Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Parasitology 133 (2005) 157–180
veterinary parasitology
www.elsevier.com/locate/vetpar

Public health aspects of dirofilariasis in the United States

J.H. Theis *

Department of Medical Microbiology and Immunology, School of Medicine, University of California, Davis, CA 95616, USA

Abstract

Coin lesions in the human lung present significant differential diagnostic problems to the physician. There are at least 20 known causes of such lesions, including neoplastic lesions, infectious diseases, and granulomas. The human medical literature contains many misconceptions about the life cycle of *Dirofilaria immitis*, including the method of entry of the infective-stage larvae and the development of the young adult worm. These misconceptions have obscured the recognition of the clinical presentation of pulmonary dirofilariasis and the potential for *D. immitis* to lodge in many other areas of the human body besides the lung. Exposure to infective larvae of *D. immitis* is more common in humans than is currently recognized. Reported cases in humans reflect the prevalence in the canine population in areas of the United States. The veterinary literature provides compelling evidence that *D. immitis* is a vascular parasite, not an intracardiac one. Its presence in the right ventricle is a post-mortem artifact, because it has never been shown to be there by echocardiography or angiography in a living dog, even though these techniques have demonstrated adult *D. immitis* in the pulmonary, femoral, and hepatic arteries; posterior vena cava; and right atrium of live dogs. Physicians have taken the name ''heartworm'' literally, believing that the worm lives in the heart and only after it dies does it embolize to the pulmonary artery. However, the coin lesion is spherical in shape, not pyramidal, as embolic infarcts to the lung in humans are known to be. The coin lesion is an end-stage result of the parasite's death in the vascular bed of the lungs and the stimulation of a pneumonitis followed by granuloma formation. This pneumonitis phase of human pulmonary dirofilariasis is often not recognized by the radiologist because of the way pneumonitis is diagnosed and treated and because the developing nodule is obscured by the lung inflammation. Serologic methods for use in humans are needed for clinical evaluations of patients with pneumonitis living in highly enzootic *D. immitis* regions. As well, epidemiological surveys are needed to determine the real extent of this zoonotic infection.

Keywords: *Dirofilaria immitis*; Heartworm; Zoonosis; Human dirofilariasis

1. Introduction

Zoonotic filarial nematode infections, other than those due to *Dirofilaria* spp. in humans, have been reported in the United States in both those with a travel history out of the country and those without such a history (Beaver et al., 1974; Scully and McNeely, 1974). By far, however, the majority of reported cases of zoonotic filariasis in the United States have involved *Dirofilaria* species, either *Dirofilaria tenuis*, a subcutaneous parasite of the raccoon (*Procyon lotor*), or *Dirofilaria immitis*, the pulmonary artery parasite of canids (Orihel and Beaver, 1965; Beaver

* Tel.: +1 530 752 3427; fax: +1 530 752 8692.
E-mail address: jhtheis@ucdavis.edu.

0304-4017/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2005.04.007

and Orihel, 1965; Neafie and Piggott, 1971; Ro et al., 1989).

From a human and veterinary medical point of view, *D. immitis* is the most important of the two recognized zoonotic species of the genus *Dirofilaria* in the United States. The public health significance of *D. immitis* is not associated with the overt clinical disease it produces in humans, but rather with the seriousness of the diseases that the radiographic findings of a coin lesion suggest might be present (Navarrete-Reyna and Noon, 1968; Schlotthauer et al., 1969; Toomes et al., 1983). The diagnostic differentials of a coin lesion include primary or metastatic neoplasia, fungal infections, hamartomas, and tuberculosis (Trunk et al., 1974; Toomes et al., 1983; Allison et al., 2004). This lesion requires an extensive clinical work-up, which, not infrequently, culminates in a thoracotomy. The cost of health-care delivery in evaluating a coin lesion may exceed $80,000 per patient and will have subjected the patient to unnecessary stress and invasive procedures if the lesion is discovered to be pulmonary dirofilariasis.

There are numerous misconceptions in the human medical literature regarding the parasitologic and pathologic aspects of pulmonary dirofilariasis. This paper will address these misconceptions, and based upon the evaluation of reports in the human and veterinary medical literature, will present an alternative hypothesis to explain the pathological sequence of events in human infections.

2. Misconceptions regarding the human host–*Dirofilaria immitis* association

2.1. Infection process

Among the many misconceptions in the human medical literature is that the infective larvae are injected into the dermis as the female mosquito feeds (Harrison and Thompson, 1965; Gershwin et al., 1974; Hoch et al., 1974; Riskin and Toppell, 1977; Robinson et al., 1977; Darrow and Lack, 1981; Kahn et al., 1983; Kochar, 1985; Lum, 1985; Ro et al., 1989; Bradham et al., 1990). In reality, however, the larvae infective to the vertebrate host are known to break out of those components of the mosquito mouthparts that remain on top of the skin while the mosquito is feeding, and far from being injected, the larvae must find their way through the hole in the skin after the female mosquito has withdrawn her fasicle. The hemorrhaged mosquito hemolymph is released when the larvae break out of the labellae or at times, the labium must remain covering the larvae until they enter the vertebrate host or the larvae lose their motility and infection fails (Fülleborn, 1908; Ewert, 1967; Ewert and Ho, 1967; McGreevy et al., 1974). Papers published in journals that physicians could be expected to read have also indicated the correct method of infection (Awe et al., 1975; Southgate and Bryan, 1975; Roy et al., 1993).

The misconception regarding the method of transmission has prevented the recognition that infective larvae transmission from mosquito to the vertebrate host is very inefficient. Based on transmission studies, Ewert and Ho (1967) have estimated that as many as 45% of the infective larvae may be lost on the surface of the skin at any one transmission event.

This misconception by the human medical community has led to the assumption that exposure to infective larvae is rare when, in fact, the recognition of such exposure remains unknown. There are no reliable commercially available serological techniques for determination of exposure of humans to infective larvae, and only those infections that result in detected lesions are reported in the literature. The vast majority of these reported detections involve the lung, an organ system that is readily surveyed by indirect, non-invasive techniques, such as chest radiographs, computed tomography (CT), and magnetic resonance imaging (MRI) scans (Table 1).

2.2. Location of adult Dirofilaria immitis

A second misconception regarding *D. immitis* is that it resides in the right ventricle, and upon its death, embolises to the pulmonary arteries (Tannehill and Hatch, 1968; Spear et al., 1968; Neafie and Piggott, 1971; Gershwin et al., 1974; Hoch et al., 1974; Robinson et al., 1977; Darrow and Lack, 1981; Kahn et al., 1983; Kochar, 1985; Lum, 1985; Ro et al., 1989; Bradham et al., 1990; Asimacopoulos et al., 1992; Roy et al., 1993).

However, the characteristic radiological pattern due to an embolic infarct of the lung is wedge- or pyramidal-shaped, not spherical as the ‘‘coin’’ lesions due to *D. immitis* in humans are all reported to be. This point has been commented on by several authors

Table 1
Characteristics of *Dirofilaria immitis* human infections

Report No.	Year gender/age (years)	Patient residence	Patient symptoms or diagnosis	Worm site	Worm sex/stage	Recovery method	Reference
1	1941 F/73	New Orleans, LA	None; classified as *D. louisianensis* later as *D. immitis*	Inferior vena cave	M/entire worm/120 mm/long tail/spiral coil	Autopsy	Faust et al. (1941)
2	1961 F/57	Detroit, MI 12 trips to SC (1958–1959)	None; rad 1958 normal; 1959 lung nodule	PAB, rt upper lobe	NR/Mature	Thoracotomy	Dashiell (1961)
3	1964 F/38	New England; no history of travel	Pleural effusion base rt lung, wheezing, afebrile, non-productive cough, 9% eos 2 cm nodule	PAB, rt lower lobe, rt middle lung field	F/Imm	Thoracotomy	Goodman and Gore (1964)
4	1965 F/NR	FL	None; routine rad; nodule	PAB, near periphery	NR	Thoracotomy	Beaver and Orihel (1965)
5	1965 F/35	LA	Nonproductive cough, pain in rt chest 2–3 days, low-grade fever	PA, lower rt lung	F/Mature	Thoracotomy	Ibid
6	1965 F/55	LA	None; rad: 3-cm nodule	PA, upper lobe lf lung area calcified	M/Mature	Thoracotomy	Ibid
7	1965 F/37	SC	Productive cough, chest pain, blood-tinged sputum, low-grade fever 2-cm nodule	PA, lf upper lobe	F/Imm	Thoracotomy	Ibid
8	1965 M/48	Southeast USA	Myalgia, fatigue, productive cough, 2-cm mass	Rt upper lobe near hilus	M/Mature	Thoracotomy	Ibid
9	1965 F/40	New Orleans, LA	None	Main PA	F/Mature/ 21.4 cm	Autopsy	Abadie et al. (1965)
10	1965 F/53	TX	None; worm not discovered in biopsy until 2 years after removal when new sections cut	PA, thrombosed branch	F/Imm	Thoracotomy Preop dx: lung tumor; postop dx: pulmonary infarct	Harrison and Thompson (1965)
11	1965 M/60	WI	None Feb 1961; chills, fever, anorexia, productive cough, 5-lb wt loss Dec 1960; antibiotics, symptoms resolved	PAB in lower lobe of rt lung	F/Imm	Thoracotomy	Ibid
12	1966 F/48	Baton Rouge, LA	Rt anterior pleuritic chest pain; rad: 2-cm coin lesion in apical section lower lobe rt lung, larger density in lingular division of upper lobe lf lung	PAB in upper lobe of lf lung	Uncertain (male?), advanced deterioration/NR	Thoracotomy. Lf lung mass removed, rt lung mass not removed	Beskin et al. (1966)
13	1967 M/53	NY	None; 1965: density; films in 1956 and 1960 normal	Med Branch of PA in rt upper lobe lung	NR/NR	Thoracotomy	Tuazon et al. (1967)
14	1968 M/43	New Orleans, LA	Acute respiratory infect 1 month before; recovered. Rads 1966: 1.6-cm lesion (not seen 1960)	Branch of PA in anterior segment lf upper lobe	NR/Imm	Thoracotomy	Tannehill and Hatch (1968)

Table 1 (*Continued*)

Report No.	Year gender/age (years)	Patient residence	Patient symptoms or diagnosis	Worm site	Worm sex/stage	Recovery method	Reference
15	1968 M/75	Houston, TX	Painful toes. 2.5-cm coin lesion in lf lung (not present in 1961)	Branch PA in upper lobe lf lung	M/Imm	Thoracotomy	Navarrete-Reyna and Noon (1968)
16	1968 F/49	FL	Routine radiograph	Small segment of PA rt lower lobe	NR/Mature	Thoracotomy	Spear et al. (1968)
17	1968 M/57	FL	Routine radiograph	Segment of PA, lf lower lobe	NR/Mature	Thoracotomy	Ibid
18	1971 F/28	San Antonio, TX	None	Branch of med PA, rt lower lobe	M/Imm	Autopsy	Neafie and Piggott (1971)
19	1971 M/34	New Haven, CT	Routine radiograph	Branch of PA, lf upper lobe	M/Imm	Thoracotomy	Ibid
20	1971 M/42	Jacksonville, FL	Routine radiograph	Branch of med PA, rt lower lobe	F/Imm	Thoracotomy	Ibid
21	1971 M/44	Orlando, FL	Routine radiograph	Branch of PA, rt upper lobe	M/Imm	Thoracotomy	Ibid
22	1971 M/48	Bethsada, MD	Routine radiograph	Branch of PA, rt upper lobe	NR/Imm	Thoracotomy	Ibid
23	1971 M/51	Coral Gables, FL	Routine radiograph	Branch of PA, lower lf lobe	M/Imm	Thoracotomy	Ibid
24	1971 M/71	Norwich, CT	Routine radiograph	Branch of PA, rt upper lobe	F/Imm	Thoracotomy	Ibid
25	1971 M/72	Hattiesburg, MS	Routine radiograph	Branch of PA, rt upper lobe	M/Imm	Thoracotomy	Ibid
26	1972 F/49	Houston, TX	None by rad; found in pulmonary lobe affected by pneumonia 11 months. Earlier. Lesion 4.5-cm. Normal rad 1964	PAB, rt lower lobe	M/Imm	Thoracotomy	Navarrete (1972)
27	1972 M/73	Port Lavaca, TX	Rt chest pain and hemoptysis, 2-cm lesion. Normal rad 1 year earlier	PAB, rt upper lobe posterior & anterior segments	M/Imm M/Mature	Thoracotomy two lesions	Ibid
28–32	1972 NR/NR		No specific data available				Lau and Pierson (1972)
33	1973 M/45	FL	Left side chest pain, fever, sweats, chills, progressive cough, hemoptysis, dyspnea 2 weeks	Small branch PA, lf lower lobe	NR/NR	Thoracotomy	Feld (1973)
34	1974 F/48	Boston, MA; briefly in FL	Hemoptysis; rad: 3 × 3 ill-defined density in lf lung	Med PA in lf midlung zone	M/Imm	Thoracotomy	Gershwin et al. (1974)
35	1974 M/56	PA	Nonproductive cough 3 months duration; rad: 1.5-cm diameter nodule (not present 5 months earlier)	PAB, lf upper lobe	Too degenerated	Thoracotomy	Hoch et al. (1974)
36	1974 M/60	PA	Productive cough, transient left chest pain, fever 4 weeks; analgesic and antibiotics ineffective. Rad: single nodule 2-cm. Previous annual rad normal	PAB in lf upper lobe	NR/NR	Thoracotomy	Ibid
37	1975		Same case reported by Gershwin et al. (1974)				Dayal and Neafie (1975)

