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A prevalence survey and risk analysis of filariosis in dogs from the Mt. Vesuvius area of southern Italy

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Abstract

A dog microfilariae prevalence and risk factor survey was conducted in 51 contiguous municipalities of the Mt. Vesuvius area (Campania region, southern Italy) in order to add data to the limited epidemiological information available regarding filarial worms in this zone. Between May 1999 and June 2000, blood samples were collected from 351 asymptomatic dogs. Blood samples were examined using a modified Knott's technique and histochemical staining in order to count and identify microfilariae. The results were subjected to statistical analysis and choroplethic municipal maps (MMs) were drawn by a geographical information system (GIS) software. Microfilariae were detected in 63 of the 351 dogs surveyed, constituting a total filarial prevalence of 17.9%. In particular, 56 dogs (15.9%) showed only microfilariae of *Dipetalonema reconditum*; three dogs (0.8%) only microfilariae of *Dirofilaria repens*; two dogs (0.6%) microfilariae of both *D. reconditum* and *D. repens* and two dogs (0.6%) microfilariae of both *Dirofilaria immitis* and *D. repens*. High *D. reconditum* prevalence was associated with hunting practice, masculine gender and older dogs. There was also a tendency to find high prevalence in dogs sampled in the afternoon.

In conclusion, the presence of microfilariae of *D. reconditum* in 92% of microfilaraemic dogs indicates that this filarial worm was the predominant filarial species in dogs in the Mt. Vesuvius area.

In addition, the general trends of the MMs showed that *D. immitis* and *D. repens* were present only in a few municipalities, whereas *D. reconditum* was widely and homogeneously spread throughout the entire study area.

Keywords: Dog; Filarial worms; Epidemiology; Italy; Geographical information system; *Dipetalonema reconditum*; *Dirofilaria* spp.

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1. Introduction

The common filarial species known to parasitize dogs in Italy are *Dirofilaria immitis* Leidy, 1856, *Dirofilaria repens* Railliet and Henry, 1911, *Dipetalonema reconditum* Grassi, 1890, and *Dipetalonema grassii* Noè, 1907.

Adult *D. immitis*, typically inhabit the pulmonary arteries and the right ventricle of the heart where they cause canine heartworm disease. *D. repens*, *D. reconditum* and *D. grassii* are reported to be nonpathogenic by Soulsby (1982), Lindemann et al. (1983), and Georgi and Georgi (1990). As adults, they usually inhabit the subcutaneous tissue. However, *D. reconditum* has also been reported in perirenal fat and *D. grassii* has also been found in the peritoneal cavity of dogs (Traldi, 1998).

In addition, it is well-known that *D. immitis*, *D. repens* and *D. reconditum* produce microfilariae that circulate in the blood of dogs, and that *D. grassii* produces microfilariae that usually circulate in the tissue lymph fluid.

D. immitis occurs worldwide in tropical, sub-tropical and temperate zones (Martin and Collins, 1985). *D. repens* occurs in the old world, in particular, throughout the Mediterranian sub-region, South Asia and sub-Saharan Africa (Lock, 1988; Pampiglione et al., 1995). *D. reconditum* occurs in North America, Europe and Africa (Quinn et al., 1997), and is also well-known as a parasite of dogs in Australia (Boreham and Atwell, 1985). *D. grassii* occurs in southern Europe, Africa (Quinn et al., 1997) and India (Balasubramanian et al., 1975).

Several studies have been published regarding the distribution and prevalence of filariosis in dogs from northern and central Italy. They tend to focus on *D. immitis* because of its pathogenicity and consequent veterinary importance. Since only two studies have been published regarding filariosis in dogs from continental southern Italy (Puccini and Abbenante, 1980; Capuano et al., 1997), limited epidemiological information is available regarding filarial worms in this zone.

For these reasons, a survey was designed to study the distribution and prevalence of filarial worms in asymptomatic dogs living in the Mt. Vesuvius area of the Campania region (southern Italy) utilizing a geographical information system (GIS) software to plan the sampling and to display the results.

