TABLE 2

Correlations between antibody isotype levels (log_{10}) to *Schistosoma japonicum* antigens and aging.

Antibody	Correlation coefficient (R) by antigen							
	AWA	PM	PM1	PM2	PM3	PM4	PM5	PM6
IgA	0.138	**0.226**[1]	0.150	**0.234**[1]	**0.335**[1]	0.117	0.113	0.004
IgE	-0.064	0.031	0.113	0.041	-0.004	-0.060	-0.027	0.016
IgG1	0.093	0.113	(-)	(-)	(-)	(-)	(-)	(-)
IgG3	**0.216**[2]	**0.325**[1]	**0.268**[1]	**0.254**[1]	**0.183**[2]	0.030	**0.223**[2]	0.090
IgG4	0.111	0.152	0.126	0.108	**0.231**[1]	0.114	**0.239**[1]	0.084

[1] $p < 0.01$, [2] $p < 0.05$.

R values for IgG1 responses to the truncated PMs are not shown.

TABLE 3

Correlations between antibody isotype levels (log_{10}) to antigens of *Schistosoma japonicum* and various markers of fibrosis.

Antibody	Correlation coefficient (*R*) by antigen							
	AWA	PM	PM1	PM2	PM3	PM4	PM5	PM6
US score								
IgA	0.143	**0.180**[2]	0.109	0.280	**0.180**[2]	0.128	0.124	0.111
IgE	0.035	-0.069	0.031	-0.020	-0.111	-0.124	-0.130	**-0.204**[2]
IgG1	**0.320**[1]	0.081	(-)	(-)	(-)	(-)	(-)	(-)
IgG3	**0.313**[1]	**0.241**[1]	0.017	0.151	0.156	0.084	0.084	-0.033
IgG4	**0.219**[2]	**0.246**[1]	**0.196**[2]	0.137	0.106	**0.186**[2]	**0.256**[1]	0.128
P-III-P								
IgA	0.027	-0.048	-0.025	**0.263**[1]	**0.194**[2]	-0.013	**0.295**[1]	**0.238**[1]
IgE	-0.049	**-0.260**[1]	0.051	-0.056	**-0.178**[2]	**-0.304**[1]	**-0.260**[1]	**-0.194**[2]
IgG1	0.097	-0.156	(-)	(-)	(-)	(-)	(-)	(-)
IgG3	**0.215**[2]	**0.292**[1]	0.025	**0.188**[2]	**0.216**[2]	0.011	**0.286**[1]	**0.182**[2]
IgG4	0.035	0.076	0.120	0.056	0.025	0.028	0.085	0.085
Type-IV								
IgA	-0.079	-0.067	0.032	0.038	-0.014	0.027	0.088	0.084
IgE	-0.030	-0.107	-0.041	-0.093	-0.062	-0.140	-0.072	0.002
IgG1	0.072	-0.057	(-)	(-)	(-)	(-)	(-)	(-)
IgG3	**0.174**[2]	0.150	0.073	0.053	0.020	-0.037	0.088	**0.177**[2]
IgG4	-0.032	-0.035	0.100	-0.031	-0.034	-0.007	0.032	-0.013

[1] $p < 0.01$, [2] $p < 0.05$.

R values for IgG1 responses to the truncated PMs are not shown.

Figure 1. Nara, *et al.*

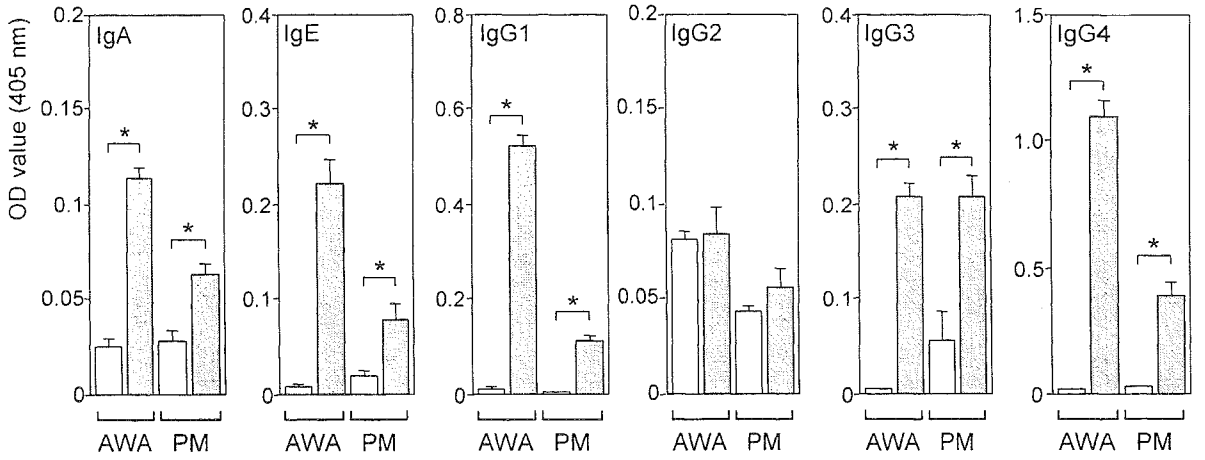


Figure 2. Nara, *et al.*

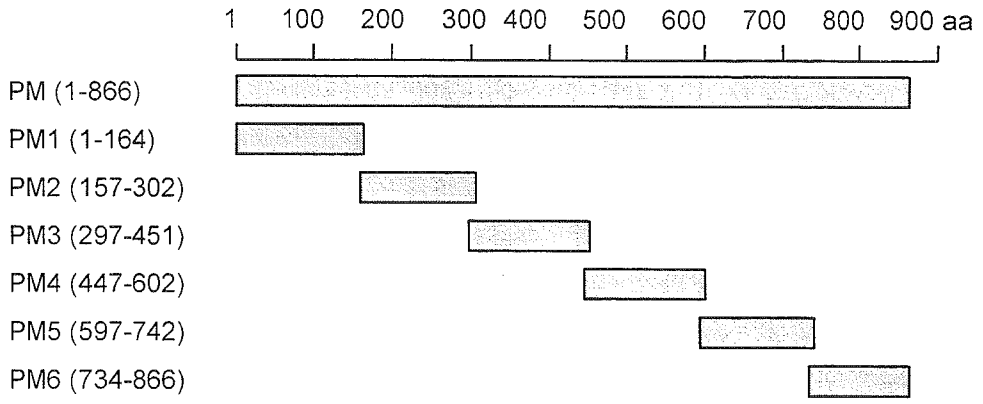
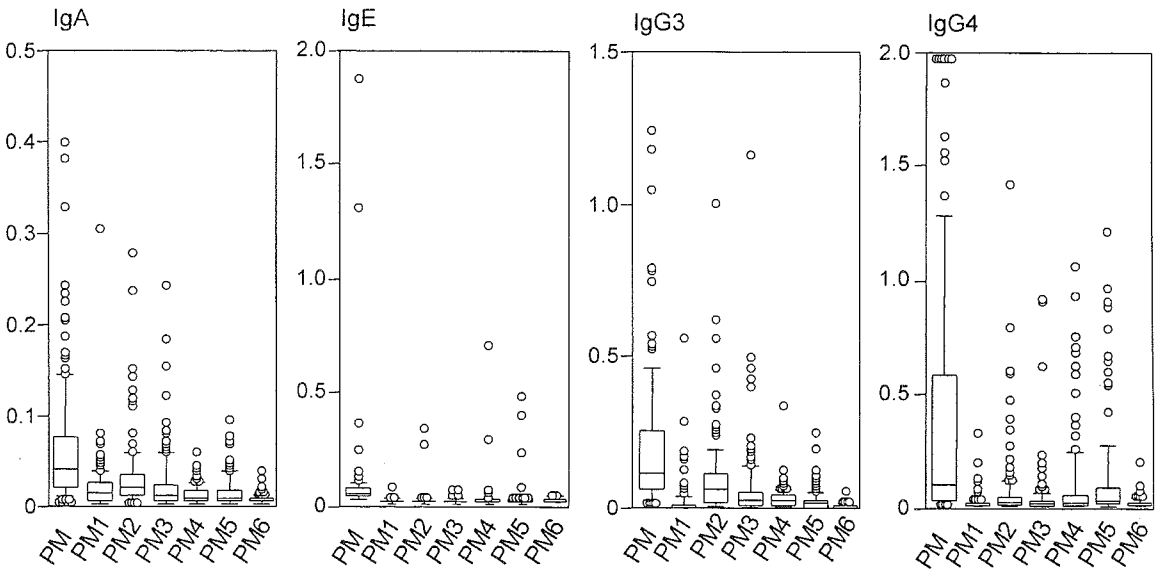


