

SHORT REPORT

Zoonotic *Dirofilaria immitis* infections in a province of Northern Spain

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(Accepted 30 June 2009; first published online 21 July 2009)

SUMMARY

Dirofilaria immitis is the causal agent of canine and feline cardiopulmonary dirofilariasis. Moreover, the existence of canine dirofilariasis implies a risk for human populations living in an endemic area in which, the parasite can cause pulmonary dirofilariasis. The Spanish epidemiological situation is not well understood, lacking data from many central and Northern provinces. In our study, epidemiological data on canine and human dirofilariasis for La Rioja (Northern Spain) have been obtained for the first time. The overall prevalence of *D. immitis* in dogs was 12% (9% of patent and 3% of occult infections), being 11·6% the seroprevalence of human residents in this province. The geographic distribution of both canine and human *D. immitis* infections in La Rioja is restricted to humid and irrigated areas near the Ebro Valley River, being absent in the rest of the province where hills and mountains predominate.

Key words: *Dirofilaria immitis*, dogs, humans, La Rioja (Spain).

Cardiopulmonary dirofilariasis caused by *Dirofilaria immitis* is a vector-borne transmitted disease. Because some mosquito vectors feed indistinctly on animal reservoirs and humans, *D. immitis* is also transmitted to humans where it causes pulmonary dirofilariasis. Thus, the existence of canine dirofilariasis represents a risk for human populations living in endemic areas and for travellers visiting these areas [1].

Canine dirofilariasis has a cosmopolitan distribution. In Europe, the highest prevalences have been observed in the Mediterranean countries such as Italy, France, Greece, and Spain [2]. Moreover the spreading of dirofilariasis to central and Northern European countries as well as suitable conditions for the development of vector populations have been observed

over recent years probably due, among other factors, to global warming and management of pets [3]. The distribution of human dirofilariasis is more difficult to assess. Most clinical cases of pulmonary dirofilariasis have been found in the USA, Japan and Australia, but in recent years, isolated cases or short series of cases have been reported with increasing frequency in other endemic countries, and in countries considered non-endemic or in which dirofilariasis has not been observed previously [4]. Clinical cases only represent a part of human infections. Seroepidemiological studies carried out in human populations living in endemic areas indicate that the incidence of infection in humans is close to that observed in canine populations of the same areas [5].

The information on the distribution and prevalence of cardiopulmonary dirofilariasis in Spain is incomplete. Canine heartworm infections have been detected in many provinces, with the exception of the

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Table 1. Seroprevalence of heartworm disease and human dirofilariasis in dogs and humans in La Rioja (Spain)

Dogs	Male			Female			Total		
	Dogs (n)	mf +	IDEXX	Dogs (n)	mf +	IDEXX	Dogs (n)	mf +	IDEXX
<2 yr	9	1	1	6	0	0	15	(1%) 1	(1%) 1
2–5 yr	17	2	3	21	1	1	38	(3%) 3	(4%) 4
>5 yr	20	2	2	27	3	5	47	(5%) 5	(7%) 7
Total	46	(10.86%) 5	(13.04%) 6	54	(7.4%) 4	(11.11%) 6	100	(9%) 9	(12%) 12

Humans	Male		Female		Total	
	Humans (n)	<i>D. immitis</i> ELISA positive	Humans (n)	<i>D. immitis</i> ELISA positive	Humans (n)	<i>D. immitis</i> ELISA positive
<20 yr	15	1	10	0	25	(0.4%) 1
20–60 yr	82	12	89	10	171	(8.8%) 22
>60 yr	31	3	24	3	54	(2.4%) 6
Total	139	(11.51%) 16	111	(11.71%) 13	250	(11.6%) 29

IDEXX, Canine Heartworm Antigen Petchek PF IDEXX test (Westbrook, USA).

central and Northern regions. The highest prevalences have been reported in the Canary Islands and Southern provinces, as well as in irrigated and wet areas of Western and North-eastern regions of the Iberian Peninsula [6]. Human pulmonary dirofilariasis has been identified only in an endemic area of Salamanca (Western) [5] and in the Canary Islands [7].

In the current study we demonstrate, for the first time, the existence of canine and human *Dirofilaria immitis* infections in La Rioja (Northern Spain), contributing to the completion of the map of the distribution of heartworm infections in Spain.

La Rioja is a Northern Province of Spain located at 42° 20' N latitude, and 02° 20' W longitude, covering a surface area of about 5.045 km². Two geographic and climatic areas can be distinguished: Zone A – the Ebro Valley in the North, including broad irrigated areas with a mean altitude of 400 m a.s.l. The most important urban nuclei are located in this zone. Zone B – the rest of the province is occupied by hills and mountains with small river valleys. The altitude is between 500 and 2000 m a.s.l. There are many small towns in this area.

