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Human Dirofilariasis in Japan

Nobuaki Akao

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 a 40-year-old women, 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 infraction 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 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 di-

Section of Enviroumental Parasitologyi Graduate School of 'Tokyo Medical and Dental Universityi Yiishima 1-5-45, Bunkyo-ku, Tbkyo, 113-8519 Japan. E-mail: ochavip@tmd.acjp

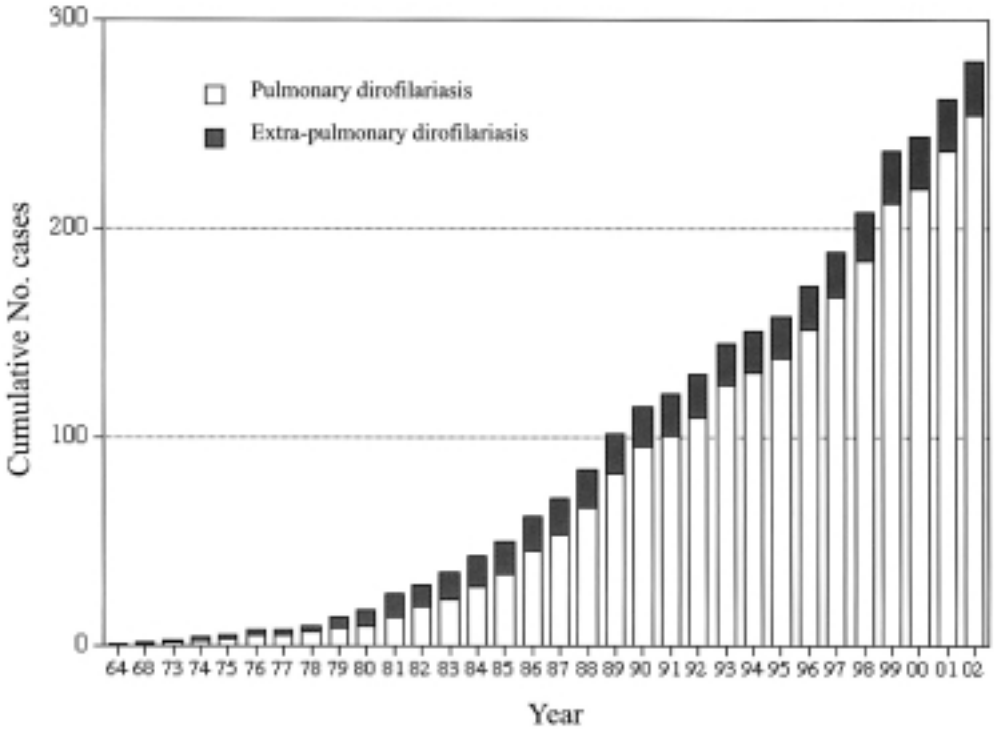

Fig. 1 Cumulative number of cases of human dirofilariasis in Japan from 1964 to 2002.

rofilariasis 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 Hosphal, 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 surgery introduced in the early 1990's has been adapted to resect the parasitic nodule provoked by *Diroflaria* 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.

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.