Figure 3. Nara, *et al.*



10. Human Dirofilariasis in Japan

Nobuaki Akao

10.1 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.

10.2 Case reports of dirofilariasis since 1964 in Japan

10.2.1 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 a 40-year-old women, were reported in New Orleans in the United States.

10.2.2 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] reviewed the clinical cases published from 1964 to 1986. A total of 41

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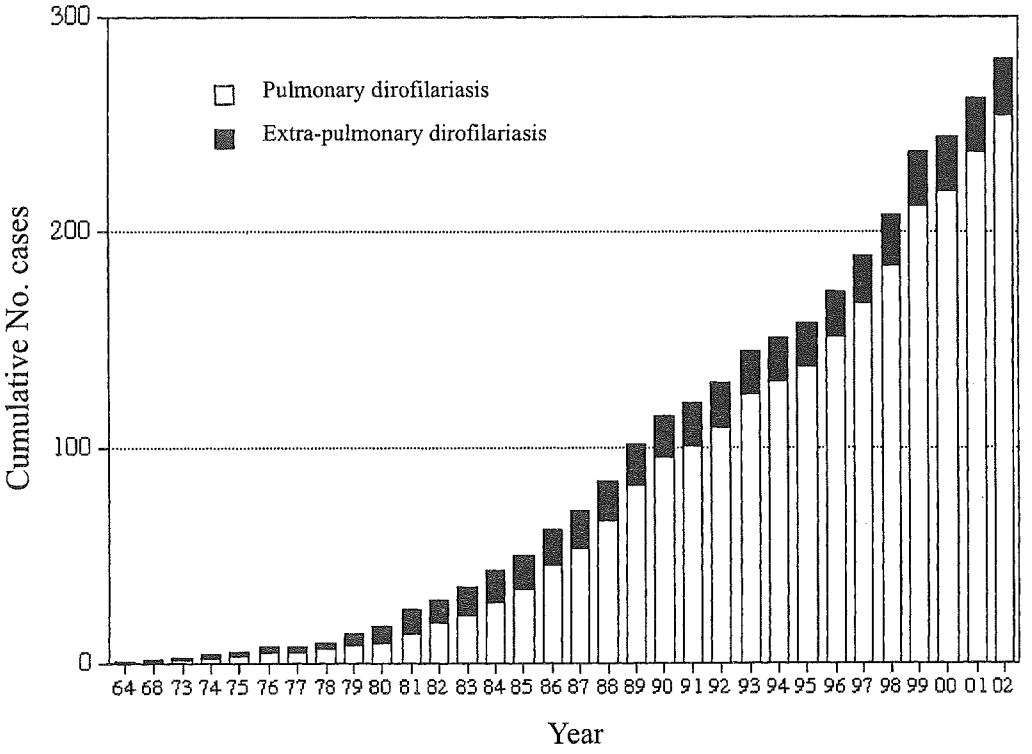

Fig. 1 Cumulative number of cases of human dirofilariasis in Japan from 1964 to 2002.

cases of pulmonary dirofilariasis were reported in this period. The coin lesions were mostly located in the right lower lobe of the affected lungs. They also observed that the most of the patients resided in the southwestern part of Japan but a few were in the northern part of Japan. They suggested that the geographical difference was attributable to the lower prevalence of microfilaremia in dogs with *D. immitis* infection in the northeastern part of Japan relative to the southwestern part, since the cumulative temperature in the northeastern part was insufficient to develop the same number of vector mosquitoes. For this reason, no cases have been reported in Hokkaido thus far, which is located in the northernmost part of Japan and has a far-colder climate than Tokyo.

The overall incidence as compiled from published cases from 1964 to 1995 was recorded by Kagei [10]. According to his report, 103 additional cases of pulmonary dirofilariasis were counted from 1986 to 1995 in Japan. These figures indicated that the patients drastically increased in number, more than doubled in 10 years. Figure 1 shows the cumulative cases of pulmonary dirofilariasis as of the end of 2002, in which the data from 1964 to 1986 and from 1986 to 1995 were quoted from Makiya *et al.* [9] and Kagei [10], respectively. The number of cases continues to increase, and since the study by Kagei [10], a total of 117 cases of pulmonary dirofilariasis have been cited in the database of Japana Centra Revuo Medicina over the last 7 years. In addition, three cases appeared in the Japanese Journal of Clinical Parasitology [11,12] and four more cases were referred to us (Dr. I. Sato, Department of Pathology, Miyagi Prefectural Hospital, personal communication). Consequently, 254 cases of pulmonary dirofilariasis have been recorded as of the end of 2002 (Fig. 1).

