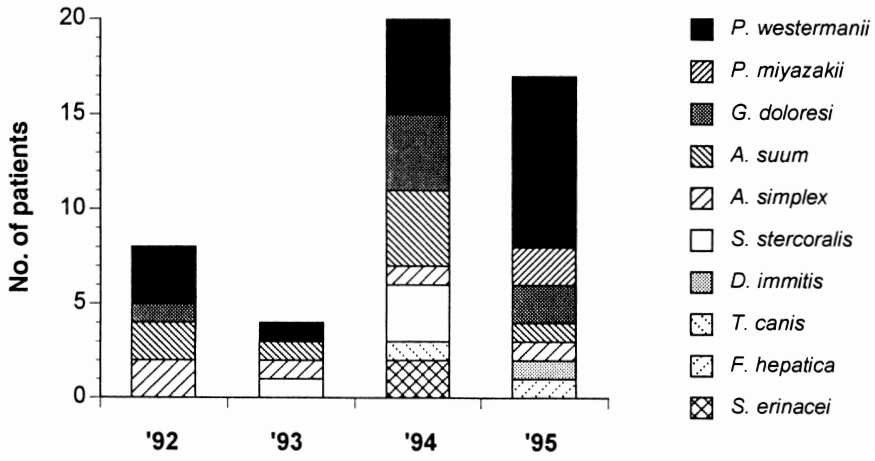

Fig. 3 Causative species of parasitic diseases in patients in Miyazaki during 1992 to 1995.

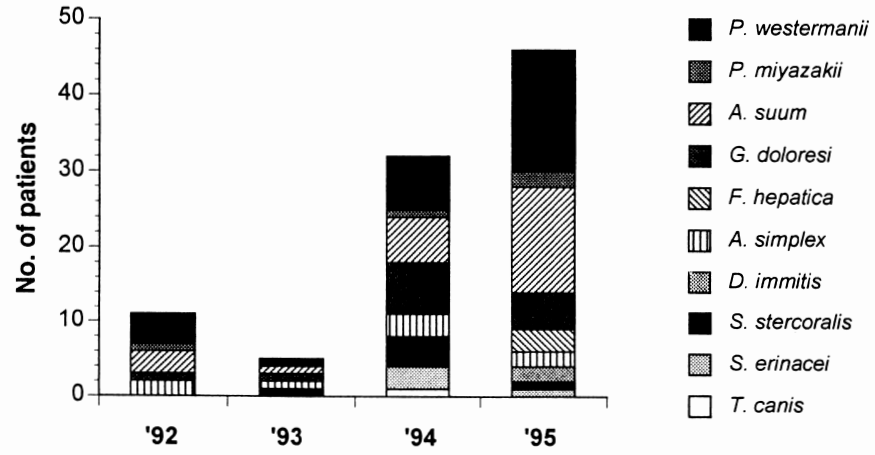

Fig. 4 Causative species of parasites in patients diagnosed at the Department of Parasitology, Miyazaki Medical College, during 1992 to 1995.

Miyazaki are included, paragonimiasis occupied somewhere between 1/3 to 1/4 of the total cases (Fig. 4). It should be noted that we are in the middle of an outbreak of swine ascariasis (Maruyama *et al.*, manuscript in preparation) and fascioliasis (Maruyama *et al.*, submitted). Though the exact reason for this outbreak remains to be solved, it should be emphasized that parasitic diseases are not disappearing at all and many more hidden cases are supposed to be present in and around Miyazaki.

Clinical features in general

In order to draw a current picture of helminthic diseases in clinical medicine in Japan, we analyzed in detail clinical manifestations of the patients appeared in 1995. In 1995, we received 129 serum samples for immunodiagnosis. In addition, 4 specimens were brought for species identification, two *Diphyllobothrium*, one *Giardia*, and one *Giardia* concurrent with *Entamoeba*. Among 129 serum samples, 45 were positive for binding to one or more parasite antigens. Serum samples examined were mostly from middle-aged people, 58.2% of them being male (Fig. 5). Age distribution of patients who were diagnosed as having parasitic diseases showed bi-phasic pattern (Fig. 6) and 55.8% of them were male. Double infection was suspected in 4 cases in dot-ELISA, and two of them were confirmed to have double infections on Ouchterlony's test and inhibition ELISA. Except for these 4 cases, all cases seemed to be an infection with one parasite species and responded well to anti-helminthic treatment. Parasite eggs or worm bodies were detected only in 2 cases, *P. westermanii* eggs in sputum of one case and *Fasciola* eggs and worm bodies in the common bile duct in the other (Maruyama *et al.* submitted).