38	1975 M/48	Houston, TX; AK 4 year before	None	Lesions bilateral lf apical lobe; rt upper lobe; PAB	NR/NR	Thoracotomy. Bacterial wedge resection both nodules	Awe et al. (1975)
39	1975 M/59	TX	None at routine rad or 1 year earlier, 2 months before admission had a transient flu-like illness	Lf lower lobe	NR/NR	Thoracotomy	Ibid
40	1976 M/64	SC	None; related to pulmonary system rad; 2-cm lesion	Peripheral PAB, lf lower lung lobe	NR/Imm	Thoracotomy 9 months. After lesion first seen	Prioleau et al. (1976)
41	1976 M/46	SC	Routine radiograph 1.5-cm lesion	Peripheral PAB, lf lower lobe	NR/Imm	Thoracotomy	Ibid
42	1976 F/59	SC	Observation for ovarian cancer, 2-cm nodule developed over 2 year period	Peripheral PAB lf lower lobe	NR/Imm	Thoracotomy	Ibid
43	1976 M/37	SC	Evaluation of peptic ulcer	Peripheral PAB in lf upper lobe	NR/Imm	Thoracotomy	Ibid
44	1976 F/76	SC	Evaluation after radical mastectomy 2 years prior; lesion static for several months after detection	Peripheral PAB in rt upper lobe	NR/Imm	Thoracotomy	Ibid
45	1977 F/67	TX	Cough, myaigia. Chest congestion	PAB in lf lower lobe	NR/NR	Thoracotomy	Riskin and Toppell (1977)
46	1977 M/66	MS	Intermittent productive cough, chest tightness, malaise, weakness 3 months and 2 weeks prior. 2-cm lesion rad	PAB, lf upper lobe	M/Imm × 2	Thoracotomy. Two lesions: 4 × 2 × 2.5-cm and 2.5 × 1.5 × 1.5-cm	Robinson et al. (1977)
47	1979 M/52	MA	No lesions in film 2 years before. Chest 1-cm lesion observed for 7 months. After diagnosis, no change in size	PAB in the lf lower lobe	NR/NR	Thoracotomy	Scully and Galdabini (1979)
48	1980 M/55	NC	Routine radiograph 1.5-cm nodule	PAB of lf upper lobe	NR/NR	Thoracotomy	Levine et al. (1980)
49	1980 M/46	GA	Routine radiograph; lesion	PAB of rt lower lobe	NR/NR	Thoracotomy	Merrill et al. (1980)
50	1980 M/47	GA	Routine radiograph 8- and 12-cm lesion	PAB rt lower lobe, two lesions	NR/NR	Thoracotomy	Ibid
51	1980 M/72	Georgia	None	PAB of upper lobe of rt lung	NR/NR	Thoracotomy	Ibid
52	1981 M/66	MA, traveled in Italy 4 months before lesion discovered	Routine radiograph: lung nodule not present 3 years previous 2-cm in diameter	PAB rt lower lobe subpleural	M/NR	Thoracotomy	Darrow and Lack (1981)
53	1981 M/67	CA	Productive cough, chest discomfort, malaise, fever 10 days; tetracycline, rad: 2-cm nodular lesion rt lung. Ampicillin, no change	PAB rt lower lobe	M/Mature	Thoracotomy	Ciferri (1981a,b)
54	1983 M/79	WI (no recent travel)	Routine rad: lesions bilateral, lf 2.0-cm and rt 2.5-cm lower lobe. Neither present 3 months before	PAB in lf and rt lower lobe	NR/NR	Bilateral thoracotomy	Kahn et al. (1983)
55	1985 F/51	AL	Rad: 3 nodules rt lower lobe 2.7 × 1.8-cm and 4.5-cm diameter	PAB rt lower lobe	NR/NR	Necropsy	Lum (1985)

Table 1 (*Continued*)

Report No.	Year gender/age (years)	Patient residence	Patient symptoms or diagnosis	Worm site	Worm sex/stage	Recovery method	Reference
56	1985 M/67	IN	No nodule on rad 2 months. Earlier 2-cm in diameter when found	PAB rt upper lobe (periphery)	NR/NR	Thoracotomy	Kochar (1985)
57	1985 M/47	IN	Pneumonitis 2 months. Cough, fever, lower rt chest pain; antibiotics: 1 year later fever, cough rt lower chest pain, hemoptysis 1 week duration. Rt sided pleural effusion, peripheral esoinophilia; thoracentesis: sanguinous fluid rt pleural cavity; antibiotics. Infiltrates cleared 3 weeks; rad: multiple bilateral nodular densities	Infarceted med branch of PA in the rt lower lobe; smaller lesion rt lower lobe 2-cm diameter	NR/NR	Thoracotomy	Ibid
58	1985 F/63	IN	Lf lower chest pain, cough, fever, rad: infiltrates in lf lung base and pleural effusions; discharged, antibiotics. Rad 3 weeks after discharge: 1.8-cm nodule in periphery of rt lower lobe	PAB in periphery of rt lower lobe	NR/NR	Thoracotomy	Ibid
59	1985 F/62	VA	Chest pain, rad: lesion rt upper lobe. Second mass in the lf upper lobe	PAB rt and lf upper lobe	NR/NR	Fine needle aspiration	Hawkins et al. (1985)
60	1985 M/52	Southeast Queensland, Australia	Routine rad: 1.5-cm lesion in rt upper lobe	PAB of rt upper lobe near periphery; worm had no internal intact tissue	NR/NR	Percutaneous needle aspiration	Kelly et al. (1985)
61	1986 M/50	CA; traveled to NC 9 months. Before pneumonitis	Non-productive cough, fever, lf pleuritic chest pain. Rad: patchy infiltrate I peripheral lf lower lung. Antibiotics. Resolved 10 days. Rad 1 month later: denser infiltrate; 2 months: nodule 2 × 2-cm in region of former infiltrate	PAB lf lower lobe	F/Imm	Thoracotomy	Smith and Schillaci (1986)
62	1989 M/58	FL	None	PAB lower lf lobe, subpleural lesion for 4 years without change	NR/NR	Thoracotomy	Ro et al. (1989)
63	1989 M/63	FL	Routine radiograph	PAB upper lobe lesion 1.5-cm subpleural	NR/NR	Thoracotomy	Ibid
64	1989 F/65	FL	Routine radiograph	PAB lower lobe lesion 1.8-cm subpleural	NR/NR	Thoracotomy	Ibid
65	1989 F/45	TX	Routine radiograph	PAB lf upper lobe 1.5-cm lesion subpleural	NR/NR	Thoracotomy	Ibid
66	1989 M/35	TX	Routine radiograph	PAB lower lf lobe 2.7-cm lesion subpleural	NR/NR	Thoracotomy	Ibid
67	1989 M/77	FL	Chest pain, loss of consciousness	PAB lf upper lobe 2.5-cm lesion subpleural	NR/NR	Thoracotomy	Ibid

68	1989 M/50	Japan 1 year before onset	Chest pain, three episodes	PAB lf upper lobe 1.5-cm lesion subpleural	NR/NR	Thoracotomy	Ibid
69	1990 F/44	SC	Bilateral nodules rt and lf lower lobes	lf and rt lower lobes	NR/NR	Thoracotomy	Bradham et al. (1990)
70	1992 F/48	TX	Lesion (0.8-cm) rt middle lobe	PAB rt middle lobes	NR/NR	Thoracotomy	Asimacopoulos et al. (1992)
71	1992 M/67	TX	1.0-cm nodule upper lf lobe	PAB upper lf lobe	NR/NR	Thoracotomy	Ibid
72	1992 F/74	TX	2-cm calcified lesion rt lower lobe	PAB rt lower lobe	NR/NR	Thoracotomy	Ibid
73	1992 F/65	TX	Routine rad: 2-cm lesion lf upper lobe	PAB lf upper lobe	NR/NR	Thoracotomy	Ibid
74	1992 M/46	TX	Cough, rad: 1-cm lesion rt lower lobe	PAB lf lower lobe	NR/NR	Thoracotomy	Ibid
75	1992 M/65	TX	Routine rad: 1-cm lesion rt lower lobe	PAB rt lower lobe	NR/NR	Thoracotomy	Ibid
76	1992 F/42	TX	Thyroid goiter; pre-op rad: lesion rt lower lobe, no pulmonary symptoms	PAB rt lower lobe	NR/NR	Thoracotomy	Ibid
77	1992 F/59	TX	1 year history mild hemoptysis; rad: lesion 2-cm rt lower lobe	PAB rt lower lobe	NR/NR	Thoracotomy	Ibid
78	1992 M/59	TX	Routine rad: lesion lf lower lobe 1.5-cm	PAB lf lower lobe	NR/NR	Thoracotomy	Ibid
79	1992 F/49	TX	Routine rad: lesion 4.5-cm rt lower lobe, history of rt lower lung, pneumonia 11 months prior	PAB rt lower lobe	NR/NR	Thoracotomy	Ibid
80	1993 M/63	CA	Routine rad: possible metastasis of transitional cell carcinoma of urinary bladder treated 4 years before; two lesions rt upper lobe 1.2-cm and 1.5-cm	PAB rt upper lobe, two worms in lesion	NR/NR	Thoracotomy	Roy et al. (1993)
81	2001 M/28	CA; OH 6 months before onset	Pain and swelling left groin, nodule papable in lf inguinal canal inferior to external inguinal ring	Spermatic artery branch supplying lf testicle	NR/NR	Left orchiectomy	Theis et al. (2001)

(Navarrete-Reyna and Noon, 1968; Feld, 1973; Scully and Galdabini, 1979).

A far more acceptable explanation of the lesion seen in pulmonary dirofilariasis in humans is that the immature adult develops in small to medium branches of the pulmonary arterial tree, where it induces a vasculitis, ultimately is killed or dies as a result of the inflammatory response, and is then incorporated into a granuloma (Table 1).

This interpretation is supported by several pieces of information. Nowhere in the veterinary or human medical literature have adult *D. immitis* ever been found in the right ventricle in a living patient. The only reports of *D. immitis* in the chambers of the heart in dogs, monkeys, and humans have come from post-mortem examinations (De Magalhaes, 1887; Brown, 1939; Otto, 1949; Kume and Itagaki, 1955; Orihel, 1961; Wong, 1974). In contrast to these post-mortem studies, neither angiography nor cardiac ultrasonography in live patients have ever reported heartworms in the right or left ventricle, yet these same papers report their presence in the pulmonary arterial branches (Hahn, 1960; Tashjian et al., 1970; Wong, 1974; Venco et al., 2001) or in the aorta and liver (Goggin et al., 1997). Studies in which the pulmonary artery was clamped prior to euthanasia, so that worms in the artery could not slip back into the right ventricle, have reported finding only worms in the pulmonary artery and none in the right ventricle at necropsy (Wilcox, 1960). Conversely, if the pulmonary artery was clamped even ''immediately'' after euthanasia, living worms were found in the right ventricle (Otto, 1949). Furthermore, using ultrasonography, many *D. immitis* were seen in the right atrium, and during diastole, heartworms extended through the right atrioventricular valve into the right ventricle (Goggin et al., 1997). Therefore, worms found in the right ventricle post-mortem could quite possibly be coming from locations other than the pulmonary artery.

The reported location of *D. immitis* adults in dogs, cats, monkeys, and humans further supports the conclusion that the term ''heartworm'' is a necropsy artifact and, in reality, this nematode is a vascular-dwelling parasite, not necessarily limited to the pulmonary arterial tree. Tomimura et al. (1969) and Hayasaki et al. (1974) reported on 13 dogs that presented with clinical signs and symptoms that included elevated body temperature, anorexia, depression, lameness, ataxia, and paralysis or hyperesthesia of the hindquarters and had *D. immitis* in the femoral artery at necropsy. In addition, *D. immitis* were found in the abdominal or thoracic aorta and the renal artery in two dogs. One dog had a patent ductus arteriosis, and all of the other dogs had a patent foramen ovale. Hayasaki et al. (1974) concluded that these defects had allowed *D. immitis* to pass from the venous side of the circulation to the arterial side. However, Liu et al. (1966) reported on a dog that had 42 *D. immitis* in the right ventricle, 35 in the right atrium and vena cava, and 24 in the main pulmonary artery and its branches at necropsy. In addition, this dog had 11 *D. immitis* in the left ventricle, 18 in the left atrium, 31 in the lumen of the aorta and the right and left internal iliac arteries, the right and left external iliac arteries, the right and left femoral arteries, and the right and left popliteal arteries. This dog had no congenital anomalies in its heart. Furthermore, all arteries in which *D. immitis* were found had roughened and thickened intima, and there was villose endarteritis in the right external iliae artery. The authors believed that this case showed that *D. immitis* could mature in the systemic arterial system as well as the pulmonary vasculature. Both male and female worms were found in the aorta, left atrium, and left ventricle. These worms were the same size as those in the pulmonary arteries, right atrium, and right ventricle. A total of 48 adult *D. immitis* were found in the aorta and left atrium and ventricle, and a total of 101 adult *D. immitis* were found in the pulmonary artery, right atrium, and right ventricle at necropsy. An angiocardiography performed just prior to death and after injection of contrast material into the right ventricle revealed the pulmonary artery was markedly dilated, the pulmonary valve was displaced ventrally, and there was distortion and filing defects in the pulmonary arterial tree, but no adult heartworms were seen in the right ventricle.