Kobayashi *et al.* [13] noted that the maximum diameter of the pulmonary lesions induced by the infarct of the worm was less than 3 cm. Therefore, a coin lesion of more than 3 cm in diameter on a chest X-ray examination should be excluded from the diagnosis of pulmonary dirofilariasis (Fig. 2). Thoracotomy, which is a high-risk procedure, used to be the only option for making a clear diagnosis prior to the 1990's. Fortunately, thoracoscopic

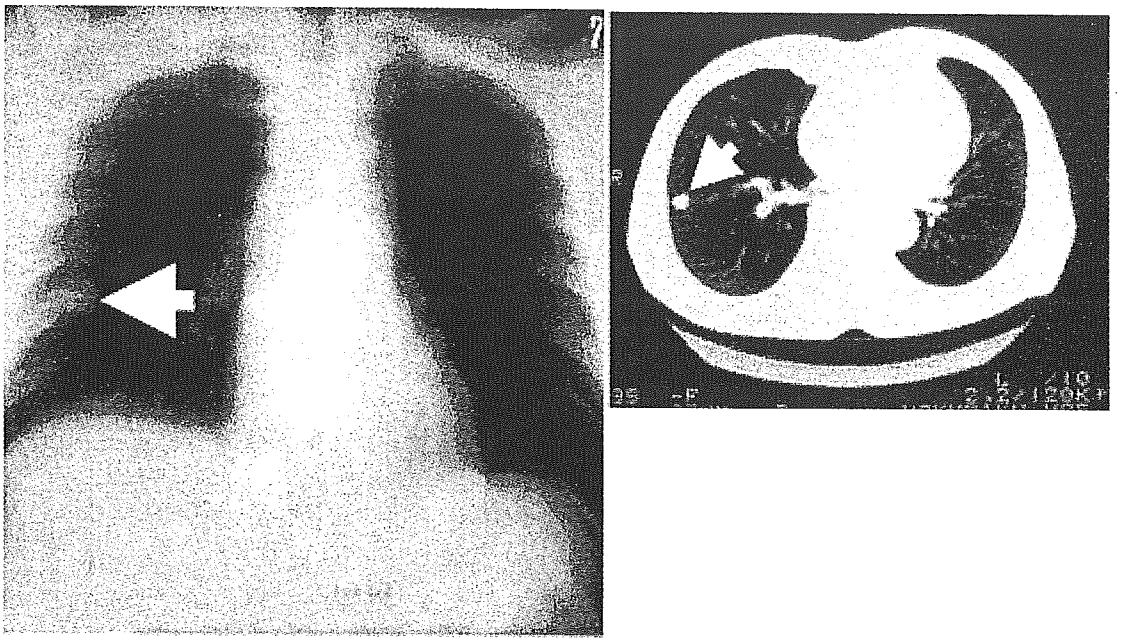

Fig. 2 Chest X-ray (left) and CT (right) appearance of a patient with pulmonary dirofilariasis. A solitary nodule called "coin lesion" is adjacent to the pleural membrane (arrow).

surgery introduced in the early 1990's has been adapted to resect the parasitic nodule provoked by *Dirofilaria* infection. Miura *et al.* [14] performed a thoracoscopic lung biopsy and observed an immature worm of *D. immitis* in the necrotic tissue of a peripheral pulmonary artery of a removed nodule. The patient, a 50-year-old male, was discharged 7 days after the medical treatment from Oita Medical University Hospital without any complications. This technique is now widely accepted as a less-invasive medical procedure and for diagnosing pulmonary dirofilariasis.

10.2.3 Cutaneous dirofilariasis

Nishimura *et al.* [15] reported the first case of cutaneous dirofilariasis in Japan. The patient, a 52-year-old female living in Ibaragi city of Osaka prefecture, was admitted to a hospital with a chief complaint of a left breast nodule of 4 days' duration. A surgical resection of the nodule was performed on 19 January 1961. A thread-like nematode of 50 mm in length and 0.21 mm in width was found in the removed tissue. From the morphological characteristics, they concluded that the worm was identical to a male *D. immitis*. Ten years later, an additional case of cutaneous dirofilariasis was reported by Otsuru *et al.* [8]. The patient, a 68-year-old male, was admitted to the Hospital of Okayama University because of a subcutaneous nodule on his right abdominal wall. Pathological specimens revealed several transverse sections of an immature female worm of *D. immitis*. Since then, 12 cases of cutaneous dirofilariasis have been reported between 1964 and 1986 [9], and nine additional cases were published between 1987 and 2002.

The parasites responsible for cutaneous nodules are thought to be *D. immitis*, except for the case described by MacLean *et al.* [16]. The patient, a 67-year-old male, living in Okinawa prefecture, which is in the southernmost part of Japan, presented with 2 cm (diameter) subcutaneous nodule which had appeared on his left anterior chest wall. The nodule was surgically removed, and pathological examination revealed several transverse sections of a worm, which was identified as *Dirofilaria repens* based on its morphological characteristics.

10.2.4 Visceral dirofilariasis

A developing immature *D. immitis* worm is occasionally found in deep inner organs, such as

the liver, uterus, and abdominal cavity. Tada *et al.* [17] reported a case of visceral dirofilariasis following a death due to bleeding in the abdominal cavity resulting from liver cirrhosis. A tumor-like mass was found embedded in the adipose tissue of the mesentery. At the central region of the nodule, they found several fragments of a female worm of *Dirofilaria* sp., probably *D. immitis*. In 1980, an additional case of extra-pulmonary dirofilariasis was found in a 74-year-old female, residing in Toyama city, in Toyama prefecture [18]. She was admitted to the Toyama Medical and Pharmaceutical University Hospital because of uterine bleeding over the past 1 year. A hysterectomy was performed and an endometrial polyp measuring 2.0 x 1.5 x 1.0 cm was seen in the rear right wall of her uterus, in which a nematode parasite was revealed by a histopathological examination. The parasite, measuring 150 to 160 μm in diameter, showed the typical appearance of a male *D. immitis*. Miyakawa *et al.* [19] reported a case of accidental identification of several transverse or oblique sections of *Dirofilaria* sp. in the liver of a 58-year-old female with colon cancer.

10.2.5 Ophthalmic dirofilariasis

The *Dirofilaria* worm has also been implicated in certain ophthalmic infections. According to the review of Kagei [10], six cases of ophthalmic dirofilariasis have been reported so far: two cases of orbital tumor, two of neuroretinitis, one of peripheral proliferative vasculitis of the fundus, and one of an eyelid legion. However, the last case did not precisely constitute ophthalmic dirofilariasis since the parasite was recovered from subcutaneous tissue from the eyelid. Moreover, there

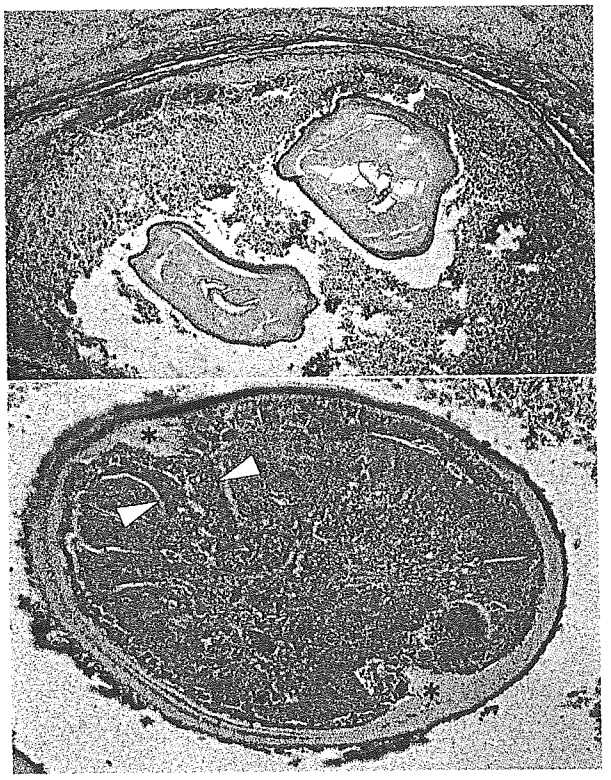

Fig. 3 Histopathologic findings of the nodule. Two transverse sections of an immature worm of *D. immtis* are seen in a small pulmonary artery (upper, Elastica van Gieson stain), and a transverse section of an immature adult worm showing large lateral chords (arrow head) with internal longitudinal ridges (*) and multilayered cuticle (bottom, HE stain).