Eosinophilia

Eosinophilia is one of the major clinical manifestations of helminthiasis. Of 129 cases in 1995, 98 were mentioned to eosinophils upon consultation, and data of peripheral blood eosinophils were available in 85 cases. As shown in Figure 7, about 20% (9/41) of patients with eosinophilia of <20%, turned out to have parasitic diseases, whereas, about 70% (30/44) of patients with eosinophilia of >20% were positive for parasitic diseases. In 6 patients, eosinophilia was the only clinical manifestation. The most striking eosinophilia we experienced so far was a 7 year-old boy infected with *P. westermanii* whose total white blood cell was 84,000/mm^3 with 91% eosinophils (Kan *et al.*, 1995).

Respiratory symptoms

Respiratory symptoms such as cough, sputum, and abnormal findings in chest X-ray could be presenting manifestations when the lung is the target organ or is on the migratory route of a parasite. Of

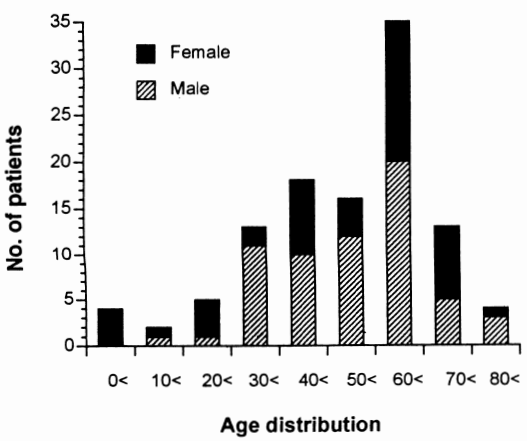

Fig. 5 Age distribution of patients whose serum were sent to the Department of Parasitology, Miyazaki Medical College, for immunodiagnosis in 1995.

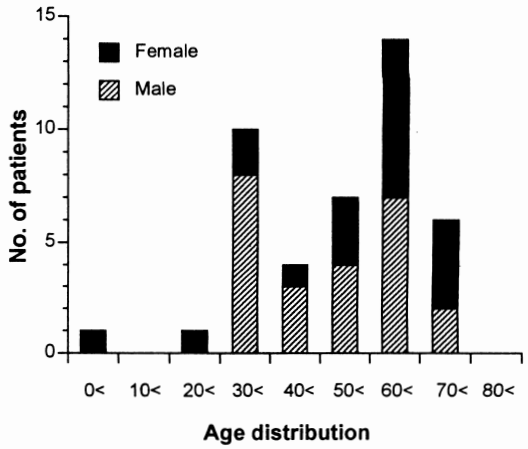

Fig. 6 Age distribution of patients who were diagnosed as having parasitic diseases in 1995.

129 cases in 1995, 45 had some of these respiratory symptoms, and 16/45 showed fever-up. Of these who had respiratory symptoms, 51.1% (23/45) of them had parasitic diseases; 13 paragonimiasis, 5 swine ascariasis, 3 gnathostomiasis doloresi, 1 sparganosis, and 1 dirofilariasis. Among patients who were diagnosed as parasitic diseases, data on eosinophils were available in 22 cases, and 95.5% (21/22) of them had eosinophilia or eosinophilic pleural effusion (Table 1). Physicians practicing in Miyazaki, at least in part, seem to have learned to suspect paragonimiasis when they encounter patients with respiratory symptoms with eosinophilia. Still, some patients were suspected to have primary or metastatic tumors in lungs. It should be noted that various kinds of parasites can cause lung symptoms. Previously, we reported nodular lung lesion caused by *G. doloresi* (Miyamoto *et al.*, 1994), pleural effusion by *Anisakis* (Matsuoka *et al.*, 1994), *Strongyloides* (Hidaka *et al.*, 1995), and *Toxocara* (Maruyama *et al.*, 1995). In 1995, we experienced cases of swine ascariasis (Maruyama *et al.*, in preparation), gnathostomiasis and sparganosis with respiratory symptoms, whose clinical features were similar to paragonimiasis. Direct demonstration of eggs or worm bodies failed or was impossible in all cases except for one with paragonimiasis.

