The Prevalence of Dog Heartworm (*Dirofilaria immitis*) Infection in Stray Dogs in Okayama, Japan

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ABSTRACT. The prevalence of dog heartworm, *Dirofilaria immitis*, was examined in stray dogs collected from 3 local public health centers in southwestern region of Okayama Prefecture, Japan during the period between October 1990 to December 1991. A total of 286 dogs (113 males and 173 females, 4.0 to 14.9 kg in body weight) were examined, and *D. immitis* infection was found in 154 (53.8%) dogs (62 males, 54.9% and 92 females, 53.2%). The monthly infection rates of *D. immitis* were 31.6 to 72.2%, and apparently lower rates (31.6 to 44.0%) were recognized between June and September. The prevalence of *D. immitis* infection in dogs and the number of heartworms per infected dog increased along with body weight of the hosts. Altogether 1,722 worms were detected from 154 dogs, and the number of worms per infected dog ranged from 1 to 57 (av. 11.2). The worms were found more in females (52.3%) than in males (47.7%). The sites of worm infestation in 154 dogs were found most in right ventricle (52.3%), followed by pulmonary artery (24.2%) and right atrium and superior vena cava (23.5%). The detected worms measured 47 to 210 mm (av. 164 mm) long in 830 males and 5 to 342 mm (av. 250 mm) long in 609 females. Unisexual worms were found in 36 (23.4%) dogs and the number of males and females per infected dog ranged from 1 to 4 (av. 1.4), and 1 to 10 (av. 2.6) respectively. The diagonal ventral ridges peculiar to *D. immitis* were recognized on the cuticular surface of the male worms by scanning electron microscope (SEM) observation.

Key words: *Dirofilaria immitis* — stray dog — prevalence — cuticular morphology — Okayama Prefecture

The adult form of *Dirofilaria immitis* (Leidy, 1856) Railliet and Henry, 1911 is ordinarily known as parasitic to heart, pulmonary artery and superior vena cava of dogs, and is well known as "dog heartworm". The adutls of *D. immitis* have also been found in fox, cat, tiger, bear, horse, weasel, seal, otter, and other mammals. The larva (microfilaria) derived from adult female is liberated in the blood of the heart and pulmonary artery and is carried throughout the body of infected animal. Some species of mosquito such as *Aedes togoi*, *A. albopictus*, *Culex pipiens pallens* in Japan are known as suitable intermediate hosts or vectors. When the blood was sucked by a vector mosquito, the microfilariae in blood of the final host develop rapidly into a third-stage or infective larvae with two moults 2 weeks after infection.

Although the infective larvae penetrate into human body through the

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salivary glands of mosquito, they never reach its full development to adult in human body because it is not likely suitable host for *D. immitis*. Nevertheless some infective larvae get into the blood circulation of human body and reach lung and subcutaneous tissue causing coin lesion and subcutaneous nodule as typical symptoms for human dirofilariasis.

Many cases of human dirofilariasis have so far been reported throughout the world including Japan. The occurrence of dirofilariasis in humans is regarded as being closely correlated with the prevalence of canine filariasis. A number of studies on the incidence and distribution of *D. immitis* infection in dogs have been made almost everywhere in Japan. On the contrary, very little is known on the prevalence of canine filariasis in Okayama Prefecture, with an exception of a rare report by Miyagawa (1927).[1)]

The present paper describes the results of a survey on the prevalence of dirofilariasis in the stray dog population of Okayama Prefecture together with SEM study of agential worms.

MATERIALS AND METHODS

The stray dogs examined were collected from 3 local public health centers, i.e., Kurashiki, Kasaoka and Sohja in southwestern region of Okayama Prefecture, Japan during the period from October 1990 to December 1991. Examinations of infected filarial worms were performed on the heart, lung and associated artery of post-mortem dogs after used in physiological experiments at Department of Physiology of our school. The sex and body weight of all dogs were recorded preparatory to the experiments. For establishing the presence of filarial worms, incision of the heart, lung and pulmonary vessel was carefully made.