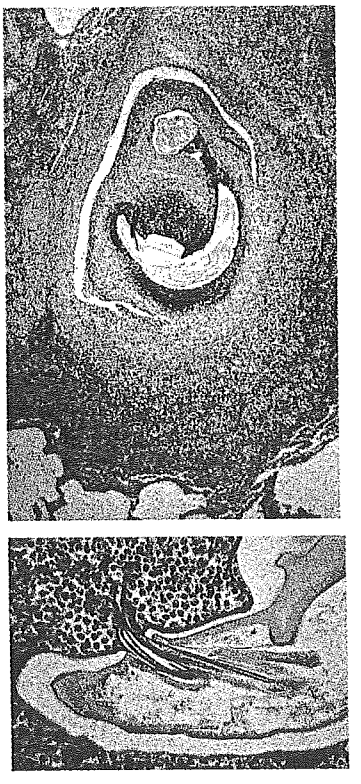

Fig. 4 Low-power view of a pulmonary infarct containing a transverse section and a longitudinal section of a mature male *D. immitis* (upper). Two spicules are clearly observable (bottom).

is no apparent evidence that the *Dirofilaria* worm is responsible for the eye pathologies in the remaining cases. The patients were suspected of having the parasitic infection based not on the pathological findings but on the clinical and serological examinations; otherwise, the authors only stated that the patient had a parasite without any evidential presentation of photographs. Therefore, it is uncertain whether these patients were frank cases of ophthalmic dirofilariasis in Japan, despite a number of cases that have appeared in the foreign literature [20,21]. In conclusion, the number of extra-pulmonary dirofilariasis cases in Japan was estimated to be 26 as of the end of 2002 (Fig. 1).

10.3 Diagnostics

10.3.1 Diagnostic morphology of zoonotic filariasis

Gutierrez [22] described the diagnostic features of zoonotic filariae in tissue sections. A review article written by Chitwood and Lichtenfels [23] also mentioned the morphological characteristics of Filaridae. Both reviews are useful for pathologists to distinguish each filarial worm from the others in pathological specimens. In Japan, Uni *et al.* [24] studied the comparative morphology of *D. urusi* and *D. immitis* in cross-sections. Yoshimura and Akao [25] investigated the cross-sectional morphology of human and zoonotic filarial worms that were found in human tissues (Figs. 3 and 4). These studies have contributed to the identification of filarial infections, including an imported case of onchocerciasis and a case of zoonotic onchocerciasis, among the Japanese [1,26].

Nagano *et al.* [27] attempted to detect the genomic DNA of *D. immitis* by polymerase chain reaction (PCR). This is a promising tool for identifying necrotizing parasites that do not show normal structures.

10.3.2 Serological investigations

Serology is an alternative method of diagnosing parasitic infections because the invading parasite cannot always be identified by pathological examination of resected tissues. Therefore, many attempts have been made to detect a specific antibody against filarial proteins. At first, filarial antigen derived from *D. immitis* was studied to diagnose bancroftian filariasis in Japan. Ishizaki *et al.* [28] prepared a defatted somatic antigen of adult *D. immitis* and adapted it to the epidemiological survey of bancroftian filariasis in an endemic area of Ehime prefecture as an intradermal test. Of 54 patients with microfilaremia, 44 showed a positive reaction and the remainder were negative, indicating that the sensitivity was unsatisfactory for a field survey. Tada and Kawashima [29] demonstrated the usefulness of a purified antigen derived from adult *D. immitis* for an intradermal skin test against bancroftian filariasis. This antigen showed extremely low cross-reactivity against the sera from eight other parasitic infections and did not show nonspecific reaction in patients with allergic diseases. Sawada and his colleagues studied the antigenic nature of a purified *D. immitis* antigen, FST, and its derivatives [30-32]. Although all these antigens were prepared for use in an intradermal test of bancroftian filariasis, they had a potential diagnostic benefit for human dirofilariasis.

The first step in making a serodiagnosis of human dirofilariasis in Japan was achieved by Tamaoki *et al.* [33], who performed several immunological tests, intradermal skin test, agar-gel diffusion, and immunoelectrophoretic analysis, that lead to a preoperative diagnosis. Sato *et al.* [34] introduced an enzyme-linked immunosorbent assay (ELISA) for the diagnosis and follow-up study of dirofilariasis. The antigen they used included a veronal-buffered saline extract of adult worms of *D. immitis* to detect specific IgG antibody. The antibody was demonstrated in the patient's serum preoperatively, but the serum also reacted with the antigen derived from adult worms of *Ascaris suum*. After operation, the IgG responses to both antigens decreased gradually, with more prominent reduction of *Ascaris* antibody. The ELISA could be useful for the post-operative follow-up in human dirofilariasis. Around the same time in the United States, Glickman *et al.* [35] demonstrated that an antibody to somatic antigen of adult *D. immitis* was detectable by indirect hemagglutination test and ELISA in eight patients with radiologically evident pulmonary nodules in whom the final diagnosis was confirmed pathologically as *Dirofilaria* sp. infection. A mixed passive hemagglutination test was also attempted to detect the IgG antibody [36].

Akao *et al.* [37] demonstrated that the

excretory-secretory (ES) products of female worms of *D. immitis* provided a more sensitive antigen than the adult somatic antigen by using an immunoblot analysis. They also suggested that a low molecular component of ES products strongly cross-reacted with the sera from non-filarial patients, and that adult somatic antigen shared this antigenic component. Nakagaki *et al.* [38] observed that, using an ELISA, the sensitivity of ES antigen was less than 50%, but periodate-treated ES (PI) antigen was superior to that of ES antigen. They also noted that not only phosphate buffer extracted antigen but also ES and PI antigens highly cross-reacted to the sera of patients with loasis, tropical eosinophila, and gnathostomiasis, suggesting that it was extremely difficult to diagnose human pulmonary dirofilariasis by ELISA. Sun and Sugane [39] isolated an immunodominant antigen of *D. immitis* from genomic DNA and established a recombinant DNA-derived fusion protein for ELISA. However, there is no report on the practical application of this antigen for human dirofilariasis to date. In conclusion, the reliability of serological tests is still questionable and further investigations are needed to identify a more specific antigen suitable for immunodiagnostics.

