Jpn. J. Trop. Med. Hyg., Vol. 23, No. 2 (1995) pp. 133-137

STUDIES ON ANNUAL CHANGES IN MICROFILARIAL PREVALENCE OF *DIROFILARIA IMMITIS* AMONG HOUSE DOGS FOR 27 YEARS IN NAGASAKI CITY, JAPAN

TSUTOMU ODA[1], OSAMU SUENAGA[2], MAKOTO ZAITSU[3], AKIO MORI[4], KENJI KUROKAWA[5], KOICHIRO FUJITA[6], YASUNORI OGAWA[6], ICHIRO YAMAZAKI[7], KUNIHIRO IIDA[7], KOUICHI DOI[7], MARIKO MINE[8] and KATSUTOMO KATO[9]

Received February 24, 1995/Accepted April 17, 1995

Abstract: The positive rate of dogs with *Dirofilaria immitis* microfilariae among house dogs was examined for 27 years from 1968 to 1994 in the southern and northern parts of Nagasaki City. Roles of the number of the main vector, *Culex pipiens pallens* and of RPSS (rate of population utilizing a sewage system) were analyzed statistically in relation to the annual changes in microfilarial prevalence. In the southern and northern parts, there was a clear correlation among three parameters of the positive rate in the dogs, the number of *Cx. p. pallens* and RPSS. The present study proved that the decrease in the positive rate of dogs in both the southern and northern parts is mainly attributable to a decrease in the number of *Cx. p. pallens*, which resulted from the decrease of the breeding places of this mosquito following the spread of the public sewage system and the improvements in the use of roads and open roadside ditches.

INTRODUCTION

Oda *et al.* (1993) previously reported that the positive rate of dogs for microfilariae of *Dirofilaria immitis* increased from 1968 to 1983 in the eastern, western and southern parts of Nagasaki City, but decreased during the same period in the northern part. Moreover, Oda *et al.* (1994a) collected female *Culex pipiens pallens*, the main vector of *Dirofilaria immitis*, from 1983 to 1989, and found that in 1983 *Cx. p. pallens* was extremely prevalent in the southern part but not in the northern part, and after 1986, the prevalence of this mosquito became low in both parts, in parallel with the spread of sewage system. In this study, we analyzed statistically the annual changes of *Dirofilaria immitis* infection rates among house dogs in southern and northern parts of Nagasaki City over the past 27 years after 1968 to clarify the role of the density of *Cx. p. pallens* and the spread of public sewage systems in reducing *Dirofilaria immitis* infection.

PLACES AND METHODS

The survey was conducted in four districts: a southern district (Tomachi) and three northern districts (Sakamoto, Takao and Yamazato). From 1983 to 1994, blood examination was conducted in April or May from about 400 registered dogs in these four districts at the time of vaccination against rabies. One drop (about 20mm^3) of blood was collected from the ear lobe of

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 Sasebo City Health Center, 1-10 Yahata-machi, Sasebo 857, Japan
4 Department of Medical Zoology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan
5 Department of Bacteriology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan
6 Department of Medical Zoology, Faculty of Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113, Japan
7 Nagasaki City Health Center, 2-34 Morimachi, Nagasaki 852, Japan
8 Scientific Data Center of Atomic Bomb Disaster, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852, Japan
9 Department of Physical Therapy, The School of Allied Medical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki 852, Japan

individual dogs. Blood samples were subjected to Giemsa staining and examined for microfilariae under a stereomicroscope.

Mosquitoes were collected using a light trap (16 watt black light) from 1972 to 1989 in Tomachi and from 1967 to 1994 in Sakamoto. Each trap was usually operated once or twice a week in the May-October period. The details of the sites and methods of mosquito collection were previously reported (Oda *et al.*, 1993; 1994a). The districts in four parts (eastern, western, southern and northern) of Nagasaki City were derived from the residential list used by Suenaga *et al.* (1971).