2. Materials and methods

2.1. Study area

The study was conducted in the Campania region of southern Italy, in 51 contiguous municipalities (2180 km^2 area) located around the base of Mt. Vesuvius near Naples (40°45′–41°03′N and 14°15′–14°45′E). These municipalities are piedmont areas overlooking the Tyrrhenian Sea, extending from 50 to 200 m above the sea level. There are no lakes; a small river, however, runs through a small tract of the low-lying areas. The climate is temperate.

2.2. Experimental animals

Blood samples from a total of 351 dogs were collected. This sample size was calculated using the formula proposed by Thrusfield (1995) for a large (theoretically "infinite") population using the following values: expected prevalence 5% (based on our previous studies on *D. repens*, Capuano et al., 1997), confidence interval (99%) and desired absolute precision (3%).

In each municipality, the number of dogs sampled was proportional to the municipal surface area. Based upon the assumption that the dog population is homogeneously distributed throughout the study area, the GIS program calculated a sample size for each municipality based upon the proportion of its surface area to the total surface area covered by the study.

The GIS software used in this study was Idrisi, distributed by "The Idrisi Project" Clark University, Graduate School of Geography, Worcester, MA, USA.

2.3. Blood samples

Blood samples from asymptomatic dogs were collected between May 1999 and June 2000. Drawing stations were established in two veterinary clinics located in the study area. Dogs were randomly selected by veterinarians from those brought to their clinic for routine health care procedures. Each veterinarian was provided with uniform materials for blood collection and transport, a copy of the trial protocol and the number of dogs to be screened in each municipality under his responsibility in the study.

A 3–5 ml of whole blood was drawn from the cephalic vein of each dog, collected in sodium citrate vacuum tubes and stored under refrigeration until analysis. All samples were obtained during the day. In addition, each dog was registered as to sex, age, weight, hair length, utilization, live together with other dogs, indoor/outdoor night status, date and time of blood sampling, and geographical origin. A questionnaire form was designed to record these data.

2.4. Laboratory procedures

Transportation time from the drawing stations to the University of Naples was 1–2 days, and all blood samples were analyzed on the day of arrival using the modified Knott's technique as reported by Balbo and Panichi (1968).

The morphometric identification of microfilariae was based on the criteria reported by Lindsey (1965) and Balbo and Panichi (1968). In addition, all microfilariae in every sample were counted. The length and width of all microfilariae in each sample were measured under $400\times$ magnification using an ocular micrometer.

In order to confirm the morphometric identification, blood samples which were Knott's positive were further analyzed to detect the somatic distribution of acid phosphatase activity. For this purpose, the polycarbonate filtration acid phosphatase histochemical staining according to Whitlock et al. (1978) was performed.

2.5. Statistical analysis

D. repens and *D. immitis* were not included in the statistical analysis because of their very low prevalence in dogs.

The individual dog data were analyzed by the logistic regression model, using the *D. reconditum* status as a dependent variable (negative = 0; positive = 1). The independent

Table 1
Profiles and parasitological results of 351 dogs tested for filariosis in the Mt. Vesuvius area between May 1999 and June 2000