10.4 Animal models for human dirofilariasis

To understand the pathophysiology and to improve the serodiagnosis of dirofilariasis in humans, several animal models have been investigated. Experimental infections with fifth-stage larvae molting in the dog were successful in rabbits, rats, and guinea pigs, while infections with third-stage larvae molting in vector mosquitoes were only successful in dogs and ferrets [40,41]. Nakagaki *et al.* [42] observed that the subcutaneous transplantation of these juvenile *D. immitis* migrated into lung arteries, resulting in pulmonary hemorrhagic infarction. They noticed that the pathological findings of the lung closely resembled the lesions of human pulmonary dirofilariasis. They are also studying the immune response of experimentally infected rabbits to develop a more precise diagnosis of human dirofilariasis (Dr. K. Nakagaki, personal communication).

10.5 Investigations of vector mosquitoes

In Japan, at least 16 species of mosquitoes are thought to play a role as a vector of *D. immitis*. Of these, *Culex pipiens pallens* and *Cx. tritaeniorhynchus* are the major species and are distributed nationwide. A detailed distribution of these vector mosquitoes and the prevalence of the infection in dogs have been described in a review article by Kagei [10].

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6 Critical Assessment of Existing and Novel Model Systems of Toxocariasis

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Introduction

Toxocariasis is a disease caused by the larval stage of *Toxocara* sp., and predominantly involves *Toxocara canis* and *T. cati*. The infectious stage larvae, which develop in the egg within 2 weeks after their excretion to the surrounding environment and mature 4 weeks after excretion, can migrate through the entire body of either a definitive or paratenic host. In paratenic hosts including human beings, the larvae cause tissue damage as either a direct or indirect effect of their presence. For example, some level of visual impairment may occur when larvae in the retina pass over the macular region, and neurologic disturbance may appear when larvae reside in the brain for a long period of time. In addition, larvae in the retina may elicit an inflammatory response resulting in serious ocular disease such as chorioretinitis or uvitis. These medical problems have been well known since 1952, when Beaver *et al.* (1952) proposed a disease syndrome characterized by chronic eosinophilia with granulomatous lesions in the liver, as reported in three young children. Since then, much effort has been invested in understanding the pathogenesis of this parasite using animal models.

Toxocariasis in Humans

Toxocariasis is clinically divided into four types of diseases: visceral, ocular, neurologic and covert types (Taylor *et al.*, 1987; Glickman and Magnaval, 1993). Visceral toxocariasis is associated with the migratory behaviour of the larvae in the early stage, in which they penetrate the intestinal wall, reach the liver, and then the lung, from where they are then distributed throughout the entire body of the host. Ocular toxocariasis is a specific form of the visceral type. This syndrome is not always accompanied by a systemic disorder, but is the type in which the disseminated larvae emerge in the retina. In some patients, a full-body or a part of a larva has been recovered from the vitreous fluid after vitrectomy (Maguire *et al.*, 1990). However, it is still unclear just how the larvae enter or the time course for this invasion after infection. Regarding neurological involvement, some previous studies have shown that neurological defects or epilepsy may be associated with *Toxocara* infection. Children who have a history of epilepsy showed a statistically significant increase in antibody against *Toxocara* antigens. Additionally, meningoencephalitis with eosinophilia and increased antibody in the cerebrospinal

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fluid is another clinical manifestation of the disease. These findings are common in neurologic toxocariasis. In contrast, the concept of covert toxocariasis is less well established. In the Midi-Pyrenees of France and Ireland, patients who had relatively non-specific symptomatology including fatigue, abdominal pain, nausea, fever, lymphoadenopathy, etc., with or without accompanying moderate eosinophilia, showed positive results for an anti-*Toxocara* antibody test (Glickman *et al.*, 1987; Taylor *et al.*, 1988). It is increasingly accepted that *Toxocara* infection could account for this syndrome. Although the variety of symptoms in human cases is a characteristic feature of the infection, our knowledge about *Toxocara* pathogenesis is fairly limited. For this reason, *Toxocara* infection has long held the attention of both parasitologists and immunologists.

Experimental toxocariasis: Existing animal models

Mice

Both inbred and outbred strains of mice are commonly used in studies of infectious disease. An outbred strain was first used in a study of the migratory behaviour of *Toxocara* larvae in 1952 (Sprent, 1952), soon after Beaver *et al.* (1952) introduced the notion of 'visceral larva migrans' by *T. canis*. Since then, many attempts were made to clarify the distribution pattern after oral administration of embryonated eggs. Embryonated eggs hatch in the upper gastrointestinal tract and then the infectious stage larvae penetrate the enteric mucosal membrane. Most of the larvae remain there until 6 h after infection, and migrate to the liver by way of the portal vein. They then remain in the liver for some time before migrating to the lung. Typically, the larvae migrate to the lung and heart; however, with repeated infection or presensitization treatment with *Toxocara* antigen, the larvae accumulate in the liver in both outbred and inbred mice. These findings, along with the fact that trapping of the larvae in the liver does not occur in congenitally athymic mice, suggest that the host immune response plays an important role in this phenomenon (Sugane and Oshima, 1983; Concepcion and Barriga, 1985; Parsons and Grieve, 1990). Thus, the mouse is a useful model for determining why the parasite is so often found in biopsy specimens of the human liver.

In general, different strains of mice show different larval distribution patterns and pathophysiological courses (Koizumi and Hayakawa, 1984). Among the inbred mice strains, BALB/c mice, but not C57BL/6 mice, are the best suited for investigations of a possible connection between allergic asthma and *Toxocara* infection (Pinelli *et al.*, 2001).

When they leave the lung, the larvae enter the systemic circulation, from which they reach the skeletal muscles and central nerve system. Interestingly, *Toxocara* larvae tend to accumulate in brain tissue and can remain alive and motile for years, resulting in behavioural changes in affected mice (Summers *et al.*, 1983; Holland and Cox, 2001). These mice also show a reduced ability in maze learning. However, little information is available on the relationship between the site of the larvae in the brain and behavioural changes in the host (Donovick and Burright, 1987; Cox and Holland, 1998). Additionally, there is no correlative evidence regarding the site where the larva was detected and a possible clinical syndrome in these mice. In fact, these findings suggest that mice are not a suitable model for neurologic toxocariasis. In spite of having the same MHC haplotype background, BALB/c and DBA mice reacted quite differently in terms of their allergic inflammation in the brain, indicating that the host response to an infection is not dictated by MHC haplotype alone (Epe *et al.*, 1994).

Studies of ocular toxocariasis have also been conducted with outbred mice (Olson, 1976; Rockey *et al.*, 1979; Ghafoor *et al.*, 1984). After oral administration of eggs, mouse eyeballs were crushed and observed microscopically. *Toxocara* larvae were observed and inflammatory changes were confirmed histologically, but the incidence was very low. Thus, the use of a mouse model for ocular toxocariasis is not recommended, since it is time-consuming to determine the migration route of the larvae to the retina and the pathogenesis of the larvae, even though useful information has been obtained from some experiments using mice.

