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STUDIES ON EPIDEMIOLOGY OF *DIROFILARIA IMMITIS* IN HOUSE DOGS IN NAGASAKI CITY, JAPAN, WITH CONSIDERATIONS ON YEARLY CHANGES IN MICROFILARIAL PREVALENCE

TSUTOMU ODA[1], OSAMU SUENAGA[2], AKIO MORI[3], KOICHIRO FUJITA[4], MAKOTO ZAITSU[5], KENJI KUROKAWA[5], TAKESHI NISHIOKA[7], TATSUYA ITOH[7] AND MARIKO MINE[8]

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Abstract: The positive rate of *Dirofilaria immitis* infection was studied in the eastern, western, southern and northern parts of Nagasaki City 3 times between 1968 and 1983. Chronological changes in the positive rate of dogs for microfilariae in these 4 parts and the roles of epidemic factors in the changes of the positive rate were evaluated. The positive rate decreased in the northern part, where houses and buildings increased annually, whereas the number of dog owners and the density of dogs decreased. Improvements in the drainage such as side ditches, which are primary breeding sites of vector mosquitoes (*Culex p. pallens*), are assumed to have led to a decrease in the mosquito density, resulting in a decrease in the positive rate of dogs. However, the positive rate increased in the eastern, western, and southern parts. The density of dogs decreased also in these parts, but it remained higher than in the northern part as the number of houses was smaller, and more people kept dogs. Also, as the sewage system did not seem to be so well developed as in the northern part, more mosquitoes might be present. The high positive rates in these parts may be ascribed to these conditions.

INTRODUCTION

The adult worm of *Dirofilaria immitis* transmitted by mosquitoes usually lives in the heart of dogs. Human infections are also known in Japan (e.g., Yoshimura 1989; Suzumiya and Nawa 1990). In Nagasaki City, house dogs were infected with this worm in high rates and the main vector was *Culex pipiens pallens* (Suenaga *et al*., 1971; Suenaga and Itoh, 1973; Suenaga *et al*., 1980). However, the relative importance of the factors actually related to the prevalence remains unknown. Therefore we conducted an epidemiological study of *D. immitis*. Herein, the changes in microfilarial prevalence from 1968 to 1983 are reported with a consideration of the factors responsible for these changes.

PLACES AND METHODS

As Suenaga *et al*. (1971) reported, Nagasaki City is divided into 44 elementary school districts, or into 4 parts, eastern, western, southern and northern. To examine the microfilariae of *D. immitis* in each division, we sampled blood from an earlobe of a dog in April and May, 1983 when the City Health Center was conducting rabies vaccination to registered dogs. A drop of blood was taken, smeared on a slide glass, and stained with 10% Giemsa's solution. The number of microfilariae in the blood specimen was counted with a stereomicroscope. The data on sex and age of examined dogs were taken from the record of Nagasaki City Health Center.

Cx. p. pallens, the main mosquito vector, were collected by a light trap with black light (20 watts) from

1. Department of General Education, the School of Allied Medical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852, Japan
2. Reference Center, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852, Japan
3. Department of Medical Zoology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan
4. Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku 113, Japan
5. Sasebo City Health Center, 1-10 Yahata-machi, Sasebo 857, Japan
6. Department of Bacteriology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan
7. Nagasaki City International Culture Hall, 7-8 Hirano-machi, Nagasaki 852, Japan
8. Scientific Data Center of Atomic Bomb Disaster, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan

Table 1 Positive rates for microfilariae of *D. immitis* in dogs with the number of registered dogs per house in the districts of Nagasaki City in 1983