Dog profile	No. of dogs tested	Parasitological results (no. of positive dogs, %)									
		D. reconditum (A)			*D. repens* (B)				*D. immitis* (C)		Total micro-filariaemia
		A	A and B	Total	B	B and A	B and C	Total	C and B	Total	
Sex											
Male	214	40 (18.7)	2 (0.9)	42 (19.6)	3 (1.4)	2 (0.9)	2 (0.9)	7 (3.3)	2 (0.9)	2 (0.9)	47 (22.0)
Female	137	16 (11.7)	–	16 (11.7)	–	–	–	–	–	–	16 (11.7)
Age (year)											
<1	31	–	–	–	–	–	–	–	–	–	–
1–7	298	48 (16.1)	2 (0.7)	50 (16.8)	3 (1.0)	2 (0.7)	2 (0.7)	7 (2.3)	2 (0.7)	2 (0.7)	55 (18.5)
>7	22	8 (36.4)	–	8 (36.4)	–	–	–	–	–	–	8 (36.3)
Weight											
Small	11	–	–	–	–	–	–	–	–	–	–
Medium	278	52 (18.7)	2 (0.7)	54 (19.4)	3 (1.1)	2 (0.7)	2 (0.7)	7 (2.5)	2 (0.7)	2 (0.7)	59 (21.2)
Large	62	4 (6.4)	–	4 (6.4)	–	–	–	–	–	–	4 (6.5)
Hair length											
Short	103	15 (14.6)	–	15 (14.6)	1 (0.9)	–	–	1 (1.0)	–	–	16 (15.5)
Medium	218	40 (18.3)	2 (0.9)	42 (19.3)	2 (0.9)	2 (0.9)	2 (0.9)	6 (2.7)	2 (0.9)	2 (0.9)	46 (21.1)
Long	30	1 (3.3)	–	1 (3.3)	–	–	–	–	–	–	1 (3.3)
Utilization											
Pet	31	–	–	–	–	–	–	–	–	–	–
Guard dog	60	–	–	–	–	–	–	–	–	–	–
Hunting dog	260	56 (21.5)	2 (0.8)	58 (22.3)	3 (1.1)	2 (0.8)	2 (0.8)	7 (2.7)	2 (0.8)	2 (0.8)	63 (24.2)
Live together with other dogs											
Yes	287	51 (17.8)	2 (0.7)	53 (18.5)	3 (1.0)	2 (0.7)	2 (0.7)	7 (2.4)	2 (0.7)	2 (0.7)	58 (20.2)
No	64	5 (7.8)	–	5 (7.8)	–	–	–	–	–	–	5 (7.8)
Outdoor night status											
Yes	340	55 (16.2)	2 (0.6)	57 (16.8)	3 (0.9)	2 (0.6)	2 (0.6)	7 (2.1)	2 (0.6)	2 (0.6)	62 (18.2)
No	11	1 (9.1)	–	1 (9.1)	–	–	–	–	–	–	1 (9.1)
Sampling time											
a.m.	77	6 (7.8)	2 (2.6)	8 (10.4)	1 (1.3)	2 (2.6)	–	3 (3.9)	–	–	9 (11.7)
p.m.	274	50 (18.2)	–	50 (18.2)	2 (0.7)	–	2 (0.7)	4 (1.5)	2 (0.7)	2 (0.7)	54 (19.7)
	Total positives	56 (15.9)	2 (0.6)	58 (16.5)	3 (0.8)	2 (0.6)	2 (0.6)	7 (2.0)	2 (0.6)	2 (0.6)	63 (17.9)

variables listed in Table 1 were tested in the multivariate model by the stepwise forward method.

At each step, the least significant variable was removed from the model until all remaining variables were significant at $P < 0.05$.

The odds ratios (OR) were calculated for the variables included in the final model.

In the first step of analysis, the risk associated with the utilization of dog (pet, guard or hunting dog) was not computed because there were no positives for *D. reconditum* in the categories “pet” and “guard dog”.

Since the variable “utilization” showed a high association with the *D. reconditum* prevalence ($P < 0.01$) in the univariate screening by the Chi-square test, it was reintroduced in the model applying “the rule of three” (Hanley and Lippmann-Hand, 1983). Specifically, the two categories “pet” and “guard dog” were conflated ($n = 91$) into the new binary variable “hunting practice” (yes = 1; no = 0) and three dogs positive for *D. reconditum* were randomly assigned a negative hunting practice value.

All the statistical analysis was performed using SPSS 10.0 software for Windows.

2.6. Data mapping

In order to display the distribution and prevalence of each filarial worm in each municipality studied, choroplethic municipal maps (MMs) (Thrusfield, 1995) were drawn using Idrisi. These maps use the municipality as the geographic unit of reference and display the following information: (1) filarial species studied; (2) total study area divided into 51 municipalities; (3) municipalities with positive dogs; (4) municipalities without positive dogs; and (5) municipal prevalence (MP).

