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THE HOST–PARASITE RELATIONSHIP IN CANINE HEARTWORM INFECTION IN A HYPERENDEMIC AREA OF ITALY

G. CAPELLI[1], G. POGLAYEN[2], F. BERTOTTI[2], S. GIUPPONI[3] AND M. MARTINI[2]

[1]Istituto di Patologia e Igiene Veterinaria, Università di Padova, Agripolis, 35020 Legnaro (Pd); [2]Dipartimento di Sanità Pubblica Veterinaria e Patologia Animale, Università di Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia (Bo); [3]Small Animal Practitioner, 35100 Padova, Italy

ABSTRACT

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From November 1990 to June 1991, 2273 *Dirofilaria immitis* were collected from 175 stray dogs euthanized in the province of Padua. The breed, sex, age and weight of each animal were noted. The worms were counted, sexed, differentiated as adults or fifth-stage larvae and weighed, and their length was measured.

The host–parasite relationship in this epidemiological situation was characterized by a high prevalence (67%) together with a low intensity (19) and abundance (13). The frequency distribution of the parasites in the population of dogs followed a negative binomial pattern ($k = 0.3177$), showing a good, stable host–parasite relationship. The major risk of infection was in late July and August, some new infections being established in October and, maybe, in November. The majority of dogs that acquired new infections were young animals, probably because of an immune response in older dogs. However, the prevalences increased ($p < 0.01$) in dogs from 1 to 5 years old, in shepherd and hunting dogs compared to the other breeds, and in large as opposed to small dogs.

Microfilaraemia (range 1–1 883 000/ml) correlated, as a trend, with the number of adult filariae, which also correlated with the age and the weight of the dogs ($p < 0.01$). No significant correlations were found between the length or weight of the adult filariae and the data from dogs.

Keywords: dog, epidemiology, *Dirofilaria immitis*, intensity, prevalence

Abbreviations: EDTA, ethylenediaminetetracetic acid; L3, L4, L5, third-, fourth- and fifth-stage larvae respectively

INTRODUCTION

For many years the Po valley has been known to be an endemic area for canine heartworm, with prevalences ranging from 30–40% to more than 90% (Balbo and Panichi, 1968; Locatelli, 1971; Tassinari and Bonsembiante, 1984; Pampiglione *et al.*, 1986; Canestri-Trotti *et al.*, 1986; Poglayen *et al.*, 1988, 1992; Di Sacco *et al.*, 1989; Genchi *et al.*, 1991). However, there are few data concerning the characteristics of the epidemiological relationship between dogs and this parasite.

The objective of the present study was to collect more information about canine heartworm infection in a stray-dog population and to assess the parameters of the host–parasite relationship in a hyperendemic area for *Dirofilaria immitis*.

MATERIALS AND METHODS

From November 1990 to June 1991, 175 stray dogs were captured and impounded in the province of Padua (Po valley). Each week 5–15 dogs were euthanized and necropsied; the heart, pulmonary artery, lungs and vena cava were removed and examined for filariae. Two samples of blood were taken from the cephalic or jugular vein of each dog using evacuated blood collecting tubes, one of which contained EDTA. The sex, age, breed and weight of the dogs were annotated.

The recovered heartworms were differentiated as adults or fifth-stage and sexed; their length was measured and the adults were weighed. The length of the oesophagus, the position of the vulva and the presence of ova in the immature filariae were recorded.

On the basis of these measurements (Orihel, 1961; Kotani and Powers, 1982) every intact immature filaria was aged and the period of the dog's infestation was assessed approximately.

Microfilaraemia was assessed by a 1 ml blood-filtration technique (Dennis and Kean, 1971). The fertility of female adult worms in amicrofilaraemic infections was assessed from the presence of microfilariae in their uteri.

Data were analysed by χ^2 tests for comparing the infection rates in different categories of dogs, and for the trends relating the dogs' age, size and breed with the rate of infection; by correlation to relate the data from the dogs and from the parasites; and finally by multiple regression, with calculation of the partial correlation coefficients, to assess the influence of independent variables, such as the weight, age and breed of the dogs, on positivity for *D. immitis*. This method allows estimation of the correlations between each independent and the dependent variables, when the effects of the other independent variables have been removed (Kleinbaum and Kupper, 1978).