Part	District No.	District Name	No. of dogs examined	No. of dogs positive	%	No. of houses (A)	No. of dogs registered (B)	No. of dogs per house (B/A)
Eastern	1	Irabayashi	3	0	0.0	9072	418	0.0461
	2	Togiya	1	0	0.0	2524	69	0.0273
	3	Katsuyama	3	3	100.0	2993	108	0.0361
	4	Shinkozen	1	0	0.0	1938	27	0.0139
	5	Kaminagasaki	4	2	50.0	4939	250	0.0506
	6	Koshima	0	—	—	6528	352	0.0539
	7	Nishizaka	49	16	32.7	2459	74	0.0301
	8	Himi	0	—	—	3280	163	0.0497
	9	Yagami	80	28	35.0	3448	122	0.0354
	10	Koga	68	28	41.2	1849	103	0.0557
	11	Toishi	56	22	39.3	1113	72	0.0647
		Total	265	99	37.4*	40143	1758	0.0438
Western	12	Inasa	69	20	29.0	5715	216	0.0378
	13	Asahi	56	13	23.2	2571	96	0.0373
	14	Akunoura	89	24	27.0	2787	116	0.0416
	15	Tategami	0	—	—	989	66	0.0667
	16	Kosakaki	0	—	—	1381	58	0.0420
	17	Fukuda	74	29	39.2	2489	101	0.0406
	18	Teguma	4	0	0.0	2063	111	0.0538
	19	Shikimi	1	0	0.0	1482	66	0.0445
		Total	293	86	29.4	19477	830	0.0426
Southern	20	Sako	19	1	5.3	3139	82	0.0261
	21	Nita	11	2	18.2	2098	84	0.0400
	22	Kitaohura	6	2	33.3	4668	248	0.0531
	23	Minamiohura	17	8	47.1	2491	127	0.0510
	24	Naminohira	24	3	12.5	1463	71	0.0485
	25	Tomachi	149	57	38.3	5044	232	0.0460
	26	Kogakura	46	22	47.8	1779	64	0.0360
	27	Doinokubi	49	29	59.2	4574	221	0.0483
	28	Fukahori	64	27	42.2	3545	104	0.0293
	29	Minami	13	5	38.5	244	16	0.0656
	30	Mogi	57	12	21.1	2049	96	0.0469
	31	Hayasaka	0	—	—	1955	131	0.0670
	32	Hiyoshi	0	—	—	343	18	0.0525
		Total	455	168	36.9*	33392	1494	0.0447
Northern	33	Zenza	53	11	20.8	2563	75	0.0293
	34	Sakamoto	32	5	15.6	4051	79	0.0195
	35	Takao	134	34	25.4	6628	227	0.0342
	36	Yamazato	30	6	20.0	5079	110	0.0217
	37	Nishishiroyama	7	1	14.3	4387	186	0.0424
	38	Shiroyama	9	2	22.2	2232	86	0.0385
	39	Nishiurakami	207	56	27.1	8692	387	0.0445
	40	Nameshi	12	4	33.3	9255	420	0.0454
	41	Nishikita	42	14	33.3	4273	166	0.0388
	42	Nishimachi	22	7	31.8	6476	299	0.0462
	43	Kawabira	7	4	57.1	2573	115	0.0447
	44	Ohzono	0	—	—	2950	61	0.0207
		Total	555	144	25.9*	59159	2211	0.0374
Sum total			1568	497	31.7	152171	6293	0.0421

*Significant (P<0.01)

1972 to 1983 in 4 points in residential area: Irabayashi (Eastern part), Inasa (Western part), Tomachi (Southern part) and Sakamoto (Northern part). In addition, the collection was made in Sakamoto-machi also from 1967 to 1971. The light trap was regularly operated from 6 PM to 7 AM (one night), once a week from May to October.

RESULTS

1. Microfilarial prevalence in house dogs in Nagasaki City

Table 1 shows the results of the blood examination of dogs in Nagasaki City in 1983. The positive rate for microfilariae was 31.7% for the whole city. The positive rate in the eastern or southern part was significantly higher than that in the northern part (x^2-test, $p<0.01$). We thought that the difference in the positive rate might be related with the dog density (the number of dogs per house). To make clear this hypothesis, the numbers of dogs per house were also examined in these parts (Table 1). As explained later, the positive rate is correlated with the dog density, which was lowest in the northern part with the lowest positive rate among the 4 parts.