Several other researchers have reported finding *D. immitis* in the systemic arteries of dogs, including one report by Hoerlein et al. (1972) of a dog presented with posterior weakness of 5–6 weeks duration. On physical examination, the dog evidenced hypersensitivity in the entire left pelvic limb, dermal hypersensitivity over the second lumbar vertebrate, and an extremely weak femoral pulse in each hind limb. Motor paresis was present in both pelvic limbs. Radiographic findings included cardiac hypertrophy,

dilation and sacculation of the pulmonary arteries, marked bronchial lymphadenopathy, pulmonary fibrosis, thromboembolic pneumonia, and hepatic density, suggesting cirrhosis. An aortogram performed by injection of contrast material into the left ventricle showed opacification of the caudal abdominal aorta, with striations and interrupted filing at the deep circumflex iliac area ventral to L7–S1. The right external and internal iliac vessels had some opacification. Femoral catheterization and retrograde injections substantiated these findings. An adult *D. immitis* was removed from each femoral artery during the catheterization. An aortotomy performed 72 h later resulted in the removal of 46 adult *D. immitis* from the internal and external iliac arteries. The dog died 15 days after surgery, and necropsy revealed that both ventricles were hypertrophied and the right ventricle was dilated. Adult *D. immitis* were present in the left ventricle in proximity to the aortic valve. Many adult *D. immitis* were found in the aorta and in the splenic, hepatic, renal, iliac, and spermatic arteries as well as the femoral arteries as far distally as the mid-tibial region. Many adult *D. immitis* were also present in the pulmonary artery and its major branches and on cut surfaces of the liver. The renal arteries showed endothelial proliferations and intimal fibrosis that resembled pulmonary arterial lesions seen with dirofilariasis.

Slonka et al. (1977) described a case that involved a dog presented with posterior paresis and paralysis extending over a 3-week period, during which progressive left limb lameness was observed. Initially, the dog was treated with phenylbutazone and discharged. The clinical condition did not improve, and the dog was referred to the UC Davis School of Veterinary Medicine. Upon examination, the dog was depressed and had an elevated temperature. The entire left hind limb was cold, firm, and swollen. The left femoral pulse was undetectable. Pain perception and tendon reflexes were absent in the left hind limb. The right hind limb was cool and paretic but had a weak femoral pulse. Microfilariae of *D. immitis* were detected in the blood, and because the dog's condition was deteriorating, the owner requested euthanasia. At necropsy, the peritoneal cavity contained dark green creamy fluid, and three *D. immitis* adults were free in the peritoneal cavity. Adult *D. immitis* also were found in the right ventricle, main pulmonary artery, smaller pulmonary arterial branches, right atrium, and caudal vena cava. The aorta contained several small worms. The left femoral artery was completely occluded by dead heartworms, extending to near the level of the stifle joint. The right femoral artery contained live and dead *D. immitis* extending to near the stifle joint. Muscles of the left limb were necrotic. The heart showed no evidence of patent ductus or foramen ovale, and the authors concluded that the immature worms had migrated into the left side of the cardiovascular system where they had developed in situ in normal fashion. The systemic arterial lesions seen in this dog were identical to those seen in the pulmonary arterial tree, indicating the worms had been in the systemic arteries for some time.

Burt et al. (1977) reported a case involving a dog that had jumped from the window of a moving car shortly before presentation to the Ohio State University Veterinary Teaching Hospital. The dog was observed to have a right hind leg lameness, with impaired proprioception and failure to bear weight when at rest. Pain could be elicited by palpation of any area on the limb but was particularly evident when the region of the stifle joint and tibia were touched. The dog had an elevated body temperature but was otherwise normal. Radiographs of the thorax were indicative of *D. immitis* infections, and microfilariae were seen in a blood examination. Angiography of the aorta and distal vessels revealed an occlusion of the right femoral artery distal to the right lateral circumflex femoral artery. A right femoral arteriotomy detected five adult *D. immitis* within the vessel. Subsequently, an angiocardiogram and caudal aortogram were performed and revealed distension of the main pulmonary artery and its branches. However, atrial and ventricular septal defects were not present, and no adult *D. immitis* were noted in the right ventricle. The aortogram revealed linear lucencies representing adult *D. immitis* in the left femoral artery and in the internal iliac and right common iliac arteries. The dog was euthanized at the owner's request, and adult *D. immitis* were present in the right ventricle, pulmonary arteries, and both femoral arteries at necropsy.

D. immitis also finds its way into the arterial system of the brain and spinal cord. Patton and Garner (1970) reported a case involving a 4-year-old dog treated for pulmonary dirofilariasis that developed grand mal seizures 3 months after treatment. The dog had profuse

salivation, dilated pupils between seizures, and leg trembling. Microfilariae were demonstrated in the blood. The dog was treated with sedatives, corticosteroids, and anticonvulsants. Three months later, the dog again developed grand mal seizures and circling to the right. The dog was euthanized and a necropsy performed. The right cerebral hemisphere was shrunken, and a live gravid female *D. immitis* was removed from the right posterior communicating artery. The heart was normal in size, and no septal defect was detected. Fifteen live adult *D. immitis* were present in the right ventricle and pulmonary arteries. Several branches of the right middle cerebral artery also contained adult non-gravid *D. immitis* that occluded the vessels. Marked fibrosis of the cerebral arterial walls containing the parasites was found. Fibrous villi arising from the intima associated with the parasite were identified. The *D. immitis* within the meningeal arteries appeared viable and were not associated with the granulomatous response that the dead *D. immitis* had stimulated. The *D. immitis* removed from the right posterior communicating artery was alive at the time of the necropsy.

A case involving a 7-year-old military dog that was euthanized because of posterior paralysis secondary to spinal cord compression was reported by Olson (1970). At necropsy, immediately after euthanasia, 14 male and 16 female *D. immitis* were present in the right ventricle. No lung pathology was noted. When the brain was removed, an adult *D. immitis* was found extending from the right internal carotid artery. Further histologic studies revealed that there were actually two adult *D. immitis* extending into branches of the artery. The only clinical signs that the dog had shown related to interference with circulation of blood to the brain was its desire to stand with its head pressed against the wall.

Three cases in dogs involving *D. immitis*-induced cerebral infarctions were described by Kotani et al. (1975). These dogs all exhibited signs of central nervous system (CNS) effects, including unstable gait, circling, roaring, visual disorders, disturbed swallowing, and coma. In each case, a single adult *D. immitis* was found in the anterior, middle, or posterior cerebral arteries.

Segedy and Hayden (1978) also reported on a cerebral vascular accident caused by *D. immitis* in a dog that presented with acute onset of quadriparesis, vomiting, and diarrhea. Examination of the blood 2 months prior to onset of signs was negative for microfilariae. The dog showed excessive salivation and signs of confusion. The dog could stand only if supported and fell to the left side when it was not. Proprioception was absent in the left rear limb and forelimb, but all spinal reflexes were normal. The dog had a very high-pitched bark. An electroencephalogram on the second day of hospitalization showed a pattern compatible with a right frontal lobe lesion. Numerous laboratory tests were conducted, including analysis of serum enzymes, cerebrospinal fluid (CSF) cultures and cytology, blood gas analysis, and complete blood count. The dog was treated for electrolyte imbalance and dehydration but developed opisthotonus and died. At necropsy, a mass of *D. immitis* was found in the pulmonary artery and its branches. The right frontal lobe of the brain was friable and slightly cavitated, and a mature *D. immitis* was found filling the middle cerebral artery. Histopathology showed extensive necrosis and malacia in the right frontal lobe.

A case involving a 2-year-old Boston terrier with a history of depression, weight loss, and hind limb and tail paresis of 3 days duration was reported by Cooley et al. (1987). The dog was dehydrated and had severe motor weakness of the tail and hind limbs. A test for *D. immitis* was positive. In spite of administration of fluid and therapy for suspected encephalitis, the dog's condition worsened, and the owner requested euthanasia. At necropsy, the right ventricle contained five adult *D. immitis,* and there was a large mass of *D. immitis* in the pulmonary artery and its branches. The left femoral artery was thrombosed and contained an incorporated degenerating *D. immitis*. The quadriceps and gastrocnemius muscles were necrotic. The brain and spinal cord had multiple foci of inflammation involving the caudate nucleus, hypothalamus, thalamus, midbrain, cerebellum, medulla, and cervical, thoracic, and lumbar spinal cord. Degenerating microfilariae were found in several areas of the brain tissue, surrounded by macrophages.

Donahoe and Holzinger (1974) described a dog and a cat with *D. immitis* in the CNS. The dog, a 5-year-old male Chihuahua from Georgia, had become irritable, vomited intermittently, bumped into furniture, and would walk into a corner of a room and stand facing the wall. Following symptomatic treatment, during

which the dog responded and then relapsed several times, it died. At necropsy, several adult *D. immitis* were found in the right ventricle of the heart, and one adult male *D. immitis* was in the lateral ventricle of the brain. The second case involved a 7-year-old cat presented because of apparent blindness. Physical examination revealed that the cat was lethargic, salivating excessively, and unsteady when walking. The cat died 6 h after being admitted to the hospital. At necropsy, a female *D. immitis* measuring 15.3 cm in length was found in a vein in the right lateral ventricle of the brain, which was filled with blood.

A case of brain involvement in a cat with *D. immitis* has also been reported (Cusick et al., 1976). The owner of this male domestic shorthair cat weighing 12 lb (5.4 kg) complained that the cat had been anorexic for 3 days and was not drinking fluids. No vomiting had been observed. The tentative diagnosis was constipation, and the cat was discharged with instruction to administer mineral oil to the animal twice daily. A few days after discharge, the cat was seen again with the same complaint. Physical examination showed the colon and rectum to be empty. The cat was discharged with prednisolone and heptacillin and a diagnosis of visceral larva migrans. The cat was presented 3 weeks later for possible euthanasia. The cat was holding its head tilted to the left, and its left ear was drooping. It was reported to be circling to the left and was ataxic in the rear quarters. Heart and lungs were normal. Tentative diagnosis was CNS disturbance, possibly caused by ascarid migration. Fecal floatation was negative for parasite ova. Approximately 1 week later, the cat was presented dead. At necropsy, an immature female *D. immitis* (8 cm) was found in the right ventricle of the brain, and hydrocephaly was found on the right side. The heart contained one male and one female *D. immitis*, both of which were sexually immature.

Ader (1979) cited a case involving a 4-year-old male cat with a history of trembling, fever, and irritability of 4 days duration. Two days before admission, the cat developed tonoclonic convulsions at a rate of two–three per hour. At admission, the cat was semicomatose. CSF contained large numbers of *Staphylococcus epidermidis* and large numbers of neutrophils. Bacterial encephalitis was suspected, and the cat was treated with chloroamphenical. By the 5th day after admission, the seizures had ceased, and the cat could stand but circled to the right. By the 9th day, the cat began to eat, and the CSF was normal. During the following month, the cat's condition became static, and euthanasia was requested. At necropsy, a single *D. immitis* (20 cm in length) was found in the intercerebral longitudinal fissure. Histopathologic studies showed cross-sections of the *D. immitis* to be surrounded by a granulomatous response. The leptomeninges were involved as well.

Mandelker and Brutus (1971) also reported encephalitis in a 2-year-old dog and a 1-year-old cat that were euthanized and necropsied. A mature female *D. immitis* was found in the lateral ventricle of the brain of the cat, but no worms were found in the heart. The dog had a *D. immitis* in the subarachnoid cavity of the brain.

Faries et al. (1974) described a case of a 1-year-old cat presented with signs of listlessness and gagging that was treated for gastroenteritis but died 2 days later. Before death, the cat exhibited muscle spasms, paddling, and pressing of its head against the wall. At necropsy, two adult female *D. immitis* were found in the right ventricle of the heart, and one was found in the left anterior cerebral artery. Eggs in embryonic development and microfilariae were seen in the uterus of all three worms. Unfortunately, the blood of the cat was not examined for microfilariae.

