(66/80) and 78.8% (63/80), respectively. The membrane-based ELISA systems appear promising but need to be investigated further for its efficacy as reliable diagnostic tests.

Keywords: *Toxoplasma* infection; Antigen; Human urine; Membrane-based ELISA; Ghana

1. Introduction

High prevalence of anti-*Toxoplasma* sero-positivity in a cross-section of the Ghanaian populace as well as evidence of congenital disease caused by *Toxoplasma gondii* infections have been reported in Ghana (Anteson et al., 1978a,b, 1980). Among the existing diagnostic tests for toxoplasmosis, the most readily available one to clinicians in Ghana is the commercial latex agglutination test (LAT), which detects mainly IgG antibodies indicative of past or old infections. According to Luft and Remington (1988), the frequent occurrence of *Toxoplasma* infection in immunocompromised patients and the difficulty in establishing the diagnosis without brain biopsy has necessitated the development of new, non-invasive methods for diagnosis of the disease. Furthermore, *Toxoplasma* antigens have been demonstrated in serum samples from acutely infected animals and humans (Raizman and Neva, 1975; Van Knapen and Panggabean, 1977; Araujo et al., 1980; Araujo and Remington, 1980; Trunen, 1983; Lindenschmidt, 1985; Asai et al., 1987) and also in urine from mice acutely infected with *T. gondii* as well as from human patients with acute toxoplasmic encephalitis (Huskinson et al., 1989). However, the methods used were rather elaborate and expensive which may not be suitable for routine examination. The risk of congenital infection, for example, in this era of rapid spread of HIV/AIDS, necessitate periodical routine examination of all females of childbearing potential, which may not be cost-effective in the Ghanaian environment. Earlier, Bosompem et al. (1996, 1997) had reported the employment of a membrane-based ELISA to detect antigens in the urine of urinary schistosomiasis patients and its convenience and cost-effectiveness in mass screening of suspected patients in the field. In this study we explored the usefulness of two membrane-based ELISA systems in diagnosing recent infection in suspected toxoplasmosis patients.

2. Patients and methods

2.1. Patients

A total of 80 Ghanaians who consented were involved in this study. Fifty-four of them were patients attending four ophthalmology clinics across the country and they included 30 females and 24 males aged 10–70 years. The patients mostly reported with aching eyes and blurred vision and presented with symptoms, such as traumatic and posterior uveitis and signs suggestive of active *Toxoplasma* retinochoroiditis and well demarcated scars upon examination (Holliman, 1990), which was defined as the presence of intraocular inflammation in association with a retinochoroidal scar and an active adjoining retinitis (Guerina et al., 1994). Twenty-two out of the 54 patients (41%) had eye lesions suggestive of active infections and others had scars suggestive of old infection.

The other 26 patients were pregnant women in their second or third trimester attending obstetrics/gynaecology clinic (OGP) and aged 22–44 years. All of them had experienced spontaneous abortions and/or stillbirth (by questionnaire), 18 of them had been sero-positive and 8 sero-negative, by a commercial LAT (according to their past medical records) 6 months to 4 years before this study.

As control 22 people, 14 males and 8 females aged 15–60 years who were considered healthy by questionnaire and screening were included in the study. All of them had tested sero-negative (by LAT) for toxoplasmosis 3 months to 2 years, before this study.

2.2. Collection and analysis of blood, urine and stool

Approximately, 3–5 ml of blood was collected from each of the individuals involved in the study. Thick and thin blood smears were prepared on microscope glass slides, processed and examined microscopically

for haemoparasites. Sera were also obtained from the blood and tested for anti-*Toxoxplasma* sero-positivity by the Dye Test (DT) and LAT.

A total of about 25–80 ml of urine was collected from the same individuals who had given blood and were also provided with containers to produce stool samples. Each urine sample was centrifuged at 400 × *g* and the deposits were examined microscopically for *Schistosoma haematobium* ova and the supernatant tested for *Toxoplasma* antigens and anti-*Toxoplasma* antibodies using a simple designed membrane-based ELISA. Stool specimens were examined for *Schistosoma mansoni* ova using the Kato method (Katz et al., 1972) and the formalin–ether sedimentation technique for the detection of other intestinal parasites' ova, cysts and oocysts of other related coccidian species like *Cryptosporidium parvum*.

2.3. Serological tests

DT (Sabin and Feldman, 1948; Beverly and Beattie, 1952; Handman and Remington, 1980) was performed with modification. Briefly, peritoneal exudates were obtained from mice inoculated with the RH strain of *T. gondii* 3 days previously. *Toxoplasma* suspensions rich mainly in extra cellular parasites were used in the test within 20 min after harvesting. Briefly, 20 μl of test sera diluted four-fold in normal saline in appropriately labelled eppendorf tubes were added with 40 μl of activator serum (normal human serum) and then 20 μl of suspension of tachyzoites in PBS (pH 7.4) in each tube and incubated in a water bath at 37 °C for 1 h after brief shaking. Then, 60 μl of 1% methylene blue dye in borate buffer (pH 11.0) were added to the tubes and allowed to stand at room temperature for 10 min. The tubes were kept at 4 °C until results were read.

The commercially available LAT (Toxotest®-MT Eiken, Tokyo, Japan) was performed according to the manufacturer's instructions.

2.4. Infection of mice

Infection of mice was carried out as by Handman and Remington (1980) with modification. Briefly, tachyzoites of the RH strain of *T. gondii* obtained from mouse peritoneal exudates (described below) were fixed in 0.1% formol–saline at 4 °C for 30 min and washed with excess PBS (pH 7.2). Eight to twelve weeks old ddY mice were bled a day before infection and sub-cutaneously injected with 2×10^3 fixed tachyzoites, and bled at days 5, 10, 15 and 20 after infection when antibody titer in DT was between 1:16 and 1:10,000. Pooled sera were stored in aliquots at −20 °C until used.

2.5. Preparation of Toxoplasma crude antigen

Toxoplasma antigen was prepared according to the method of Araujo and Remington (1980) with modification. Crude tachyzoite antigen was prepared using *T. gondii* tachyzoites from the peritoneal exudates of mice infected 3 days earlier with the RH strain. Pooled exudates were centrifuged at 1000 × *g* for 10 min in a refrigerated centrifuge at 4 °C. The sediment containing cells and parasites was re-suspended in cold PBS (pH 7.2), washed three times with PBS and filtered (Remington et al., 1970). Parasites were pelleted and frozen at −30 °C overnight. The frozen pellet containing approximately 2×10^7 tachyzoites was re-suspended with distilled water and the suspension subjected to repeated freezing (−80 °C for 30 min) and thawing before sonicating on ice using a Branson Sonifier 250 (Branson Sonic Power Company, CT, USA) set at 60 cycles for 45 s. The resulting suspension was centrifuged at 1000 × *g* for 20 min and then at 3600 × *g* for 30 min. After demonstrating the presence of *Toxoplasma* antigens by Ouchterlony (1976), protein concentration was estimated at 0.8 mg/ml using Bio-Rad Protein Assay Reagent according to the manufacturer's protocol (Bio-Rad Laboratories, CA, USA). Aliquots of the preparation were stored at −30 °C until used.

2.6. Urinary antigen detecting ELISA procedure

The urinary antigen detecting ELISA (UAgE) was performed according to Bosompem et al. (1997) with modification as follows. Methanol wetted polyvinylidene difluoride (PVDF) membrane (Atto Company Ltd., Tokyo, Japan) strips were incubated in test urine for 36 h at 4 °C, rinsed with Tris-buffered saline (TBS) (50 mM Tris and 150 mM NaCl, pH 7.2) and then blocked for 45 min in blocking solution consisting of 5% skimmed milk/TBS. The strips were transferred into *T. gondii* infected mouse serum (1:100) for 1 h, washed three times each by 5-min incubation in TBS and then incubated in goat anti-mouse polyclonal

IgG + A + M conjugated to horse-raddish peroxidase (HRPO, 1:5000, Catalog no. 1999, Sigma–Aldrich, Japan) for 1 h. The strips were washed three times each by 10-min incubation in TBS and then incubated in substrate solution [98.65% (w/v) (3,3-diaminobenzidine), 0.25% (v/v) H_2O_2 and 15.4% (w/v) $NiCl_2$ in TBS] for 1 min. A bluish black reaction represented positive results while negative results remained colourless. The assay was run at room temperature and reagents were diluted in 0.1% blocking solution, unless otherwise stated.

2.7. Detection of type of immunoglobulins bound by Toxoplasma antigens

The UAgE described above was repeated with multiple membrane strips incubated in each urine sample. At the conjugate incubation level, strips from each sample were distributed into appropriately diluted goat anti-mouse monoclonal IgG-, IgA- and IgM-HRPO (Catalog no. 1999, Sigma–Aldrich, Japan), respectively.

2.8. Urinary antibody detecting sandwich ELISA procedure

The urinary antibody detecting ELISA (UAbE) was performed as described above with the following modifications. Briefly, methanol wetted PVDF membrane strips were incubated in test urine for 36 h at 4 °C. Strips were removed and rinsed with TBS and blocked. They were then rinsed in TBS and transferred into *T. gondii* tachyzoite lysate diluted appropriately in PBS (5 μg/ml) for 1 h at room temperature. They were washed three times each by 5-min incubation in TBS and then incubated in *T. gondii* infected mouse serum and the procedure from here was the same as for the antigen detecting system.

