

## Pulmonary Dirofilariasis -Clinicopathological Study-

HIROSHI HIRANO,<sup>1,2</sup> TOMOHIKO KIZAKI,<sup>2</sup> TERUMASA SASHIKATA,<sup>2</sup>  
and TAKEO MATSUMURA<sup>3</sup>

<sup>1</sup> Department of Pathology, Toneyama National Hospital, 5-1-1 Toneyama,  
Osaka 560-8552, Japan

<sup>2</sup> Department of Pathology, Hyogo Medical Center for Adults, 13-70 Kitaoji-cho,  
Akashi 673-8558, Japan

<sup>3</sup> Faculty of Health Science, Hyogo University, 2301 Shinzaike Hiraoka-cho,  
Kakogawa 675-0195, Japan

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**Abbreviations:** PD; pulmonary dirofilariasis, *D. immitis*; *Dirofilaria immitis*, IHA; indirect hemagglutination, ELISA; enzyme-linked immunosorbent assay, *D. repense*; *Dirofilaria repense*

**Pulmonary dirofilariasis (PD), caused by *Dirofilaria immitis* (*D. immitis*), the dog heartworm, is not common in humans, though we recently encountered 4 cases. Chest X-ray images from annual health examinations showed a single spherical nodule in the inferior or middle portion of the right lung in each patient. None of the patients showed any clinical symptoms and had no contact with dogs. Hematological results in 3 of the cases were within normal limits, while mild eosinophilia was found in one. Serological tests for the Anti-*Dirofilaria* antibodies were not performed. There were no characteristic clinical manifestation of PD in any of the patients, however, we consider it important to keep a diagnosis of PD in mind, when we experienced these cases, they present no characteristic clinical manifestations. Pathologically, macroscopic findings showed well-circumscribed nodules that were round peripheral lesions in lungs. Histological results revealed coagulation necrosis with fibrosis and granulation in the nodule edge, which contained inflammatory cells. By means of silver staining, the worm structures in the nodules could be identified well, and the quadrant cells in the sections were numbered about 30. Immunohistochemically, the somatic muscle tissues were stained with anti-*Dirofilaria* antibody. These findings indicated that the pulmonary lesions in all 4 cases were due to *D. immitis*.**

Dashiell first described human pulmonary infarction due to *dirofilaria immitis* (*D. immitis*) in 1961.<sup>4</sup> Recently, it has been diagnosed more frequently because of developments in diagnostic techniques.<sup>2,15</sup> Patients with pulmonary dirofilariasis (PD) are typically asymptomatic and indication usually occurs after a coin lesion is revealed on a chest X-ray image.<sup>1,2,17</sup> Clinical manifestations of PD are occasionally similar to those of lung cancer, however it is important to differentiate them. Most reports have indicated that PD can not be diagnosed without operation, though a few have noted that a definitive diagnosis of PD was obtained from histological observations of biopsy specimens.<sup>8,15</sup> Since a thoractomy would be more dangerous than the PD itself, a minimal resection without resecting parenchymal

lung tissue or using anti-helminthic medication is adequate for diagnosis.

Identification of the worms is accomplished by recognition of the structures after silver staining. Tsukayama et al.<sup>17</sup> and Naefie and Pigott<sup>10</sup> previously reported characteristic features of *D. immitis*, are valuable resources for diagnosis. However, the worm structure deteriorates if there is a long duration before recognition, making accurate identification difficult. In addition, other types of dirofilaria have structures and size of similar to *D. immitis* and the differentiation among them is occasionally difficult.<sup>12,13</sup>

Herein we present the clinical features of 4 cases of PD before surgery, along with more detailed results of the *D. immitis* specimens from microscopic analysis. In addition, immunohistological studies were performed using rabbit serum that was hyperimmunized with *D. immitis*.

## MATERIALS AND METHODS

### *Clinical cases:*

Case 1. A 55-year-old man, who worked as a doorkeeper at a department store, was found to have a 25-mm noncalcified coin lesion in the right lower field during a routine chest x-ray examination taken at his company. He was completely asymptomatic.

Case 2. A 55-year-old woman, who worked as a housewife, was found to have a 14 X 9-mm sized noncalcified tumor with a thick-walled cavity in her right lower field at a periodic examination (Fig. 1a,b). She was free of symptoms.

Case 3. A 60-year-old man, who was not working at the time, was found with a 31 X 26-mm noncalcified coin lesion in the right middle lung field on a regular physical examination (Fig. 1c, d). He was asymptomatic.