Paralysis of varying degrees has also been reported due to *D. immitis* in the cerebrospinal canal. Luttgen and Crawley (1981) reported a case involving a 7-year-old mixed poodle presented 18 h after developing acute paralysis in the hind limbs. The dog had been very active prior to the time she was found unable to walk on her hind legs. Tentative diagnosis was thoracolumbar disc herniation, and steroids were given. Upon presentation to Auburn University Small Animal Clinic, no abnormalities were noted in the chest, and the dog had a temperature of 102.6 F. Neurologic examination revealed absence of locomotion, and static muscle tone was absent in both hind limbs. Radiographs were taken of the cervical, thoracic, and lumbar spine, and a myelogram was performed. The contrast media failed to flow caudally to T7–T8. The owner requested exploratory surgery. At surgery, while removing tissue from the epidural space, a 16-cm long *D. immitis* was removed from the spinal cord at the level of T12. Following surgery, blood was examined and found to contain many *D.*

immitis larvae. Granulation tissue removed from the epidural space contained microfilariae. The dog recovered from the posterior paralysis and after physical therapy, regained use of its hind limbs.

A report by Shires et al. (1982) involved a 3.5-year-old dog with acute pelvic limb paralysis. Neurologic examination indicated that the lesion was at the level of L5. The pectoral limb reflexes and cranial nerve functions were normal. Cisternal myelography showed that the water-soluble contrast media did not flow beyond the level of the midbody of T11. Lumbar myelography then provided evidence of cord compression from T11 to L5. Surgical intervention to achieve decompression was instituted immediately. In removing the dorsal laminae and articular facets of L2–L3 on the left side, a 24-cm long *D. immitis* was removed from the epidural space. The worm was later identified as an unfertilized female. Seven days after surgery, there was no neurological improvement. Ten days after surgery, the owners requested euthanasia. Necropsy showed that there was no area of decreased diameter of the spinal canal. Histologic examination of the cord showed damage from T7 caudally. There were 30–40 adult male and female *D. immitis* in the right atrium, right ventricle, and pulmonary arteries. Since the female was unfertilized, migration into the epidural space presumably occurred before sexual maturity was reached at around 68 days after infection. On the basis of the worm's length, it was estimated that the worm was at least 190 days old. There was no evidence of migration down the cord.

A case of recurring tetraparesis in a mature Pomeranian that had been treated for *D. immitis* infection 6 months before the onset of tetraparesis and had been on heartworm preventive medication since becoming amicrofilaremic was reported by Blass et al. (1989). Sixteen days before referral, the dog developed signs of neck pain and hind limb weakness, which progressed to non-ambulatory tetraparesis. Treatment with prednisone by the referring veterinarian resulted in the dog regaining the ability to walk 3 days after treatment began. However, episodic tetraparesis persisted, and the dog was referred to the School of Veterinary Medicine at Louisiana State University. Neurologic examination localized the lesion to the cranial aspect of the cervical region. Radiographically, the vertebral canal was normal. Lumbar and cisternal myelography localized the lesion at C2–C3 and indicated that it was an extradural mass dorsal in location. A presumptive diagnosis of extramedullary neoplasia was made. Surgical intervention was undertaken and a hemilaminectomy was performed at C2–C3. A tightly coiled parasite was found in the epidural space. The worm was 20 cm long and was identified as a non-gravid female *D. immitis*. Examination of the blood post-surgically for microfilariae was negative. The dog recovered rapidly after surgery and was discharged 4 days later. Three weeks after treatment, the dog was free of pain and only had mild weakness of the hind limbs.

Intraocular *D. immitis* has also been reported by Schnelle and Jones (1945). A large white worm was seen in the anterior chamber of 4-year-old dog presented because of a diffuse opacity of the right eye. The worm had rapid whipping movements and would disappear at times through the pupillary opening into the posterior chamber. Examination of the dog's blood did not show any microfilariae nor were microfilariae seen in a sample of the aqueous fluid removed from the eye and centrifuged. Surgery was performed, and a worm 12 cm in length, identified as an immature male *D. immitis*, was removed.

Beller (1962) reported a case in which the owner of a 7-year-old dog noted a 2-week history of the dog periodically acting startled for no apparent reason. Lacrimation began soon after in the left eye. The owner noted a foreign object in the eye. On examination by the veterinarian, a motile worm about 7.5 cm long was seen in the anterior chamber. The parasite evidenced photosensitivity and became actively motile when light was focused on the eye. Direct blood smears were negative for microfilariae. The worm was removed surgically and later identified as an immature female filarial worm, most likely *D. immitis*. Unfertilized ova were present in the uterus.

Bellhorn (1973) reported a case of ocular infection in a 16-month-old dog that developed an irritable right eye that had a diffuse corneal edema with episcleral vascular congestion, mild iritis, and a rapidly moving parasite in the anterior chamber. At surgery, the cornea was thickened, and a parasite approximately 7 cm long was removed and later identified as a male *D. immitis*. Nine months after surgery, the dog had circulating microfilariae and was treated for *D. immitis* infection. The left eye was normal throughout the course of treatment and the dog never had signs or

symptoms of systemic disease normally attributed to *D. immitis*.

Thornton (1978) reported four cases of ocular dirofilariasis in a 2-year period. Each dog was presented with a history of epiphora, pawing and rubbing the affected eye, and photosensitivity. Examination of the affected eye in each dog revealed an immature *D. immitis* floating freely in the anterior chamber. In each case, the cornea was opaque, and there appeared to be an increase in intraocular pressure. All of the worms were removed surgically, and the cornea cleared without scarring.

Carastro et al. (1992) noted that *D. immitis* is the most common reported canine intraocular parasite. They reviewed 21 such cases reported in dogs from Arkansas (1), Alabama (2), Illinois (5), Louisiana (4), Maryland (2), Mississippi (2), and Michigan, Missouri, New York, Ohio, Virginia (1 each). The dogs ranged from less than 6 months to 10 years of age. None of the dogs presented with symptoms of pulmonary dirofilariasis. Eleven did not have immunodiagnostic tests for *D. immitis* infection. Fourteen were microfilariae-negative by concentration techniques at the time of presentation. Thoracic radiographs were not done for 12 of the dogs. Nine were positive by immunodiagnostic techniques. Eight (all that were done) had pulmonary artery and right ventricular changes characteristic of *D. immitis* infection.

Schnelle and Jones (1945) also reported *D. immitis* from an interdigital abscess in the left hind foot of a dog presented because of recurrent lameness. When the abscess was incised, a live parasite 8 cm in length, identified as an immature female *D. immitis*, emerged from the wound. The dog was euthanized, and at necropsy the right atrium, right ventricle, and branches of the pulmonary arteries contained a large mass of *D. immitis*.

Elkins and Berkenblit (1990) reported a case involving the space between the third and fourth digits of the left rear foot. The dog would not bear weight on the left rear leg. There was a 5-cm diameter fluctuant swelling palpated between the toes. A Knott test was negative for microfilariae, but an ELISA test for *D. immitis* antigen was positive. Thoracic radiographs were normal. Surgical exploration of the lesion produced a filariform worm later identified as an adult male *D. immitis*. Two weeks after surgery, the dog was bearing weight on the foot, and the surgical incision had healed. Four weeks following surgery, an ELISA test was again positive, and the dog was treated for adult *D. immitis*.

Many of the authors of the papers cited above, and others, have reported these cases as examples of aberrant locations of *D. immitis* (Bistner and Sheirr, 1970; Otto, 1974). However, given the large number of worms discovered in these locations, the extensive and expensive work-ups carried out in many of these cases, the clinical signs and symptoms, which gave no indication of pulmonary dirofilariasis, even when this infection was later discovered in the dog, the size of the worms found in these other arterial locations, and the presence of both immature and mature *D. immitis* in these locations, what may well be ‘‘aberrant’’ is the fact that the owners were willing to pay for the procedures that eventually revealed the presence of *D. immitis*. It would be interesting to know how many veterinarians and owners would have been willing to perform or support further testing or diagnostic work-ups had any of those dogs presented only with characteristic signs and symptoms of pulmonary dirofilariasis with confirmation of *D. immitis* infection through serological or microfilarial testing. In the cases of CNS lesions or tetraparesis, how many owners would normally decide on euthanasia, which would have left the cause of the dog’s disease to go completely undiagnosed?

When a dog diagnosed with pulmonary dirofilariasis is treated but subsequently dies, how many veterinarians would be in a position to do more than examine the lungs and heart for evidence of worms? The relative ease and the non-invasive methods available for examination of the lungs for *D. immitis* may well account for the belief that the pulmonary arteries are the ‘‘normal’’ location of *D. immitis* when, in fact, it is only in that position that the infection is most easily detected.

Wong (1974) conducted studies on *Macaca* species of monkeys by injecting third-stage larvae subcutaneously. Necropsy of the infected monkeys 1–12 months after inoculation, followed by soaking of subcutaneous and muscle tissue revealed that at 3 months after inoculation, worms could be found inside subcutaneous nodules that were surrounded by intense eosinophil infiltration. Beyond 2 months, only one monkey had a live female worm in the muscle. Monkeys treated with prednisone, however, had *D.*

immitis in the branches of the pulmonary arteries as well as the right heart at necropsy at 10 and 15 months post-inoculation. The worm, a live adult female, measured 14.5 cm in length in the right ventricle and extended into the pulmonary artery at the post-mortem examination. Histopathologic studies on the lung tissue showed evidence of pulmonary arterial thrombosis with two degenerating *D. immitis* adults incorporated in the thrombus. There was fibrous thickening of the entima and hypertrophy of the muscles in the walls of the infected arteries. Radiographs of two prednisone-treated monkeys showed densities 2 cm in diameter that resembled the ''coin'' lesions seen in human cases involving the pulmonary arteries. Angiocardiography for two of the prednisone-treated monkeys 7 months post-inoculation showed longitudinal filling defects, some 1 mm in diameter, within the right, left, and main pulmonary arteries. These were considered to be heartworms, but no worms were seen in the right ventricle while the monkeys were still alive.

Johnsen et al. (1972) conducted experimental infections in gibbons. Third-stage larvae were administered, and four animals were necropsied 6–14 months after inoculation. Triangular areas of increased density extended from the hilus to the inferior margin of the lower lobe of the right lung. These densities were still visible, and live worms were recovered at necropsy from all four gibbons inoculated. Microfilariae were present in the female worms in some cases. Most of the worms recovered were females, although portions of a male were recovered from one gibbon. Histologically, the pulmonary arterial tree showed the lesions were similar to those produced in dogs, including intimal proliferation and thickening. Dead worms were associated with thrombi and granulomatous reactions in the affected arteries. In some areas, these extended into the arterial wall and involved the full thickness of the artery, extending into the surrounding lung and producing an eosinophilic pneumonitis.

These are the only two studies found for *D. immitis* in primates. They show that in a live animal, worms may be detected in the pulmonary arteries but are not seen in the right ventricle. Pulmonary thrombosis similar to that seen in humans can be induced; in some primates, it is not necessary to immunosuppress them in order for adult *D. immitis* to develop, and eosinophilic pneumonitis can result from death of the worms in pulmonary arteries with extension of the resulting inflammation into the surrounding lung parenchyma.

3. Dirofilariasis in extrapulmonary sites in humans

Although the vast majority of human cases of *D. immitis* infection have been detected in the lung, as with dogs, this may be due to the ease with which the lung can be surveyed. Involvement of other areas of the human, however, are reported, including the same regions that have been reported in dogs and cats.

Dobson and Welch (1974) reported that six of seven children with acute dirofilariasis showed cranial involvement manifested by epileptiform seizures and/or eosinophilic meningitis. One of the children had a confirmed ocular infection. Although most of these cases were diagnosed based on immunological tests, the authors concluded that *D. immitis* was the cause of the eosinophilic meningitis.

Moorehouse (1978) cited a case of intraocular *D. immitis* infection in a human. The worm measured 8.3 mm in length and was removed from the anterior chamber. The author concluded that the eye infection probably resulted from the normal migration of the developing worm in the SC tissues.

Tada et al. (1979) found a granuloma embedded in the adipose tissue of the mesentery in a 74-year-old man. The mass measured 3 cm × 1 cm × 1 cm and was attached to surrounding tissue. The morphology of the worm was well preserved and was diagnosed as a *Dirofilaria*, possibly *D. immitis*.

Theis et al. (2001) found an immature male *D. immitis* in a branch of the left testicular artery of a 28-year-old man that presented with a 3-cm nodule palpable in the left inguinal canal that was initially suspected of being an inguinal hernia. The lesion involved the left testicle and was thought to be a tumor. The testicle was removed and histological studies identified an immature male *D. immitis* in the artery. The identification was confirmed by polymerase chain reaction analysis of a segment of the worm recovered from the excised testicle.

A case reported by Kim et al. (2002) involved a 39-year-old man with a non-specific hepatic nodule in

segment VI. Computed tomography showed the lesion to be 2 cm × 1.3 cm. Resection of the nodule produced a 1.1 cm nodule that was found to contain sections of a nematode identified as an immature male *D. immitis*.

Taken as a whole, these papers indicate that many different arteries besides those in the lungs may be the site of infection and development of *D. immitis* in dogs, cats, and primates, including humans. There are many of these cases scattered through the literature, and there is clear evidence that the worms in some of these cases developed in these sites and did not arrive there due to septal defects in the heart.