2.9. Determination of optimal working dilutions of murine serum and goat anti-mouse horse-raddish peroxidase conjugate used in the membrane-based ELISA

Pooled murine *T. gondii* infected serum was tested in membrane-based ELISA as follows. Briefly, *Toxoplasma* tachyzoite lysate-captured PVDF membrane strips were rinsed in TBS, pH 7.2 for 2 min and different strips incubated with test serum and then goat anti-mouse polyclonal (IgG + IgA + IgM) horse-raddish peroxidase conjugate in a checkerboard, from 1:50 to 1:1000 and 1:1200 to 1:20,000 at two-fold dilutions in blocking solution, respectively. The respective dilutions that gave the most intense colour reactions by visual assessment were used in the membrane-based ELISA. The procedure was repeated using goat anti-mouse monoclonal (IgG); (IgA); and (IgM) horse-raddish peroxidase conjugate separately to ascertain the inclusion of all the necessary immunoglobulins and in sufficient amounts using visual assessment of the intensity of colour reactivity.

2.10. Sensitivity and specificity of the tests

The percentage sensitivity and specificity of LAT and membrane-based tests were calculated, using DT as the gold standard, as follows (see Bosompem et al., 1996):

$$\text{sensitivity (\%)} = \frac{\text{number of individuals positive by the test that were also positive by DT}}{\text{total number of individuals testing positive by DT}} \times 100$$

$$\text{specificity (\%)} = \frac{\text{number of individuals negative by the test that were also negative by DT}}{\text{total number of individuals testing negative by DT}} \times 100$$

3. Results

3.1. Results of stool, blood smears and urine deposit examination

Two out of the 26 (7.8%) pregnant women and 36 out of the 54 (66.7%) ophthalmology (22 active and 14 past infection) patients, submitted stool samples, the overall being 38/80 (47.5%). None of the 38 stool samples were found to contain any *S. mansoni* eggs, however, ova of

hookworms were identified in 3 (7.9%) ophthalmology (2 active and 1 past infection) patients' samples. *Plasmodium falciparum* was the only haemoparasite detected in blood smears from 5/80 (6.3%) individuals including four ophthalmology (3 active and 1 past infection) patients and one pregnant woman. *S. haematobium* ova were demonstrated in urine sample deposits of 6/80 (7.5%) ophthalmology (3 active and 3 past infection) patients who included 2 that were also *P. falciparum* positive. No cysts or oocysts were detected in any of the 62 faecal samples collected.

None of the parasites mentioned above were found in any of the 22 stool specimens from the normal controls except 2 of them that had ascariasis.

3.2. Detection of anti-Toxoplasma antibody by DT, LAT and UAbE

Results of the membrane-based tests are presented in Fig. 1. The use of $NiCl_2$ in the membrane-based ELISA resulted in a bluish black positive reaction, which was easier to differentiate from a negative result, than a brown positive result without its addition. Thus, membrane strips that appeared coloured after incubation in test urine samples with haematuria demonstrated clearly as positives (strips d and e) or negatives (strips j and k). Control strips (strips l and m) remained colourless. In both systems, positive results showed varying intensity of colouration of membrane strips, which were graded from strong to weak positive.

Prevalence of anti-*Toxoplasma* antibodies in sera and urine as determined by the three different tests, is presented in Table 1. The overall prevalence values were not statistically different ($P > 0.05$). Three samples, which were DT-positive could not be determined clearly as positive or negative by LAT, 2 of them were from obstetrics and 1 from ophthalmology (past infection) patients, respectively.

Table 1
Prevalence of anti-*Toxoplasma* antibody as determined by the three different tests

Test	Source of sample	Positive	Negative	Ind[a]	Prevalence (%)
DT[b]	OP[c]	47	7	0	87.0
	OGP[d]	19	7	0	73.1
LAT[e]	OP	46	7	1	85.2
	OGP	17	7	2	65.4
UAbE[f]	OP	48	6	0	89.0
	OGP	21	5	0	80.8

[a] Indeterminate: positivity or negativity could not be determined.
[b] Sabin–Feldman Dye Test.
[c] Samples from ophthalmology patients ($n = 54$).
[d] Samples from pregnant women attending obstetrics/gynaecology clinic ($n = 26$).
[e] Latex agglutination test.
[f] Urinary antibody detecting ELISA.

Urinary antibody detection by UAbE as compared with DT and LAT from obstetrics/gynaecology (OGP) as well as ophthalmology (active and past infection) patients' samples is presented in Table 2. As shown in Table 2, 17/22 (77.3%) suggestive active cases and 30/32 (93.8%) of the past-toxoplasmosis cases were confirmed. Also, 19/26 (73.1%) of the pregnant women showed positive serology. Seven out of eight OGP that were previously sero-negative remained so during this study.

Using DT as gold standard, the global sensitivity and specificity of LAT and UAbE on the total num-

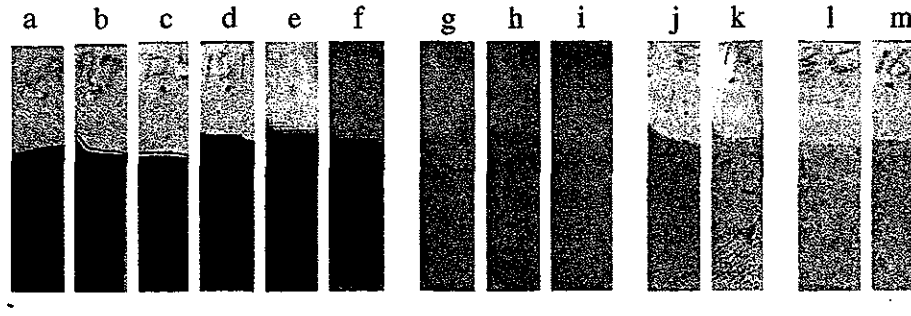

Fig. 1. Results of membrane-based ELISA showing colourization of PVDF membrane strips. Strips (a–f) indicate strong positive results; strips (g–i) indicate very weak positive results; strips (j and k) were incubated with urine from negative samples; strips (l and m) were not incubated with urine.

Table 2
Results of UAbE as compared with DT and LAT

Test	Result	UAbE[a]						Total ($n=80$)
		OP-active infection[b] ($n=22$)		OP-past infection[c] ($n=32$)		OGP[d] ($n=26$)		
		Pos[e]	Neg[f]	Pos	Neg	Pos	Neg	
DT[g]	Pos	17	0	24	6	16	3	66
	Neg	5	0	2	0	5	2	14
LAT[h]	Pos	17	0	23	6	15	2	63
	Neg	5	0	2	0	5	2	14
	Ind[i]	0	0	1	0	1	1	3

[a] Urinary antibody detecting ELISA.
[b] Samples from ophthalmology patients with active toxoplasmosis.
[c] Samples from ophthalmology patients with past toxoplasmosis.
[d] Samples from pregnant women attending obstetrics/gynaecology clinic.
[e] Number positive by the tests from that group of patients.
[f] Number negative by the tests from that group of patients.
[g] Sabin–Feldman Dye Test.
[h] Latex agglutination test.
[i] Indeterminate: positivity or negativity could not be determined.

ber of samples examined in this study (including the 80 toxoplasmosis suspected group of patients and the 22 normal individuals) were determined. The sensitivity and specificity for LAT were 96.0% (63/66) and 92.0% (33/36), and for UAbE, 86.4% (57/66) and 67.0% (24/36), respectively.

All the 22 control samples were negative for DT, LAT and UAbE.

3.3. Detection of *Toxoplasma* urinary antigens by UAgE

The UAgE detected antigens in 78.0% (42/54) and 42.3% (11/26) of the ophthalmology and obstetrics/gynaecology urine samples, respectively, and an overall prevalence of 66.3% (53/80) as shown in Table 3. The 42 UAgE-positive samples included 100% (22/22) of patients that presented with eye disorders suggestive of active *Toxoplasma* infection and 62.5% (20/32) of the suggestive past infection cases.

Results of UAgE as compared with DT are shown in Table 4. All of the 11 UAgE-positive obstetrics/gynaecology patients were also DT-positive and antigens from 7 of them recognized murine-IgGs only, mostly in trace amounts whilst 1 bound both murine-IgA and IgG. In addition, six (five ophthalmology active infection and one OGP) samples, which were negative by both DT and LAT were positive by UAgE and bound by both murine-IgA and IgG (four samples), and IgG only (two samples). All six were positive for urinary antibodies. Samples from five out of seven ophthalmology (active infection) patients all of which were negative by both DT and LAT were positive by UAgE, and the immunoglobulins bound by the antigens were murine-IgA and/or IgG. Two of them were positive for both murine-IgA and IgG and, three for IgG only. On the other hand, all seven samples tested positive for urinary antibodies.