2. Comparison of positive rates in dogs by sex and age in the 4 parts of the city

The positive rates were compared according to sex and age in the 4 parts in Table 2. The rates were calculated only for dogs with an exact record of age. The positive rate is generally lower at any age for either sex in the northern part of the city than in the other 3 parts. This means that the lower prevalence in the northern part was not due to different age distribution nor sex ratio in the dogs examined.

3. Correlation between positive rate and dog density

We examined the correlation between positive rate and dog density in districts in Figure 1, where points were plotted only when the number of dogs examined was over 10. The microfilaria positive rate was positively correlated with dog density in the city (correlation coefficient 0.4163, $0.01<p<0.05$).

4. Yearly changes in positive rates of the dogs in the 4 parts of the city

The yearly changes in positive rates in the 4 parts were examined by comparing the results reported by Suenaga *et al.* (1971; 1980) with the present data by the linear trend test of Armitage (1955) (Table 3). In the

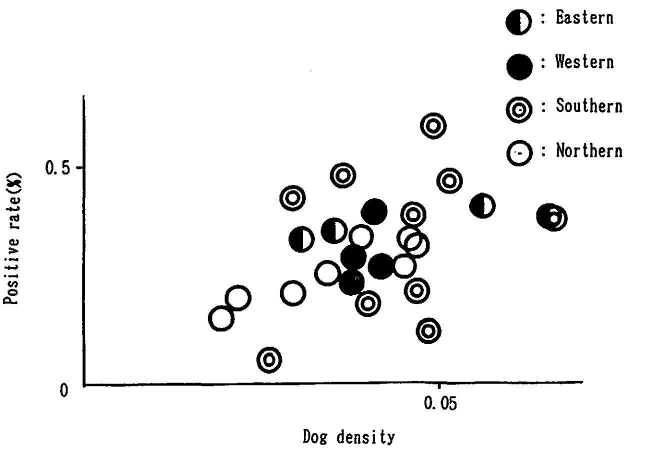

Figure 1 Relation between dog density and positive rate of dogs in districts in Nagasaki City in 1983.
* Points were plotted only when the number of dogs examined was over 10.

northern part, the total positive rate of 40.5% in 1968 clearly decreased to 25.9% in 1983, while the rate increased in the eastern, western and southern parts.

In 1977 and 1983, the results of positive rates in the 4 parts were obtained in spring, but in 1968 the results were composed of data taken in spring and autumn. The positive rate calculated only for spring was 34.6%, 19.5%, 21.6% and 41.8% in the eastern, western, southern and northern parts, respectively, there being not much difference between the results obtained only in spring and those obtained in spring and autumn combined (Table 1).

5. Yearly changes in the number of the registered dogs in Nagasaki City

Figure 2 shows the yearly change in the total number of dogs registered in whole Nagasaki City. The number of these dogs decreased gradually from 1968 to 1983, with some variations. Changes in numbers of dogs and houses in each of 4 parts of Nagasaki City are shown in Table 4. In each part, the decrease in the number of dogs was apparent, while the number of houses increased.

6. Yearly changes of the relation between positive rate and dog density in the 4 parts

As the numbers of houses and registered dogs in each district were available for 1968 and 1983, the dog density (number of dogs per house) was calculated. The relation between the microfilaria positive rate in dogs and the dog density in each district in 1968 was compared with that in 1983 in Figure 3. The positive rate

Table 2 Number and percentage of dogs positive for *D. immitis* microfilariae by age and sex in 4 parts in Nagasaki City, 1983