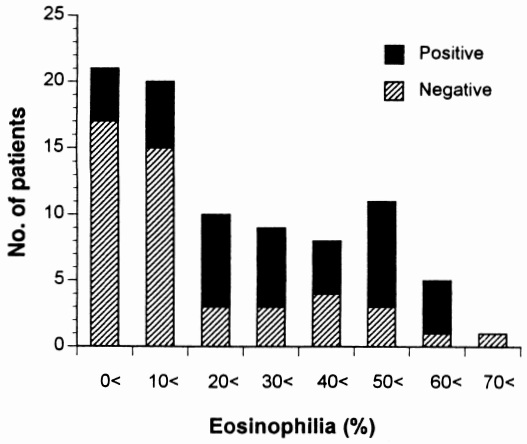

Fig. 7 Eosinophilia and the number of patients with or without parasitic diseases in 1995. Closed columns represent number of patients diagnosed as having parasitic diseases.

Abdominal symptoms

Apparent abdominal symptoms like abdominal pain, vomiting, and diarrhoea were noted only in 4 patients upon consultation in 1995. Among these patients, two of them had an infection with *Fasciola*. Our experience before 1995 also suggests that abdominal symptoms are not major ones in parasitic diseases today in Japan. Although we experienced patients with prominent protein loosing enteropathy in capillariasis (Nawa *et al.*, 1988), metagonimiasis (Ichiki *et al.*, 1990), and strongyloidiasis (Tanaka *et al.*, in press), these cases, that could be diagnosed readily in fecal examinations, were rare. Colonic

Table 1 Frequency of patients having parasitic diseases and their clinical manifestations with reference to eosinophilia

	Respiratory Symptoms	Abdominal Symptoms		Others	None	Total
		Hepatic lesion	Others			
Eo↑	21/32*	8/9	2/6	4/17	7/20	41†/84
(>20%)	(11/15)	(7/8)	(2/4)	(4/10)	(6/7)	(30/44)
Eo→	1‡/7	0	0	0/5	0/2	1/14
Eo (?)	1‡/6	0/1	0	2§/12	0/12	3/31
Total	24/45	8/10	2/6	6/34	7/44	45/129

Eo: eosinophil count

* Figures represent number of parasite positive cases/total cases.
Figures in brackets are the patients having eosinophilia of >20%.

† One case had both respiratory symptoms and hepatic mass due to *A. suum* infection.

‡ *P. westermanii* infection

§ One case each of *G. doloresi* and *S. stercoralis* infection

ileus due to a heavy infection with *A. lumbricoides* (Oshikawa *et al.*, 1992) or invasion of larval *G. doloresi* (Seguchi *et al.*, 1995) are extraordinary cases. In spite of low frequency of patients having abdominal complaints in 1995, nodular or mass lesion(s) in liver were frequently disclosed by abdominal scan such as ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI). Ten patients had been pointed out to have solitary or multiple nodular lesion in liver that were detected either in US or CT, and sometimes confirmed in MRI. Many of them were suspected to have primary or metastatic tumor and 3 patients received liver biopsy. Quite notably, nine of them had marked eosinophilia and 8/9 were revealed to have some helminthic diseases by immunodiagnosis (Table 1); 5 swine ascariasis, 2 fascioliasis, and 1 paragonimiasis. One exceptional case was found to be T cell-type malignant lymphoma. Biopsy specimens taken from the 3 patients, all of whom turned out to have swine ascariasis, showed eosinophilic granuloma or eosinophilic abscess. Invading larvae were not seen in the sections.

Other symptoms

Other occasional symptoms that made physicians to consult us were intraocular inflammation, anaemia of unknown origin, arthralgia, intracranial tumor, erythema, and edema of lower extremities. None of sera from these cases had detectable antibodies to any parasites. Two cases of sensory disturbance with eosinophil infiltration in cerebrospinal fluid were strongly suspected infection with some kind of nematode parasites, though we could not conclude with confidence that their symptoms were due to parasitic infections.

Discussion

The present study showed that parasitic helminthiases were not disappearing in and around Miyazaki. We started accumulating data of serological diagnosis for parasitic diseases in 1986. We focused on helminthic infections, since imported tropical diseases or STD were not major problems in rural areas like Miyazaki. In fact, most patients we experienced were born, raised, and living in and around Miyazaki, and their life-style was more or less conservative and following traditional style especially in eating and cooking. Only a small portion of them had an experience of overseas travel. Therefore, our accumulated data on parasitic diseases in the last decade seem to be an representative of domestic parasitic diseases in Japan.