Table 3
Detecting *Toxoplasma gondii* urinary antigens in samples from ophthalmology and obstetrics patients using the membrane-based ELISA (UAgE)

Source of sample	UAgE[a]		Prevalence (%)
	Positive	Negative	
OP-active infection[b] ($n=22$)	22	0	100.0
OP-past infection[c] ($n=32$)	20	12	62.5
OGP[d] ($n=26$)	11	15	42.3
Total ($n=80$)	53	27	66.3

[a] Urinary antigen detecting ELISA.
[b] Samples from ophthalmology patients with active toxoplasmosis.
[c] Samples from ophthalmology patients with past toxoplasmosis.
[d] Samples from pregnant women attending obstetrics/gynaecology clinic.

Table 4
Results of UAgE as compared with DT

Test	Result	UAgE[a]						Total ($n=80$)
		OP-active infection[b] ($n=22$)		OP-past infection[c] ($n=32$)		OGP[d] ($n=26$)		
		Pos[e]	Neg[f]	Pos	Neg	Pos	Neg	
DT[g]	Pos	17	0	17	10	11	11	66
	Neg	5	0	3	2	0	4	14
Total		22	0	20	12	11	15	80

[a] Urinary antigen detecting ELISA.
[b] Samples from ophthalmology patients with active toxoplasmosis.
[c] Samples from ophthalmology patients with past toxoplasmosis.
[d] Samples from pregnant women attending obstetrics/gynaecology clinic.
[e] Number positive by the tests from that group of patients.
[f] Number negative by the tests from that group of patients.
[g] Sabin–Feldman Dye Test.

Serum and urine samples from one patient tested positive for all the tests and the antigens recognized murine-IgM in trace amounts in addition to IgA (++) and IgG (+) (data not shown). The individual involved was a 30-year-old male ophthalmology (active infection) patient who presented with traumatic uveitis. All the 22 urine samples from control individuals were negative by UAgE.

3.4. Probable influence of detected parasites on UAbE and UAgE

All the three ophthalmology (active infection) patients with *S. haematobium* infection, one of whom was also habouring *P. falciparum*, were positive for UAbE and UAgE but one was negative for DT. Two of the other three *S. haematobium* infected ophthalmology (past infection) patients were negative for both UAbE and UAgE and the remaining one who had *P. falciparum* infection in addition was UAbE positive only, although all three were sero-positive. Two of the ophthalmology (active infection) hookworm infested patients' samples were both positive by UAbE and UAgE but one was sero-positive and the other sero-negative. The remaining one (past infection) was sero-negative but positive by UAbE and UAgE.

4. Discussion

Detection of toxoplasmosis in the early stages of infection could pave the way for early treatment and consequently effective control of the disease. In addition, a relatively simple, cheap and easy-to-perform test could encourage routine mass screening of people, especially, women of potential childbearing age which will help control congenital transmission as well as to determine the sero-conversion rate among such women in Ghana. The present study is the first to employ membrane-based ELISA to detect *Toxoplasma* antigens and anti-*Toxoplasma* antibodies from the urine samples of such a large number of patients since Huskinson et al. (1989) reported the detection of *Toxoplasma* antigens in urine samples from patients with acute toxoplasmic encephalitis and acquired immunodeficiency syndrome in preliminary studies.

In this study, the two membrane-based ELISA systems were successfully used for the detection of *Toxoplasma* antigens and anti-*Toxoplasma* antibodies in the urine of infected individuals. Although sensitivity of UAbE (86.4%) compared with DT (gold standard) on one hand and with LAT (96.0%) on the other, was not statistically different ($P>0.05$), its specificity (67.0%) was quite low and might not be considered as a reliable diagnostic method for *Toxoplasma* infection, nevertheless, its performance could be enhanced in further studies. It was useful for comparative purposes in this study. For example, in the present study, detection of anti-*Toxoplasma* antibodies by UAbE in the urine samples of seven patients who were anti-*Toxoplasma* antibody sero-negative by both DT and LAT could be due to IgM and/or IgA antibodies which is suggestive of ongoing infections as has been reported by Bessieres et al. (1992) that, IgA antibodies associated with IgM

antibodies indicate acute infection because they are not usual in acquired immunity and very rare in chronic infections. They further reported that the presence of IgA together with IgG antibodies in circulation indicate recent acquired infection because IgA antibodies persist over 3 or 4 months following infection in acquired toxoplasmosis and low titers of IgG antibody are usual in patients with active toxoplasmic chorioretinitis, and IgM antibody cannot usually be detected.

Direct statistical comparison of the performance of UAgE with DT on one hand and with LAT on the other may be erroneous since the respective tests are detecting different components in circulation. Moreover, antibodies once produced against antigens are known to remain in circulation for a prolonged period even after drug therapy whilst antigens gradually reduce in quantity and go out of circulation. However, detection of *Toxoplasma* antigens in urine from patients whose sera were negative for anti-*Toxoplasma* antibodies is suggestive of early stage of fresh (or primary) infection, which was evident in this study. In other words, DT sero-negative serum samples that were positive by UAbE and/or UAgE could possibly be due to a putative active infection (IgM/IgA+ but IgG−). Additionally, in the UAgE system, antigens from samples that indicated weak positive results bound to murine-IgM and IgA antibodies, which appear in circulation earlier than IgG. Among the patients whose sera were positive for both DT and LAT but negative for urinary antigens by UAgE were two with high IgG titers (1:1024–1:2048). This is expected because both DT and LAT are reported to measure primarily IgG antibodies low titers of which may persist for life whilst some patients have persistently high titers (e.g. 1:1000–1:4000) for years. Consequently, the titer does not correlate with the severity of illness (Anderson and Remington, 1975). The comparable efficacy of DT and LAT in determining seropositivity was evident in this study (Table 1).

Considering the high prevalence of parasitic diseases like falciparum malaria, schistosomiases and soil-transmitted helminthiasis in Ghana, and more so with the existence of a urinary antigen detecting membrane-based dipstick assay for urinary schistosomiasis (Bosompem et al., 1997), it was necessary to clarify possible cross-reactions due to any of these parasites' antigens. None of the parasites detected do seem to have influenced the results of the membrane-based tests since urine samples from some individuals with mixed parasitic infections were negative for those tests.

Cryptosporidiosis is in Ghana but no comprehensive studies have been conducted. Addy and Aikins-Bekoe (1986) recorded a prevalence of 12.9% (61/474) in children from 2 to 60 months old and prior to our study a hospital-based research conducted by Otchere in 2001, using the same method on watery and normal formed faecal specimens from Ghanaian patients, yielded no oocysts (personal communication). That no oocysts were detected in any of the stool samples by the formalin–ether sedimentation technique was not surprising but does not rule out also inclusion of patients with antigenemia for, and/or antibodies against other relevant coccidian species like *C. parvum*, *Isospora belli* and *Cyclospora cayetanensis*. Current research methods like the use of fluorescein isothiocyanate-labelled anti-parasite monoclonal antibodies, SDS-PAGE and Western blotting techniques as well as ELISA could be employed to detect parasite antigens (Smith, 1998; Ungar, 1990) in a more elaborate study.

The 22 normal samples were confirmed by the study as sero-negative and could therefore be considered as true controls.

In this study, the UAbE and UAgE systems appear to have good prospects as their outcome are consistent with the intentions of development. However, their sensitivity and specificity need to be enhanced, and detailed cross-reactivity studies conducted with respect to infections from other coccidian species, using well-defined and larger study populations, especially, for obstetrics/gynaecology patients.

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CASE REPORT

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Strongyloidiasis in a patient with acquired immunodeficiency syndrome

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Abstract Rhabditiform larvae, transforming larvae from rhabditiform to filariform, and eggs of *Strongyloides stercoralis* were identified in the sputum of a Thai woman with acquired immunodeficiency syndrome (AIDS), and stool microscopy also showed a heavy load of rhabditiform larvae of *S. stercoralis*. She was treated with 12 mg ivermectin once a day for 2 days for the strongyloidiasis, with good therapeutic results being obtained. Strongyloidiasis may be a curable disease through the use of an appropriate therapy, even in a patient with AIDS.

Key words *Strongyloides stercoralis* · HIV · Ivermectin

Introduction

Strongyloidiasis stercoralis is an infectious disease caused by *Strongyloides stercoralis*, a small intestinal nematode, and is usually symptomatic or asymptomatic in immunocompetent persons. This parasitic infection is distributed widely in tropical and subtropical areas. Okinawa Prefecture and the southwestern part of Kagoshima Prefecture, which are subtropical areas, have been known as endemic regions in Japan, but strongyloidiasis is uncommon and Japanese physicians are unfamiliar with this infectious disease outside the areas mentioned above. Some patients coinfected with human immunodeficiency virus (HIV) and *S. stercoralis* have been reported.[1,2] The effectiveness of ivermectin against strongyloidiasis in immunocompetent patients is well known; however, its usefulness has not been well studied in regard to strongylodiasis in patients coinfected with HIV. Here we present a case of strongyloidiasis treated with ivermectin in an acquired immuno deficiency syndrome (AIDS) patient.