Part	Sex	No. and %	Age							Total
			0	1-2	3-4	5-6	7-8	9-10	11≦	
Eastern	Male	Dogs examined	0	65	40	28	18	9	7	167
		Dogs positive	0	17	12	11	10	6	6	62
		% positive	0	26.2	30.0	39.3	55.6	66.7	85.7	37.1
	Female	Dogs examined	0	31	19	16	10	7	12	95
		Dogs positive	0	7	7	8	4	4	7	37
		% positive	0	22.6	36.8	50.0	40.0	57.1	58.5	38.9
Western	Male	Dogs examined	1	51	43	35	13	15	16	174
		Dogs positive	0	8	21	15	7	4	6	61
		% positive	0	15.7	48.8	42.9	53.8	26.7	37.5	35.1
	Female	Dogs examined	0	37	33	15	12	11	8	116
		Dogs positive	0	7	6	5	1	4	2	25
		% positive	0	18.9	18.2	33.3	8.3	36.4	25.0	21.6
Southern	Male	Dogs examined	3	99	72	43	30	11	14	272
		Dogs positive	0	25	36	18	16	7	9	111
		% positive	0	25.3	50.0	41.9	53.3	63.6	64.3	40.8
	Female	Dogs examined	1	54	42	32	15	19	8	171
		Dogs positive	0	10	16	14	3	7	3	53
		% positive	0	18.5	38.1	43.8	20.0	36.8	37.5	31.0
Northern	Male	Dogs examined	3	95	72	65	42	29	29	335
		Dogs positive	1	15	22	25	17	10	9	99
		% positive	33.3	15.8	30.6	38.5	40.5	34.5	31.0	29.6
	Female	Dogs examined	1	76	41	41	24	20	16	217
		Dogs positive	0	5	6	13	6	6	7	43
		% positive	0	6.6	14.6	31.7	25.0	30.0	43.8	19.8

Table 3 Yearly changes in *D. immitis* positive rates of dogs in 4 parts in Nagasaki City from 1968 to 1983

Part	1968*			1977*			1983			Increase (+) or decrease (−) in prevalence 1968 to 1983
	No. of dogs examined	No. of dogs positive	Positive rates (%)	No. of dogs examined	No. of dogs positive	Positive rates (%)	No. of dogs examined	No. of dogs positive	Positive rates (%)	
Eastern	675	194	28.7	328	113	34.5	265	99	37.4	+**
Western	337	68	20.2	334	98	29.3	293	86	29.4	+**
Southern	639	137	21.4	504	178	35.3	455	166	36.9	+**
Northern	719	291	40.5	555	174	31.4	555	153	25.9	−**

* : Cited from Suenaga *et al.* (1971, 1980).
** : Significant $P<0.001$

decreased in parallel with the decrease of the dog density in the northern part, but in the other parts the opposite tendency was generally observed.

7. Yearly changes in number of *Cx. p. pallens* females at 4 points

In Figure 4, the mean number of *Cx. p. pallens* females per night was shown as an annual population index. The mosquito population was not large from

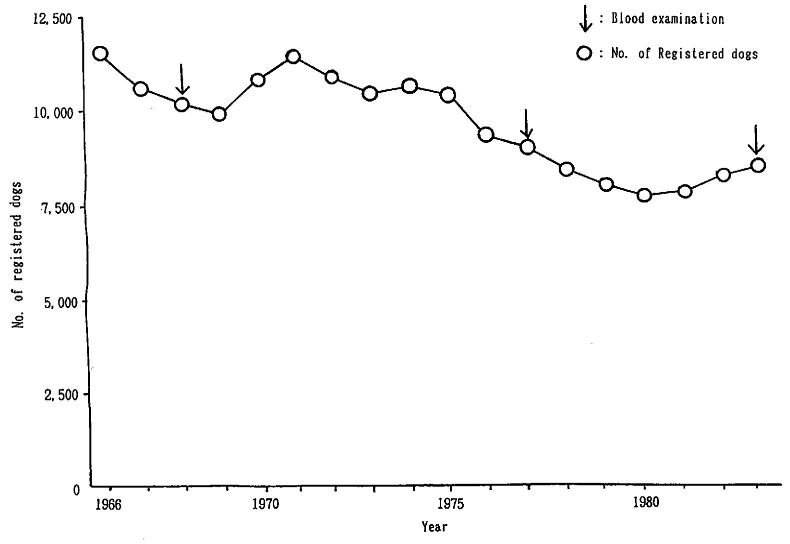

Figure 2 Yearly changes in the total number of registered dogs in Nagasaki City.