The influence of maternal infection on offspring has been the subject of study with murine toxocariasis. In mice infected during pregnancy, larvae were found in the uterus, placenta and foetus (Lee *et al.*, 1976), and there was a predictable decrease in litter size in female mice with

Toxocara infection (Akao *et al.*, 1990; Reiterova *et al.*, 2003).

Numerous immunological and immunopathological studies of *Toxocara* infection in mice have also been performed in the last two decades. Among them, larval trapping in the liver of presensitized hosts is an interesting phenomenon (Sugane and Oshima, 1983; Concepcion and Barriga, 1985; Kayes, 1997). This event might remind us why *Toxocara* larvae are frequently observed in the liver of human visceral toxocariasis. Eosinophilic granuloma formation in the liver was found to be regulated by the host TH1/TH2 response, and eosinophils play an essential role in the pathology of infected C57BL/6 mice (Takamoto *et al.*, 1997). However, eosinophils do not play a significant role in the expulsion and killing of *T. canis* larvae in infected mice (Sugane *et al.*, 1996). Furthermore, the presence of IgE antibody to excretory-secretory products of *T. canis* has been monitored during infection, and allergic asthma in murine models has been studied (Buijs *et al.*, 1994; Dent *et al.*, 1997).

To interpret the findings from these experimental studies, it is very important to know the precise count and administration method of the eggs in each experiment. In this context, the work done by Oshima (1961), was an important advance in this field. Oshima described a standard method for the oral inoculation of eggs and specified that all equipment used in their preparation should be siliconized and that the albuminoid coat of the egg should be removed. It is also important that the number of eggs be counted in a statistically valid manner so that this and other techniques, taken together, will ensure reproducible results.

In conclusion, while mice provide a very informative model for studying the contribution of genetic diversity to *Toxocara* infection and the distribution of larvae after infection, the mouse model cannot provide a complete understanding of all aspects of *Toxocara* infection.

Rats

The utility of the rat model is similar to that of the mouse model; however, the reports on experimental toxocariasis of rats are limited. The pattern of migration of larvae in rats is similar to that in mice (Lescano *et al.*, 2004) and in one study, rats infected with *T. canis* showed a decline in learning ability of maze (Olson and Rose, 1966). Rats infected with *Toxocara* have also been used to demonstrate eosinophilic chemotactic activity in bronchoalveolar lavage fluid and eosinophils-mediated cardiomyopathy (Fujimoto *et al.*, 1990; Schaffer *et al.*, 1992; Okada *et al.*, 1996). Ocular infections have also been reported in infected rats (Burren, 1972), but occurred less commonly than in mice.

Guinea pigs

In allergic asthmatic children, a high prevalence of antibody to *Toxocara* antigens has been reported worldwide (Oteifa *et al.*, 1998). To better understand the factors involving the onset of this disease, guinea pigs are frequently used due to their high responsiveness of bronchial refraction to antigen (Buijs *et al.*, 1995). Collins and Ivey (1975) reported that IgE antibody in infected guinea pigs was evident using homologous passive cutaneous anaphylaxis tests. Ocular inflammation was induced by intravitreal infection (Rockey *et al.*, 1979); however, guinea pigs are considered to be an inappropriate model for the study of ocular toxocariasis due to their atypical immune response (Ghafoor *et al.*, 1984; Fenoy *et al.*, 2001).

Hamsters

Very little information is available on toxocariasis in the hamster (Burren, 1972). Since hamsters are frequently used to investigate airway hyper responsiveness or inflammation to foreign materials, it would be helpful to understand their possible allergic response to *Toxocara* infection.

Rabbits

Since, with rabbits, blood samples can easily be taken once or twice a week, they have frequently been used to investigate the time course of antibody production during infection. Specific IgG antibody against excretory-secretory antigens of *T. canis* was first detected in the serum after the

5th day of infection and reached its peak at 2 weeks post-infection. Thereafter, the level of antibodies remained high for a long period of time (Fernando, 1968; Kondo *et al.*, 1981; Smith *et al.*, 1982). By contrast, eosinophil counts in the peripheral blood reached their peak at 4 weeks after infection, and decreased gradually to the normal level after 10 weeks of infection. Immunoblot analysis has also been performed in rabbits to examine changes in the antigen recognition in infected rabbits and to identify the specific antigen moieties in larval excretory-secretory products (Akao *et al.*, 1982).

Primates

The genetic homology between human beings and primates has made the primate model of toxocariasis an attractive option for studies of the pathogenesis of toxocariasis (Fernando *et al.*, 1970; Fernando and Soulsby, 1974; Tomimura *et al.*, 1976; van Knapen *et al.*, 1982). In the cynomolgus macaque, *Macaca fascicularis*, the haematologic and serologic changes were similar to those observed in children with VLM, and some individuals (three out of 16 macaques) developed neurologic signs such as ataxia and nystagmus (Glickman and Summers, 1983). Despite intensive studies using oral inoculation of eggs, intraocular lesions associated with larval migration have not been observed, although intraocular inoculation with larvae did cause inflammatory changes. Histopathologically, *Toxocara* larvae can survive for at least 10 years after infection in rhesus monkeys (Beaver, 1969).

Despite these advantages over other animals, primates tend to be nervous and difficult to handle for experimental purposes. Moreover, studies using primates are much more expensive and controversial than those using other animals.

Chickens, Pigs and other mammals

Visceral toxocariasis was thought to be a disease affecting younger children who accidentally ingested *Toxocara* eggs, even though ocular toxocariasis can occur in older children or in individuals of any age (Glickman and Magnaval, 1993). In 1989, a new infection route of toxocariasis was reported (Nagakura *et al.*, 1989). Twin brothers, aged 21 years, were admitted to the hospital due to fever, nausea and myalgia with urticaria of both lower legs. They had eaten raw chicken liver and meat 12 days before admission. Eosinophilia, elevation of total IgE and *T. canis* specific IgG antibodies were confirmed by a laboratory examination. In another case, a 26-year-old woman presented to the hospital complaining of fever, headache and a dry cough. Laboratory examination revealed eosinophilia, elevated concentration of IgE and positive for *T. canis* specific IgG. A *Toxocara* larva was detected in a small brown itchy nodule on her left ankle (Aragane *et al.*, 1999). Before the onset, the patient had a history of eating raw beef liver. Similar cases have been reported from Switzerland (Sturchler *et al.*, 1990), North America (Salem and Schantz, 1992) and Spain (Espana *et al.*, 1993). In addition, experimental studies revealed that *Toxocara* larvae tend to accumulate in the liver of chicken (Taira *et al.*, 2003) and quail (Pahari and Sasmal, 1990; Maruyama *et al.*, 1994). We assure, therefore, that table fowls play an important role in the transmission of toxocariasis.

