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Canine filarial infections in Tuscany, central Italy

M. Magi[1], L. Guardone[1], M.C. Prati[2], G. Tozzini[1], B. Torracca[1], G. Monni[1] and F. Macchioni[1]*

[1]Dipartimento di Patologia Animale, Profilassi e Igiene degli Alimenti, Pisa University, Viale delle Piagge 2, 56124 Pisa, Italy: [2]Scuola Normale Superiore di Pisa, Italy; INFN Sezione di Pisa, Italy

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Abstract

This study was conducted in order to investigate the epidemiology of filarial species in a region of central Italy (Tuscany) in dogs that did not undergo prophylaxis for filariasis. From 2007 to 2009, 630 blood samples were collected from 40 kennels throughout the regional territory. Samples were analysed with Knott's modified test and with an enzyme-linked immunosorbent assay (ELISA) for the detection of *Dirofilaria immitis* antigens, those positive for microfilariae were also subjected to histochemical staining for acid phosphatase activity to validate the identification of the species. An overall elevated prevalence of filariasis (23.2%) was found. *Dirofilaria immitis* and *D. repens* were found to be the dominant species, with similar prevalences (12.5% and 12.1% respectively), while *Acanthocheilonema reconditum* was less common (1.9%). Results of this study indicate that monitoring for filariasis should be kept at a high level. A constant monitoring of the canine sanitary status should also be carried out for the protection of human health, considering the zoonotic potential of filarial worms.

Introduction

Dogs can be infected by several species of filarial worms; species found in Italy are *Dirofilaria immitis*, *Dirofilaria* (*Nochtiella*) *repens*, *Acanthocheilonema* (syn. *Dipetalonema*) *reconditum* and, less frequently, *Acanthocheilonema* (syn. *Dipetalonema*) *dracunculoides* and *Cercopithifilaria* (syn. *Acanthocheilonema*) *grassii*. These species can also infect cats and wild animals, as well as foxes and wolves, and occasionally humans. Filarial nematodes differ in the localization of adult parasites: *D. immitis* adult stages live in the right side of the heart and in the pulmonary artery, *D. repens* lives in the subcutaneous tissue, while *A. reconditum*, *A. dracunculoides* and *C. grassii* live in the peritoneal cavity, in adipose tissue and in intramuscular spaces, respectively. Their life cycle is indirect, with definitive vertebrate hosts and arthropods as intermediate hosts, also acting as vectors. After mating, adult female worms release microfilariae into the haematic circulation of the definitive host. Arthropods, in which first-stage larvae complete their development to the infective third stage, are mosquitoes belonging to genera *Culex* and *Aedes* for *Dirofilaria* spp., and fleas (*Ctenocephalides* spp.) and ticks (*Rhipicephalus* spp.) for *Acanthocheilonema* spp. and *C. grassii*.

Dirofilaria immitis and *D. repens* are filarial species of major concern in Europe for both canine and human health (Simón *et al.*, 2009). *Dirofilaria immitis* is the aetiological agent of canine heartworm disease and it is the most pathogenic species, as it can be lethal; in dogs *D. repens* is considered less dangerous and causes pruriginous dermatitis. In Italy current data on canine dirofilariasis confirms the expanding trend of parasites as observed in the rest of Europe (Genchi *et al.*, 2009). Today *D. immitis* is present, not only in the hyperendemic area of the Po River Valley, but also in the northern part of Italy (Lombardy and Friuli Venezia Giulia), in central Italy (Umbria, Abruzzo and Tuscany), in the south (Campania, Apulia and Calabria) and in the islands (Sicily and

*Fax: 0502216941
E-mail: fmacchion@vet.unipi.it

Table 1. Dogs classified according to districts and detected filarial species; k = number of kennels sampled; n = number of dogs sampled.

Districts	*k*	*n*	*D. immitis*	*D. repens*	*A. reconditum*	*D. immitis* *D. repens*	*D. immitis* *A. reconditum*	*D. repens* *A. reconditum*
Arezzo	2	27	0	4	0	2	0	0
Firenze	8	162	11	13	4	5	0	1
Grosseto	7	141	22	20	0	4	0	0
Livorno	4	27	2	10	1	1	0	3
Lucca	4	26	2	4	0	0	0	0
Massa-Carrara	2	60	1	0	0	0	0	0
Pisa	5	55	10	0	0	0	3	0
Pistoia	4	41	7	0	0	0	0	0
Prato	1	31	5	3	0	0	0	0
Siena	3	60	2	4	0	2	0	0
Total	40	630	62 (9.8%)	58 (9.2%)	5 (0.8%)	14 (2.2%)	3 (0.5%)	4 (0.6%)

Sardinia). *Dirofilaria repens* is distributed in almost all of the Italian territory.

Acanthocheilonema reconditum has a worldwide distribution in dogs (Quinn *et al.*, 1997). In Europe it has been reported in Serbia (Tasić *et al.*, 2008), in Spain (Aranda *et al.*, 1998), in Greece (Vakalis & Himonas, 1997), in Austria (Hinaidy *et al.*, 1987) and in Italy (Cringoli *et al.*, 2001). *Acanthocheilonema dracunculoides* has rarely been reported: in Europe it has been found in dogs in The Netherlands, Greece, Spain and Portugual (Bolio *et al.*, 2004). In Italy it was found in foxes in Latium and Tuscany (Cancrini, 1986; Magi *et al.*, 2009) and for the first time it has been reported in a dog in Sicily (Giannetto *et al.*, 2003). *Cercopithifilaria grassii* was discovered in 1907 in a dog from Rome (Noè, 1908), subsequent sporadic reports have occurred (India: Balasubramanian *et al.*, 1975; Greece: Vakalis & Himonas, 1997). In Italy microfilariae were found in the blood of a dog from Sicily (Giannetto *et al.*, 2004) and of a cat from the Aosta Valley (Tarello, 2004).