RESULTS

Dog population

A total of 117 (67%) dogs were found to be infected, 110 with adult filariae and 7 with only L5s. The prevalence increased significantly in older dogs (Figure 1), with maxima of 87.5% in 3–5-year-old dogs, 87% in larger dogs (Figure 2) and 89% in shepherd and hunting dogs (Figure 3). There were no significant differences between the sexes. As expected, there were positive correlations between age and size and between size and breed of the dogs, but the partial correlation coefficients demonstrated that each of these factors acted independently. Therefore a dog's age,

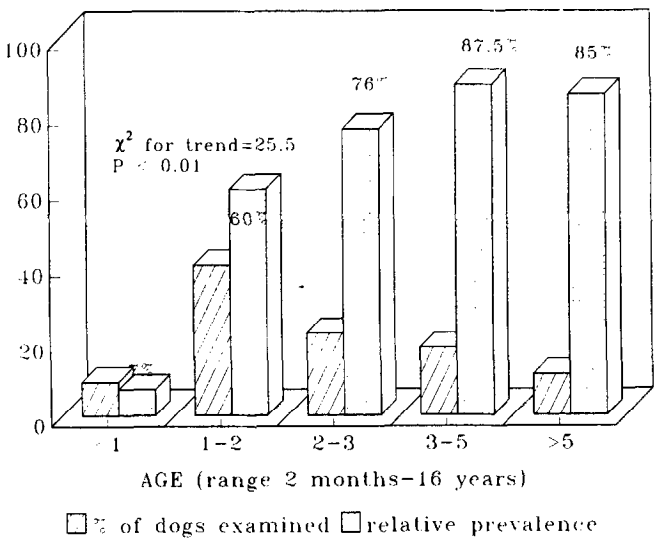

Figure 1. Prevalence of dirofilariosis according to the age of the dogs

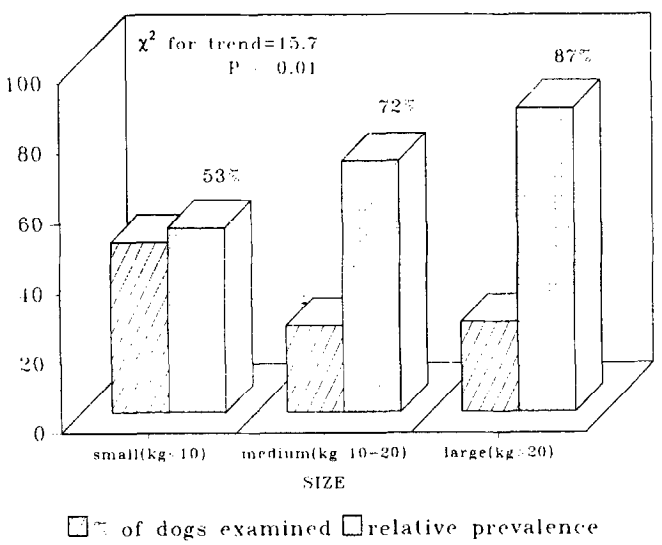

Figure 2. Prevalence of dirofilariosis according to the size of the dogs

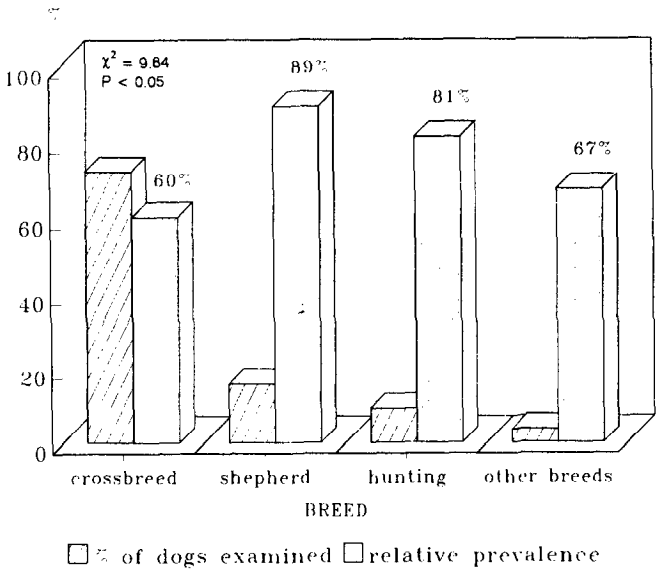

Figure 3. Prevalence of dirofilariosis according to the breed of the dogs

size and breed can be considered as different and separate risk factors, at least in this epidemiological situation.

Heartworm population

Of the 2273 heartworms collected from the infected dogs (Figure 4), 93.5% were adults and 6.5% L5s or immature. There was no difference in the percentage of male and female worms.