In a pig model, Taira *et al.* (2003, 2004) demonstrated that no clinical signs developed in infected pigs, although most of the larvae were recovered from the lungs and there were numerous white spots in the liver due to the continuous migration of the larvae. Although few in number, the larvae were detected in various organs and tissue. Therefore, they suggested that the experimental infection of pigs may be a useful model of covert toxocariasis in humans (Taira *et al.*, 2004). Furthermore, Helwigh *et al.* (1999) stated that the pig was a useful non-primate model for human visceral larva migrans, since *T. canis* larvae migrated well and induced a strong immunological response in the pig.

New Model for Human Toxocariasis: Mongolian gerbils, *Meriones unguiculatus*

Mongolian gerbils are known to be susceptible to a variety of parasites, including *Brugia pahangi*, *Strongylus stercoralis*, *Nippostorongylus brasiliensis* and *Entamoeba hystolytica* (Horii *et al.*, 1993; Nolan *et al.*, 1993; Campbell and Chadee, 1997). However,

with the exception of the study of Burren (1972), no studies have evaluated the usefulness of the Mongolian gerbil as an animal model of toxocariasis. Unfortunately, since Burren was unable to detect larvae in the ocular chamber, he concluded that the Mongolian gerbil was an unsuitable animal model for ocular toxocariasis, and since then, no similar report has been published on this species. Several species of animals, including mice, rabbits, guinea pigs and monkeys, have been evaluated pathologically; however, the incidence of ocular infection is low and eosinophilic infiltration is rarely observed through oral inoculation.

In 1998, Mongolian gerbils assumed a more important role in *Toxocara* and toxocariasis research when they were found to have a high susceptibility to ocular infection not only by *T. canis* (Takayanagi *et al.*, 1998; Takayanagi *et al.*, 1999); but also by *T. cati* (Akao *et al.*, 2000). After oral inoculation of eggs (approximately 1000 eggs/gerbil), the retinas of gerbils were observed with an ophthalmoscope, which was specifically adapted for observing the fundi of small animals. This new tool provided valuable insight into the pathogenesis of *Toxocara* infection.

Ocular toxocariasis in Mongolian gerbils

A motile larva was clearly observed in the retina as early as 3 days after infection in Mongolian gerbils, and the incidence of retinal involvement was at least 80% in infected gerbils. A maximum of three migrating larvae was seen in one eye at the same time, and on rare occasions, migrating larvae were found bilaterally. Once a larva appeared in the eye, it was present until the end of the observation period, 158 days after inoculation.

Haemorrhagic lesions and exudative lesions with or without migrating larvae were consistently found in gerbils after 3 days of infection. Figure 6.1 shows typical ophthalmoscopic findings. In hemorrhagic lesions, four different types of changes:

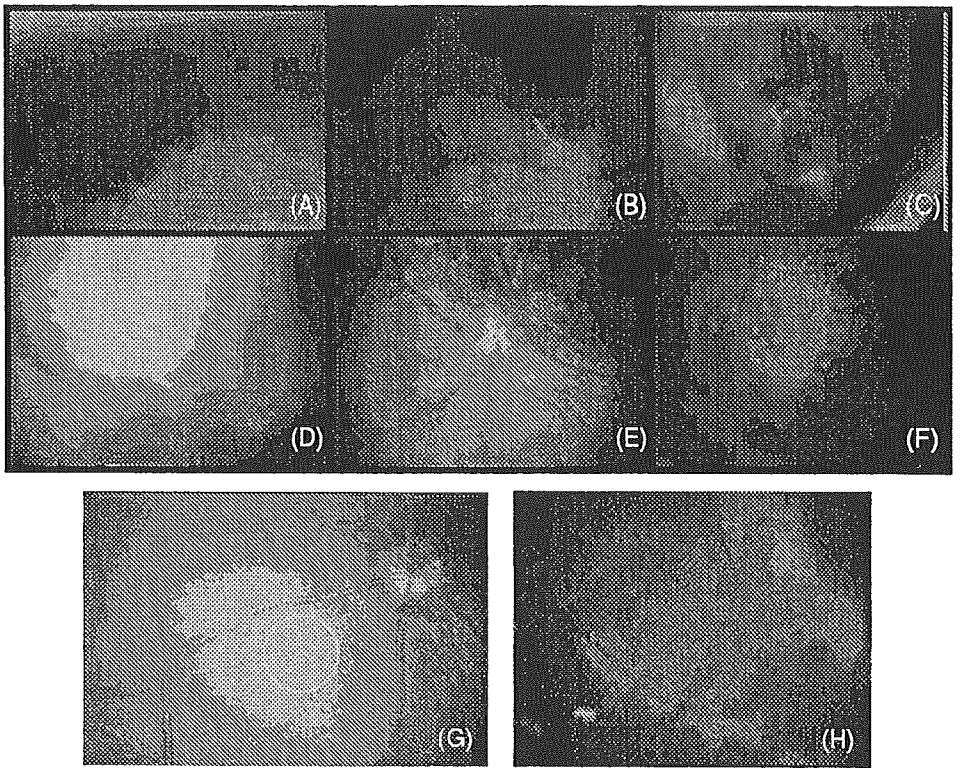

Fig. 6.1. Haemorrhagic changes of the retinas in Mongolian gerbils infected with *Toxocara canis*. (A) Deep seated retinal haemorrhage. (B) Superficial retinal haemorrhage. (C) Three deep seated haemorrhages in the peripheral region. (D) Optic papilla is covered with a large vitreous haemorrhage. (E) Optic papilla is covered with a superficial haemorrhage. (F) White centred small retinal haemorrhages. (G) A motile larva on the retina. (H) White exudative lesions around vessels.

vitreous haemorrhage, superficial retinal haemorrhage, deeply seated retinal haemorrhage and white centred small retinal haemorrhage, were seen in the fundi. Histhopathologically, haemorrhagic lesions and proliferative changes of the retina were observed (Fig. 6.2). White exudative lesions around the vessel walls suggest vasculitis consisting of eosinophils and lymphocyte infiltration. Table 6.1 shows the results of ophthalmoscopic observations and the incidence of lesions in 46 gerbils. Migrating larvae just beneath the retina often left bright, whitish-yellow restiform traces on the retina. A large vitreous haemorrhage was absorbed within 7 days and left behind small, brilliant, yellowish particles. Fortunately, the dark-grey fundi of the gerbils made it easy to detect the motile white larvae of *T. canis* on the retina (Fig. 6.1). In contrast, ophthalmologic changes are difficult to detect in BALB/c mice, while their albino fundi made the larvae difficult to identify. Figure 6.3 shows the predilection sites of the haemorrhagic lesions that consist of large (larger than one optic disk diameter) and small (smaller than one optic disk diameter) sizes. There was no significant difference in the incidence of lesions between the right eyes and left eyes, but the lesions appeared to emerge more in the peripheral region than in the central region, and more in the horizontal region than in the vertical region.