RESULTS

1. Annual changes in microfilarial prevalence of *Dirofilaria immitis* among house dogs

Table 1 shows the annual changes of the positive rate (percentage of dogs having microfilariae) in each of the four districts during the 1968-1994 period. The annual changes in positive rate were examined by the linear trend test of Armitage (1955). The positive rate of dogs generally decreased in all districts, although some fluctuations were noted. This decrease during the 27 years was significant ($P<0.01$) in all districts.

2. Incidence of new infection among the dogs

The incidence of new infection in each year from 1984 was calculated as in Table 2 by dividing the number of dogs which turned from negative to positive for microfilariae by the number of dogs examined among those which had been negative in the previous year. In each district, this incidence decreased from year to year (Table 2). Fig. 1 shows the relationship between the incidence of new infection and the percentage of dogs having microfilariae for the southern part (Tomachi) and the northern part (data in Sakamoto, Takao and Yamazato were combined) of Nagasaki City. In both parts of the city, there was a significant positive correlation between the positive rate of dogs and the incidence of new infection ($P<0.01$). Thus the percentage of dogs having microfilariae decreased clearly from year to year owing to the reduction of new infection in both the southern and northern parts of the city.

3. Annual changes in positive rates of dogs, the number of *Cx. p. pallens* and the rate of public sewage system

Fig. 2 shows annual changes in the positive rate of dogs, the number of *Cx. p. pallens* females caught and the rate of human population utilizing a public sewage system (RPSS). As described previously (Oda *et al.*,

Table 1 Annual changes in microfilarial prevalence of *Dirofilaria immitis* among house dogs in southern and northern parts in Nagasaki City

Year	Southern part		Northern part							
	District name		District name						Total	
	Tomachi		Sakamoto		Takao		Yamazato			
	No. dogs examined	No. (%) of positive dogs	No. dogs examined	No. (%) of positive dogs	No. dogs examined	No. (%) of positive dogs	No. dogs examined	No. (%) of positive dogs	No. dogs examined	No. (%) of positive dogs
1968	100	34(34.0)	42	15(35.7)	64	30(46.9)	123	61(49.6)	229	106(46.3)
1977	120	42(35.0)	33	15(45.5)	43	14(32.6)	41	13(31.7)	117	42(35.9)
1983	149	57(38.3)	32	5(15.6)	134	34(25.4)	32	6(18.8)	198	45(22.7)
1984	137	41(29.9)	63	17(27.0)	161	41(25.5)	46	7(15.2)	270	65(24.1)
1985	107	44(41.1)	52	16(30.8)	74	14(18.9)	55	8(14.6)	181	38(21.0)
1986	129	41(31.8)	76	23(30.3)	57	9(15.8)	30	6(20.0)	163	38(23.3)
1987	127	36(28.3)	73	13(17.8)	103	13(12.6)	36	5(13.9)	212	31(14.6)
1988	137	36(26.3)	70	24(34.3)	177	30(16.9)	39	5(12.8)	286	59(20.6)
1989	155	41(26.5)	46	14(30.4)	148	26(17.6)	57	7(12.3)	251	47(18.7)
1990	125	24(19.2)	74	10(13.5)	164	24(14.6)	59	3(5.1)	297	37(12.5)
1991	51	7(13.7)	86	8(9.3)	166	21(12.7)	61	4(6.6)	313	33(10.5)
1992	145	26(17.9)	83	10(12.0)	154	17(11.0)	56	3(5.4)	293	30(10.2)
1993	74	11(14.9)	32	0(0.0)	78	7(9.0)	30	1(3.3)	140	8(5.7)
1994	137	20(14.6)	56	3(5.4)	149	16(10.7)	54	2(3.7)	259	21(8.1)

Table 2 Number and percentage of dogs infected newly with *Dirofilaria immitis* microfilariae