The detected worms were counted and the body weights of the worms were measured. The sex of filarial worms can easily be recognized by their size and cork-screw appearance of the caudal end of the male. So that special attention was paid to anterior and posterior regions of the worms, although the full extent of each worm was also examined. A limited number of worms were studied with a SEM for morphological observation of cuticular markings after the worms were prepared by the ordinary technics.

RESULTS AND DISCUSSION

Incidence of canine filariasis in the stray dogs

Number of dogs examined, seasonal occurrence of canine filariasis and rates of heartworm infection were summarized in Table 1.

A total of 286 dogs (113 males and 173 females, 4.0 to 14.9 kg in body weight, Table 2), were examined for the filarial infection, and 154 dogs (62 males and 92 females) were found positive. The infection rates ranged from 31.6% (August) to 72.2% (December) with a mean of 53.8%. Although there was some difference in the infection rates according to the stages surveyed, remarkably low rates was recognized during 4 months from June to September. No significant difference in the infection rates was found between males (54.9%) and females (53.2%). By the study, 1,722 worms were detected from 154 dogs

TABLE 1. Seasonal occurrence of *D. immitis* infection in stary dogs in Okayama Prefecture

Dates examined	No. of dogs examined		No. of dogs infected		Infection rates (%)	No. of worms per dog min.	max.	total	average
'90. Oct.	16	M 9, F 7	9	M 4, F 5	56.3	1	57	113	12.6
Nov.	22	M 4, F 18	13	M 3, F 10	59.1	1	47	145	11.2
Dec.	18	M 8, F 10	13	M 6, F 7	72.2	1	42	167	12.8
'91. Jan.	8	M 1, F 7	5	M 0, F 5	62.5	1	12	41	8.2
Feb.	12	M 5, F 7	8	M 4, F 4	66.7	1	17	59	7.4
Mar.	19	M 8, F 11	12	M 4, F 8	63.2	1	30	154	12.8
Apr.	29	M 7, F 22	17	M 4, F 13	58.6	1	31	206	12.1
May	28	M 11, F 17	16	M 7, F 9	57.1	1	40	185	11.6
June	21	M 7, F 14	7	M 4, F 3	33.3	4	40	102	14.6
July	25	M 14, F 11	11	M 6, F 5	44.0	1	33	87	7.9
Aug.	19	M 8, F 11	6	M 4, F 2	31.6	5	32	83	13.8
Sep.	21	M 15, F 6	7	M 6, F 1	33.3	1	17	53	7.6
Oct.	18	M 10, F 8	12	M 6, F 6	66.7	1	34	117	9.8
Nov.	19	M 3, F 16	11	M 2, F 9	57.9	1	35	159	14.5
Dec.	11	M 3, F 8	7	M 2, F 5	63.6	1	20	51	7.3
Total	286	M 113, F 173	154	M 62 (54.9), F 92 (53.2)	53.8	1	57	1722	11.2

* F=female, M=male.

TABLE 2. The incidence of *D. immitis* infection in dogs in relation to body weight

Body weight of dogs (Kg)	No. of dogs		Infection rates (%)	No. of worms per dog		
	examined	infected		min.	max.	average
4.0- 4.9	3	1	33.3	1		1.0
5.0- 5.9	27	12	44.4	1	35	8.0
6.0- 6.9	74	35	47.3	1	34	9.8
7.0- 7.9	85	49	57.6	1	40	11.2
8.0- 8.9	45	26	57.8	1	42	12.2
9.0- 9.9	25	14	56.0	1	47	10.7
10.0-10.9	15	7	46.7	2	19	9.4
11.0-11.9	6	6	100	6	57	18.3
12.0-12.9	4	3	75.0	13	30	20.0
13.0-13.9	1	0	0	—	—	—
14.0-14.9	1	1	100	29		29.0
Total	286	154	53.8	1	57	11.2

and the number of worms per infected dog ranged from 1 to 57 with a mean of 11.2 (Tables 1, 2). The sex distribution of the examined worms was found more in females (900 or 52.3%) than in males (822 or 47.7%).