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Case report

A 46-year-old Thai woman, who came to Japan in 1998 and married a Japanese man, was admitted to a hospital in Tokyo complaining of fever and dyspnea on June 16, 2002. She was diagnosed as having pneumonia and treated with meropenem, but her condition did not improve. She was referred to our hospital on June 21, 2002, because her serum anti-HIV antibody was positive and *Pneumocystis carinii* pneumonia was suspected. She also complained of soft, but not bloody, stool (1–3 times/day) on admission; however, the onset date of her soft stool was unknown. She did not complain of nausea or vomiting.

On admission to our hospital, she was alert; her height was 153 cm, body weight 45 kg, body temperature 37°C, blood pressure 110/60 mmHg, pulse rate 102/min and regular, respiratory rate 40/min, and SpO_2 was 82% in room air. There was no jaundice, and cervical lymph nodes were not palpable, but oral candidiasis was found. Heart sounds were normal, and mild coarse crackles were audible on her right-side chest. Abdominal examination revealed no tenderness, normoactive bowel sounds, and no ascites. Pretibial edema were absent.

White blood cell count on admission was 10800/mm^3 (neutrophils 96.0%, lymphocytes 2.6%, monocytes 1.0%, eosinophils 0.4%), red blood cells were 387 × 10^4/mm^3, hemoglobin was 9.4 g/dl, and hematocrit was 29.7%. Plasma HIV-RNA levels and CD4$^+$ lymphocytes were 1.5 × 10^5 copies/ml and 3/mm^3, respectively. The numbers of white blood cells (with eosinophil percentages) were as follows: 5900/mm^3 (2%) on June 24, 7600/mm^3 (2%) on June 26, 11200/mm^3 (0%) on June 28, 6500/mm^3 (0%) on July 4, and 4400/mm^3 (3%) on July 8. Serum levels of total protein,

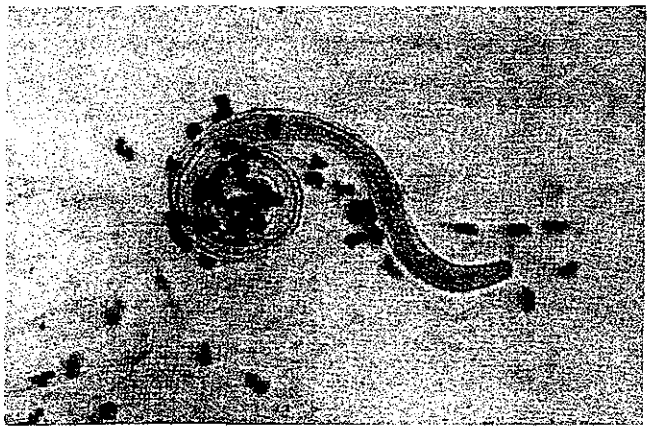

Fig. 1. Transforming larva from rhabditiform to filariform in sputum. Papanicolaou stain, ×182

albumin, aspartate transaminase, lactate dehydrogenase, potassium, iron, C-reactive protein, immunoglobulin G, immunoglobulin A, and β-D-glucan on admission were 7.7 g/dl, 2.5 g/dl, 71 IU/l, 782 IU/l, 128 mEq/l, 15 mg/dl, 10.3 mg/dl, 2050 mg/dl, 732 mg/dl, and 153.7 pg/ml, respectively. Cysts of *P. carinii* were identified in her sputum, and moreover, rhabditiform larvae, transforming larvae from rhabditiform to filariform (Fig. 1), and embryonated ova of nematodes were all identified in her sputum under Papanicolou staining. Stool microscopy showed a heavy load of rhabditiform larvae, and occult blood examination in her stool was positive. The mean size of the rhabditiform larvae found in her sputum with Papanicolou staining was 200 × 20 μm. An esophagus with a club-shaped anterior portion and a posterior bulbus was found in the rhabditiform in her sputum, with a buccal cavity that is short and of a small diameter. A longer esophagus was found in the transforming larvae, but a posterior bulbus was not identified. The rhabditiform in her stool had a genital primordium halfway down the midgut, an esophagus with a club-shaped anterior portion and a posterior bulbus, as well as a short buccal cavity. They were thought to be larvae and ova of *S. stercoralis*.

She was then diagnosed as having acquired immunodefieciency syndrome (AIDS) with *P. carinii* pneumonia, oral candidiasis, and strongyloidiasis. Sulfamethoxazole trimethoprim and predonisolone were administered from June 21, 2001, for the treatment of *P. carinii* pneumonia, and clotrimazole was administered orally for the oral candidiasis. She was treated with a single oral dose of 12 mg ivermectin on June 24 and again on July 1, 2002. Her defecation rate changed to between 0 and 2 times daily beginning June 27, 2002. Ivermectin caused no adverse side effects. Her condition significantly improved, and she was discharged on July 16, 2002; a highly active antiretroviral therapy with sanilvudine, lamivudine, and efavirenz was started at an outpatient clinic beginning August 15, 2002. *S. stercoralis* was not found in her stool by a direct smear method on November 11, 2002, or by a direct smear method, filter paper culture method, or ordinary agar plate culture method on February 6, 2003.

Discussion

There are two possible explanations for the findings of larvae and eggs of *S. stercoralis* in the sputum from our patient. First, eggs were expelled by matured worms in the lungs where larvae were developing. Second, her sputum was contaminated with duodenal juice. In our patient, not only intestinal strongyloidiasis but also pulmonary strongyloidiasis may have been present, as she did not complain of nausea or vomiting.

It has been reported that eosinophils play an important role in protecting the host against fulminant strongyloidiasis,[3] and eosinopenia may be associated with a poor prognosis of strongylodiasis.[3–5] If strongyloidiasis had been overlooked in our patient, her prognosis might have been poor, because her eosinophil count was low or normal. Further studies of the relationship between eosinopenia and strongyloidiasis are anticipated, however.

Ivermectin is the most useful drug for the treatment of strongylodiasis in immunocompetent conditions,[6,7] and appears to be promising for the treatment of strongyloidiasis in patients coinfected with HIV.[8,9] Because the number of cases is few, however, the effectiveness of ivermectin against strongyloidiasis in patients infected with HIV has not been fully studied. The disappearance of *S. stercoralis* and its symptoms after the administration of ivermectin in our patient may indicate that strongylodiasis is a curable disease, even in patients with AIDS, and at the same dose as for patients infected with *S. stercoralis* without HIV. Our patient is thought to have become infected with *S. stercoralis* outside Japan, because she had not been to any *S. stercoralis* endemic areas in Japan. She probably acquired the causative organism in Thailand. Our present report indicates that it is important to investigate stool and sputum for *S. stercoralis* in HIV-positive patients who have been in a *S. stercoralis* endemic area and are complaining of diarrhea, because strongyloidiasis may be curable through the use of an appropriate therapy, even in a patient with HIV.

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LARVA MIGRANS BY *BAYLISASCARIS TRANSFUGA*: FATAL NEUROLOGICAL DISEASES IN MONGOLIAN JIRDS, BUT NOT IN MICE

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ABSTRACT: Raccoon roundworms (*Baylisascaris procyonis*) and other *Baylisascaris* species cause patent or latent larva migrans (LM) in a variety of mammals and birds, including humans. It is not clear whether LM by *Baylisascaris transfuga*, roundworms of bears, is associated with clinical neurological disorders. To clarify this issue, ICR and BALB/c mice as well as Mongolian jirds (*Meriones unguiculatus*) were orally inoculated with 2,000–5,000 embryonated eggs of *B. transfuga*. In mice, the ascarid caused symptomatic LM of limited extent and duration, whereas the infection was fatal in jirds; i.e., they exhibited general signs such as severe depression and emaciation on days 8–11 postinfection (PI) and died, or they developed progressive and fatal neurological disorders after day 14 PI. Histological examination showed *B. transfuga* larvae in the brain of all mice and jirds examined, and the larvae collected from them developed to a size comparable with that of *B. procyonis*. There existed, however, critical differences in host reactions against larvae localized in the brain of mice and jirds; *B. transfuga* larvae found in mice were surrounded by granulomatous reactions and immobilized, whereas larvae found in jirds were free from any host reaction and mobile, causing extensive malacia.

Because of its zoonotic impact on animals and humans, larva migrans (LM) by the raccoon roundworm (*Baylisascaris procyonis*) has been investigated extensively using laboratory rodents as well as accidentally infected domestic or wild animals. Because raccoons (*Procyon lotor*) are endemic in North America, a dozen human cases of *B. procyonis* LM with severe or fatal neurological manifestations, as well as dozens of patients with visual disorders related to diffuse unilateral subacute neuroretinitis (DUSN) caused by this ascarid, have been documented almost exclusively from that continent (Huff et al., 1984; Fox et al., 1985; Goldberg et al., 1993; Cunningham et al., 1994; Boschetti and Kasznica, 1995; Park et al., 2000; Rowley et al., 2000; Gavin et al., 2002; Kazacos et al., 2002; Mets et al., 2003; reviewed by Kazacos, 1997, 2001). Recent and progressive naturalization of imported raccoons in other continents is ominous of an expansion of the disease worldwide in the near future. Raccoons exported to West Germany and Russia in the mid-1930s became feral, expanding their distribution into Germany, France, The Netherlands, Turkestan, Azerbaijan, Uzbekistan, Kirgiz, Belorussia, Ukraine, and Caucasia. In these countries, raccoons cause ecological, economic, and public health problems. For example, a human case of DUSN and serologically suspected human cases of asymptomatic LM caused by *B. procyonis* have been reported in Germany (Küchle et al., 1993; Conraths et al., 1996). More than 20,000 raccoons had been exported to Japan as pets during the past 3 decades until 2000, and several thousand raccoons have become feral in this country (Miyashita, 1993; Kawanaka et al., 2001). After the report of *B. procyonis* causing fatal infection in rabbits kept at a wildlife park where visitors, including infants and children, had common access to an egg-contaminated rabbitry and had close contact with infected rabbits (Sato et al., 2002; Sato, Kamiya et al. 2003; Furuoka et al., 2003), this ascarid LM became an important public health problem in Japan as well.