Year	Southern part		Northern part							
	District name		District name						Total	
	Tomachi		Sakamoto		Takao		Yamazato			
	No. dogs* examined	No. (%) of dogs newly infected.	No. dogs* examined	No. (%) of dogs newly infected.	No. dogs* examined	No. (%) of dogs newly infected.	No. dogs* examined	No. (%) of dogs newly infected.	No. dogs* examined	No. (%) of dogs newly infected.
1984	46	8(17.4)	20	4(20.0)	57	4(7.0)	12	1(8.3)	89	9(10.1)
1985	40	8(20.0)	26	3(11.5)	25	0(0.0)	19	1(5.3)	70	4(5.7)
1986	36	6(16.7)	25	2(8.0)	24	1(4.2)	12	0(0.0)	61	3(4.9)
1987	49	4(8.2)	32	3(9.4)	28	0(0.0)	15	0(0.0)	75	3(4.0)
1988	58	4(6.9)	32	1(3.1)	61	5(8.2)	20	0(0.0)	113	6(5.3)
1989	63	4(6.3)	25	3(12.0)	79	7(8.9)	28	1(3.6)	132	11(8.3)
1990	66	4(6.1)	20	1(5.0)	70	1(1.4)	30	0(0.0)	120	2(1.7)
1991	14	1(7.1)	40	1(2.5)	87	1(1.1)	29	0(0.0)	156	2(1.3)
1992	21	0(0.0)	44	1(2.3)	89	1(1.1)	31	1(3.2)	164	3(1.8)
1993	70	1(1.4)	33	0(0.0)	75	2(2.7)	28	0(0.0)	136	2(1.5)
1994	79	1(1.3)	36	0(0.0)	64	1(1.6)	30	0(0.0)	130	1(0.8)

*No. dogs examined : Number of dogs examined among those which had been negative in the previous year.

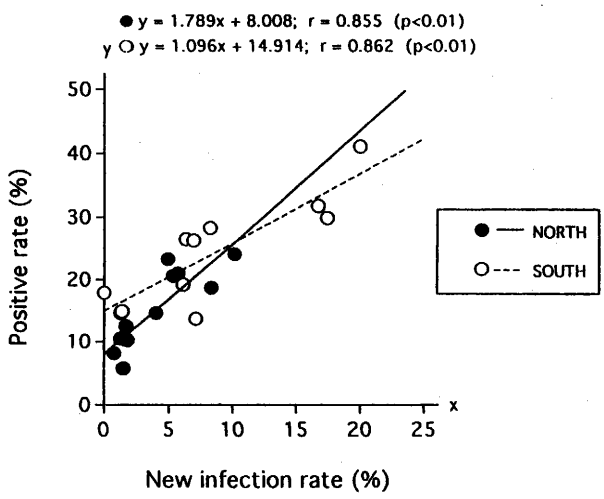

Figure 1 Relationship between positive rate of dogs and new infection rate in southern and northern parts in Nagasaki City.

1994a), the numbers of *Cx. p. pallens* females in southern and northern parts were obtained at Tomachi and Sakamoto, respectively. The RPSS for Tomachi was regarded as the RPSS for the southern part of Nagasaki City, and the RPSS's for the northern part was calculated by averaging the RPSS for Sakamoto, Takao and Yamazato. In the southern part, the positive rate of dogs remained unchanged between 1968 and 1983 but it decreased gradually thereafter, the number of *Cx. p. pallens* was considerably high until about 1980 but it was