Case 4. A 64-year-old woman, who also worked as a housewife, visited our hospital complaining of vomiting and dizziness. A chest X-ray film revealed partial atelectasis in the right lower lobe (Fig. 1e, f). She had no pulmonary symptoms.

A preoperative diagnosis of PD was not made in any of these patients, and none had contact with a dog as their pet. Eosinophilia was present in 1 patient (case 4). Useful information of PD could not be obtained from cytological and histological examinations, and serologic tests of *D. immitis* were not performed. Three patients (case 1, 2, and 3) underwent thoractomy and one (case 4) had a wedge resection. In all except case 4, the surgeons could not determine dirofilariasis by gross appearance or physical examination.

### *Immunohistochemistry*

The largest cut surface of each resected pulmonary portion was used for histologic and immunohistochemical examinations. Tissues were fixed in 15% formalin and embedded in paraffin. We made 2-10 blocks in each case and the embedded tissues were cut into 5 µm sections and stained with hematoxylin-eosin. Immunohistochemical staining was performed using an avidin biotin peroxidase complex method kit (Vecstain, Burlingame, USA). We used antibodies against alpha-smooth-muscle actin (Dako, Kyoto, Japan) and *D. immitis*, and serum from a rabbit hyperimmunized with *D. immitis* was used as the anti-Dirofilaria antibody.

### *Pathological findings*

On gross examination, the 1 to 2.5cm infarcts were found to be firm and well-circumscribed nodules, that were round rather than wedge-shaped and peripheral, however, none were pleura-based (Fig. 2a-d). Histological examinations revealed coagulation

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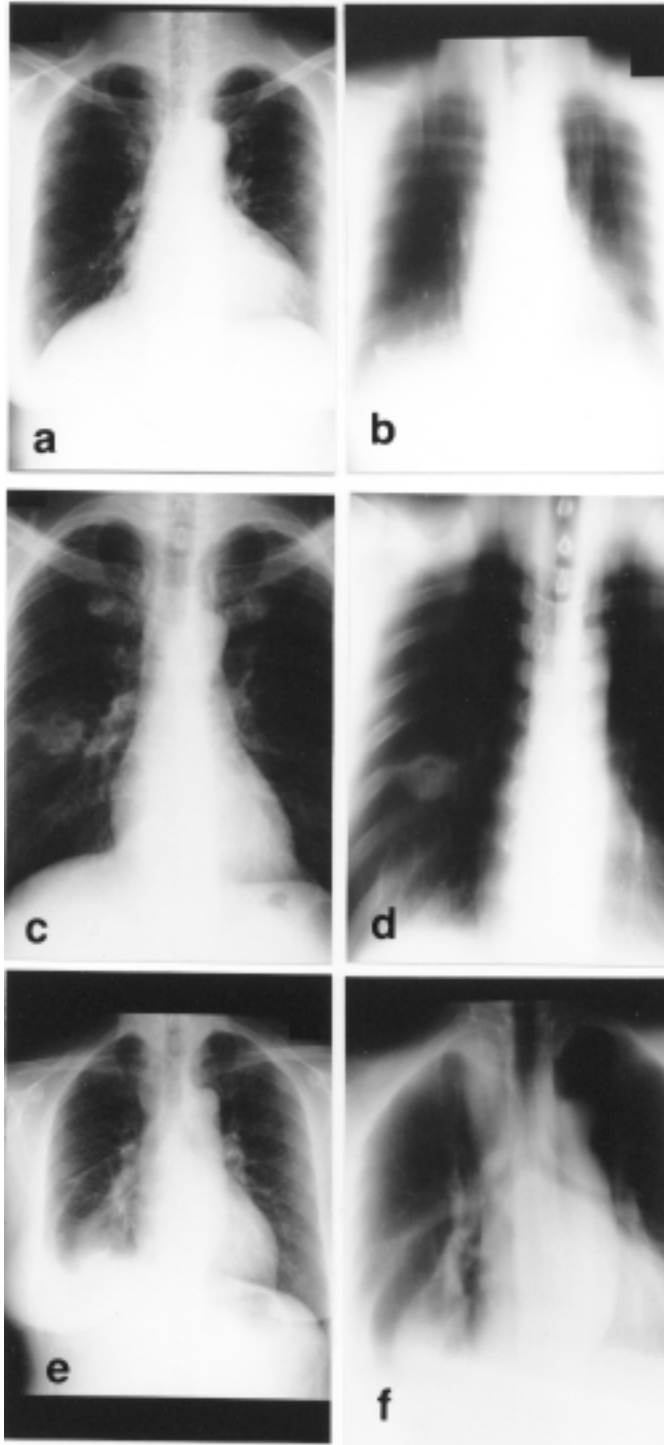


Fig. 1. Abnormal shadows of chest X-ray (a,c,e) and tomography (b,d,f) images. (a,b) case 2, (c,d) case 3, and (e,f) case 4.