The MP (%) was determined as follows:

$$\text{MP} = \frac{\text{number of positive dogs in the municipality}}{\text{number of dogs examined in the total study area}} \times 100$$

3. Results

The profiles and the parasitological results of tested dogs are shown in Table 1.

Microfilariae were detected in 63 (17.9%) of the 351 dogs sampled and classified as belonging to three different species: *D. reconditum, D. repens*, and *D. immitis*.

Microfilariae of *D. reconditum* were present in 58 dogs (16.5%). *D. reconditum* occurred as a single infection in 56 (15.9%) dogs and together with microfilariae of *D. repens* in two dogs (0.6%). Microfilariae of *D. repens* were present in seven dogs (2.0%). *D. repens* occurred as a single infection in three dogs (0.8%), together with microfilariae of *D. reconditum* in two dogs (0.6%), and together with microfilariae of *D. immitis* in two dogs (0.6%). Microfilariae of *D. immitis* were only present in two dogs (0.6%), in both cases with microfilariae of *D. repens*.

The length and width measurements of identified microfilariae are reported in Table 2.

The average number of microfilariae detected in positive blood samples was 10/ml for *D. reconditum* (3 (min)–32 (max)), 39/ml for *D. repens* (17 (min)–100 (max)) and 125/ml for *D. immitis* (100 (min)–150 (max)).

Table 2
Measurements of microfilariae detected by modified Knott's technique

Microfilarial species	Length (μm)		Width (μm)	
	Mean	S.D.	Mean	S.D.
D. immitis	311.3	9.5	5.96	0.15
D. repens	366.2	12.1	6.40	0.31
D. reconditum	265.2	10.1	5.01	0.49

Table 3
Results of multivariate analysis[a]

Dog profile (independent variable name)	Standard error	*P*-value	OR	95% Confidence interval	
				Inferior	Superior
Hunting practice[b]	0.622	0.000	8.979	2.653	30.382
Age	0.458	0.001	4.808	1.960	11.790
Sex	0.333	0.035	2.015	1.049	3.871

[a] Significant association between *D. reconditum* prevalence and dog profiles.
[b] See Section 2, for this variable.

Histochemical staining showed an acid phosphatase distribution similar to that described by Balbo and Abate (1972) and Whitlock et al. (1978): the microfilariae of *D. immitis* exhibited two distinct red-staining spots (one at the excretory pore and one at the anal pore); the microfilariae of *D. repens* exhibited only one staining red spot at the anal pore;

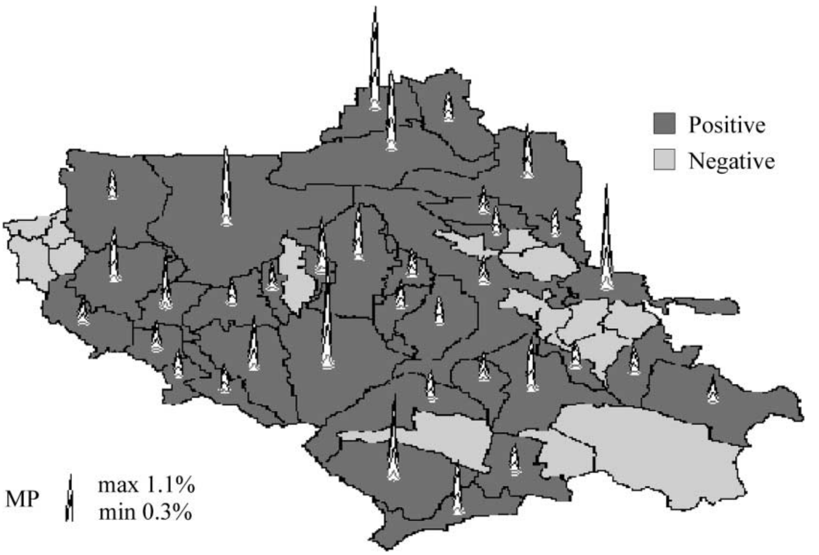

Fig. 1. Mt. Vesuvius area municipalities with dogs parasitized by *D. reconditum* and MP.