Most of the worms, adult or immature, were found in the right heart or vena cava rather than in the lungs or pulmonary artery, but the percentage of larvae recovered from the lungs (32%) was almost double that for adults (19%) from the same organ.

Heartworms were found in both locations in 53% of the infected dogs, only in the heart/vena cava in 44%, and only in the lungs/pulmonary artery in 3%. Figures 5 and 6 show the lengths and the weights of male and female adult filariae; there was no difference in the development of the worms collected from the different organs. As a curiosity, it was calculated that the dog with the largest number of worms (99) in its heart contained about 11.5 g of filariae.

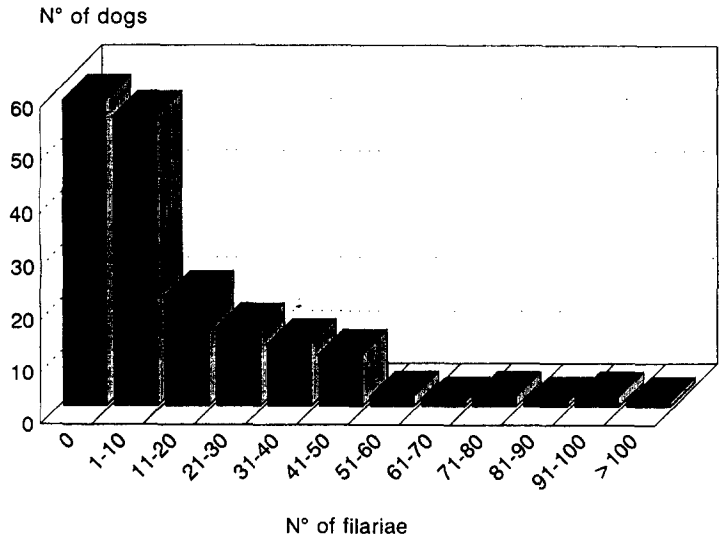

Figure 4. Distribution of the 2273 filariae in the population of dogs

Host–parasite relationship

Despite the high prevalence (67%), the intensity (mean number of parasites in the infected dogs) and abundance (mean number of parasites in all the dogs examined) values were quite low, being 19 and 13, respectively. The number of filariae recovered from the dogs ranged from 1 to 117. The distribution of these worms in the dog population followed a negative binomial pattern (Figure 4), with a high degree of aggregation ($k = 0.31$). In particular, 76 of the positive dogs (65%) contained fewer than 20 filariae, 32 dogs (27%) contained between 20 and 50, and only 9 dogs (8%) contained more than 50. Since the survey began in late November and continued through to June, it was possible to detect the new infections established in the previous summer by collecting the immature worms that had reached the final location. In all, 123 L5s were found in 29 dogs, including both new infections in previously negative dogs, in which only L5s were recovered (8%), and infections that overlapped with an older one (24%). The period of the dogs' infections and the rates of infection calculated per month are reported in Table I. A high percentage of new infections was found in late July and August, with a maximum of 56% in the first half of August, the period when most mosquitos are present. A considerable rate of infection was recorded in October and one dog may also have been infected in November. These findings should be considered in planning prophylactic measures. Data from May and June were not available because the L3s inoculated into the host during this period had probably already reached maturity by the beginning of the period in which the study was conducted.