Oishi (1986)[2] recently has given a comprehensive review on the literature on dog heartworms. According to his description, the infection rates of *D. immitis* in dogs in all regions of Japan are as follows: 2.0 to 73% in Hokkaido (Kawamata *et al.*, 1977),[3] 20 to 60% in Honshu, 3.8 to 50% in Shikoku and Kyushu (Kitazawa *et al.*, 1963)[4] and 1.8 to 65% in Okinawa Prefecture (Suenaga *et al.*, 1976[5]; Asato *et al.*, 1985[6]). As noted above, Miyagawa (1927)[1] reported that 34.8% of dogs were found positive to *D. immitis* infection in Okayama Prefecture. However, no detailed investigation has reported in the prefecture.

In the present study, the infection rates of filarial worms in dogs is shown in Table 1. Very little change is found in the infection rates as compared with the previous data reported in Okayama Prefecture (Miyagawa, 1927).[1] The underlying causes of lower infection rates between June to September (Table 1) may be considered whether the juvenile worms invaded to the dogs did not fully develop to adult form or the dogs did not have frequent contacts with vector mosquitoes having infective larvae. It is believed that the immature larvae may have been multiplied in the mosquito body during summer months of June to September.

Regarding the number of *D. immitis* worms per infected animal in Japan, Kitaura *et al.* (1961)[7] reported that a total of 230 worms were found in one dog, and Oishi (1986)[2] reported finding a total of 209 worms in a dog.

TABLE 3. Distribution of dirofilarial worms in infected dogs

Dates examined	No. of worms found	No. of worms found in		
		R・V	P・A	R・A and VCS
'90. Oct.	113	64	6	43
Nov.	145	79	31	35
Dec.	167	85	37	45
'91 Jan.	41	18	22	1
Feb.	59	24	23	12
Mar.	154	70	51	33
Apr.	206	110	40	56
May	185	88	49	48
June	102	49	9	44
July	87	53	24	10
Aug.	83	43	26	14
Sep.	53	28	21	4
Oct.	117	61	21	35
Nov.	159	85	51	23
Dec.	51	43	6	2
Total	1722	900	417	405
(%)		(52.3)	(24.2)	(23.5)

* R・V=right ventricle, P・A=pulmonary artery, R・A=right atrium, VCS=superior vena cava

However, such severe infection with *D. immitis* in dog does not seem to occur frequently. In the past cases, the number of *D. immitis* worms found in each infected dog ranged from 14 to 76 (Oishi, 1986)[2] which is similar to our results (Table 1). It is commonly said that sex ratio of filarial worms in dogs is the same in both sexes or slightly higher in female than in male. With unknown reasons, the worms found in infected dogs were more in female than in male in our investigation.

The relationship between the incidence of *Dirofilaria* infection and the body weight of dogs were summarized in Table 2. In the present study, most dogs examined were medium-sized, possibly under 2 to 3 years of age, ranging from 6.0 to 7.9 kg in body weight, and large ones over 11.0 kg were no more than 12. Both the incidence of *D. immitis* infection and the number of worms per infected dog correlated with the body weight of dogs. As have already pointed out by the earlier workers, it is assumed that the increase in the number of filarial worms which invaded into the heart and lung of host animals is

extremely proportional to the frequency of whether they lived through the summer. Yasuda *et al.* (1989)[8] had made successive observation on *D. immitis* infection among house dogs in Sapporo City, and stated that the microfilarial infection was found in 13.5% of 165 dogs kept in the house, and 21.5% of 153 dogs bred outdoor. According to his comments, recent *D. immitis* infection tends to occur more frequently in indoor pet dogs, and the developmental mechanism of dirofilariasis in dogs seems more complicated today compared with the past. Needless to say that the incidence of *Dirofilaria* infection in the dogs kept outdoor is evidently higher than that of indoor pets.