After the first reports of *B. procyonis* LM in Japan, histological slides of ascarid LM in zoo animals were sent to our laboratory for identification of the causative species (Sato, Matsuo et al., 2003). One example was Japanese macaques (*Macaca fuscata*) kept in a safari park, which shared living space with American black bears (*Ursus americanus americanus*) harboring *B. transfuga*. In contrast to *B. procyonis*, only a few studies have been conducted on *B. transfuga* LM, although larval migration to the viscera, eyes, and brains has been demonstrated in laboratory mice (Sprent, 1955; Papini et al., 1994; Papini, Lo Piccolo, and Casarosa, 1996; Papini, Renzoni, Lo Piccolo, and Casarosa, 1996) and rabbits (Papini, Demi, and Croce, 1996), suggesting the zoonotic potential of this ascarid species.

In this study, we conducted several experiments to determine the clinical and histological changes of LM caused by *B. transfuga*, *B. procyonis*, and *Toxocara canis* in mice and Mongolian jirds (*Meriones unguiculatus*), which has been reported to be the best laboratory model for DUSN caused by *B. procyonis* larvae and other ascarid ocular LM (Takayanagi et al., 1999; Akao et al., 2000, 2003). The results suggest that *B. procyonis* LM is fatal to both rodent species, whereas *B. transfuga* LM is fatal only in jirds but not in mice. This difference in clinical manifestations is ascribed to different host reactions against *B. transfuga* larvae in the central nervous system (CNS) of jirds and mice; specially jirds could not encapsulate and immobilize invading larvae, whereas mice could.

MATERIALS AND METHODS

Animals

Mice of outbred (ICR) and inbred (BALB/c) strains and Mongolian jirds were bred in the Institute for Animal Experiments, Hirosaki University School of Medicine. They were housed in plastic boxes and provided with commercial pellets (MF; Oriental Yeast Co., Ltd, Tokyo, Japan) and water ad libitum. After oral inoculation of embryonated eggs of ascarids, rodents were kept in a closed room to avoid environmental contamination with eggs or biohazards to other workers. Equipment used to keep infected rodents was subsequently cleaned in boiling water.

Parasites

Adult *B. transfuga* were expelled after anthelmintic treatment from a polar bear (*Ursus maritimus*) kept in a zoological garden. *Baylisascaris procyonis* was obtained from raccoons in a wildlife park, where LM due to this ascarid was found in kept rabbits in 2000 (Sato et al., 2002). *Toxocara canis* was collected from naturally infected dogs after the administration of anthelmintics. Fertile eggs were collected from the uteri of adult females and incubated for 1 mo at 27 C to obtain embryonated eggs, then kept at 4 C until used.

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TABLE I. Fate of larva migrans caused *Baylisascaris transfuga*, *Baylisascaris procyonis*, and *Toxocara canis* in mice and jirds.

Parasite	Animal	Eggs inoculated*	Rate of clinical disease	Date of the first signs of disease	Date of death or killing due to severe clinical disease	Date of the end of the experiment
B. transfuga						
	ICR mice (21-wk-old, male)	2,000	1/7	21	—	60
	BALB/c mice (12-wk-old, female)	2,000	3/8	21	28†	56
	Jirds (7-wk-old, female)	5,000	12/12	1	9.8 ± 1.1 (8–11)	11
	Jirds (9-wk-old, female)	4,000	13/13	20.3 ± 4.2 (14–28)	25.8 ± 3.7 (21–31)	31
B. procyonis						
	ICR mice (16-wk-old, male)	200	5/7	14.0 ± 2.2 (10–21)	25.6 ± 3.6 (21–31)	60
	BALB/cmice (8 to 12-wk-old, female)	100‡	24/24	13.0 ± 2.7 (9–18)	16.0 ± 3.4 (12–21)	56
	BALB/c mice (12-wk-old, female)	200	5/6	22.6 ± 3.1 (21–28)	23.2 ± 2.7 (22–28)	56
	BALB/c mice (12-wk-old, female)	100	2/6	31.5 ± 5.0 (28–35)	34.0 ± 8.5 (28–40)	56
	Jirds (11 to 13-wk-old, female)	100‡	16/16	13.6 ± 2.3 (9–15)	16.6 ± 3.4 (12–21)	21
	Jirds (8-wk-old, female)	200	10/12	19.2 ± 7.5 (15–39)	27.1 ± 5.9 (21–39)	60
	Jirds (7-wk-old, male)	200	13/13	21.7 ± 5.0 (14–31)	30.3 ± 11.9 (21–60)	60
T. canis						
	ICR mice (14-wk-old, male)	2,000	0/7	—	—	77
	BALB/c mice (12-wk-old, female)	2,000	0/6	—	—	56
	Jirds (9-wk-old, female)	2,000	0/15	—	—	93

* Embryonated eggs with viable larvae were used to infect rodents in all experiments, but *B. procyonis* eggs except for those marked by (‡) were preserved in vitro for more than 1 yr before use.
† One mouse only.
‡ Explanation appears in the footnote marked by *.

Infection, monitoring of clinical manifestations, and necropsy

Rodents were inoculated orally with embryonated eggs using a metal gastric sonde under light anesthesia (Table I). Approximate numbers of eggs used for infection were estimated after counting eggs with mobile larvae inside per unit volume. Rodents were observed daily to check the onset and progression of clinical signs. When the clinical condition deteriorated, i.e., spinning around the longitudinal axis, continuous circling or lateral recumbency, rodents were killed on that day. Animals not exhibiting clinical signs after inoculation with eggs of *Baylisascaris* spp. were killed on days 56 or 60 PI. A part of mice and jirds infected with *T. canis* were treated with immunosuppressive agents such as prednisolone or anti-T cell monoclonal antibody (mAb) mentioned below or after day 71 PI, and killed on days 77 and 93 PI, respectively. Animals were skinned and dissected. Pieces of the intestinal wall, liver, lungs, heart, kidney, femoral muscle, and whole parts of the brain were fixed in 10% neutral-buffered formalin. Other parts of the body were minced and digested in physiological saline containing 0.8% pepsin and 0.8% HCl at 37 C for 3–4 hr. Digested material was passed through a 125-μm metal mesh sieve and washed several times by simple sedimentation using physiological saline.

Immunosuppressive treatments

To evaluate the effects of immunosuppressive treatment on clinical manifestations associated with chronic *T. canis* LM, 4 of each of the mice and jirds were injected subcutaneously with 4 mg of prednisolone tertiary-butylacetate (Suspension of Codelcortone®-T.B.A., Merck & Co., Inc., Rahway, New Jersey) on day 71 PI. Similarly, 4 jirds were treated with anti-jird T cell murine mAbs, HUSM-M.g.13 of IgG1 isotype and HUSM-M.g.14 of IgG2a isotype, from day 71 PI. The epitope recognized by these mAbs is identical to HUSM-M.g.15 of IgG2b isotype, which has been used for successful depletion of functional T cells from jirds (Sato et al., 2000; Sato et al., 2004). Preparation of semipurified mAbs from mouse ascitic fluid and injection schedule were based on the methods described previously (Sato et al., 2000), except for the injection dose of 0.5 mg protein/injection. Complement-fixing mAb HUSM-M.g.14 exhibits the same effects as HUSM-M.g.15, but complement-unfixing mAb HUSM-M.g.13 exhibits no immunosuppressive effect and was used as a negative control mAb to assess the effect of T-cell depletion by mAb HUSM-M.g.14.

Histological examination

Formalin-fixed tissue blocks were dehydrated in a series of alcohol, cleared in xylene, and embedded in paraffin. Sections, 5 μm thick, were stained with hematoxylin and eosin.

Measurements of recovered larvae

Morphological examination and measurements of ascarid larvae were conducted using parasites collected by artificial digestion of body muscles and viscera. Measurements were calculated with the aid of camera lucida.

Statistical analysis

Data are expressed as mean ± SD with range values provided in parentheses. Differences between 2 groups were examined for significance using the Student's *t*-test. A P value less than 0.05 denoted the presence of statistical significance.