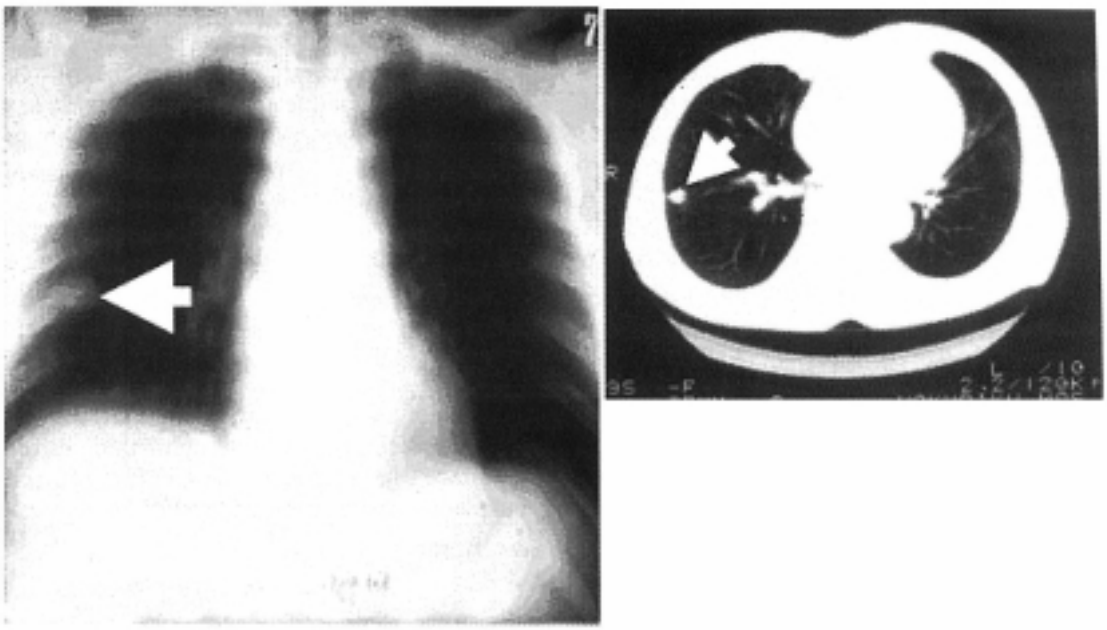

Fig. 2 Chest X-ray (left) and CT (right) appearance of a patient with pulmonary dirofilariasis. A solitary nodule called "coin lesion" is adjacent to the pleural membrane (arrow).

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.

2.4 Visceral diroflariasis

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 l.0cm 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.

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 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).

3 Diagnostics

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.

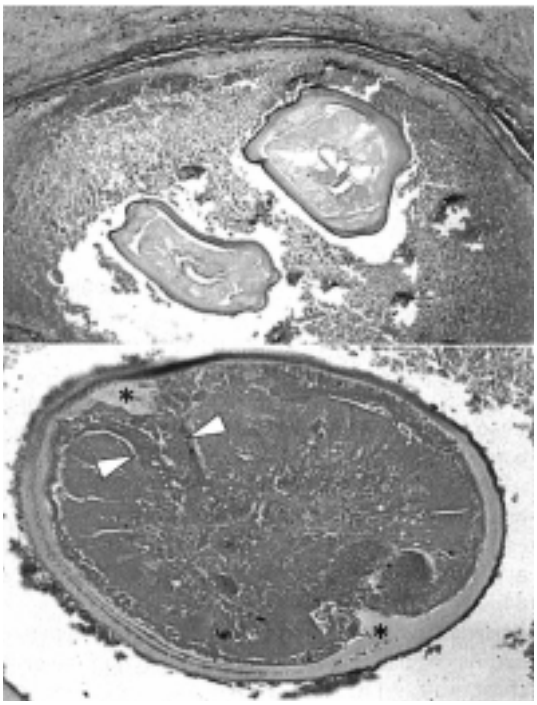

Fig. 3 Histopathologic findings of the nodule. Two transverse sections of an immature worm of *D. immtis* are seen in a small pulmonary artery (upper, Elastica van Gieson stain), and a transverse section of an immature adult worm showing large lateral chords (arrow head) with internal longitudinal ridges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

3.2 Serological investigations

Serology is an alternative method of diagnosing parasitic infections because the invading parasite cannot always be identified by pathologyical 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.

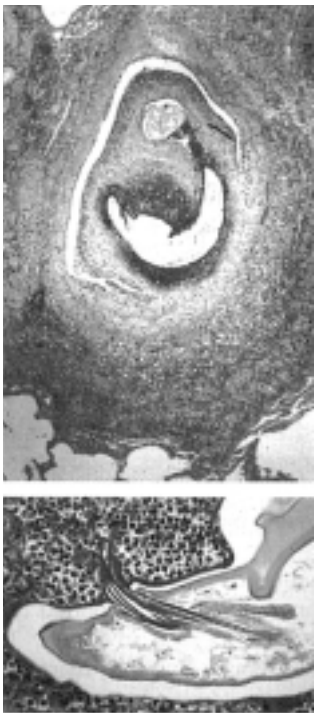

Fig. 4 Low-power view of a pulmonary infarct containing a transverse section and a longitudinal section of a mature male *D. immitis* (upper). Two spicules are clearly observable (bottom).

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, Glickrnan *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 immunodiagnosis.

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).

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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