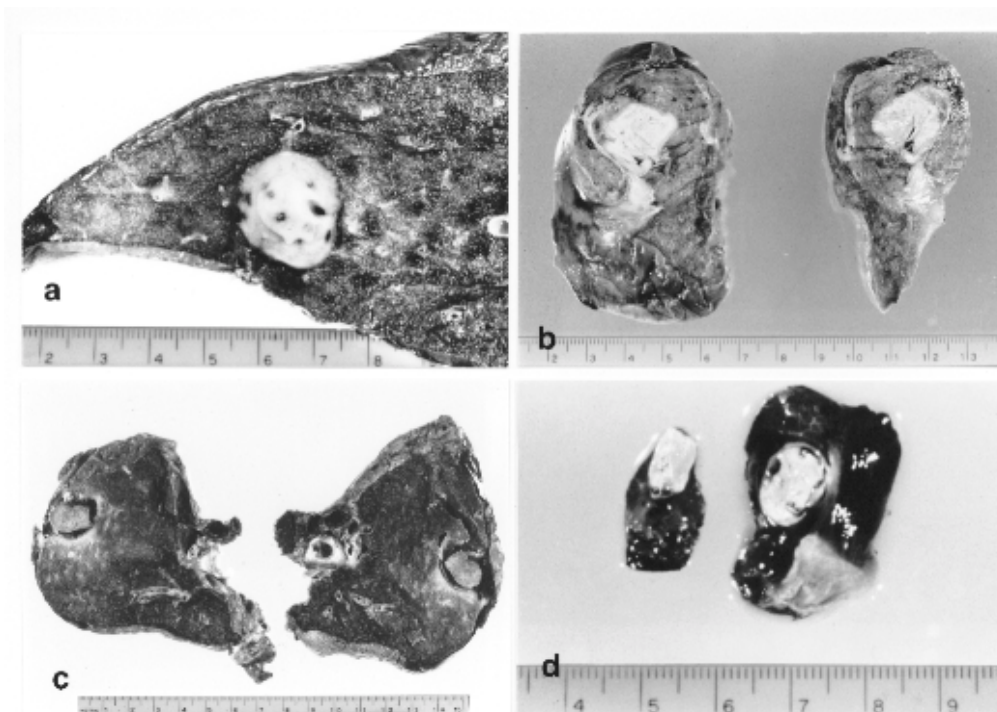


Fig. 2. Macroscopic appearance of pulmonary nodules. (a) case 1, (b) case 2, (c) case 3, and (d) case 4.

necrosis in the center of each nodule, while the edge of the lesion consisted of fibrotic and inflammatory granulations, with lymphocytes, histiocytes, and occasionally giant cells (Fig. 3a). The worm structures were recognized in the arteries (Fig. 3b). The worms had a longitudinal internal cuticle, muscular structure, internal coelom, intestinal tract, and genital tract (Fig. 3c,d). We counted the number of somatic muscular cells, which was divided by 4 and termed a quadrant. The number of quadrants in our cases was 30. In the immunohistochemistry of anti-*Dirofilaria* antibodies examinations, the somatic muscle fiber were strongly stained and the cuticle weakly (Fig. 4a,b). These muscular fibers were not reactive with alpha-smooth muscle actin.

## DISCUSSION

The first report of human infection from *Dirofilaria* was in 1887, when de Magalhaes reported finding worms in the left ventricle of a male child from Rio de Janeiro, Brazil.<sup>16</sup> In 1961, Dashiell first described human pulmonary infarction due to *D. immitis*,<sup>4</sup> and in 1969, the first case of human PD in Japan was reported by Yoshimura. Since then, there has been a rapid increase in the number of patients with PD, and 84 cases have been recorded in literature.<sup>11</sup>

The life cycle of *D. immitis* requires a definitive host, usually a dog, and a vector-intermediate host, generally a mosquito.<sup>6,14</sup> Other possible definitive hosts are wolves, coyotes, and foxes. In humans, the larva cannot mature to a sexually mature form, thus they are a 'dead-end' host if infected with infectious forms from a mosquito. However, mosquitoes are nearly ubiquitous, thus the possibility of infections in the humans exists.