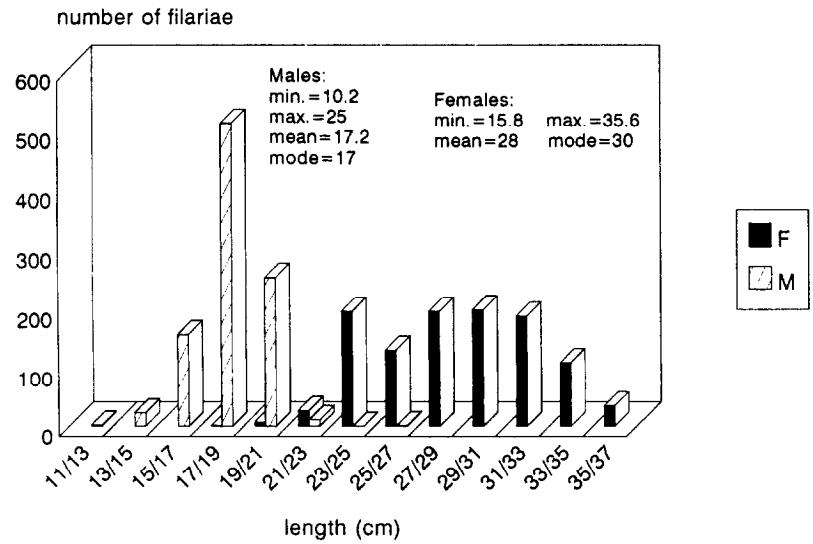

Figure 5. Lengths of adult female and male filariae

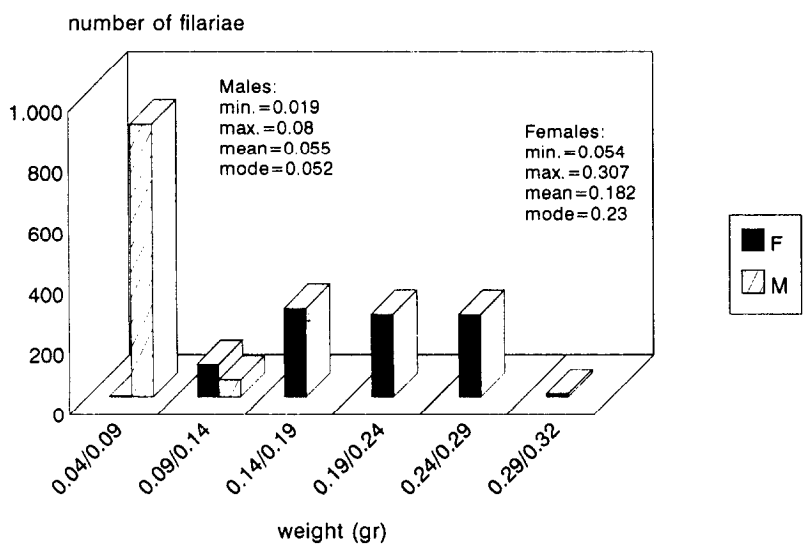

Figure 6. Weights of adult female and male filariae

TABLE I
Percentage of new infections established in the previous summer per month (123 larvae in 29 dogs)

Month[a]	Number examined[b]	Number positive (%)	Number of L5s (%)
July	32	2 (6)	2 (1.6)
July	32	9 (28)	26 (2)
Aug.	32	18 (56)	54 (44)
Aug.	32	7 (22)	17 (14)
Sept.	42	4 (10)	4 (3)
Sept.	30	1 (3)	10 (8)
Oct.	21	2 (10)	2 (1.6)
Oct.	29	5 (17)	7 (6)
Nov.	59	1 (2)	1 (0.8)

[a]First and second halves of the month respectively

[b]Dogs examined between 70 and 120 days after the latest possible infection

TABLE II
Fertility of the female adult filariae in 13[a] dogs with occult dirofilariosis

Dog's age (years)	No. of filariae F	No. of filariae M	Fertile female worms (%)
1	2	1	–
1.5	2	1	–
1.5	2	1	–
1.5	1	1	–
2	26	25	18 (69)
2	8	6	7 (87.5)
2	1	1	–
2.5	18	14	6 (33)
3.5	2	6	1 (50)
4	14	17	6 (43)
5	17	10	13 (76)
7	4	4	–
13	4	1	1 (25)

[a]Fertility not determined in two of the 15 amicrofilaraemic dogs

TABLE III
Microfilaraemic dogs negative for adults at the necropsy

Dog's age	Microfilariae/ml	Serology	Note
2 months	14	–	
3 months[a]	640	+	
3 months[a]	13	+	
1 year	7	–	Only L5
1 year	40	–	Only L5
1 year	1	–	
2.5 years	113	–	
12 years	1780	–	Artery lesions

[a]Sibling (brother) puppies

Most of the dogs (62%) in which a new infection developed were less than 3.5 years old, probably because of an immune response in older dogs.

Microfilaraemia ranged from 1 to 1 883 000 per ml of blood.

Occult dirofilariosis occurred in 30 dogs (26% of the infected animals and 17% of all dogs); 15 of these harboured only one worm or only one sex, 9 dogs carried fewer than 8 adult filariae and 6 carried more than 10. The fertility of the female heartworms in amicrofilaraemic dogs is shown in Table II. In no case were all the females present in any dog fertile. Extreme dilution of the microfilariae is possible in dogs with only a few adult couples, but most of the other 22 microfilaraemic dogs containing fewer than 8 adult filariae had more than 1000 microfilariae per ml of their blood.

In 8 dogs (7% of infected animals and 4% of all the population tested) there were microfilariae in the blood but no adult filariae were found at the necropsy (Table III).