1972 to 1983, in the eastern, western and southern parts, though population varied with year. In Sakamoto (northern part) where the mosquitoes were collected from 1967 to 1983, the density was also low since 1971.

DISCUSSION

We studied the infections of *D. immitis* in registered dogs in the eastern, western, southern and northern parts of Nagasaki City in 1968, 1977 and 1983, and found a significant difference between the northern part and the other 3 parts. In the northern part, the positive rate had decreased, whereas in the other 3 parts, it had increased markedly. As one of the factors causing this phenomenon, introduction of Ivermectin, an effective

Table 4 Changes in numbers of dogs and houses in 4 parts between 1968 and 1983

Part	Dog and House	1968*	1983
Eastern	No. registered dogs (A)	3,666	1,758
	Dog density (A/B)	0.1212	0.0438
	No. houses (B)	30,243	40,143
Western	No. registered dogs (A)	1,428	830
	Dog density (A/B)	0.0932	0.0426
	No. houses (B)	15,316	19,477
Southern	No. registered dogs (A)	2,491	1,494
	Dog density (A/B)	0.1034	0.0447
	No. houses (B)	24,085	33,392
Northern	No. registered dogs (A)	3,733	2,211
	Dog density (A/B)	0.1050	0.0374
	No. houses (B)	35,566	59,159

*Cited from Suenaga *et al.* (1971, 1980)

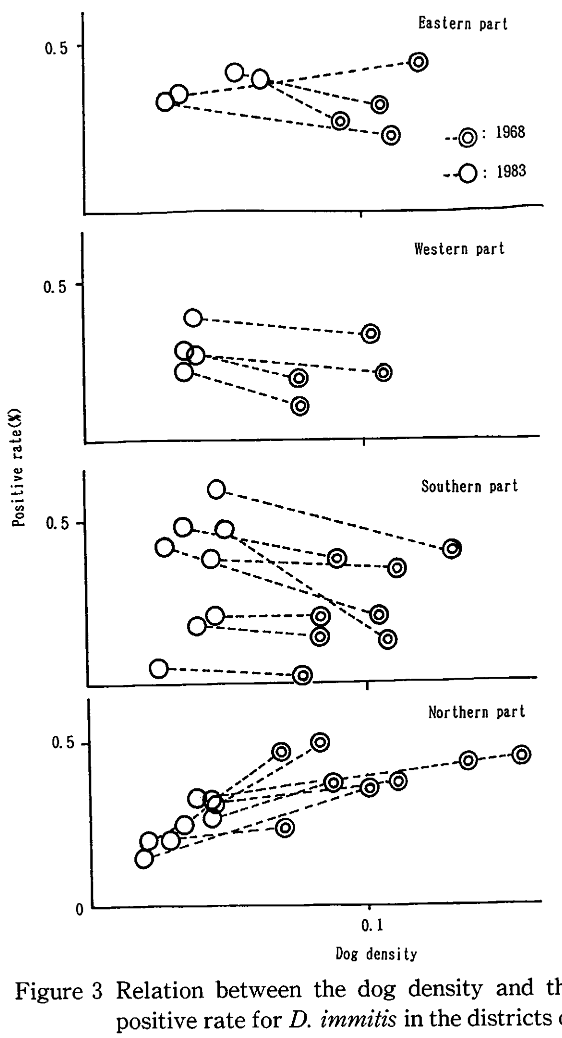

Figure 3 Relation between the dog density and the positive rate for *D. immitis* in the districts of Nagasaki City in 1968 and 1983.