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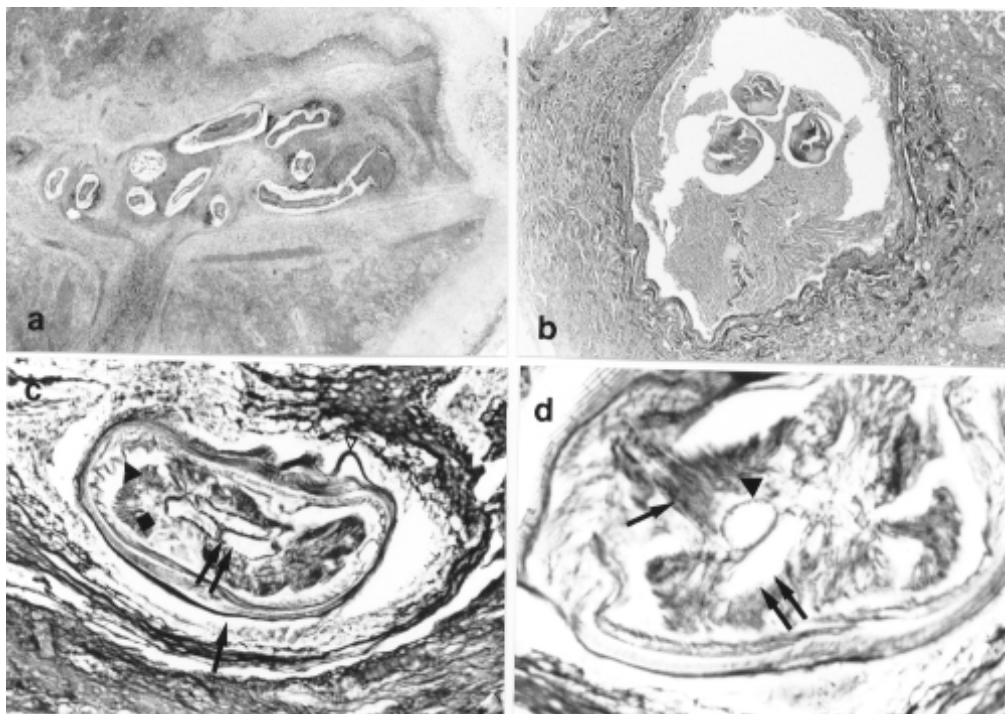


Fig. 3. Microscopic appearance of a resected lung, (a) Many worms can be seen in the center of necrosis, surrounded by fibrosis and granulation tissue (Masson Trichome stain). (b) Three worms in the pulmonary artery (Elastica von Gieson stain). (c) Oblique transverse section of a worm showing the cuticle (arrow), lateral cord (closed triangle), somatic fiber (closed square), longitudinal ridge (open triangle). Three tubules (double arrows) in the center correspond to the intestine and the gonad (silver stain). (d) Higher manifestation of sections revealing the detail structures; somatic fiber (arrow), intestine (closed triangle), and the gonad (double arrows) (silver stain).

According to most previous descriptions,<sup>1,2,17</sup> the most common manifestation of PD are asymptomatic nodule discovered on a chest X-ray film, though a few symptomatic patients have complained cough, chest pain, and occasionally hemoptysis.<sup>1,2,14</sup> Our all patients with PD present asymptomatic non-calcified, solitary pulmonary nodules 2.5cm or less in diameter. According to a previous report,<sup>15</sup> peripheral eosinophilia was present in 20% of the patients, though only 1 out of 4 patients showed eosinophilia.

Indirect hemagglutination (IHA) and enzyme-linked immunosorbent assay (ELISA) procedures have been used for diagnosis of PD.<sup>5,13</sup> Glickman et al<sup>5</sup> investigated the serologic findings of IHA and ELISA in 8 patients and found that 5 had diagnostic high IHA to *D. immitis* and 6 positive findings by ELISA. Serological methods may be useful for a PD diagnosis, however, we did not perform the serological tests for PD. An operation is advised for accurate diagnosis, especially for patients that have a possibility of malignancy. However, a thoractomy would not be necessary if a pre-surgical diagnosis of PD could be made, and a minimal resection without resecting parenchymal lung tissue or using antihelminthic drugs would be adequate. As described above, there was no characteristic clinical manifestation for a preoperative diagnosis of PD. Therefore, it was important to keep a diagnosis of PD in mind when we encountered the presented cases.