The sites of infection of 1,722 worms found were mostly in right ventricle (900 or 52.3%), followed by pulmonary artery (417 or 24.2%) and right atrium and superior vena cava (405 or 23.5%) (Table 3, Fig. 1). Oishi (1986)[2] reported that 66.9% of 717 worms were found in right ventricle, 11.2% in pulmonary artery, 10.1% in right atrium and 8.6% in superior vena cava. Kihara and Okamura (1975)[9] investigated that they found mostly in ventricle. According to their results, 79.6% was found in right ventricle, 15.2% in pulmonary artery, 7.8% in right atrium and 1.6% in superior vena cava.

In our study, the parasitism of unisexual worms was recognized in 36 (23.4%) of 154 dogs, and the number of male and female worms per infected dog ranged from 1 to 4 (av. 1.4) and from 1 to 10 (av. 2.6), respectively. Oishi (1986)[2] reported that the parasitic incidence of bisexual worms is 84%, and that of unisexual worms is 16%. It thus appears that the incidence and distribution of filarial worms in dogs of Okayama Prefecture are scarcely different from those of the results in past studies in Japan.

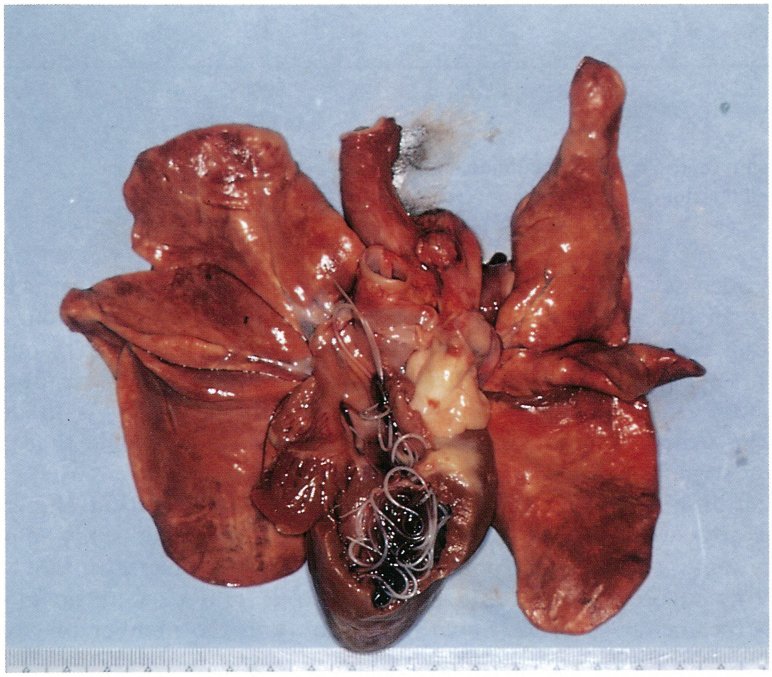

Fig. 1. Heart and lung of an infected stray dog showing the adult worms of *Dirofilaria immitis* appearing from incision in the right ventricle (ventral view).

Morphological aspects of filarial worms

The adults of filarial worms were long and slender in form and milk-white in color. Adult male worm was rather shorter than female, gradually tapering toward the caudal end with a coiled tail. The body length of 1,439 worms (830 males and 609 females) was 47 to 210 mm (av. 164 mm) in the males and 55 to 342 mm (av. 250 mm) in the females. The mean values of maximum width measured 0.6 mm in the males and 0.9 mm in the females. Oishi (1986)[2] reported that the average length of the adults was 172 mm (156 to 188 mm) in 86 males and 276 mm (244 to 308 mm) in 103 females. The average length of worms found in our study was slightly shorter than that of the preceding descriptions. It is conceivable that the apparent size difference is perhaps to be correlated with ages of examined dogs.