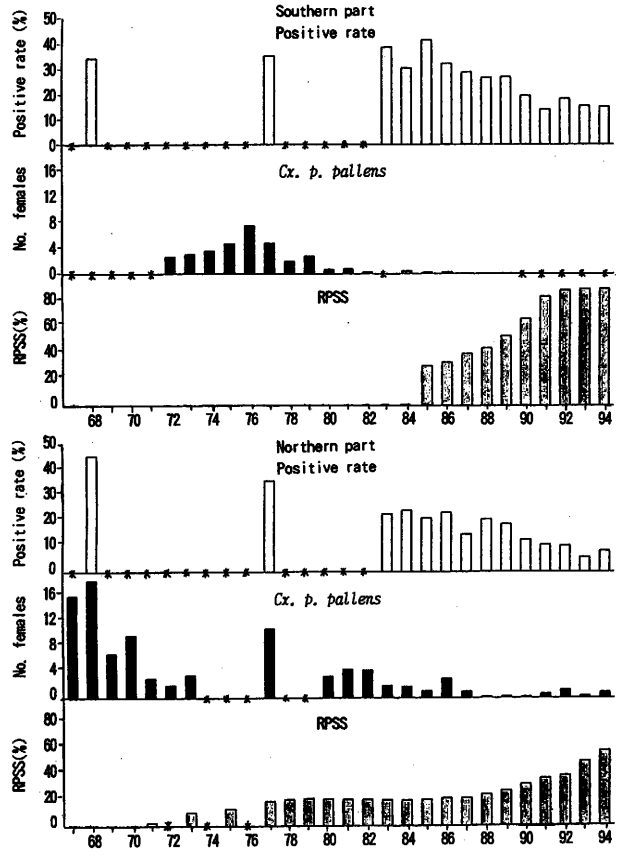

Figure 2 Annual changes in positive rate of dogs, mean number of *Culex pipiens pallens* per night and rate of population utilizing a sewage system (RPSS).
*Data not available

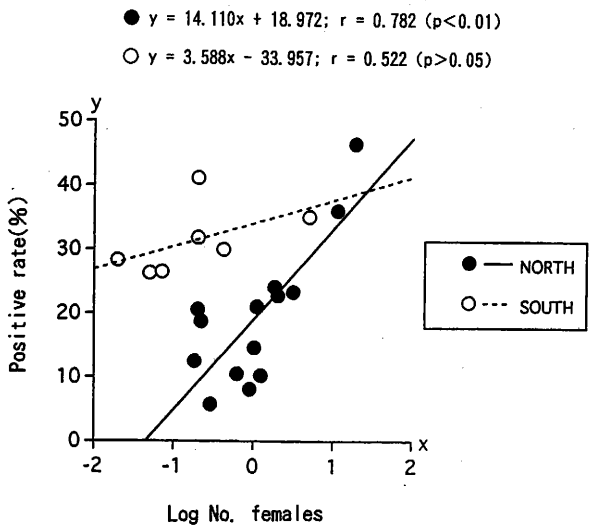

Figure 3 Relationship between positive rates of dogs and number of *Culex pipiens pallens* females.

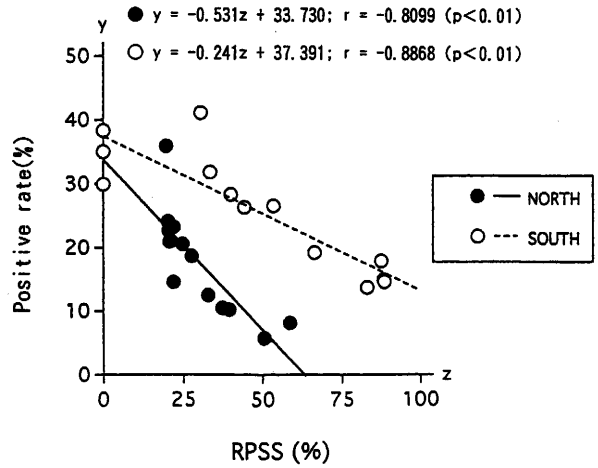

Figure 4 Relationship between positive rates of dogs and RPSS in southern and northern parts in Nagasaki City.
RPSS : Rate of population utilizing a sewage system.