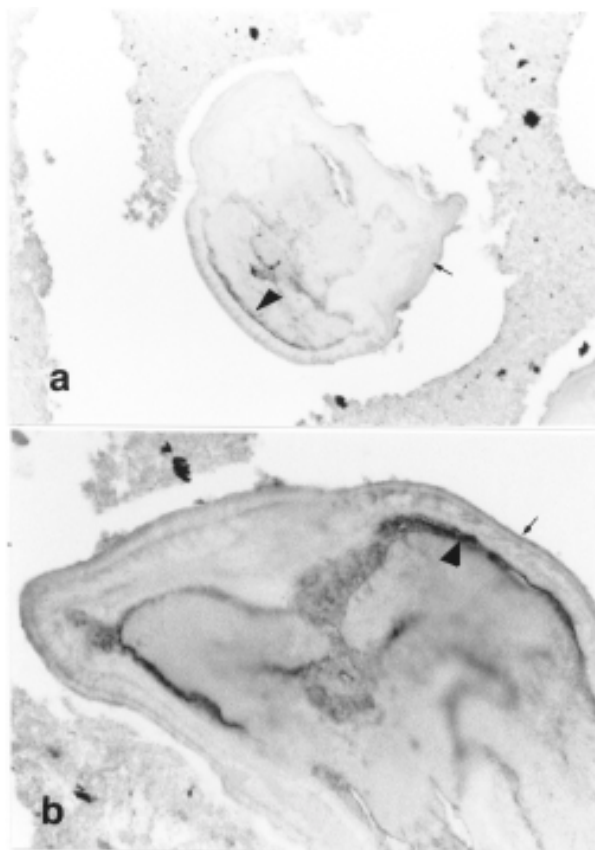


Fig. 4. (a) (b) Cuticle, stained weakly (arrow), and somatic muscle fibers, stained strongly (arrowhead) (anti-Dirofilaria antibody).

Well-defined pulmonary lesions with necrosis are considered to be a differential diagnosis of PD, which includes carcinoma, tuberculosis, and fungal infections,<sup>1,3,9,14,15</sup> therefore recognition of the worm structure is essential. Silver staining is the most useful method for showing recognizable worm structures, such as a smooth cuticle, well-developed musculature, internal coelom, and intestinal and genital tracts.<sup>7,10,17</sup> Regarding this point, Naeffie and Pigott<sup>10</sup> has described the worm structure of *D. immitis* and noted the following diagnostic features: (1) a thick laminated cuticle composed of three layers, (2) external transverse striation, (3) an internal longitudinal ridge, and (4) abundant somatic muscle tissue. Tsukayama et al.<sup>17</sup> also noted that the existence of worms in the pulmonary artery was an additional diagnostic feature. The structure found in our patients fulfilled all of the features described above.

The worm structures have a tendency to degenerate over a period of time, however, since the muscular tissues of dirofilaria are well-developed, even if the worm structure has deteriorated and cannot be identified in detail, the remnants retain the somatic muscle structures, which allows for counting the number of muscular cells and layers. In addition, *Dirofilaria repens* (*D. repense*) occasionally invades the human subcutaneous or mucosal tissue as a parasite,<sup>7</sup> though pulmonary nodules due to *D. repense* have been rarely reported.<sup>12</sup> Further, it is difficult to discriminate *D. immitis* from *D. repense* because the morphology and size of *D. immitis* are similar to those of *D. repense*. Counting the number

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of muscular cells per a quadrant is widely used for analysis of the worm structures,<sup>14</sup> and it is important to count the muscular cells when identifying *D. immitis* structures. The number of muscular cells per a quadrant in *D. immitis* and *D. repense* are 30 and 15, respectively. We counted approximately 30 per a quadrant in each of the present patients, which indicated PD due to *D. immitis*.

We used immunohistochemical methods, which did not show a reaction with alpha-smooth muscle actin, but did with the serum of a rabbit hyperimmunized with *D. immitis*. As a result, findings of the present cases suggested PD due to *D. immitis*. However, there are problems with a diagnosis of PD due to *D. immitis* using this antibody. To our knowledge, there are no other descriptions of PD identified with immunohistochemistry for the anti-*Dirofilaria* antibody. As for serologic diagnosis of PD, Glickman et al. analyzed PD with *D. immitis* by using a crude antigen prepared from adult *D. immitis*.<sup>5</sup> In their report, a cross-reactivity of ELISA with serum from patients of other nonfilarial parasitic infections appeared. Therefore, in the immunohistochemical examination, the anti-*Dirofilaria* antibody may induce cross-reactivity with other nonfilarial parasitic infections. To evaluate immunohistochemical efficiency using the anti-*Dirofilaria* antibody, other *Dirofilaria* and nonfilarial parasites, in addition to *D. immitis*, should be analyzed.

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