Ming *et al.* (1978)[10] studied the cuticular morphology of 5 *Dirofilaria* species and suggested that SEM would be a good tool for a detailed study of specific cuticular morphology. They further reported that the cuticular pattern of ridges and striation on the ventral surface of the caudal region of adult male of *D. immitis* is quite unique than that of the other *Dirofilaria* species. Therefore, the cuticular observation of the present worms was undertaken by SEM in order to classify species. The characteristic SEM pictures were shown in Fig. 2. The cuticular markings of the anterior end showed no appreciable difference between male and female. A small circular mouth was recognized in the middle of the apical most portion, and its surroundings were slightly swelled. Two pairs of papillae and a single pair of amphids were also recognized on the apical surface. They were peripherally arranged at some distance from the mouth (Fig. 2a). The morphological features of the cuticular markings in male were as follows.

On the region of short distance behind the anterior end, only the transverse cuticular striation was present and they were arranged horizontally with approximately 1.5 μm intervals, where no longitudinal ridge existed on the cuticular surface (Fig. 2b). On the midbody region, the logitudinal ridges were begun to appear on the dorsal cuticular surface, but it was somewhat discontinued (Fig. 2c). Whereas, on the dorso-caudal region, longitudinal ridges altered over irregular pattern to almost consecutive form swelled primarily to the longitudinal direction (Fig. 2d). On the ventro-caudal region just in front of the posterior end, ventral ridges lined to an oblique ventro-lateral direction, and these ridges tapering down to a narrow point at its caudal extremity, and they disappeared entirely from this level caudally (Fig. 2e). On the most posterior region, 5 pairs of anal papillae on each side of the cloaca which are commonly said to be the adanal and postanal papillae were found (Fig. 2f). Neither preanal central papilla situated closely in front of the cloaca nor postdeirid located on the left dorso-lateral surface of the caudal region were confirmed.

As noted above, adult worms of the genus *Dirofilaria* are parasitic in the heart and lung of dog and other mammals. Many species belonging to *Dirofilaria* have been reported in past. Among them, 9 species such as *Dirofilaria immitis*, *D. repens* Railliet and Henry, 1911, *D. striata* (Molin, 1858), *Dipetalonema reconditum* (Grassi, 1889), *D. dracunculoides* (Cobbold, 1870), *D. grassi* (Noë, 1907), *Brugia pahangi* (Buckley and Edeson, 1965), *B. patei* (Buckley, Nelson and Heisch, 1958) and *B. ceylonensis* (Jayewardene,