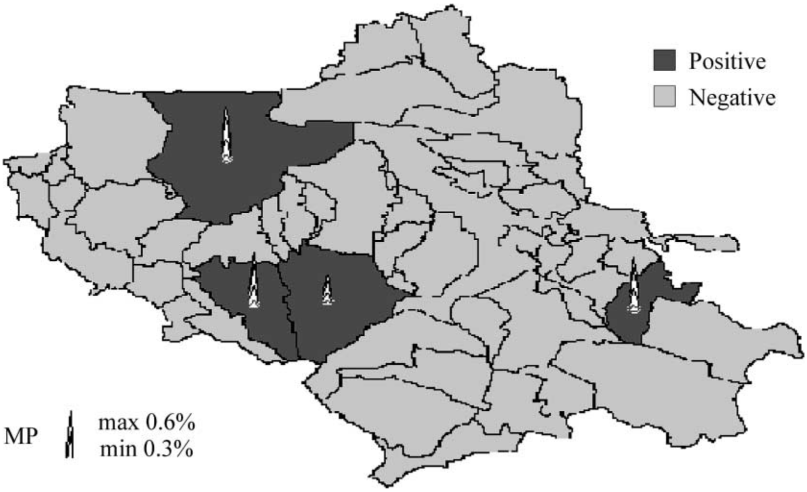

Fig. 2. Mt. Vesuvius area municipalities with dogs parasitized by *D. repens* and MP.

and the microfilariae of *D. reconditum* exhibited a diffuse pink staining throughout the body, not correlated with a well-defined anatomical structure.

The significant relationship between dog profiles and *D. reconditum* prevalence is shown in Table 3. The utilization, age and sex resulted as risk factors, i.e. hunting dog, older dog and male dog had a higher rate of infection. Particularly, the hunting practice showed the strongest association with *D.reconditum* prevalence ($P < 0.01$). Associations were not found for weight, hair length, night outdoor status and presence of other dogs. The variable

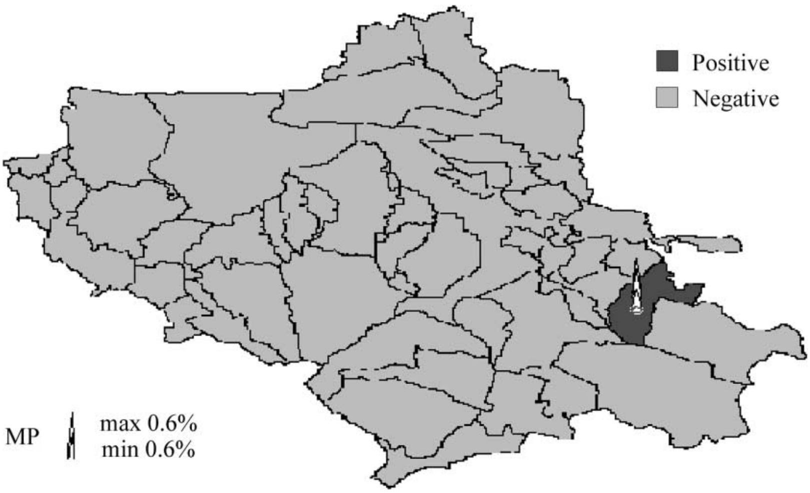

Fig. 3. Mt. Vesuvius area municipalities with dogs parasitized by *D. immitis* and MP.

"time of sampling" was the last variable removed from the multivariate model, showing a tendency to be associated with *D.reconditum* prevalence ($P = 0.093$).

The MMs in Figs. 1, 2 and 3 show the following: (1) dogs parasitized by *D. reconditum* were present in 36 of the 51 municipalities studied and the MP range was between 0.3 and 1.1%, (2) dogs parasitized by *D. repens* were present in only four municipalities and the MP range was between 0.3 and 0.6% and (3) dogs parasitized by *D. immitis* were present in only one municipality having an MP of 0.6%.

4. Discussion and conclusion

Our present survey, carried out in the Mt. Vesuvius area of the Campania region (southern Italy), has shown low values for the prevalence of *D. immitis* (0.6%) and *D. repens* (2.0%) in the dog population sampled. The prevalence for *D. reconditum*, however, was fairly high (16.5%).