4. Development of *Dirofilaria immitis* in its vertebrate host

The development and migration of *D. immitis* following natural and experimental infections has been reported in detail in at least two papers (Kume and Itagaki, 1955; Orihel, 1961). These studies indicate that neither the location of the migrating larvae nor their size supports an exclusive intracardiac destination. The studies also support the hypothesis that the pulmonary arteries are the site of development because the worms are too small at the time they enter the right ventricle to maintain themselves in that location and are instead swept into the distal branches of the pulmonary arteries. Cases in which worms have migrated into vessels on both the arterial and venous side of the circulation some distance from the lungs suggest that the real destination of these larvae are the blood vessels per se, and the infrequency of their discovery in extrapulmonary sites is not because such locations are ‘‘aberrant’’ but rather because other locations have not been routinely searched for *D. immitis*.

Kume and Itagaki (1955) conducted experimental infections in 19 dogs, with each dog receiving between 101 and 2,921 infective larvae by injection either s.c. or i.v. and necropsies conducted from 23 to 287 days after infection. The first worms were found in submuscular membranes, sc tissue, fat tissue, subserosa, and muscles of the whole body 67–73 days after 1164 infective larvae were administered s.c.

The dogs had been injected s.c. on the left neck, left abdomen, and left tarsus, yet larvae were found in tissues along the midline and the right side of the infected dogs as well as on the left side. The larvae found were less than 5.0 cm in length, and the smallest was 1.1 cm. Dogs injected i.v. with 627–1171 infective larvae had no worms found in any locations at 101–116 days after inoculation. The lymphatic system also was examined as a route out of the skin in natural transmission, and no evidence of larval invasion of the lymphatics was found between 2 and 53 h after feeding of infective mosquitoes. The greatest concentration of larvae was in the submuscular membranes 67–112 days after infection. The intermediate site for development of the larvae appears to be the submuscular membrane, and the larvae reach these sites from the dermis via tissue migration. What’s more, the larvae in this intermediate location were found all over the dogs’ bodies, and there was no indication that the larvae had tended to migrate towards the thorax of the dog. No worms of the size found in the submuscular membranes were ever found in the thorax, pericardium, or bronchi. In addition, if young worms were artificially inserted into other sites, such as the abdominal cavity or the thorax, they did not migrate to the heart but continued their development at the initial site of implantation. This indicates that location in the pulmonary arterial tree is not an essential feature for the worm to develop beyond the young larval stage.

Between 85 and 120 days after infection, the worms had moved from the intermediate sites of development. They had not reached the pulmonary arteries by 85 days, yet none were found in the intermediate locations after 120 days. The length of worms found in the intermediate locations ranged from 3.2 to 11.0 cm in length. If a worm measuring 3.2 cm were to enter a vessel on the venous side of the systemic circulation, the first capillary bed it would encounter is that of the pulmonary arterial system where it would be mechanically stopped from going further. If such a worm was to enter the arterial side of the circulation when it was 3.2 cm in length, it would be arrested in the arterioles of the artery and most likely, continue its development there, as shown by the artificial implantation of young worms in the thorax and abdomen.

Orihel (1961) studied the morphology of *D. immitis* larvae developing in the dog following inoculation of third-stage larvae obtained from the mouth parts of

Anopheles quadrimaculatus. The dogs were necropsied at specific times after the single infection. The skin of each dog was soaked in warm saline for 6 h. The body cavities, muscles, and viscera were also examined for worms, and the tissues soaked in saline and the sediment examined. After 90 days, only fourth-stage larvae were found, and the largest of these measured 2 cm. Orihel only found these larvae in the right side of the heart, but all of his examinations were performed post-mortem. Given that in the living dog examined by echocardiography or angiography, no *D. immitis* have ever been seen in the right ventricle, coupled with the fact that *D. immitis* has no attachment organs by which to fix itself to the endocardium, it seems unlikely that worms 2 cm or less in length could avoid being swept out of the right ventricle during systole. Wilcox (1960) reported that the primary location of *D. immitis* was in the pulmonary arteries, not the right ventricle. Adcock (1961) showed that frequently large numbers of *D. immitis* extended far out into the finer branches of the pulmonary arterial tree. Knight (1977) reported that the right caudal artery is preferentially affected in canine dirofilariasis. Buoro and Atwell (1983) provided evidence to indicate that the angle of deviation of the right pulmonary artery from the pulmonary trunk was larger and more ventral than that of the left, and the right pulmonary artery had a greater mean diameter (8.70 mm) than the left (7.60 mm). These authors concluded that the outflow track from the right ventricle follows a curved pathway, and the right pulmonary artery could be expected to receive more blood flow than the left. Thus, most of the larvae contained in the ejection fraction would be expected to enter the right caudal artery, as this is the largest branch of the right pulmonary artery. Atwell et al. (1986) conducted studies in which one dead female adult *D. immitis* was inserted into the anterior vena cava of 10 puppies through an incision in the jugular vein. Two puppies were necropsied at 1, 3, 5, 7, and 9 days after surgery. The lungs were infused with 10% formalin via the trachea, and the heart and lungs were placed in 10% buffered formalin for at least 4 days before dissection of the heart and lungs. At the time of dissection, all 10 filariae were found in the right pulmonary artery; none were present in the right ventricle. This means that the adult female worms, nearly 20 cm in length, were ejected from the right ventricle into the pulmonary artery. Fourth-stage larvae are one-tenth this length, and could be expected to be expelled from the right ventricle with little difficulty.

Considering *D. immitis* in humans has been found in nearly all of the anatomical locations that it has been reported in dogs, cats, and other primates, it would appear that the migration and final resting site of the fifth-stage immature adult follows an identical sequence. The young larvae may migrate in the SC tissues some distance from their site of entry; many may die in the SC tissues where they are incorporated into granuloma that are small and not clinically apparent (Orihel, 1961; Wong, 1974). At the end of the fourth stage, the larvae enter vessels, and the vessels they enter determine where they will ultimately lodge. The walls of the veins may provide an easier entry, but veins and arteries are anatomically often in close proximity. If a fourth-stage larva enters an artery, it will not reach the venous side of the circulation and will likely develop in the branch of the arterial tree where it lodges. On the other hand, if a fourth-stage larva enters a vein anywhere in the peripheral vascular system, it will arrive in the lungs. Therefore, the preponderance of *D. immitis* in the pulmonary artery and its branches is not because this is the preferred site of development, but rather because veins carry blood towards the heart, and the pulmonary arterial tree is the first capillary bed that will arrest the movement of such larvae.

5. Differential diagnosis of the coin lesion in humans

As mentioned above, the significance of pulmonary dirofilariasis in humans is not due to its production of overt disease. As reported by several reviews, fewer than 50% of the patients with pulmonary dirofilariasis have symptoms at the time the coin lesion is discovered (Moorehouse et al., 1971; Neafie and Piggott, 1971; Neafie et al., 1976; Robinson et al., 1977; Ciferri, 1982; Kochar, 1985; Ro et al., 1989; Asimacopoulos et al., 1992) (Table 1).

Coin lesions pose a considerable problem to the physician where diagnosis is concerned (Seybold, 1964; Zelch et al., 1973; Toomes et al., 1983). At least 20 different pathological conditions have been found

to cause coin lesions (Seybold, 1964). These causes can be grouped into five major categories: primary malignant tumors, metastatic tumors, benign tumors, cysts, and inflammatory granulomas. Coin lesions generally range between 1 and 3 cm in diameter (Seybold, 1964; Toomes et al., 1983; Liptay, 1999). Those proven to be due to *D. immitis* have ranged from 1 to 4.5 cm in diameter (Table 1). The vast majority of patients (74% in a review of 955 cases), regardless of the ultimate diagnosis are asymptomatic at the time the coin lesion is discovered (Toomes et al., 1983). Few coin lesions larger than 5 cm in diameter are benign (Seybold, 1964). However, 27% of coin lesions 1–4 cm in diameter are malignant (Sharp and Kinsella, 1950). Bronchoscopy, bacteriologic examination of the sputum, or culture of transtracheal washings, are usually not contributory. Examination of transtracheal washings or brushing for malignant cells, if negative, does not rule out a malignancy because carcinoma in the periphery of the lung is the least likely of all bronchogenic cancers to exfoliate malignant cells (Seybold, 1964). The vast majority of coin lesions due to *D. immitis* are located in the peripheral areas of the lungs, and all lobes of the lung may be involved in primary or metastatic malignant coin lesions as are coin lesions caused by *D. immitis* (Seybold, 1964; Toomes et al., 1983) (Table 1). Approximately, one out of every 500 chest radiograms taken shows a newly formed solitary pulmonary nodule (those having a diameter of 3 cm or less) (Lillington, 1997; Liptay, 1999). Each year in the United States, 150,000 patients are found to have such a lesion (Liptay, 1999). Lesions larger than 3 cm are referred to as masses and have a distinctly higher proportion of malignant diagnoses. Lesions due to *D. immitis* may also fall into this size category (Table 1). Newly detected solitary nodules are considered by most physicians to be malignancies until proven otherwise (Lillington, 1997; Liptay, 1999).

The accuracy in diagnosing solitary pulmonary nodules by radiologic assessment has not changed significantly in over 30 years (Liptay, 1999). Between 25 and 40% of malignant nodules are classified as benign using radiologic characteristics of the nodule alone (Edwards and Cox, 1962; Gurney et al., 1993).

The overall 5-year survival rate in patients diagnosed with bronchogenic carcinoma is roughly 15% (Lillington, 1997; Henschke et al., 1999). However, the resection of solitary nodular bronchogenic carcinoma has a 5-year survival rate of 50%, and if the nodule is 1 cm or less at the time of resection, the 5-year survival rate increases to about 80% (Lillington, 1997). Therefore, the surgeons' recommendations, in cases of solitary pulmonary nodules of the size produced by *D. immitis*, are most often a wedge resection of the lesions via thoracotomy (Table 1). This technique has the advantage of providing the pathologist with ample material for diagnostic procedures and also may be curative (or at least improve the patient's chance of surviving 5 or more years) if the lesions prove to be neoplastic.

However, thoracotomy carries some surgical risk. Mortality rates between 0.3 and 7.3% at surgery have been reported (Jackman et al., 1969; Cummings et al., 1986). Benign and infectious granulomas do not require immediate resection and may never require such an approach. These types of nodules may be observed by CT scans over time for evidence of change without risking surgical intervention (Lillington, 1997). Alternatively, thoracoscopic biopsy or resection may be used to obtain a sample of the lesion for diagnosis with much less risk of morbidity and mortality compared to open thoracotomy for wedge resection (Liptay, 1999; Swanson et al., 1999). Although there have been two papers published in which pulmonary dirofilariasis was diagnosed by percutaenous needle aspiration, the size of the granuloma in comparison with the size of the worms in such granulomas makes their inclusion in the biopsy fortuitous rather than predictable (Hawkins et al., 1985; Kelly et al., 1985) and complications from such an approach do occur (Peters and Kubitschek, 1984; Westcott, 1988; Grogan et al., 1990; Gruden and Stern, 1994).

The human medical literature indicates that physicians believe that *D. immitis* initially comes to reside in the right ventricle and, upon its death, is embolized to the pulmonary artery. As has been noted above from studies in dogs, this belief is unfounded. In fact, several authors have remarked that the lesion seen in human pulmonary dirofilariasis does not radiologically resemble an embolic infarct, which is pyramidal in shape, whereas the coin lesion is spherical (Feld, 1973; Navarrete-Reyna and Noon, 1968; Kochar, 1985; Smith and Schillaci, 1986; Bradham et al., 1990; Asimacopoulos et al., 1992).

A much more likely sequence of events is that the fourth-stage larvae reaches the fine branches of the pulmonary artery and upon moulting to the fifth stage, induces a vasculitis and then dies, stimulating a granuloma. Many papers note that some time prior to discovery of the coin lesion, the patient had signs and symptoms of a pneumonitis and was, in some cases, diagnosed as having pneumonitis, and was treated with antibiotics as an outpatient. Only at follow-up, after the lung had cleared, was the coin lesion discovered (Goodman and Gore, 1964; Tannehill and Hatch, 1968; Navarrete, 1972; Feld, 1973; Hoch et al., 1974; Kochar, 1985; Smith and Schillaci, 1986).

6. The vascular reaction to *Dirofilaria immitis*

The lesions in the pulmonary arterial branches in dogs supports this sequence of events (Schaub and Rawlings, 1980). Adcock (1961) reported pulmonary arteritis to be present in 42 dogs infected with *D. immitis* and, in all cases, the worms were still alive, whereas in the arteries with granuloma formation, all the worms were dead. Atwell et al. (1986) inserted one dead adult female *D. immitis* into each of 10 puppies. Although all worms were inserted into the anterior vena cava at necropsy 1, 3, 5, 7, and 9 days post-insertion, all worms were found in the pulmonary artery. Microscopic pathology 1 day after placement showed endarteritis, periartenitis, and hemorrhage within the arterial wall. There was extensive perivascular inflammatory edema and hemorrhage in the surrounding alveoli. By Day 5 after placement, active fibroblast proliferation and lung consolidation due to granulomatous inflammation was seen in the interstitial tissue adjacent to the artery containing the dead worm. By Days 7 and 9, the arterial walls were indistinct, and the lung parenchyma around the infected vessel showed more advanced granulomatous reaction, but the remaining lung parenchyma remained unaffected. Atwell et al. (1985) inserted flexible polyvinyl chloride threads, similar in size, shape, and flexibility, to adult female *D. immitis*, into the pulmonary arteries of dogs and showed that the intima of the pulmonary arteries had lesions nearly identical to those reported by Adcock (1961) from live filarial worms in dogs' pulmonary arteries. Atwell concluded that mechanical irritation from the threads was responsible for the endothelial and intimal changes, just as Adcock (1961) had postulated that the response to contact with live *D. immitis* had induced such changes.