RESULTS

Clinical features

As shown in Table I, 14 experiments of ascarid LM were conducted to compare the pathogenecity of *B. transfuga*, *B. procyonis*, and *T. canis* larvae in mice and jirds. Clinical signs ascribed to *B. transfuga* LM were rarely noticed in mice; there was 1 case of continuous circling among 7 infected ICR mice and 3 cases of locomotor incoordination among 8 infected BALB/c mice. Except for 1 fatal case, all other mice showed full recovery from these conditions within 1 wk. In contrast, *B. transfuga* LM caused fatal infection in jirds. One group of 7-wk-old jirds inoculated orally with 5,000 eggs exhibited roughened hair coats within a few days after infection, became highly emaciated, and died by day 11 PI. Another group of 9-wk-old jirds inoculated orally with 4,000 eggs survived the acute phase of the disease but had clinical neurological signs such as torticollis, spinning around the longitudinal axis, and continuous

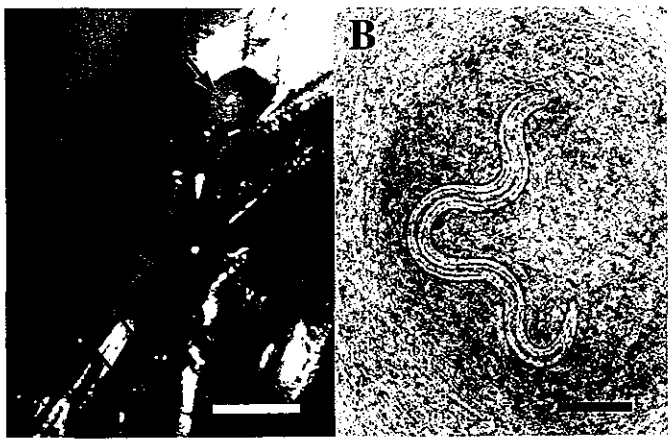

FIGURE 1. Granulomatous lesions elicited by *Baylisascaris transfuga* larva migrans. **A.** Granulomas of variable sizes (arrows) in the fascia lata femoris. Bar = 2.0 mm. **B.** Image of a press specimen of 1 granuloma containing the larva. Bar = 200 μm.

circling or lateral recumbency on days 14–28 PI, and died by day 31 PI. *Baylisascaris procyonis* LM induced severe neurological signs in both mice and jirds, and the infection was usually fatal for both host species. No neurological sign associated with *T. canis* LM was detected in either mice or jirds.

Gross and histological findings

Examination of jirds that died from acute *B. transfuga* LM by day 11 PI showed fibrinous exudate trapping numerous larvae in the peritoneal cavity with marked pulmonary hemorrhage and edema. No granulomatous lesion could be detected in these jirds. The other group of jirds that died from neurological disorders caused by *B. transfuga* LM had disseminated granulomatous lesions on the serosal surface of abdominal and thoracic organs, particularly the posterior part of the ileum, cecum, and colon, as well as in the subcutaneous tissue or on the surface of muscles of the trunk (Fig. 1). Mice also had numerous disseminated granulomas in various organs, similar to jirds. Similarly, mice and jirds with *B. procyonis* LM had several granulomatous lesions. No gross granulomatous lesion was noticed in mice and jirds with *T. canis* LM. No gross lesion was found in the brain of any mice or jirds with *B. transfuga*, *B. procyonis*, and *T. canis* LM, even though some animals manifested severe neurological signs.

Histological lesions associated with ascarid LM in organs other than the CNS were characterized by formation of granuloma around migrating larvae, albeit the cellular composition varied in animals infected with different ascarid species or examined on different days of infection. The most conspicuous histological differences were noted in the CNS, i.e., localization of the lesions and host reactions surrounding ascarid larvae. Figure 2 depicts the distribution of malacia, gliosis, and larvae themselves in a series of sections from the frontal cerebrum to the cerebellum and pons of jirds with *B. transfuga*, *B. procyonis*, and *T. canis* LM. *Baylisascaris procyonis* larvae caused malacic lesions, usually in the tissues beneath the aqueductus cerebri and the ventriculus quartus cerebri, whereas malacia and subsequent accumulation of foamy macrophages in association with *B. transfuga* larval migration were prominent in the tissues above the ventriculus quartus cerebri or in the cerebellar medulla. *Baylisascaris procyonis* larvae in jirds and mice or *B. transfuga* larvae in jirds were found free from host reactions or adjacent to malacic foci (Fig. 3A, B), whereas *B. transfuga* larvae in mice were surrounded or embedded in granulomatous reactions (Fig. 3C, D). Histological examination strongly suggested that the larvae were immobilized in these locations, although they were alive and well developed. *Toxocara canis* in mouse CNS induced little tissue damage or host reaction (Fig. 3E), whereas the ascarid in jird CNS caused disseminated foci of foamy macrophage accumulation (Fig. 3F), indicating that they caused substantial damage to the nervous tissue. There was no difference in clinical manifestations or histological changes in the CNS between immunosuppressed and competent mice or jirds with chronic *T. canis* LM.

The size of migrating larvae

Table II compares measurements of 3 ascarid species mainly from infected jirds. The average worm length and maximum width of *B. procyonis* larvae collected from infected rodents were always larger than those of *B. transfuga* larvae. Although differences in these values were sometimes statistically significant when comparisons were made between samples collected at the same time, the values significantly overlapped with each other.

DISCUSSION

Baylisascaris includes species of roundworms in Carnivora, Rodentia, and Marsupialia as follows: *B. columnaris* (Leidy, 1856) of skunks, *B. devosi* (Sprent, 1952) of martens and fishers, *B. laevis* (Leidy, 1856) of marmots and ground squirrels, *B. melis* of badgers, *B. procyonis* (Stefanski et Zarnowski, 1951) of raccoons, *B. schroederi* (McIntosh, 1939) of giant pandas, *B. tasmaniensis* (Sprent, 1970) of carnivorous marsupials, and *B. transfuga* (Rudolphi, 1819) of bears. Among them, in addition to *B. procyonis*, which is a well-known cause of zoonotic LM in birds and mammals, including humans, with neurological manifestations (Kazacos et al., 1981, 1984, 1985; Kazacos, 1997, 2001), *B. columnaris* and *B. melis*, followed by *B. devosi*, *B. tasmaniensis*, and *B. transfuga*, are considered potential causes of LM when a substantial number of eggs are ingested (Sprent, 1953b, 1955; Matoff and Komandarev, 1965; Sprent et al., 1973; Kazacos, 2001). The lower pathogenecity of the latter 5 species is manifested by delayed onset of neurological disorders and no progression of disease to death, with occasional recovery of affected mice. The distinct pathogenecity of *B. procyonis* from other *Baylisascaris* spp. is explained partly by the faster growth rate and larger size of larvae on tissue migration as well as their predilection for the CNS, in contrast to the predilection of *B. devosi* for the anterior carcass musculature and that of *B. tasmaniensis* and *B. transfuga* for the intestinal wall or mesentery (Sprent, 1952, 1953a; Tiner, 1953a, 1953b; Matoff and Komandarev, 1965; Berry, 1985; Kazacos, 2001). According to Sprent (1952), Matoff and Komandarev (1965), and a series of studies by Papini et al. (Papini and Casarosa, 1994; Papini, Lo Piccolo and Casarosa, 1996; Papini, Renzoni et al., 1996), the number of embryonated *B. transfuga* eggs (2,000, 4,000, or 5,000 eggs/animal) used for