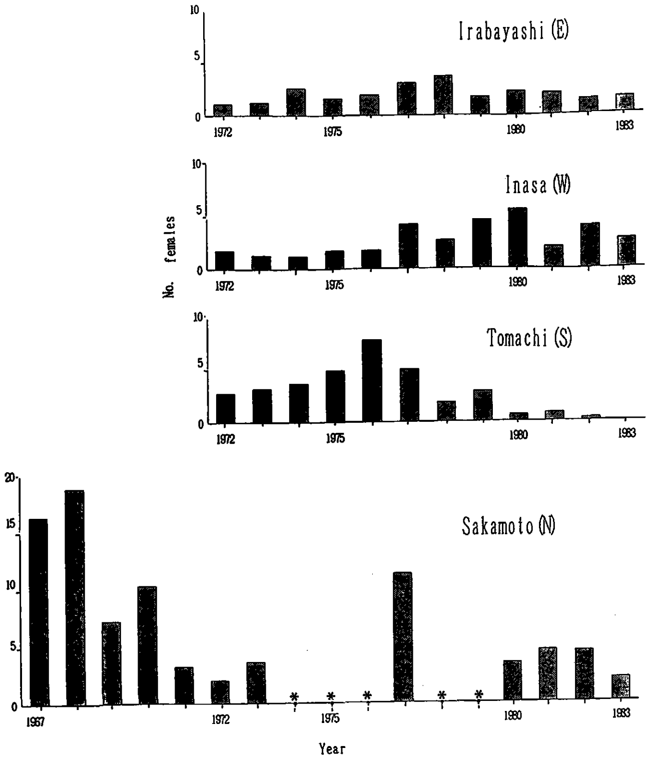

Figure 4 Yearly changes in the number of *Cx. p. pallens* females per night in Nagasaki City. E, W, S and N show eastern, western, southern and northern part respectively.
* The collection by light trap was not made.

preventive drug for *D. immitis*, was considered, but it can not be a reason for the decrease in the positive rate in the northern part since 1968 because its introduction was 1988. The present result showed that mosquito density was scarcely different in all the parts after 1972. But the density decreased in the northern part from 1968 to 1972, and thus, this decrease in mosquito density may be related to the decrease of the rate.

Dog density, which is an important factor responsible for infection (Wada *et al*., 1989), decreased apparently in all 4 parts, but the positive rate decreased in the northern part and increased in the other 3 parts. As shown in Table 4, the number of houses in the northern part, which was the largest among the parts, had increased. This implies that many new residential areas had developed in this part, where it is assumed that the space to keep dogs outside was limited and there was some environmental complaint against dogs. Therefore, many dogs may have been kept within the house, and prevented from attack of the vector mosquito, *Cx. p. pallens*, which occurs in the breeding sites such as side ditches. Furthermore, when we took dog's blood, we had an impression that in the northern part more dog owners became to take measures to prevent exposure of their dogs to mosquitoes by, for example, letting them within the house at night and providing mosquito repellents. In the northern part, the increase in houses and office buildings was associated with the development of the sewage system thus decreasing drainages such as side ditches, according to the Department of Sewage System in Nagasaki City. Such improvements in the sanitary environment are considered to have been a factor in the decrease of the *Cx. p. pallens*. These conditions are assumed to relate to the decrease in the positive rate in the northern part.

On the other hand, the positive rate increased in eastern, western and southern parts. The density of dogs increased also in these parts, but it remained higher than in northern part as the number of houses was smaller, and more people kept dogs. In addition, it seems that the sewage system was poorly developed in these parts, because the number of houses was smaller than in northern part. From this we assumed that as the drainage has not been so well developed as in the northern part, more mosquitoes would be present in the 3 parts, though this result was not clearly shown by the data of light trap. According to dog owners, more dogs seem to be bred outdoors. Therefore it is suspected that dogs had more chance to be attacked by mosquitoes even if mosquito density was low in these parts.

As for the role of other mosquitoes in the transmission of *D. immitis*, Suenaga and Itoh (1973) reported that *Aedes albopictus* may be the secondary important vector, and *Cx. tritaeniorhynchus* may also be some bearing on the transmission in Nagasaki City. However, there is no evidence that these mosquitoes increased in the 3 parts in particular, and they are not considered to have contributed much to the increase of positive rate. The number of stray dogs may be another factor influencing the transmission. Their number, as given by the number of arrested dogs (unpublished data), decreased with years, therefore stray dogs are not an important factor for the increase of positive rate.

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