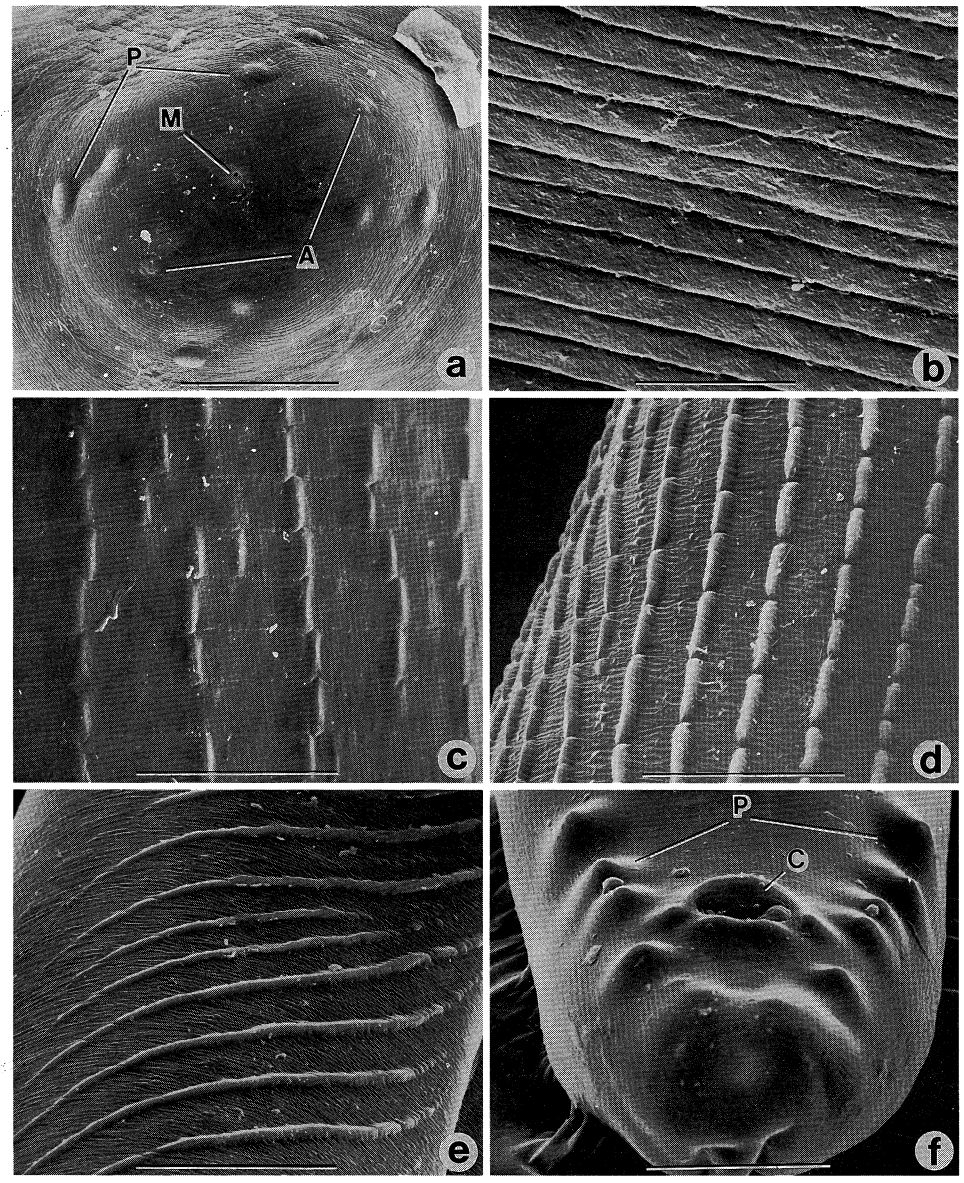

Fig. 2. Scanning electron micrographs showing the cuticular markings of an adult male of *Dirofilaria immitis*. (a) Anterior end, apical view (Scale bar=50 μm); (b) Transverse striations on the region of a short distance behind the anterior end (Scale bar=6.0 μm); (c) Longitudinal ridges on the dorso-middle region (Scale bar=30 μm); (d) Longitudinal ridges on the dorso-caudal region (Scale bar=60 μm); (e) Diagonal ventral ridges immediately in front of the posterior end (Scale bar=50 μm); (f) Ventro-posterior end, caudal view (Scale bar=50 μm)
A: amphids, C: cloaca, M: mouth, P: papillae

1962) have been found in spontaneously infected dogs (Oishi, 1986).[2)] Especially *D. immitis* is known to be medically significant for human health

and hygiene.

In Japan, Kikuchi *et al.* (1973)[11] was first to study the cuticular morphology of male and female worms of *D. immitis* by SEM, and thereafter, Uni (1978)[12] observed surface structure of *D. immitis* and *D. ursi* adults also by SEM. As indicated above, Ming *et al.* (1978)[10] reported the detailed cutiuclar morphology of 5 species of *Dirofilaria* worms by SEM. In conclusion, *D. immitis* worm is characterized by the presence of diagonal ventral ridges on the cuticular surface of adult male. The morphological characteristics of cuticular marking patterns in the present specimens are essentially identical to those described by Kikuchi *et al.* (1973)[11], Uni (1978)[12] and Ming *et al.* (1978).[10] Therefore, the worms studied in the present report are identified as *Dirofilaria immitis* (Leidy, 1856) Railliet and Henry, 1911, and no other species was recognized.

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