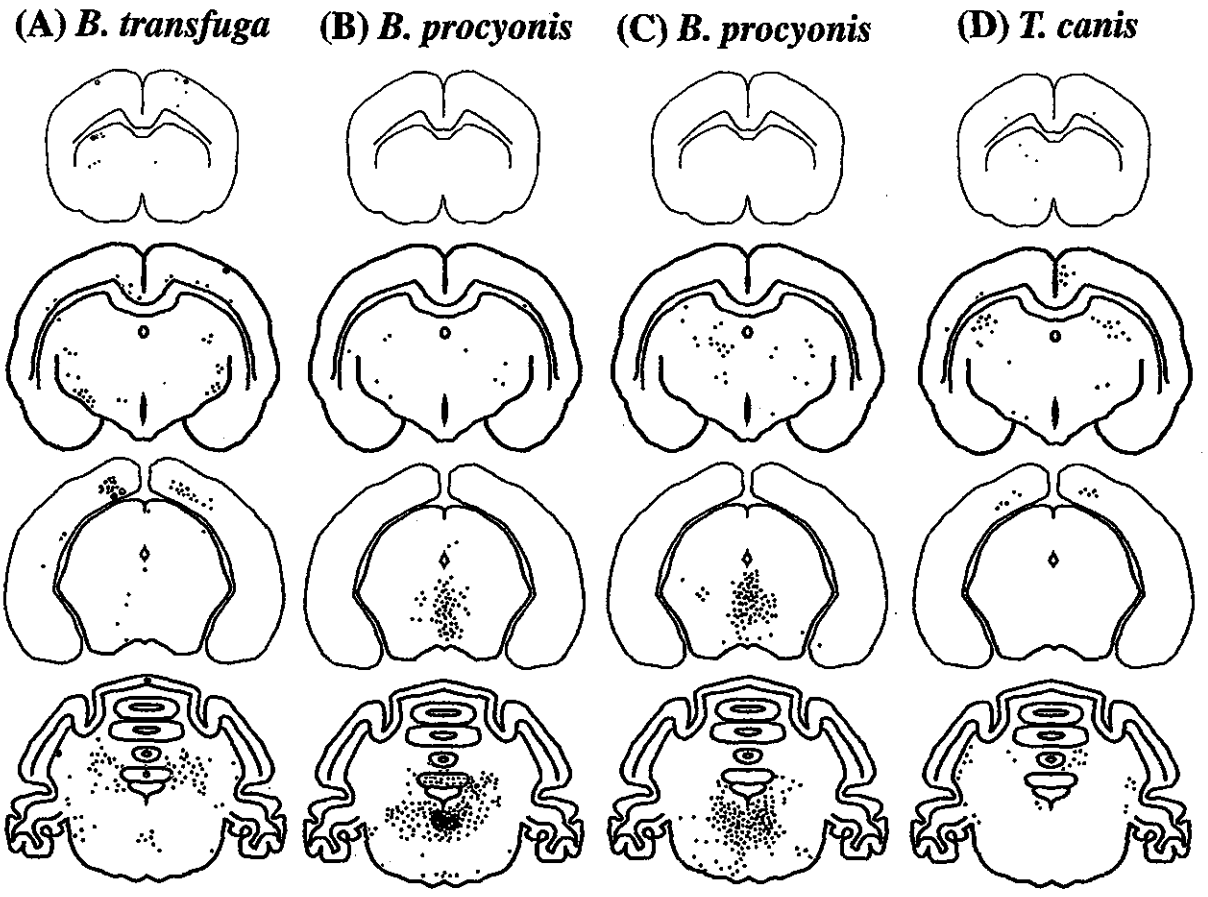

FIGURE 2. Diagram showing distribution of larvae (red dots) and histopathological changes such as malacia and gliosis (green dots) or granuloma formation (blue dots) associated with larva migrans caused by *Baylisascaris transfuga* (**A**), *Baylisascaris procyonis* (**B**, **C**) and *Toxocara canis* (**D**) in jirds. Serial transverse sections at 4 levels of the brains as shown here were prepared from 21, 23, and 15 jirds inoculated orally with embryonated eggs of *B. transfuga* (4,000 eggs), *B. procyonis* (100 eggs for B; 200 eggs for C) and *T. canis* (2,000 eggs), respectively. The foci detected on these sections were plotted accumulatively for each ascarid species. Note the distinct distribution of histological lesions in the brains of jirds infected with different ascarid species.

infection in this study was determined to be 10, or more, times the number of *B. procyonis* eggs (100 or 200 eggs/animal).

In this study, a group of jirds died on days 8-11 PI and were found at necropsy to have numerous fibrin-trapped larvae in the peritoneal cavity and marked pulmonary hemorrhages. Sprent (1952) noted that a considerable number of larvae migrated to the lungs within the first day, at which time the lungs showed hemorrhagic spots and patches that increased to a uniform congestion by the third day. After oral infection with 2,000 or 5,000 eggs, Matoff and Komandarev (1965) also noted numerous larvae in the peritoneal cavity until day 7 PI, then the number of localized larvae diminished markedly. They also noticed that encapsulation of live larvae was evident also in the subcutaneous tissues of the trunk, which was also found in our study. In the same study, Matoff and Komandarev (1965) found multiple larvae in the CNS throughout the observation period of up to day 22 PI. Sprent (1955) reported that *B. transfuga* larvae, 1.0 on average, were found in CNS of mice orally infected with 2,000-30,000 eggs. These authors did not notice neurological disorders in their mice despite the presence of multiple *B. transfuga* larvae in CNS tissues.

Papini and Casarosa (1994) investigated whether *B. transfuga* larvae in the CNS can cause neurological manifestations in mice. They argued that inoculation of high doses of infective eggs could induce neurological disorders in mice; they found neurological signs in 1 of 40 mice (2.5%), 2 of 30 mice (6.7%), and 2 of 10 mice (20.0%) inoculated with 3,000, 4,000, and 50,000 eggs, respectively, and 16–26 larvae were detected in the CNS of these 5 mice. On the basis of these results, Papini and Casarosa (1994) concluded that *B. transfuga* larvae were less pathogenic, compared with *B. procyonis*, *B. columnaris*, and *B. melis*, and ascribed lower pathogenicity of *B. transfuga* larvae partly to their smaller sizes attained on migration in mice. As shown in this study, the size of *B. transfuga* larvae found in laboratory rodents was comparable or overlapped significantly with that of highly pathogenic *B. procyonis* larvae, although larval size is commonly referred to as a critical factor in determining the outcome of ascarid neural LM. In addition to the predilection of the larvae to the CNS, the critical factor responsible for induction of neurological diseases in laboratory rodents seems to be host reactions against *Baylisascaris* spp. larvae in the CNS; when migrating larvae are encapsulated and immobilized in the brain, the affected rodent does not show neurological signs, as observed in mice with *B. transfuga* LM. Sheppard and Kazacos (1997) described 1 asymptomatic white-footed mouse (*Peromyscus leucopus*) with neural LM by *B.*

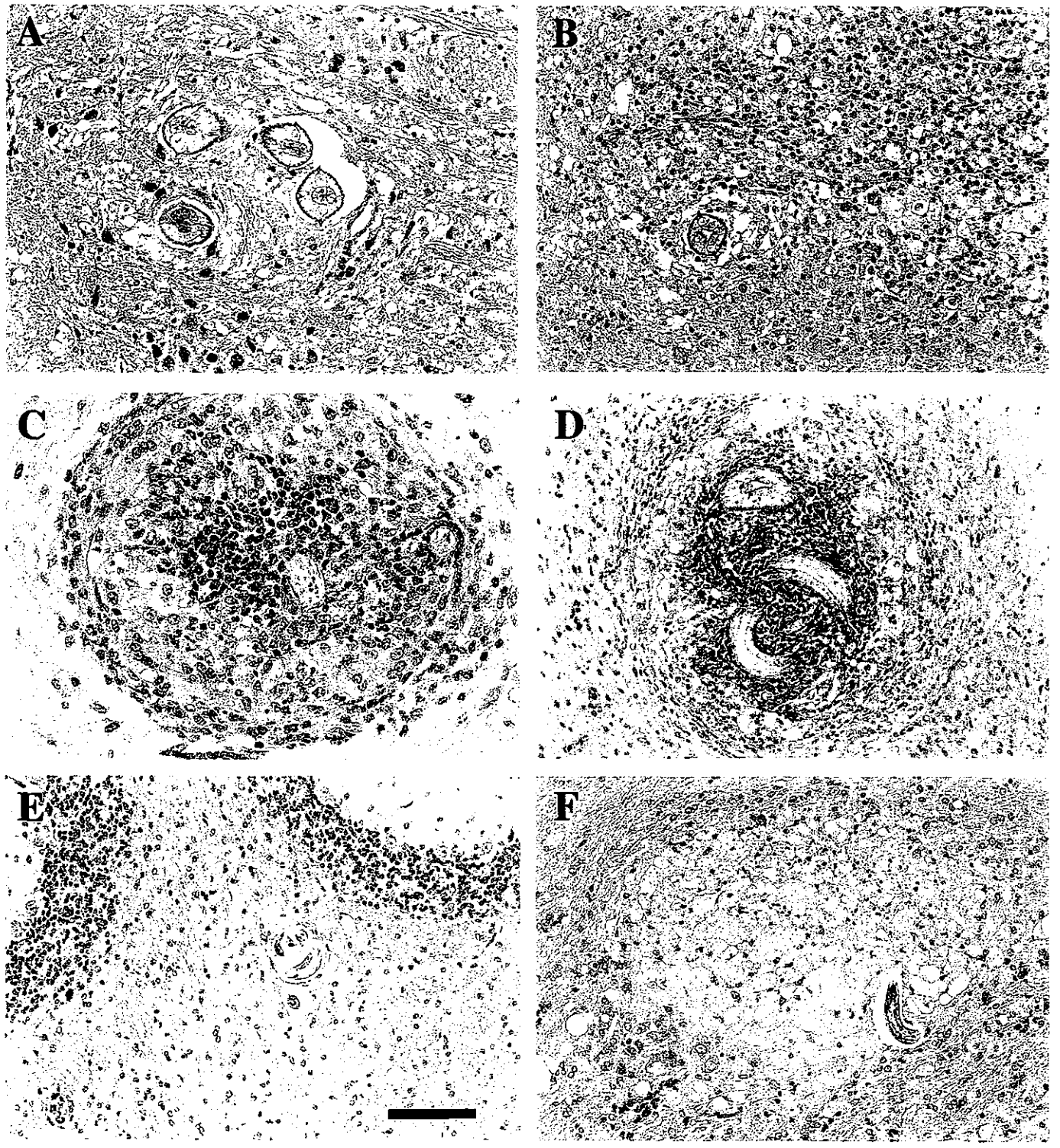

FIGURE 3. Histological changes associated with neural larva migrans by *Baylisascaris transfuga*, *Baylisascaris procyonis*, and *Toxocara canis* in jirds and mice. **A.** *Baylisascaris procyonis* larva in the jird cerebellum on day 31 PI. **B.** *Baylisascaris transfuga* larva and malacia with replacing accumulation of foamy macrophages in the jird cerebellum on day 9 PI. **C.** Granuloma immobilizing a *B. transfuga* larva in the hippocampus of an ICR mouse on day 28 PI. **D.** Granuloma immobilizing a *B. transfuga* larva in the cerebrum of an ICR mouse on day 60 PI. **E.** *Toxocara canis* larva in the cerebellum of an ICR mouse on day 77 PI. **F.** *Toxocara canis* larva and malacia with replacing accumulation of foamy macrophages in the jird cerebrum on day 93 PI. All figures except for (C) are at the same magnification; bar shown on (E) = 50 μm for (C); and 100 μm for other photographs.