Most of the *D. immitis* identified from coin lesions in the human lung have been immature adults recently moulted from fourth-stage larvae (Table 1). Release of moulting fluid may well induce or contribute to the initiation of vasculitis, and antigens from the dead worm may continue to stimulate the vascular inflammation, leading to escape of fluid from the vessel producing the interstitial edema and pleural effusion reported in many cases (Table 1). This, in turn, can produce the symptoms (cough, chest pain, and occasionally hemoptisis) reported in human cases. Peripheral eosinophilia and infiltration of eosinophils into the granuloma have been reported in some cases and suggests IgE production by the host in response to the antigens released from the dead worm (Lau and Pierson, 1972; Prioleau et al., 1976; Lum, 1985) (Table 1). These coin lesions may remain stable in size for at least 4 years, and therefore, may be discovered by routine chest radiograms long after the infection has taken place (Ro et al., 1989). Patients might not remember having signs and symptoms indicative of a pneumonitis by the time the end-stage granuloma is discovered.

The microscopic vascular lesions seen in humans with pulmonary dirofilariasis, including villious intimal hyperplasia and fibroblastic proliferation, are strikingly similar to those described in dogs, indicating that the worms have been present and alive long enough to stimulate such a response (Beskin et al., 1966; Neafie and Piggott, 1971; Lau and Pierson, 1972; Prioleau et al., 1976; Robinson et al., 1977; Scully and Galdabini, 1979; Lum, 1985; Ro et al., 1989; Asimacopoulos et al., 1992).

The medical literature continually refers to pulmonary dirofilariasis as a ''rare'' occurrence in humans; however, its infrequent diagnosis might be based on the fact that pulmonary dirofilariasis is not considered in patients with pneumonitis, but is considered only in those with discovered coin lesions (the end-stage of infection) that are evaluated for the cause of the nodule. Even when the nodule is resected, the discovery and proper identification of *D. immitis* may not occur. Many sections of the granuloma are often needed to find the worm because the part containing the worm may be trimmed off during

preparation of the nodule for histologic sectioning (Harrison and Thompson, 1965; Beskin et al., 1966; Prioleau et al., 1976; Scully and Galdabini, 1979; Ro et al., 1989). The worm sections may be overlooked even when present (Navarrete-Reyna and Noon, 1968). The etiology of the coin lesion may go undiagnosed. In a series of 900 such lesions reported in one hospital in Virginia over a 5-year period (1995–2000), 24 granulomas removed by thoracotomy went undiagnosed as to cause (Warren, S., Theis, M.D., personal communication). The worms, if found, may be misdiagnosed (Beaver and Orihel, 1965; Navarrete, 1972). Green et al. (1994) have described a non-specific fluorescent whitener stain that improved the recognition of *D. immitis* in granulomas, even when only fragments of the worm were present in 20 cases of pulmonary dirofilariasis collected from Houston, Texas hospitals.

7. The need for serologic tests to diagnose *Dirofilaria immitis* in humans

If a more accurate evaluation of human *D. immitis* infection is to be achieved, indirect tests must be developed and applied to people living in highly enzootic areas of canine *D. immitis* infections. These tests should be used to evaluate patients with pneumonitis as well as those with coin lesions.

Currently, there are no commercially available serologic tests for this purpose. However, experimental indirect hemagglutination and ELISA tests have been used on sera from human patients with histologically confirmed pulmonary dirofilariasis (Glickman et al., 1986) and in seroepidemiological studies. The accuracy of these tests clearly indicates that with careful selection of the *D. immitis* antigens used, specific antibody detection tests are possible (Ambrose-Thomas and Truong, 1974; Welch and Dobson, 1974; Konishi, 1989; Alvarez et al., 1990; Simon et al., 1991; Muro et al., 1991; Kurniawan et al., 1993), just as it was possible to develop such tests for the diagnosis of canine dirofilariasis (Grieve et al., 1981, 1983; Hamilton et al., 1983; Glickman et al., 1984; Weil et al., 1985; Tamashiro et al., 1985). The antigens chosen for such tests in humans must be shown not to react with antibody in humans against *Toxocara canis*, *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Ancylostoma duodenale*, and *Necator americanus*. All of these nematodes have a migratory pathway that potentially exposes humans to antigens and will, therefore, stimulate an antibody response. In regions outside the United States, antibody to taxonomically more distantly related helminths, such as *Taenia solium* and *Clonorchis sinensis*, may have to be shown to be non-cross-reactive with the *D. immitis* antigen.

If the human medical community was supplied with such a test, the extent of human infection with *D. immitis* would be more accurately evaluated, and the health-care cost of differentiating *D. immitis* granulomas from more significant lesions could be greatly reduced. Currently, the differential diagnosis of pulmonary dirofilariasis costs $80,000 or more per patient. In addition, it exposes the patient to unnecessary surgery, which carries a risk of mortality. If a physician knew that a patient lived in an area of enzootic canine *D. immitis*, had a pneumonitis up to as long as 4 years before a coin lesion was discovered, and had a specific and sensitive serologic test to evaluate the presence of antibody to *D. immitis*, a positive serologic test would justify a more conservative approach to the diagnosis of the coin lesion (Lillington, 1997, 2001) as a result of improvement in imaging techniques, CT scans, positron emission tomography (PET) scans, and MRI are replacing the plain radiogram in the evaluation of solitary pulmonary nodules (SPN) (Siegelman et al., 1980; Lillington, 2001). These techniques provide the ability to detect much smaller nodules, measure them more accurately, and determine their morphological characteristics (Mulshine and Henschke, 2000; Lillington, 2001). This information, if supportive, can justify a ‘‘wait-and-watch’’ approach to monitoring SPN, which in the case of granulomatous coin lesions, do not usually require a thoracotomy. This more conservative approach is less costly and carries less risk to the patient (Lillington, 2001). At the same time, the discovery of significant human infection via the application of these serological tests in epidemiological studies could provide the veterinary medical profession with a public health justification for obtaining financial support to control the infection in dogs. From data presented in Table 2 and Fig. 1, it is clear that the preponderance of coin lesions reported to date are from humans living in states where canine

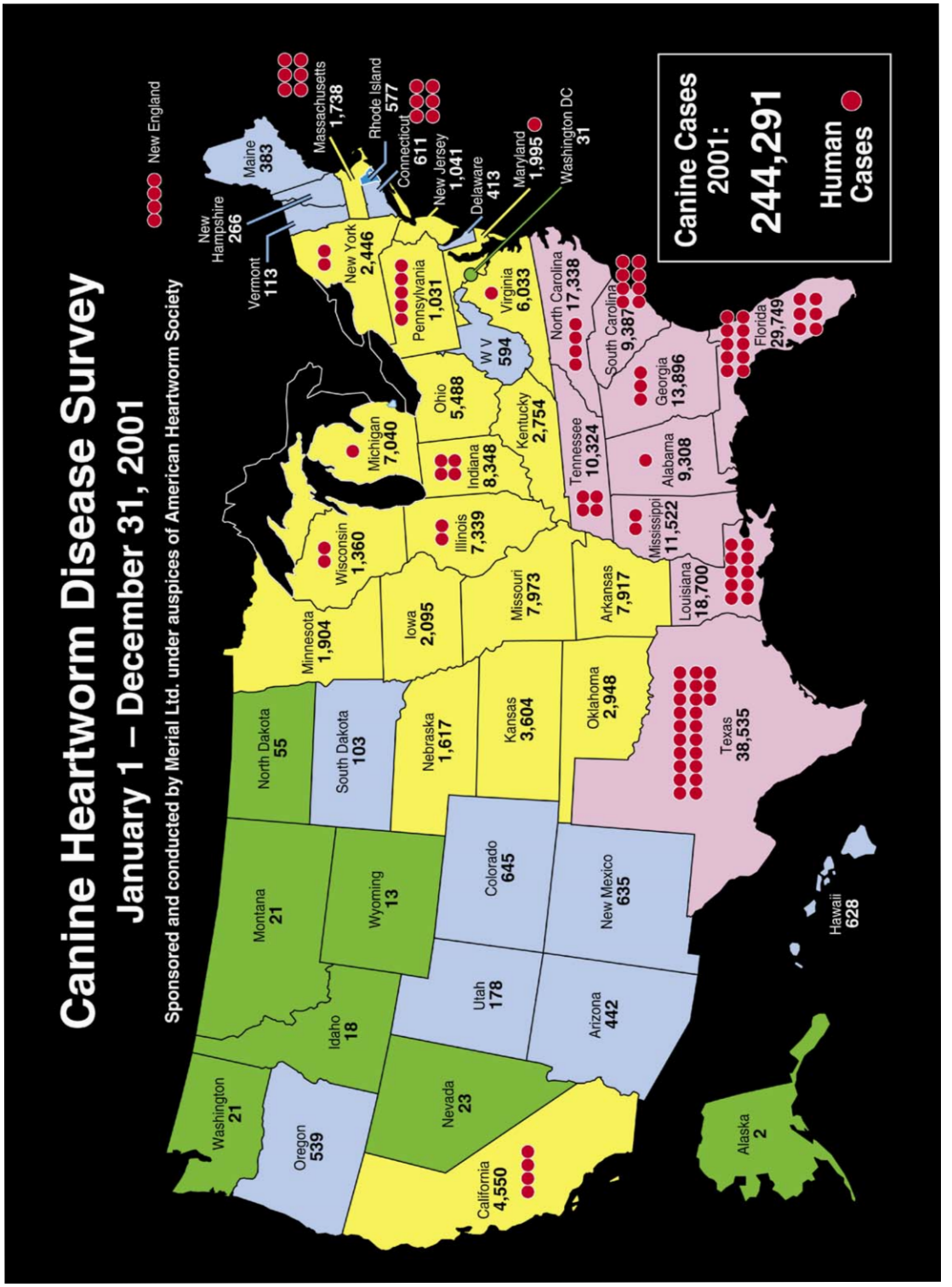
Canine Heartworm Disease Survey
January 1 – December 31, 2001
Sponsored and conducted by Merial Ltd. under auspices of American Heartworm Society
New England
Maine 383
New Hampshire 266
Vermont 113
Massachusetts 1,738
Rhode Island 577
Connecticut 611
New Jersey 1,041
Delaware 413
Maryland 1,995
Washington DC 31
New York 2,446
Pennsylvania 1,031
W V 594
Virginia 6,033
North Carolina 17,338
South Carolina 9,387
Georgia 13,896
Florida 29,749
Alabama 9,308
Mississippi 11,522
Louisiana 18,700
Tennessee 10,324
Kentucky 2,754
Ohio 5,488
Indiana 8,348
Michigan 7,040
Illinois 7,339
Wisconsin 1,360
Minnesota 1,904
Iowa 2,095
Missouri 7,973
Arkansas 7,917
Oklahoma 2,948
Texas 38,535
Kansas 3,604
Nebraska 1,617
South Dakota 103
North Dakota 55
Montana 21
Wyoming 13
Colorado 645
New Mexico 635
Arizona 442
Utah 178
Nevada 23
Idaho 18
Washington 21
Oregon 539
California 4,550
Alaska 2
Hawaii 628
Canine Cases 2001: 244,291
Human Cases

Fig. 1.

Table 2
Occurrence of human cases of *Dirofilaria immitis* by State

Texas	23
Florida	16
Louisiana	10
South Carolina	8
Connecticut	6
Massachusetts	6
Pennsylvania	5
California	4
Indiana	4
New England	4
North Carolina	4
Tennessee	4
Georgia	3
Illinois	2
Mississippi	2
New York	2
Wisconsin	2
Alabama	1
Maryland	1
Michigan	1
Southeastern U.S.	1
Virginia	1
Total	110

dirofilariasis is highly enzootic. However, such lesions in humans are being reported more frequently from many states where *D. immitis* in dogs is less common. This trend is likely to continue unless *D. immitis* in dogs is brought under control and greatly reduced. At a conservative $80,000 per patient in medical costs, the 110 reported cases of human pulmonary dirofilariasis in the United States alone carry a cost of $8.8 million.

References

Abadie, S.H., Swartzwelder, J.C., Holman, R.L., 1965. A human case of *Dirofilaria immitis* infection. Am. J. Trop. Med. Hyg. 14, 117–118.

Adcock, J.L., 1961. Pulmonary arterial lesions in canine dirofilariasis. Am. J. Vet. Res. 22, 655–662.

Ader, P., 1979. Heartworm (*Dirofilaria immitis*) in the brain of a cat – Review and case report. California Veterinarian 33, 23–25.

Allison, R.D., Vincent, A.L., Greene, J.N., Sandin, R.L., Field, T., 2004. Infectious pulmonary nodules mimicking lung carcinoma. Infect. Med. 21, 181–186.