All canine filarial species have the potential to infect humans; *D. immitis* can cause pulmonary nodules, *D. repens* ocular and subcutaneous nodules and pruriginous dermatitis (Pampiglione & Rivasi, 2007; González-Miguel *et al.*, 2010) and *A. reconditum* can affect the eye (Huynh *et al.*, 2001). The aim of this study is to investigate the prevalence and the distribution of different filarial species in an area of central Italy (Tuscany).

Materials and methods

The study was carried out between April 2007 and December 2009 in Tuscany, a region with natural breeding sites for a great variety of mosquito species, such as *Culex pipiens*, *Culex modestus*, *Anopheles maculipennis*, *Coquilletidia richardii* and *Aedes albopictus* (Cancrini *et al.*, 2006), which have all proved to be suitable vectors of dirofilarial nematodes (Cancrini & Gabrielli, 2007).

Blood samples were collected from 630 dogs; 40 kennels were involved in the study (see table 1). The sample size was chosen so that the number of subjects enrolled in each municipality was roughly proportional to the surface area of the municipality itself. Animals were housed in outdoor kennels located in different towns in every district of Tuscany. They were all autochthonous, older than 6 months of age and had not undergone any prophylactic treatment for filariasis. Dogs were sampled in kennels, as most privately owned dogs currently undergo chemoprophylaxis. Individual data about sex, age, breed, living habitat and geographical origin were recorded. Each blood sample was divided into two fractions: 1 ml of whole blood was mixed with 9 ml of 2% formaldehyde solution for the detection of circulating microfilariae; and at least 2 ml were collected in tubes with anticoagulant, in order to obtain plasma for *D. immitis* antigen detection. Samples were submitted to the Department of Animal Pathology, Prophylaxis and Food Hygiene of Pisa University.

The identification of microfilariae species was carried out using modified Knott's technique (Lindsey, 1965). In order to confirm the morphometric identification, blood samples which were Knott's positive were further analysed using the naphthol AS-TR-phosphate method for acid phosphatase activity as described by Barka (1966). This staining distinguishes microfilariae of *D. immitis*, *D. repens*, *A. reconditum* (Chalifoux & Hunt, 1971; Balbo & Abate, 1972) and *A. dracunculoides* (Ortega-Mora *et al.*, 1989) by four different patterns, depending on the different activity of the acid phosphatase enzyme: *D. immitis* microfilariae have two red areas corresponding to the excretory pore and the anal pore; *D. repens* microfilariae only have one red area corresponding to the anal pore; *A. reconditum* microfilariae show a reddish colour on the whole body and *A. dracunculoides* microfilariae show a very intense red colour on the central part of the body, at the anal pore and at the excretory pore.

Samples were also subjected to an enzyme-linked immunosorbent assay (ELISA) for the detection of antigens of *D. immitis* with the Canine Heartworm Antigen kit (DiroCHEK®, Synbiotics, Kansas City, Missouri, USA). The procedure was conducted following instructions contained in the kit.

Results were subjected to statistical analysis, using the χ^2 test, and were considered significant if $P < 0.05$.

Results and discussion

Amongst 630 samples tested, 146 were positive for filariasis (prevalence 23.2%; 95% confidence interval: 19.9–26.5%). *Dirofilaria immitis* was found in 79 cases (12.5%), *D. repens* in 76 (12.1%) and *A. reconditum* in 12 (1.9%).

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Several cases of double infestations were found; the detailed situations in each district are summarized in table 1. The difference amongst the prevalence of the first two species in the various districts is highly significant ($P < 0.0001$). No sex- and age-related differences were observed.

Filarial infections in Italy have, in recent years, shown an expanding trend, as reported for all Europe (Genchi *et al.*, 2005). The prevalence (23.2%) of filariasis in untreated dogs found in this study, shows that in Tuscany the parasitic pressure and the risk of infestation are high. *Dirofilaria immitis* and *D. repens* were the dominant species, showing widespread diffusion throughout the region and similar prevalence values (12.5% and 12.1%, respectively), whereas *A. reconditum* (1.9%) was less common and was found in only three districts (Firenze, Pisa and Livorno). Different intermediate hosts and the easy control of fleas and ticks in contrast to dirofilariasis vectors could explain the lower prevalence of *A. reconditum*. The widespread diffusion in Tuscany of mosquito species, potential vectors of dirofilariae (Cancrini *et al.*, 2006), including *Ae. albopictus*, might have played a determining role in the presence of dirofilariasis. The identification of species achieved with the modified Knott's technique was validated by the acid phosphatase histochemical staining.

The ELISA test for *D. immitis* antigens was positive for 65 dogs, 42 of these were also found to be positive using Knott's modified test, while 23 were negative for the presence of *D. immitis* microfilariae (occult filariasis): this could be due to a unisex infection, a prepatent period or an immune-mediated clearance of microfilariae. On the other hand, 10 animals were found to be positive for *D. immitis* microfilariae using Knott's method and acid phosphatase staining but negative for antigen detection; this could be explained by the immune-mediated clearance of antigen–antibody complexes.

This study shows that the monitoring of filariasis should be kept at a high level because, despite the common use of prophylaxis, parasites are widespread. Dogs' sanitary status should be constantly monitored, for the protection of both animal and human health, taking into consideration the zoonotic potential of filarial worms, in particular *D. repens*. A complete diagnosis with the correct species identification is needed in order not to underestimate the presence of species that are not detectable using serological tools. Finally, awareness of prophylaxis, accurate diagnosis and correct treatment by veterinary surgeons are important.

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