TABLE II. Measurements of *Baylisascaris transfuga*, *Baylisascaris procyonis*, and *Toxocara canis* recovered from mice or jirds (in mm).

Parasite	Host	Day of collection	No. of worms examined	Worm length	Maximum worm width	Length of esophagus	Length of tail
B. transfuga							
	ICR mice	28	10	1.33 ± 0.14 (1.15–1.54)	0.070 ± 0.018 (0.071–0.093)	0.207 ± 0.033 (0.187–0.247)	0.124 ± 0.0017 (0.099–0.148)
	ICR mice	60	9	1.23 ± 0.28 (0.76–1.58)	0.061 ± 0.018 (0.039–0.088)	0.199 ± 0.039 (0.154–0.258)	0.120 ± 0.0021 (0.088–0.154)
	Jirds	26	10	1.28 ± 0.13 (1.08–1.52)	0.065 ± 0.013 (0.044–0.077)	0.217 ± 0.021 (0.187–0.253)	0.098 ± 0.0011 (0.082–0.115)
	Jirds	31	10	1.38 ± 0.13 (1.11–1.58)	0.079 ± 0.012 (0.060–0.099)	0.199 ± 0.046 (0.115–0.247)	0.113 ± 0.0013 (0.099–0.137)
B. procyonis							
	Jirds	26	7	1.41 ± 0.18 (1.17–1.57)	0.071 ± 0.010 (0.060–0.088)	0.199 ± 0.012 (0.187–0.214)	0.117 ± 0.0012 (0.099–0.132)
	Jirds	31	9	1.57 ± 0.07 (1.48–1.65)	0.084 ± 0.008 (0.071–0.093)	0.228 ± 0.011 (0.214–0.253)	0.120 ± 0.0016 (0.104–0.154)
	Jirds	60	8	1.59 ± 0.12 (1.39–1.79)	0.078 ± 0.010 (0.066–0.093)	0.231 ± 0.008 (0.220–0.242)	0.126 ± 0.0013 (0.104–0.143)
T. canis							
	Jirds	93	7	0.37 ± 0.02 (0.33–0.40)	0.021 ± 0.004 (0.016–0.027)	0.068 ± 0.009 (0.055–0.077)	0.062 ± 0.0005 (0.055–0.066)

procyonis due to encapsulation and immobilization of a single larva invading the CNS. Although *B. procyonis* larvae are rarely encapsulated in the CNS of rodent hosts and a single larva can induce clinical signs, the formation of granuloma around the larvae is a common finding in human and non-human primate cases of LM with encephalitis (Kazacos et al., 1981; Garlick et al., 1996; Kazacos, 1997; Rowley et al., 2000). In humans, it is speculated that CNS tissue damage by cytotoxic eosinophil granule proteins may contribute to the neurologic symptoms of *B. procyonis* infection (Hamann et al., 1989; Moertel et al., 2001). In rodent hosts, infiltration or accumulation of eosinophils around ascarid larvae, including *B. procyonis*, is not prominent; therefore, we might exclude possible modification or aggravation of the lesion by infiltrated eosinophils.

In mice with *T. canis* LM, a substantial number of larvae (1.1–6.1% relative to the number of inoculated eggs) were detected in the CNS, particularly the telencephalon and the cerebellum (Holland and Cox, 2001; Good et al., 2001). Although neural LM by *T. canis* is suggested to affect social behavior of affected mice, it is often difficult to detect neurological or locomotory changes associated with the infection (Sprent, 1953a, 1955). Indeed, we could not find any clinical signs except for roughened hair coats and depression in the acute phase of infection. In the mice in this study, histological examination showed no evidence of tissue damage, despite the localization of larvae in that area. In contrast, *T. canis* larvae localized in the CNS of jirds was associated with the appearance of several foci of foamy macrophages, indicating tissue damage. At this time, we could not find any clinical signs related to these histological lesions in these jirds. However, the size of larvae does not seem to be associated directly with distinct pathogenecity of different ascarid species as found in different clinical outcomes of *B. procyonis* and *B. transfuga* neural LM in mice. We are interested in defining the neural pathogenecity of ascarid larvae of different species by characterizing the biochemical properties of the parasite surface in association with host immune reactions. In addition, the histological sequels to migration of *T. canis* larvae in CNS tissues of mice and jirds are intriguing because the same ascarid larvae cause no tissue damage in mice CNS, but induce clear, but latent, tissue damage manifested solely by replacing accumulation of foamy macrophages.

Although distinct pathogenecity of *B. transfuga* larvae compared with *B. procyonis* larvae was demonstrated in this study, the morphological features and dimensions of the larvae were quite similar. It is practically very difficult to identify the causative agent for natural LM cases caused by *Baylisascaris* spp. An example is the primate cases mentioned in the Introduction. In addition, the distribution of feral raccoons is expanding and their population is increasing in mountainous Japan, where wild bears *Ursus arctos* and *Ursus thibetanus*, harboring *B. transfuga*, are common (Uni et al., 1995). To diagnose or determine the prevalence of *B. procyonis* LM in zoo and wild animals, a new approach involving molecular biology for differentiation of *B. procyonis* from *B. transfuga* might be critical, which should provide complementary data to routine parasitological methods involving morphological examinations. When referring to expanding distributions of raccoons worldwide, including Japan, efforts of continuous and critical monitoring of the

disease in the regions where raccoons are endemic or feral become an important public health issue.

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Zoonotic Filariasis in Japan

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1. Introduction

Human filariasis is mainly caused by the parasites, *Wuchereria bancrofti* and *Brugia malayi*, whose adults live in the lymphatic vessels of humans. In Japan, bancroftian filariasis was once endemic, but has been completely eradicated from the country. Although imported cases of filariasis are occasionally reported [1,2,3], no autochthonous case has been identified in recent years. By contrast, more than 10 cases of filariasis of animal origin are diagnosed annually in Japan. The most important parasite responsible for zoonotic filariasis in Japan is *Dirofilaria immitis*, the canine heartworm. The adult worms reside in the pulmonary arteries and the right ventricle, resulting in severe heart failure, which may cause sudden death of the affected dog. Humans can also be infected with *D. immitis* by a mosquito bite, but the larvae are unable to reach maturity in humans or primates, which are unsuitable hosts. Infected people present either pulmonary infarct or a subcutaneous nodule. The parasite is also occasionally observed in a deep inner organ. Hence, it is frequently confused with malignant tumor.

Human dirofilariasis, therefore, can be categorized into two groups: pulmonary and extra-pulmonary dirofilariasis. Extra-pulmonary dirofilariasis is classified further into four groups: cardiovascular, subcutaneous, visceral, and ophthalmic dirofilariasis. In this article, we focus on the studies of zoonotic filariasis that have been carried out by Japanese researchers in Japan.

2. Case reports of dirofilariasis since 1964 in Japan

2.1.Cardiovascular dirofilariasis

The filarial parasite of animal origin was first found in the left ventricle of a Brazilian boy (Magelhaes, 1887). Later, the worms were identified as adult male and female worms of *D. immitis* by Faust *et al.* [4]. This was a very unusual case in which the invading worm survived and grew into maturity in a human, just as it would do in the definitive host, Canidae. To date, only four cases of cardiovascular dirofilariasis have been reported worldwide; one of these was in Japan. Takeuchi *et al.* [5] found two slender nematodes in the heart and inferior vena cava of a 36-year-old Japanese male who died of liver cirrhosis. The worms were incidentally found through an administrative autopsy, and there was no evidence that the worms were involved as a cause of death. Both worms were identified as non-gravid adults females of *D. immitis*. The other two cases, a 73- and 40-year-old woman, were reported in New Orleans in the United States.

2.2. Pulmonary dirofilariasis

In Japan, pulmonary dirofilariasis, the most common type of human dirofilariasis, was first found in Kanazawa city in 1968 [6]. The patient was a 42-year-old male high school teacher. He was admitted to the hospital because of loss of consciousness for 20 minutes following his morning stretching routine. Chest X-ray examination revealed a coin lesion in his left lower lobe. Under the diagnosis of tuberculosis or lung cancer, a thoracotomy was carried out. Histopathological examination showed a pulmonary infarction caused by a premature female of *D. immitis*. Six years later, two additional cases of pulmonary dirofilariasis were independently reported by Fuse *et al.* [7] and Otsuru *et al.* [8].

Thereafter, many clinical cases were noticed every year. Makiya *et al.* [9]