In Italy, the overall country-wide prevalence, based upon the surveys published by Italian investigators between 1910 and 2000, is 20.5% for *D. immitis*, 12.7% for *D. repens*, and 1.5% for *D. reconditum*.

D. immitis occurs throughout the northern regions of Italy with an average prevalence of 24%; however, in the central and southern regions, it is rarely present and has an average prevalence of 3.1% (Guerrero et al., 1989). In the present survey, the low prevalence of *D. immitis* together with the fact that the two dogs infected with microfilariae of *D. immitis* were not natives of the Mt.Vesuvius area, but had come from northern Italy (endemic area), confirms the rare diffusion of *D. immitis* in southern Italy.

D. repens is more homogeneously spread throughout Italy; it is found in several regions of the north, centre and south of the country, with prevalence values ranging from 1.4% in Apulia (Puccini and Abbenante, 1980) to 32.6% in Sicily (Cancrini and Scaglione, 1984). The prevalence value for *D. repens* reported in our present survey, 2.0%, is one of the lowest values reported in Italy. Moreover, it is lower than the 5% previously reported for other areas of the Campania region (Capuano et al., 1997).

D. reconditum, the predominant filarial species in this survey, has previously been reported in nine different Italian regions with prevalence values ranging from 0.9% in Emilia Romagna (Canestri-Trotti et al., 1988) to 9% in Piemonte (Balbo and Panichi, 1968).

It is important to note that the *D. reconditum* prevalence (16.5%) reported in our survey is the highest ever reported in Italy. By comparing this value to those in other recent studies of *D. reconditum* in the Mediterranian area, one sees that it is similar to the 15.8% reported by Ortega-Mora et al. (1991) for Soria province (Spain), but is higher than the 3.7% reported by Aranda et al. (1998) for the Llobregat area of Barcelona (Spain) and the 6% reported by Papazahariadou et al. (1994) for Thessaloniki province (northern Greece).

The statistical analysis performed by the logistic regression model showed that hunting practice, increasing age and masculine gender were risk factors for *D. reconditum* infection. The first two factors are probably related to the filarial life-cycle which, in Italy, involves fleas and ticks as intermediate hosts. Dog owners and veterinarians know very well that dogs used for hunting have a higher probability of being infected by ticks than pets and guard dogs.

The present study has not found any association between *D. reconditum* positivity and live together, in contrast to the results reported by Balbo and Panichi (1968) and Theis et al. (1999). This lack of association, together with the association between *D. reconditum* positivity and hunting practice, stresses the role of the tick in the transmission of this parasite in the Mt. Vesuvius area.

The increase in *D. reconditum* prevalence in older dogs has also been reported by Theis et al. (1999). This is probably due to the fact that older dogs are more easily infected because of many past flea and tick infestations. In general, immunity against these arthropods is incomplete and does not guarantee protection against new infestations.

It is more difficult to explain why males are more subject to infection than females. This observation has also been reported by Theis et al. (1995) and merits further attention. The higher *D. reconditum* positivity in dogs sampled in the afternoon suggests, as has been reported for *D. immitis* (Traldi, 1998), that microfilariaemia varies in the blood during the course of the day.

The GIS software employed in the present study and in other territorial parasitological surveys (Cringoli et al., 1996, 2000a,b) was useful for the planning of sampling procedures and for clearly and quickly displaying the spatial distribution of filarial worms in the study area. In particular, the MMs showed that *D. immitis* and *D. repens* are present only in a few municipalities, whereas *D. reconditum* is widely and homogeneously spread throughout the study area.

In conclusion, the findings in this survey indicate that *D. reconditum* is the predominant filarial species in dogs in the Mt. Vesuvius area. The presence of the microfilariae of *D. reconditum*, reported above as a nonpathogenic filarial worm, in 92% of microfilaraemic dogs in the study area emphasizes the need to identify the particular species of microfilariae before initiating chemoprophylactic and/or chemotherapeutic strategies.

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