Statistical analysis showed that the length and weight of the adult filariae were not correlated with the number of adults or with the age or size of the dogs, except for a very weak, but nevertheless significant, correlation ($r = 0.06$, $p < 0.05$) between the weight of adult worms and the age of the dogs. However, positive and significant correlations ($p < 0.01$) existed between the number of adult filariae in each dog and the age ($r = 0.51$) and size ($r = 0.42$) of the dogs. There was a positive correlation ($r = 0.75$, $p < 0.01$) between the density of the microfilariae and the number of adult filariae found in the host, contrasting with the findings by other authors (Otto, 1978; Tanaka and Atwell, 1991) in similar populations, where lower burdens were observed to be more likely to be microfilaraemic.

DISCUSSION

This study confirmed the province of Padua and the Po valley as a hyperendemic area of *D. immitis* infection.

The host–parasite relationship in the population of dogs studied was characterized by a high prevalence and incidence accompanied by low intensity and abundance and by an aggregated distribution of parasites in the host population, fitting a negative binomial pattern, which is characterized by a large number of hosts with few parasites and a low number of heavily infected hosts. As is expected under natural conditions and without any known human intervention, the host–parasite relationship seems to be stable (Anderson and May, 1978).

As in previous Italian research (Poglayen *et al.*, 1988; Genchi *et al.*, 1988), the prevalence and intensity of the infection increased in older and larger dogs. These findings can be explained as resulting from the longer time of exposure in old dogs and the larger area of exposure in larger animals, both of which factors will result in a greater risk of mosquito bites.

Two suppositions can be made concerning breed: there may be a difference in susceptibility to the infection in certain breeds or, more likely, shepherd and hunting dogs are more often kept outdoors, day and night, and are therefore subjected to a major risk of infection.

In view of the large size of the gravid adult heartworms, it seems unlikely that some worms were overlooked in the dogs which had microfilariae in their blood but in which no adults were found, even though this cannot be excluded in individual cases. A possible explanation of this finding is that the microfilariae had survived the death of the adults, especially in older dogs or, since the history of these dogs was not known, in those in which adulticidal treatment had previously been performed. However, three of these 'uninfected-microfilaraemic' dogs were 2–3 months old. In these cases passage of microfilariae from an infected mother to her fetus may have occurred (Mantovani and Jackson, 1966). In two of these puppies, adult filarial antigens were also detected in the serum.

Some of the observations in this study, such as the increase of prevalence and intensity in older dogs and the fact that development of the worms was not dependent on the dogs' ages, might point to the conclusion that there is no natural protective immunity response in dogs living in endemic areas. However, Grieve and colleagues (1988), Abraham and colleagues (1988) and Abraham and Grieve (1991), demonstrated experimentally the presence of protective immunity against larval *D. immitis*, capable of killing L3s and of retarding the development of L4s. The number of infective larvae inoculated into a dog in an endemic area is probably much higher than the number of parasites that actually develop, suggesting the presence of regulation of the population, acting generally in the first part of the infection. The aggregated parasite distribution, the low values of intensity and abundance and the observation that the majority of new infections developed in young animals confirm this hypothesis. Evidently, some larvae have the ability to evade the immune system and to reach the heart, even in older dogs.

When ageing the L5s, the possibility that the immune response of the host might

interfere with the growth of the larvae and/or with the time of migration was considered. So far as we are aware, there is no evidence that the migration of larvae to the heart was delayed beyond 90 days after inoculation or that L5s, and therefore adults, arrive at their final location at a different stage of development in immunized dogs. Experimental research (Kume and Itagaki, 1955; Orihel, 1961; Kotani and Powers, 1982) has shown that most immature worms arrive at the final location by 70 days after inoculation and that the migration is completed by day 90. Kotani and Powers (1982) suggested that those worms still in the intermediate location after day 110 would never reach the heart. In the present study, no immature worms were found in the final location after 12 March, that is more than 117 days from the latest possible infection in the first half of November.

Other immune mechanisms, such as control of the fertility of adult worms, do not seem to occur as a rule in this population, since microfilaraemia was correlated with the number of adult filariae. This latter observation cannot simply be transferred to general veterinary practice, where the association of high microfilarial count with heavy worm burdens is common, because two different populations of dogs must be considered. In domestic dogs other factors, such as therapy, prophylaxis and different conditions of exposure, are involved in the host–parasite relationship, so that, in any individual case of dirofilariosis, the circumstances must be investigated carefully.