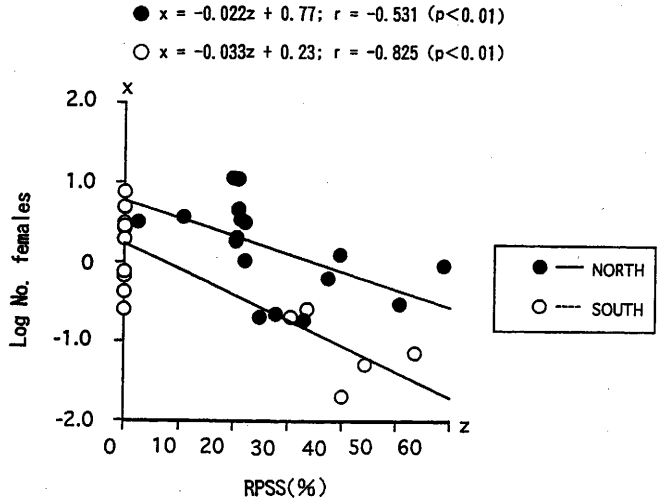

Figure 5 Relationship between number of *Culex pipiens pallens* females and RPSS.
RPSS : Rate of population utilizing a sewage system.

quite low after that year, and the RPSS increased sharply after about 1985. These patterns of annual changes in the three parameters for the northern part were approximately equal to those observed in the southern part, excepting that the percentage of dogs having microfilariae began to decrease earlier in the northern part (around 1977) than in the southern part, and that the number of *Cx. p. pallens* caught in the northern part began to decrease in about 1977. These features of the northern part probably reflect the fact that public sewage systems began to spread in about 1970.

To analyze the relationships among the positive rate of dogs, the number of *Cx. p. pallens* and RPSS, we calculated a correlation coefficient and a regression line between two of the three parameters (Fig. 3-5). In the southern part, the correlation between the positive rate of dogs and the number of *Cx. p. pallens* was not strong, probably because the number of available data in pair was not sufficient. However, it is noteworthy that the percentage of positive dogs in this part had a significant correlation with the RPSS ($P<0.01$) as in Fig. 4. A significant correlation between the number of *Cx. p. pallens* and the RPSS was also noted ($P<0.01$), as shown in Fig. 5.

In the northern part, a clear correlation was noted in any combination of two among three parameters of the positive rate of dogs, the number of *Cx. p. pallens* and RPSS (Fig. 3-5). From these results, it was concluded that the decrease in the positive rate of dogs in both the southern and northern parts is attributable to a decrease in number of *Cx. p. pallens,* which was an outcome of a decrease of the breeding places of this mosquito following the spread of public sewage systems.

DISCUSSION

The present study revealed correlations among the positive rate of dogs, the number of female *Cx. p. pallens* caught and the RPSS, and that the correlation was particularly strong between the positive rate of dogs and the RPSS. In view of these findings, the decrease in the percentage of positive dogs observed apparently in both parts of this city after 1983 may be explained as follows. In public sewage systems, the sewage including all house

sewage but excluding rain water is directly sent to the sewage processing plant. Under this sewage system, clean rain water containing little organic substance in open road side ditches does not constitute significant oviposition sites of mosquitoes. As a result, the sewage system reduced the number of breeding places of *Cx. p. pallens*. The number of breeding places of this mosquito was further reduced by the improvement of roads and open roadside ditches, in addition to the development of public sewage systems. Accordingly the number of female *Cx. p. pallens* decreased, and the transmission of *Dirofilaria immitis* to dogs by mosquitoes was reduced.

Contrarily, the positive rate of dogs increased greatly during the 1968-1977 period, in Koga, Toishi, Fukuda and Hayasaka (the four newly populated residential areas at margins of Nagasaki City) (Suenaga *et al.*, 1980). This is explained by the fact that no public sewage systems were available in any of these four districts at that period. The lack of sewage systems probably resulted in the formation of many breeding places of *Cx. p. pallens*, leading to the increase in the percentage of positive dogs by a large number of vector mosquitoes.