Alvarez, A.M., Sanchez, M.C., Martin, J.M., Martin, F.S., 1990. Seroepidemiological studies on human pulmonary dirofilariasis in Spain. Ann. Trop. Med. Parasitol. 84, 209–213.

Ambrose-Thomas, P., Truong, T.K., 1974. Application of the indirect fluorescent antibody test on sections of adult filariae to the serodiagnosis, epidemiology, and post-therapeutic surveillance of human filariasis. Ann. Trop. Med. Parasitol. 68, 435–451.

Asimacopoulos, P.J., Katras, A., Christie, B., 1992. Pulmonary dirofilariasis. The largest single-hospital experience. Chest 102, 851–855.

Atwell, R.B., Buoro, I.B.J., Sutton, R.H., 1985. Experimental production of lesions in canine pulmonary arteries similar to those produced by *Dirofilaria immitis* infection. Vet. Rec. 116, 539–541.

Atwell, R.B., Sutton, R.H., Buoro, I.B.J., 1986. Early pulmonary lesions caused by dead *Dirofilaria immitis* in dogs exposed to homologous antigens. Br. J. Exp. Pathol. 67, 395–405.

Awe, R.J., Mattox, K.L., Alvarez, A., Stork, W.J., Estrada, R., Greenberg, S.D., 1975. Solitary and bilateral pulmonary nodules due to *Dirofilaria immitis*. Am. Rev. Respir. Dis. 112, 445–449.

Beaver, P.C., Horner, G.S., Bilos, J.Z., 1974. Zoonotic onchocercosis in a resident of Illinois and observations on the identification of *Onchocerca* species. Am. J. Trop. Med. Hyg. 23, 595–607.

Beaver, P.C., Orihel, T.C., 1965. Human infection with filariae of animals in the United States. Am. J. Trop. Med. Hyg. 14, 1010–1029.

Beller, J., 1962. Parasite in eye of a dog. Sm. An. Clin. 2, 681–683.

Bellhorn, R.W., 1973. Removal of intraocular *Dirofilaria immitis* with subsequent corneal scarring. Am. Anim. Hosp. Assoc. J. 9, 262–264.

Beskin, C.A., Colvin, S.H., Beaver, P.C., 1966. Pulmonary dirofilariasis cause of pulmonary nodular disease. J. Am. Med. Assoc. 198, 197–199.

Bistner, S., Sheirr, B., 1970. Aberrant location of *Dirofilaria immitis*. Vet. Med./SAC 65, 867–870.

Blass, C.E., Holmes, R.A., Neer, T.M., 1989. Recurring tetraparesis attributable to a heartworm in the epidural space of a dog. J. Am. Vet. Med. Assoc. 194, 787–788.

Bradham, R.R., Locklair, P.R., Grimball, A., 1990. Bilateral pulmonary nodules caused by *Dirofilaria immitis*. Ann. Thorac. Surg. 50, 312–313.

Brown, H.W., 1939. Observations on the dog heartworm *Dirofilaria immitis*. North Am. Vet. 20, 49–55.

Buoro, I.B.J., Atwell, R.B., 1983. Angles at branching and the diameters of pulmonary arteries in relation to the distribution of pulmonary lesions in canine dirofilariasis. Res. Vet. Sci. 35, 353–356.

Burt, J.K., Lipowitz, A.J., Harris, J.A., 1977. Femoral artery occlusion by *Dirofilaria immitis* in a dog. J. Am. Vet. Radiol. Soc. XVIII, 166–169.

Carastro, S.M., Dugan, S.J., Paul, A.J., 1992. Intraocular dirofilariasis in dogs. Compend. Contin. Educ. Pract. Vet. 14, 209–217.

Ciferri, F., 1981a. Human pulmonary dirofilariasis in the west. West. J. Med. 134, 158–162.

Ciferri, F., 1981b. Diagnosis of human pulmonary dirofilariasis. West. J. Med. 134, 547.

Ciferri, F., 1982. Human pulmonary dirofilariasis in the United States: A critical review. Am. J. Trop. Med. Hyg. 31, 302–308.

Cooley, A.J., Clemmons, R.M., Gross, T.L., 1987. Heartworm disease manifested by encephalomyelitis and myositis in a dog. J. Am. Vet. Med. Assoc. 190, 431–432.

Cummings, S.R., Lillington, G.A., Richard, R.J., 1986. Managing solitary pulmonary nodules: The choice of strategy is a "close call". Am. Rev. Respir. Dis. 134, 453–460.

Cusick, P.K., Todd, K.S., Blake, J.A., Daley, W.R., 1976. *Dirofilaria immitis* in the brain and heart of a cat from Massachusetts. Am. Anim. Hosp. Assoc. J. 12, 490–491.

Darrow, J.C., Lack, E.E., 1981. Solitary lung nodule due to *Dirofilaria immitis* (dog "heartworm"). J. Surg. Oncol. 16, 219–224.

Dashiell, G.F., 1961. A case of dirofilariasis involving the lung. Am. J. Trop. Med. Hyg. 10, 37–38.

Dayal, Y., Neafie, R.C., 1975. Human pulmonary dirofilariasis. A case report and review of literature. Am. Rev. Respir. Dis. 112, 437–443.

De Magalhaes, P.S., 1887. Descripcào de uma especie de filarias encontradas no coracào humano, precedida de uma contribuicâo para o estudo de filariose de Wucherer e do respectivo parasita aduto, a Filaria Bancrofti (Cobbold ou Filaria sanguinis hominis (Lewis). Rev dos cursos praticos e theorico de faculd de Med. do Rio de Janeiro 3, 193–214.

Dobson, C., Welch, J.S., 1974. Dirofilariasis as a cause of eosinophilic meningitis in man diagnosed by immunofluorescence and arthus hypersensitivity. Trans. R. Soc. Trop. Med. Hyg. 68, 223–228.

Donahoe, J.M.R., Holzinger, A., 1974. *Dirofilaria immitis* in the brains of a dog and a cat. J. Am. Vet. Med. Assoc. 164, 518–519.

Edwards, W.M., Cox, R.S., 1962. The solitary nodule (coin lesion) of the lung: An analysis of 52 consecutive cases treated by thoracotomy and a study of preoperative diagnostic accuracy. Am. J. Radiol. 88, 1020–1042.

Elkins, A.D., Berkenblit, M., 1990. Interdigital cyst in the dog caused by an adult *Dirofilaria immitis*. J. Am. Anim. Hosp. Assoc. 26, 71–72.

Ewert, A., 1967. Studies on the transfer of infective *Brugia pahangi* larvae from vector mosquitoes to the mammalian host. Trans. R. Soc. Trop. Med. Hyg. 61, 110–113.

Ewert, A., Ho, B.C., 1967. The fate of *Brugia pahangi* larvae immediately after feeding by infective vector mosquitoes. Trans. R. Soc. Trop. Med. Hyg. 61, 659–662.

Faries, F.C., Mainster, M.E., Martin, P.W., 1974. Incidental findings of *Dirofilaria immitis* in domestic cats. Vet. Med./Sm. An. Clin. 69, 599–600.

Faust, E.C., Thomas, E.P., Jones, J., 1941. Discovery of human heartworm Infection in New Orleans. J. Parasitol. 27, 115–122.

Feld, H., 1973. *Dirofilaria immitis* (dog heartworm) as a cause of a pulmonary lesion in man. Radiology 108, 311–312.

Fülleborn, F., 1908. Über versuche an Hundefilarien und deren Übertragung durch Mücken. Arch. Für Schiffs Tropen Hyg., Beiheft 8 (12), 331–343.

Gershwin, L.J., Gershwin, M.E., Kritzman, J., 1974. Human pulmonary dirofilariasis. Chest 66, 92–96.

Glickman, L.T., Grieve, R.B., Breitschwerdt, E.B., Mika-Grieve, M., Patronek, G.J., Domanski, L.M., Root, C.R., Malone, J.B., 1984. Serologic pattern of canine heartworm (*Dirofilaria immitis*) infection. Am. J. Vet. Res. 45, 1178–1183.

Glickman, L.T., Grieve, R.B., Schantz, P.M., 1986. Serologic diagnosis of zoonotic pulmonary dirofilariasis. Am. J. Med. 80, 161–164.

Goggin, J.M., Biller, D.S., Rost, C.M., DeBay, B.M., Ludlow, C.L., 1997. Ultrasonographic identification of *Dirofilaria immitis* in the aorta and liver of a dog. J. Am. Vet. Med. Assoc. 210, 1635–1637.

Goodman, M.L., Gore, I., 1964. Pulmonary infarct secondary to *Dirofilaria* larvae. Arch. Int. Med. 113, 702–705.

Green, L.K., Ansari, M.Q., Schwartz, M.R., Ro, J.Y., Alpert, L.C., 1994. Non-specific fluorescent whitener stains in the rapid recognition of pulmonary dirofilariasis. A report of 20 cases. Thorax 49, 590–593.

Grieve, R.B., Lok, J.B., Glickman, L.T., 1983. Epidemiology of canine heartworm infection. Epidemiol. Rev. 5, 220–246.

Grieve, R.B., Mika-Johnson, M., Jacobson, R.H., Cypress, R.H., 1981. Enzyme-linked immunosorbent assay for measurement of antibody responses to *Dirofilaria immitis* in experimentally infected dogs. Am. J. Vet. Res. 42, 66–69.

Grogan, D.R., Irwin, R.S., Channick, R., Raptopoulos, V., Curley, F.J., Bartter, T., Corwin, R.W., 1990. Complications associated with thoracentesis. A prospective, randomized study comparing three different methods. Arch. Intern. Med. 150, 873–877.

Gruden, J.F., Stern, E.J., 1994. Bilateral pneumothorax after percutaneous transthoracic needle biopsy. Chest 105, 627–628.

Gurney, J.W., Lyddon, D.M., McKay, J.A., 1993. Determining the likelihood of malignancy in solitary pulmonary nodules with bayesian analysis. Part I: theory. Radiology 186, 405–413.

Hahn, A.W., 1960. Angiocardiography in canine dirofilariasis. II Utilization of a rapid film change technique. J. Am. Vet. Med. Assoc. 136, 355–358.

Hamilton, R.G., Scott, A.L., D'Antono, R., Levy, D.A., Adkinson, N.F., 1983. *Dirofilaria immitis*: Performance and standardization of specific antibody immunoassays for filariasis. Exp. Parasitol. 56, 298–313.

Harrison, E.G., Thompson, J.H., 1965. Dirofilariasis of human lung. Am. J. Clin. Pathol. 43, 224–234.

Hawkins, A.G., Hsiu, J.G., Smith, R.M., Stitik, F.P., Siddiky, M.A., Edwards, O.E., 1985. Pulmonary dirofilariasis diagnosed by fine needle aspiration biopsy. A case report. Acta Cytol. 29, 19–22.

Hayasaki, M., Oishi, I., Kobayashi, S., Kume, S., 1974. Seven cases of paradoxical embolism due to canine filariae. J. Jpn. Vet. Med. Assoc. 27, 2–9.

Henschke, C.I., McCauley, D.I., Yankelevitz, D.F., Naidich, D.P., McGuinness, G., Miettinen, O.S., Libby, D.M., Pasmantier, M.W., Koizumi, J., Altorki, N.K., Smith, J.P., 1999. Early lung cancer action project: overall design and findings from baseline screening. Lancet 354, 99–105.

Hoch, W.S., Wershba, M.E., Patchefsky, A.S., 1974. Pulmonary dirofilariasis. J. Thorac. Cardiovasc. Surg. 67, 159–163.

Hoerlein, B.F., Horne, R.D., Wood, J.P., Bartels, J.E., Hoff, E.J., 1972. An aortic occlusion in a dog caused by heartworms. Auburn Vet. 28, 71–81.

Jackman, R.J., Good, C.A., Clagett, O.T., Woolner, L.B., 1969. Survival rates in peripheral bronchogenic carcinomas up to four

centimeters in diameter presenting as solitary pulmonary nodules. J. Thorac. Cardiovasc. Surg. 57, 1–8.

Johnsen, D.O., DePaoli, A., Tanticharoenyos, P., Diggs, C., Gould, D.J., 1972. Experimental infection of the Gibbon (*Hylobates lar*) with *Dirofilaria immitis*. Am. J. Trop. Med. Hyg. 21, 521–527.

Kahn, F.W., Wester, S.M., Agger, W.A., 1983. Pulmonary dirofilariasis and transitional cell carcinoma benign lung nodules mimicking metastatic malignant neoplasms. Arch. Intern. Med. 143, 1259–1260.

Kelly, W.T., Roszkowski, A., Firouz-Abadi, A.A., Zimmerman, P.V., 1985. Pulmonary dirofilariasis diagnosed by computerised tomography scan controlled percutaneous needle aspiration. Aust. N. Z. J. Med. 15, 656–657.

Kim, M.K., Kim, C.H., Yeom, B.W., Park, S.H., Choi, S.Y., Choi, J.S., 2002. The first human case of hepatic dirofilariasis. J. Korean Med. Sci. 17, 686–690.

Knight, D.H., 1977. Heartworm heart disease. In: Brandly, C.A., Cornelius, C.E. (Eds.), Advances in Veterinary Science and Comparative Medicine, vol. 21. Elsevier, London, pp. 107–149.