REFERENCES

Abraham, D. and Grieve, R.B., 1991. Passive transfer of protective immunity to larval *Dirofilaria immitis* from dogs to balb/c mice. *Journal of Parasitology*, **77**, 254–257

Abraham, D., Grieve, R.B., Mika-Grieve, M. and Seibert, B.P., 1988. Active and passive immunization of mice against larval *Dirofilaria immitis*. *Journal of Parasitology*, **74**, 275–282

Anderson R.M. and May, R.M., 1978. Regulation and stability of host/parasite population interactions. 1. Regulatory processes. *Journal of Animal Ecology*, **47**, 219–247

Balbo, T. and Panichi, M., 1968. La filariosi del cane: Epizoologia e diagnosi di laboratorio. *Nuova Veterinaria*, **44**, 18–32.

Canestri-Trotti, G., Pampiglione, S. and Visconti, S., 1986. Ricerche sulle diffusioni delle filariosi canine in alcune provincie della Pianura Padana. *Annali Istituto Superiore di Sanità*, **22**, 449–452

Dennis, D.T. and Kean, B.H., 1971. Isolation of microfilariae: report of a new method. *Journal of Parasitology*, **57**, 1146–1147

Di Sacco, B., Madonna, M. and Dominoni, S., 1989. Epidemiology of canine heartworm infection in Italy. *Proc. 13th Conference of the World Association for the Advancement of Veterinary Parasitology*, August 1989, Berlin

Genchi, C., Traidi, E., Di Sacco, B. and Benedetti, M.C., 1988. Epidemiologia della filariosi cardiopolmonare del cane in Italia. In: F. Stanga and A. Vezzoni (eds), *Proc. Fourth Seminar SCIVAC, Filarosi*, Milan, 1988, (SCIVAC Publications, Cremona, Italy), 53–63

Genchi, C., Vezzoni, A. and Di Sacco, B., 1991. La dirofilariosi cardiopolmonare del cane: epidemiologia e profilassi. *Veterinaria*, **5**, 2–11

Grieve, R.B., Abraham, D., Mika-Grieve, M. and Seibert, B.P. 1988. Induction of protective immunity in dogs to infection with *Dirofilaria immitis* with chemically-abbreviated infections. *American Journal of Tropical Medicine and Hygiene*, **39**, 373–379

Kleinbaum, D.G. and Kupper, L.L., 1978. *Applied Regression Analysis and Other Multivariate Methods*, (Duxbury Press, North Scituate, Mass.)

Kotani, T. and Powers, K.G., 1982. Developmental stages of *Dirofilaria immitis* in the dog. *American Journal of Veterinary Research*, **43**, 2199–2206

Kume, S. and Itagaki, S., 1955. On the life-cycle of *Dirofilaria immitis* in the dog as a final host. *British Veterinary Journal*, **111**, 16–24

Locatelli, A., 1971. Sulle filariosi animali in provincia di Pavia. *Parassitologia*, **13**, 197–202

Mantovani, A. and Jackson, R.F., 1966. Transplacental transmission of microfilariae of *Dirofilaria immitis* in the dog. *Journal of Parasitology* **52**, 116

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

Otto, G.F., 1978. The significance of microfilaremia in the diagnosis of heartworm infection. In: H.C. Morgan, G.F. Otto, R.F. Jackson and C.H. Courtney (eds), *Proceedings of the Heartworm Symposium, 1977, Atlanta, Georgia* (Veterinary Medicine Publishing Inc., Bonner Springs, Kansas), 22–30

Pampiglione, S., Poglayen, G. and Capelli, G., 1986. Distribuzione geografica delle filariosi canine. *Parassitologia*, **28**, 297–300

Poglayen, G., Capelli, G., Roda, R., Calvi, N. and Zanangeli, A., 1988. Indagini sulla diffusione della filariosi del cane in alcuni comuni dell'Emilia Romagna. *Proc. Fourth Seminar SCIVAC: Filariosi*, Milan, 1988, (SCIVAC Publications, Cremona, Italy), 67–78

Poglayen, G., Martini, M., Bomben, L. and Roda, R., 1992. Updating of the occurrence of canine heartworm disease in northern Italy. *VIth European Multicolloquium of Parasitology*, The Hague, 1992, 114

Tanaka, K. and Atwell, R.B., 1991. The humoral response in natural *Dirofilaria immitis* infections in dogs. *Australian Veterinary Journal*, **68**, 396–399

Tassinari, M. and Bonsembiante, P., 1984. Le filariosi cardiopolmonari del cane. *Bollettino AIVPA*, **23**, 5–18

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