If the number of *Cx. p. pallens* in both the southern and northern parts remains unchanged or decreases from the current level on, the positive rate of dogs will further decrease. The current number of *Cx. p. pallens* may have already approached critical vector density, as pointed out by Wada *et al.* (1984), but this needs to be further studied. As pointed out in a previous paper (Oda *et al.*, 1994a), it is also likely that secondary vectors (*Culex tritaeniorhynchus* and *Aedes albopictus*) will play a relatively more important role in the future, following a decrease in the number of *Cx. p. pallens*.

The number of stray dogs may be another factor influencing the transmission. The number, as given by the number of arrested dogs (unpublished data) decreased in about 1972 and thereafter remained little changed in Nagasaki City. From this, stray dogs are not considered to be an important factor for the decrease of positive rate.

According to our questionnaire survey in 1989 and 1993, the number of households which keep dogs indoors has been increasing (Oda *et al.*, 1994b). It is not clear whether this change contributed greatly to the reduction in positive rate of dogs.

It is known that the preventive drugs of diethylcalbamazine and ivermectin which has recently been developed have the excellent effects against *Dirofilaria immitis*. However, according to our questionnaire survey in 1989 and 1993, the percentage of dog owners who used the preventive drugs was not high (Oda *et al.*, 1994b). Therefore, these drugs do not seem to contribute greatly to reducing *Dirofilaria immitis* infection.

ACKNOWLEDGMENTS

Professor Y. Wada, Department of Medical Entomology, Institute of Tropical Medicine, Nagasaki University kindly read this manuscript and provided detailed suggestions. Dr. T. Fukuda, President, and members of the Society of Veterinarians in Nagasaki City really cooperated with our research. The staff in Department of Sewage System in Nagasaki City gave us information for sewage system. We wish to express our sincere gratitude to these persons.

REFERENCES

1) Armitage, P. (1955): Test for linear trends in proportions and frequencies, Biometrics 11, 375-386
2) Oda, T., Suenaga, O., Mori, A., Fujita, K., Zaitsu, M., Kurokawa, K., Nishioka, T., Itoh, T., and M. Mine (1993): Studies on epidemiology of *Dirofilaria immitis* in house dogs in Nagasaki City, Japan with considerations on yearly changes in microfilarial prevalence, Jpn. J. Trop. Med. Hyg., 21, 231-237
3) Oda, T., Suenaga, O., Fujita, K., Zaitsu, M., Kurokawa, K., Ogawa, Y., Yamazaki, I., Iida, K., and M. Mine (1994a): Comparision of population of vector mosquitoes of *Diofilaria immitis* and their natural infection rates in southern and northern parts of Nagasaki City, Japan, Jpn. J. Trop. Med. Hyg., 22, 196-202
4) Oda, T., Suenaga, O., and M. Mine (1994b): A questionnaire survey of living environments of house dogs and measures taken by dog owners to prevent zoonotic parasite, *Dirofilaria immitis* infection in Nagasaki City, Japan, Bull. Sch. Allied. Med. Sci., Nagasaki Univ., 8, 9-16
5) Suenaga, O., Itoh, T., and T. Nishioka (1971): Studies on the filarial prevalence among house dogs and the mosquito vectors in Nagasaki City, Western Japan. 1. On the microfilarial prevalence among house dogs in Nagasaki City, Trop. Med., 12, 169-178 (in Japanese with English summary)
6) Suenaga, O., Nishioka, T., and T. Itoh (1980): On the transition of canine heartworm prevalence among dogs in Nagasaki City, Western Japan. 1. The filarial prevalence among dogs in the city, Trop. Med., 22, 35-45. (in Japanese with English summary)
7) Wada, Y., Tsuda, Y., and O. Suenaga (1984): Transmission dynamics of *Dirofilaria immitis* in a southwestern part of Japan, Trop. Med., 31, 35-47