The drastic increase in the sample number in 1992 was probably due to the change of our assay procedures. The introduction of dot-ELISA in 1991 as a primary screening enabled us to respond quickly to attending physicians and surgeons (within 2–3 hours), so that they learned a merit to consult us about their patients. The reason for the next increase in 1995 was clearly due to an increase in number of samples coming from outside Miyazaki as shown in Fig. 2. Increase in the total number of samples did not result in decrease in the proportion of positive samples. Although precise reason for this increase in absolute and relative number of positive cases remains to be clarified, it is likely that physicians became aware of the presence of parasitic diseases and hidden cases have been disclosed. Alternatively, it is also possible that parasitic diseases are actually increasing in these areas. Since people are less aware of parasitic diseases than before, basic procedures seem to be decomposing in handling possible infectious sources such as food materials or manure.

Most cases we diagnosed so far were characterized by eosinophilia with lung and/or liver involvement. Lung lesion was seen in paragonimiasis, swine ascariasis, dirofilariasis, and gnathostomiasis, and liver lesion was noted in ascariasis, fascioliasis, and paragonimiasis. The data about causative species indicate that most parasitic helminthiases today in rural Japan fall into parasitic zoonoses, which cause visceral larva migrans (VLM) in a broader sense. VLM is a syndrome caused by the migration of larval parasites in deeper parts of the body (Beaver and Jung, 1984). It was first described and named by Beaver *et al.* (1952). Subsequently the terms toxocariasis and VLM have become almost synonyms, though a number of ascarid and non-ascarid parasites have been known to cause VLM (Beaver, 1969; Katz *et al.*, 1982; Beaver and Jung 1984). Although paragonimiasis and fascioliasis are not classified as VLM, these parasites exhibit extensive migration and tissue invasion. Clinically, VLM is

characterized by eosinophilia and eosinophilic granulomas in various organs. In our cases, eosinophilia was also the most important manifestation to note. Since the patients were mostly middle-aged, malignant tumors were the most important differential diagnosis. Our results suggest that if a patient with lung and/or liver lesion had eosinophilia, especially >20%, a parasitic disease should be a primary suspect. Occasionally patients who had no symptoms were pointed out to have eosinophilia (Ogata *et al.*, 1995), or multiple nodular lesions in liver with eosinophilia (Maruyama *et al.*, in preparation) in a regular check-up (7/20 in Table 1). It seems quite likely that substantial portion of apparently healthy people could have parasitic infections if they had marked eosinophilia. Characteristic in our patients was that most of the cases could not have been diagnosed by only searching for eggs or worm bodies in excreta and secretions. It is practically impossible to demonstrate parasite larvae in VLM, and even in tissue parasite infections such as paragonimiasis and fascioliasis cases, in which worms are supposed to produce eggs in the human body, parasite eggs were not detected in most of the cases. This might be due to the small number of worms parasitizing the patients. Although Miyazaki is endemic for various kinds of parasitic diseases, heavy infections are, as long as we know, becoming rare.

When direct demonstration of eggs and worm bodies are difficult, serological tests are the only and most reliable way to reach diagnosis. Immunological diagnosis have been developed for various kinds of parasitic helminthiasis (Beaver and Jung, 1984; Tsuji, 1990). As a consequence of relatively large number of patients, serological tests for toxocariasis and anisakiasis have been most extensively studied (Glickman *et al.*, 1978; DeSavigny *et al.*, 1979) and diagnosis kits are now commercially available for these two diseases (for toxocariasis: Jacquir *et al.*, 1991; for anisakiasis: Yagihashi *et al.*, 1990). However, as we have shown in the present study, parasitic diseases in Japan are sporadic and caused by various kinds of species, some of which are unexpected. If limited kinds of kits are employed which are sufficiently specific to exclude cross reactivities, some patients would remain undiagnosed and treated in an inadequate way. We experienced a case of paragonimiasis that took more than 3 years before the final diagnosis. The patient had been treated with steroids on the diagnosis of pulmonary infiltration with eosinophilia and allergic granulomatous angiitis until a *P. miyazakii* worm was dissected out from a biopsy specimen of skin (Okamoto *et al.*, 1993). In order to prevent such devastating cases, clinicians have to be prepared for parasitic diseases, and they have to have a screening kit which is not very specific but covers various parasitic diseases broadly, rather than a monospecific diagnosis kit. Because it is more important for clinicians to recognize that their patients have a parasitic disease, though they are not necessary to identify the exact causative species. If such a standard screening kit for parasitic diseases were available, primary physicians can immediately test sera from patients with eosinophilia, which would save enormous money and time. Then, parasitologists would be consulted when primary physicians obtain positive or ambiguous results and further investigation is needed.

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