Kochar, A.S., 1985. Human pulmonary dirofilariasis. Report of three cases and brief review of the literature. Am. J. Clin. Pathol. 84, 19–23.

Konishi, E., 1989. Yearly change in antibody level to *Dirofilaria immitis* (Spirurida: Filariidae) among humans exposed naturally to infected mosquitoes. J. Med. Entomol. 26, 489–490.

Kotani, T., Tomimura, T., Ogura, M., Yoshida, H., Mochizuki, H., 1975. Cerebral infarction caused by *Dirofilaria immitis* in three dogs. Jpn. J. Vet. Sci. 37, 379–390.

Kume, S., Itagaki, S., 1955. On the life cycle of *Dirofilaria immitis* in the dog as the final host. Br. Vet. J. 111, 16–24.

Kurniawan, A., Yazdanbakhsh, M., Ree, R.V., Aalberse, R., Selkirk, E., Partono, F., Maizels, R.M., 1993. Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis. J. Immunol. 150, 3941–3950.

Lau, T., Pierson, K.K., 1972. Dog heartworm infection in man. Lab. Med. 3, 41–43.

Levine, S.E., Mossler, J.A., Woodard, B.H., 1980. *Dirofilaria immitis*: A zoonosis of clinical concern. Southern Med. J. 73, 749–754.

Lillington, G.A., 1997. Management of solitary pulmonary nodules. How to decide when resection is required. Postgrad. Med. 101 (3), 145–150.

Lillington, G.A., 2001. Solitary pulmonary nodules: New wine in old bottles. Curr. Opin. Pulm. Med. 7, 242–246.

Liptay, M.J., 1999. Solitary pulmonary nodules. Treatment options. Chest 116 (6, Suppl.), 517S–518S.

Liu, S.K., Das, K.M., Tashjian, R.J., 1966. Adult *Dirofilaria immitis* in the arterial system of a dog. J. Am. Vet. Med. Assoc. 148, 1501–1507.

Lum, G.B., 1985. An unusual etiology of multiple pulmonary nodules. Invest. Radiol. 20, 574–578.

Luttgen, P.J., Crawley, R.R., 1981. Posterior paralysis caused by epidural dirofilariasis in a dog. J. Am. Anim. Hosp. Assoc. 17, 57–59.

Mandelker, L., Brutus, R.L., 1971. Feline and canine dirofilarial encephalitis. J. Am. Vet. Med. Assoc. 159, 776.

McGreevy, P.B., Theis, J.H., Lavoipierre, M.M.J., Clark, J., 1974. Studies on filariasis. III. *Dirofilaria immitis* emergence of infective larvae from the mouthparts of *Aedes aegypti*. J. Helminthol. 48, 221–228.

Merrill, J.R., Otis, J., Logan, W.D., Davis, M.B., 1980. The dog heartworm (*Dirofilaria immitis*) in man. An epidemic pending or in progress? J. Am. Med. Assoc. 243, 1066–1068.

Moorehouse, D.E., Abrahams, E.W., Stephens, B.J., 1971. Human pulmonary dirofilariasis in Queensland. Med. J. Aust. 58 (2), 1230–1233.

Moorehouse, D.E., 1978. *Dirofilaria immitis*: A cause of human intra-ocular infection. Infection 6, 192–193.

Mulshine, J.L., Henschke, C.I., 2000. Prospects for lung-cancer screening. Lancet 355, 592–593.

Muro, A., Cordero, M., Ramos, A., Simon, F., 1991. Seasonal changes in the levels of anti-*Dirofilaria immitis* antibodies in an exposed human population. Trop. Med. Parasitol. 42, 371–374.

Navarrete, A.R., 1972. Pulmonary dirofilariasis. Chest 61, 51–55.

Navarrete-Reyna, A., Noon, G., 1968. Pulmonary dirofilariasis manifested as a coin lesion. Report of a case and review of the literature. Arch. Pathol. 85, 266–271.

Neafie, R.C., Connor, D.H., Meyers, W.M., 1976. Dirofilariasis. In: Binford, C.H., Connor, D.H. (Eds.), Pathology of Tropical and Extraordinary Diseases. Armed Forces Institute of Pathology, Washington, pp. 391–396.

Neafie, R.C., Piggott, J., 1971. Human pulmonary dirofilariasis. Arch. Pathol. 92, 342–349.

Olson, R.C., 1970. *Dirofilaria immitis* in the cerebral arteries of a dog. J. Parasitol. 56, 203–204.

Orihel, T.C., 1961. Morphology of the larval stages of *Dirofilaria immitis* in the dog. J. Parasitol. 47, 251–262.

Orihel, T.C., Beaver, P.C., 1965. Morphology and relationship of *Dirofilaria tenuis* and *Dirofilaria conjuctive*. Am. J. Trop. Med. Hyg. 14, 1030–1043.

Otto, G.F., 1949. Heartworm in dogs. North. Am. Vet. 30, 181–189.

Otto, G., 1974. Occurrence of the heartworm in unusual locations and in unusual hosts. In: Proceedings of the Heartworm Symposium. pp. 6–8.

Patton, C.S., Garner, F.M., 1970. Cerebral infarction caused by heartworms (*Dirofilaria immitis*) in a dog. J. Am. Vet. Med. Assoc. 156, 600–605.

Peters, J., Kubitschek, K.R., 1984. Clinical evaluation of a percutaenous pneumothorax catheter. Chest 86, 714–717.

Prioleau, W.H., Parker, E.F., Bradham, R.R., Gregorie, H.B., 1976. *Dirofilaria immitis* (dog heartworm) as a pulmonary lesion in humans. Ann. Thorac. Surg. 21, 382–385.

Riskin, W., Toppell, K.L., 1977. Pulmonary dirofilariasis: Another coin lesion. Southern Med. J. 70, 502–503.

Ro, J.Y., Tsakalakis, P.J., White, V.A., Luna, M.A., Chang-Tung, E.G., Green, L., Cribbett, L., Ayala, A.G., 1989. Pulmonary dirofilariasis: The great imitator of primary or metastatic lung tumor. A clinicopathologic analysis of seven cases and a review of the literature. Hum. Pathol. 20, 69–76.

Robinson, N.B., Chavez, C.M., Conn, J.H., 1977. Pulmonary dirofilariasis in man. A case report and review of the literature. J. Thorac. Cardiovasc. Surg. 74, 403–408.

Roy, B.T., Chirurgi, V.A., Theis, J.H., 1993. Pulmonary dirofilariasis in California. West. J. Med. 158, 74–76.

Schaub, R.G., Rawlings, C.A., 1980. Pulmonary vascular response during phase of canine heartworm disease: scanning electron microscopic study. Am. J. Vet. Res. 41, 1082–1089.

Schlotthauer, J.C., Harrison, E.G., Thompson, J.H., 1969. Dirofilariasis – An emerging zoonosis? Arch. Environ. Health 19, 887–890.

Schnelle, G.B., Jones, T.C., 1945. *Dirofilaria immitis* in the eye and in an interdigital cyst. J. Am. Vet. Med. Assoc. 107, 14–15.

Scully, R.E., McNeely, B.U., 1974. Case records of the Massachusetts General Hospital weekly clinicopathological exercises Case 26-1974. N. Engl. J. Med. 291, 35–42.

Scully, R.E., Galdabini, J.J., 1979. Case records of the Massachusetts General Hospital weekly clinicopathological exercises Case 13-1979. N. Engl. J. Med. 300, 723–729.

Segedy, A.K., Hayden, D.W., 1978. Cerebral vascular accident caused by *Dirofilaria immitis* in a dog. J. Am. An. Hosp. Assoc. 14, 752–756.

Seybold, W.D., 1964. Solitary or ''coin'' lesions of the lung. Analysis of 2258 recorded cases. Postgrad. Med. 36, 424–430.

Sharp, D.V., Kinsella, T.J., 1950. The significance of the isolated pulmonary nodule. Minnesota Med. 33, 886–888.

Shires, P.K., Turnwald, G.H., Qualls, C.W., King, G.K., 1982. Clinical reports: Epidural dirofilariasis causing paraparesis in a dog. J. Am. Vet. Med. Assoc. 180, 1340–1343.

Siegelman, S.S., Zerhouni, E.A., Leo, F.P., Khouri, N.F., Stitik, F.P., 1980. CT of the solitary pulmonary nodule. Am. J. Radiol. 135, 1–13.

Simon, F., Muro, A., Cordero, M., Martin, J., 1991. A seroepidemiologic survey of human dirofilariasis in western Spain. Trop. Med. Parasitol. 42, 106–108.

Slonka, G.F., Castleman, W., Krum, S., 1977. Adult heartworms in arteries and veins of a dog. J. Am. Vet. Med. Assoc. 170, 717–719.

Smith, L.S., Schillaci, R.F., 1986. Pulmonary dirofilariasis in humans – pneumonitis that evolved to a lung nodule. West. J. Med. 145, 516–519.

Southgate, B.A., Bryan, J.H., 1975. Human pulmonary dirofilariasis entry method of infective larvae. Chest 68, 274.

Spear, H.C., Daughtry, D.C., Chesney, J.G., Gentsch, T.O., Larsen, P.B., 1968. Solitary pulmonary lesion due to *Dirofilaria*. N. Engl. J. Med. 278, 832–833.

Swanson, S.J., Jaklitsch, M.T., Mentzer, S.J., Bueno, R., Lukanich, J.M., Sugarbaker, D.J., 1999. Management of the solitary pulmonary nodule. Role of thoracoscopy in diagnosis and therapy. Chest 116 (6, Suppl.), 523S–524S.

Tada, I., Sakaguchi, Y., Eto, K., 1979. *Dirofilaria* in the abdominal cavity of a man in Japan. Am. J. Trop. Med. Hyg. 28, 988–990.

Tamashiro, W.K., Powers, K.G., Levy, D.A., Scott, A.L., 1985. Quantitative and qualitative changes in the humoral response of dogs through the course of infection with *Dirofilaria immitis*. Am. J. Trop. Med. Hyg. 34, 292–301.

Tannehill, A.W., Hatch, H.B., 1968. Coin lesions of the lung due to *Dirofilaria immitis*. Report of a case. Dis. Chest 53, 369–371.

Tashjian, R.J., Liu, S.K., Yarns, D.A., Das, K.M., Stein, H.L., 1970. Angiocardiography in canine heartworm disease. Am. J. Vet. Res. 31, 415–435.

Theis, J.H., Gilson, A., Simon, G.E., Bradshaw, B., Clark, D., 2001. Case report: Unusual location of *Dirofilaria immitis* in a 28-year-old man necessitates orchiectomy. Am. J. Trop. Med. Hyg. 64, 317–322.

Thornton, J.G., 1978. Heartworm invasion of the canine eye. Mod. Vet. Prac. 59, 373–374.

Tomimura, T., Funahashi, N., Kotani, T., Oka, T., Mochizuki, H., Noda, S., Nomura, K., 1969. Studies on paradoxical embolization with *Dirofilaria immitis* in dogs. Kiseichugaku Zasshi 18, 265–280.

Toomes, H., Delphendahl, A., Manke, H.G., Moykopf, I.V., 1983. The coin lesion of the lung. A review of 955 resected coin lesions. Cancer 51, 534–537.

Trunk, G., Gracey, D.R., Byrd, R.B., 1974. The management and evaluation of the solitary pulmonary nodule. Chest 66, 236–239.

Tuazon, R.A., Firestone, F., Blaustein, A.U., 1967. Human pulmonary dirofilariasis manifesting as a ''coin'' lesion. A case report. J. Am. Med. Assoc. 199, 103–104.

Venco, L., Genchi, C., Colson, P.V., Kramer, L., 2001. Relative utility of echocardiography, radiography, serologic testing and microfilariae counts to predict adult worm burden in dogs naturally infected with heartworms. In: Lee, R.E., Ward, S.E. (Eds.), Recent Advances in Heartworm Disease Symposium, 2001. American Heartworm Society, Batavia, IL, pp. 111–124.

Weil, G.J., Malane, M.S., Powers, K.G., Blair, L.S., 1985. Monoclonal antibodies to parasite antigens found in the serum of *Dirofilaria immitis*-infected dogs. J. Immunol. 134, 1185–1191.

Welch, J.S., Dobson, C., 1974. The prevalence of antibodies to *Dirofilaria immitis* in aboriginal and Caucasian Australians. Trans. R. Soc. Trop. Med. Hyg. 68, 466–472.

Westcott, J.L., 1988. Percutaneous transthoracic needle biopsy. Radiology 169, 593–601.

Wilcox, H.S., 1960. Pulmonary arteriotomy for removal of *Dirofilaria immitis* in the dog. J. Am. Vet. Med. Assoc. 136, 328–338.

Wong, M.M., 1974. Experimental dirofilariasis in macaques susceptibility and host responses to *Dirofilaria immitis*, the dog heartworm. Trans. R. Soc. Trop. Med. Hyg. 68, 479–490.

Zelch, J.V., Lalli, A.F., McCormack, L.J., Belovich, D.M., 1973. Aspiration biopsy in diagnosis of pulmonary nodule. Chest 63, 149–152.