A variety of lesions were found in gerbil eyes after infection; however, no eosinophilic

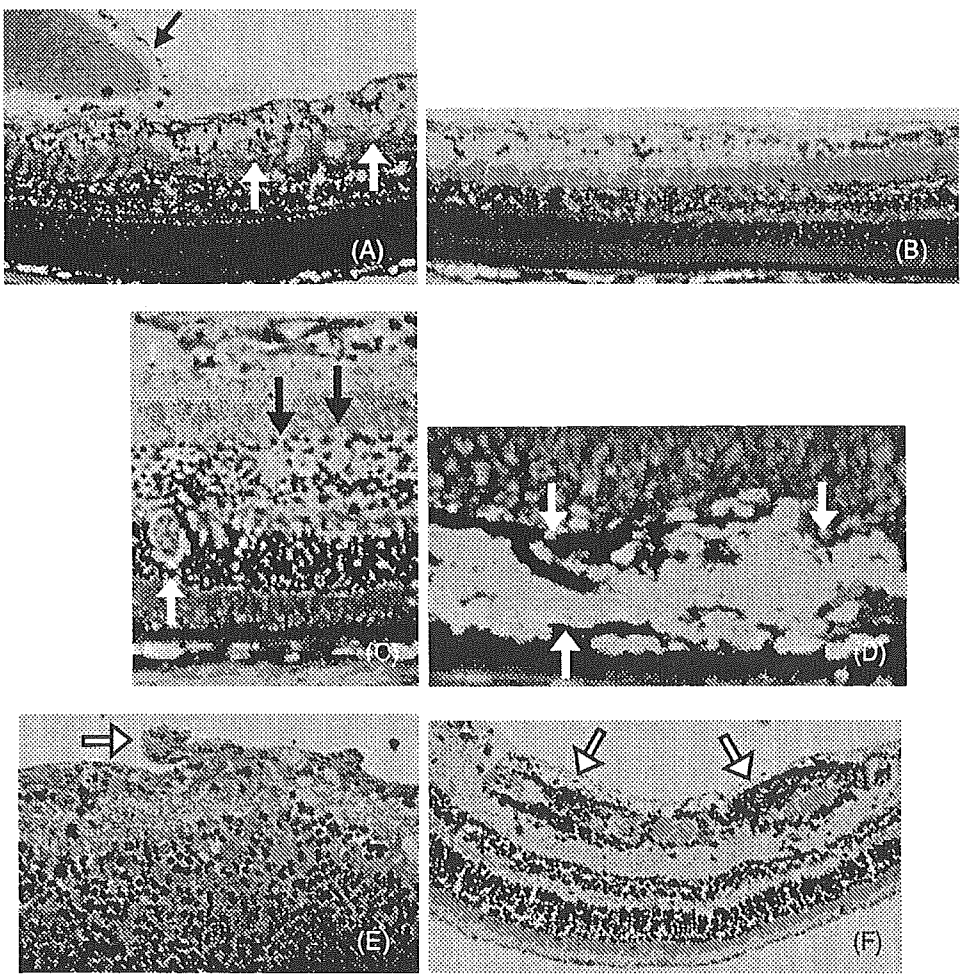

Fig. 6.2. Histopathological changes of the retinas in Mongolian gerbils infected with *Toxocara canis*. (A) Vitreous (white arrows) and superficial (black arrow) haemorrhages. (B) Diffused superficial haemorrhage. (C) Haemorrhage of outer nuclear layer (black arrows) and a transverse section of *T. canis* larva (white arrow). (D) Haemorrhage in the pigment epithelium (white arrows). (E) Proliferative change (arrow) of nerve fibre layer (F) Vasculitis with lymphocyte and eosinophil infiltration (arrows).

Table 6.1. Ophthalmoscopic characteristics and the frequency of incidence of lesions in gerbils infected with *T oxocara canis**.

	Within 7 days	Until 35 days
Larvae	30 (65)	37 (80)
Vitreous haem.	1 (2)	5 (11)
Superficial retinal haem.	19 (41)	27 (59)
Deeply-seated retinal hae	33 (72)	41 (89)
Exudative lesions	21 (46)	37 (80)
Vasculitis	3 (7)	25 (54)

*46 infected gerbils were observed. Parenthesis indicates% of the affected gerbils.

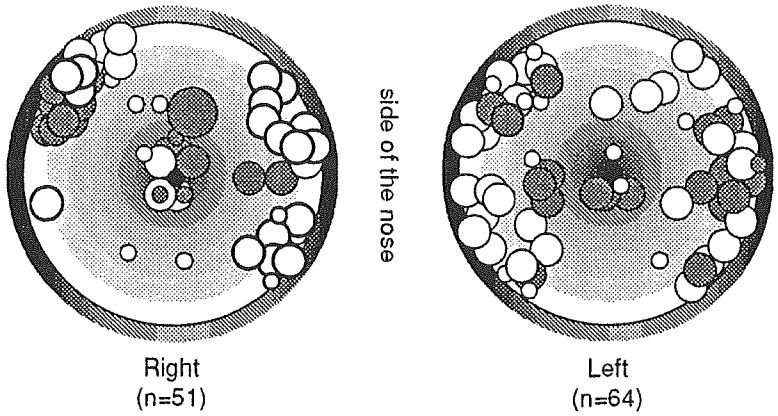

Fig. 6.3. Predilection sites of haemorrhagic lesions in infected gerbils. White circles indicate deep-seated haemorrhage and black circles indicate superficial retinal haemorrhage. The size of circle represents a diameter of the lesion when the lesion is smaller than that of one optic disk (white circle) and larger (black circle).

granuloma, which is the most frequent finding with human ocular toxocariasis, was observed either ophthalmoscopically or histopathologically. Alba-Hurtado *et al.* (2000) examined gerbil eyes histopathologically after oral inoculation of eggs and found granulomatous lesions in the retina 60 days after infection, which was their last day of observation. This finding is in marked contrast to our own. We found that, once the larvae entered the eye, they survived and were observable under an ophthalmoscope for at least 158 days post-infection. Thus, we suggest that granulomatous lesion would not occur as long as the larvae are motile.

It has been hypothesized that the migration of larvae to the eye occurs via the following routes: (i) through the arteries from the internal carotid artery to the ophthalmic artery, retinal central artery, or ciliary artery; (ii) through the brain to the optic nerve; and (iii) through the brain to the cerebrospinal fluid space, and then to the choroids. We observed a larva that emerged from the edge of the ora serrata. Additionally, since choroidal haemorrhage was the most frequent observation in the early ocular findings and was often observed simultaneously with motile larvae, the third route is the most likely to be used. To assess the possibility of the second route of migration, we tested whether larvae could arrive in the eye via the optic nerve if motile larvae were directly inoculated into the brain. Approximately 300 larvae that were maintained aseptically in a culture medium were inoculated intracranially through the cranial bone using a 23-gauge needle (Hayashi *et al.*, 2003). From 6 days after inoculation, either vitreous or choroidal haemorrhages were found in the gerbils by ophthalmoscopy. These lesions were sometimes accompanied by a larva. Pathological examination confirmed that larvae were migrating in the optic nerve of the gerbils 6 days after inoculation and two larvae were found in the optic chiasma (Fig. 6.4). These results clearly indicated that *Toxocara* larvae are able to migrate from the