Blood samples were taken from dogs ($n=100$) and humans ($n=250$) in 49 localities of the province. Dog samples were taken randomly from animals that received routine veterinary attention during summer 2006. Forty-two samples were from male animals and 58 from female animals. The numbers by age group for dogs was: <2 years ($n=15$), 2–5 years ($n=40$) and >5 years ($n=45$). Human serum samples were taken

randomly from people seeking medical attention for causes other than dirofilariasis, during the same period as for dogs. Of the humans samples 120 were from men and 130 from women. The numbers by age group for humans was: <20 years ($n=25$), 20–60 years ($n=171$) and >60 years ($n=54$).

The presence of microfilariae and circulating antigens of *D. immitis* were analysed in the dog samples. To identify canine *D. immitis* infections, blood samples were analysed by the Canine Heartworm Antigen Petchek PF IDEXX test (Westbrook, USA) for the detection of *D. immitis* circulating antigens, according to the manufacturer's instructions. A modified Knott technique as described by Acevedo *et al.* [8] was used to determine the presence or absence of microfilariae.

Human samples were analysed with an ELISA test to detect the specific IgG antibody response using adult *D. immitis* somatic antigens as described by Simón *et al.* [5] with some modifications. Briefly, 96-well microplates were coated with 0.8 µg of an extract of *D. immitis* adult worms (DiSA). All serum samples were analysed at a 1:100 dilution and the secondary antibody (anti-human IgG peroxidase-conjugated; Merck, Germany) was used at 1:4000 dilution. Optical densities (ODs) were measured at 492 nm in an Easy Reader (Bio-Rad Laboratories, USA). The cut-off (OD=0.8) was established calculating the mean value ± 3 s.d. of 20 serum samples from clinically healthy blood donors living in a *D. immitis*-free area.

Twelve out of the 100 dog samples analysed were positive using the IDEXX test and nine of these had microfilariae in their blood. Thus the overall

prevalence of *D. immitis* is 12%; of which 9% were microfilaraemic and 3% amicrofilaraemic infections. All these dogs were companion animal living habitually outdoors in both rural and small urban areas from Zone A (Ebro Valley). The ages of the 12 seropositive dogs were: <2 years ($n=1$), 2–5 years ($n=4$) and >5 years ($n=7$). The prevalence detected in males was 13.04% and in females 11.11%. Nine out of 12 positive ELISA test kits analysed were positive using the Knott technique indicating a prevalence of 9% of microfilaraemic infections (Table 1).

In humans, 29/250 serum samples were positive using the ELISA test, indicating a seroprevalence of 11.6% (Table 1). The highest seroprevalence was observed in the 20–60 years age group (8.8%), followed by the >60 years group (2.4%), and the <20 years group (0.4%). All positive samples belonged to individuals living in Zone A. No differences in seroprevalence were observed between males and females in humans (11.51% and 11.71%, respectively).

This is, to our knowledge, the first report on *Dirofilaria immitis* in the canine and human populations of La Rioja (Spain). From the technical point of view the use of the IDEXX test allowed the specific identification of *D. immitis* infections in dogs, while the Knott test indicated which of these are microfilaraemic or amicrofilaraemic infections.

The results of the epidemiological studies previously carried out in Spain, reviewed by Montoya *et al.* [6], show a broad distribution of heartworm infection, with high prevalence in the Canary Islands, some Southern provinces of Andalucía and well defined areas in the Tormes River Valley (Salamanca, Western Spain) and the Ebro River Delta (Tarragona, Northwest Spain). Moreover, this review reveals the absence of dirofilariasis in many provinces of the central and Northern parts of the Iberian Peninsula. Nevertheless, in a recent epidemiological study we have detected, for the first time, canine heartworm infections in one of the provinces where dirofilariasis has not been previously recorded (La Coruña) [9], suggesting the necessity of implementing studies in areas lacking data to complete the epidemiological picture of heartworm infection in Spain.

In this way, the results obtained in the current work demonstrate the existence of canine dirofilariasis in the Ebro Valley in La Rioja, where broad irrigated areas give suitable conditions for the development of mosquito vector populations during summer. The prevalence observed is quite similar to those reported in other Northern endemic areas of Spain (Zaragoza,

Barcelona, Galicia), but lower than those of areas located in the centre and South, like the Tormes River (Salamanca) where the prevalence is 33% and Huelva, 36.7% [6]. Because no previous data are available, we do not know whether these differences are due to a recent introduction of dirofilariasis in La Rioja and other Northern areas, or if it is a consequence of the climatic and social characteristics extant in each area.

The existence of canine dirofilariasis is a risk factor for human populations living in endemic areas. Positive serology of specific anti-*D. immitis* antibodies demonstrates contact with the parasite [5]. Our results show that the human population of La Rioja is subject to a similar risk of *D. immitis* infection as the canine population, because prevalence in dogs and seroprevalence in humans are very similar. In Spain, only other two studies have been carried out on human dirofilariasis: Salamanca where seroprevalence is 22% [5, 10] and Tenerife island with a prevalence of 48% [7]. As in La Rioja, there exists some correlation between the prevalence of dirofilariasis in canine and human populations in each area.

In conclusion, our results demonstrate the presence of *D. immitis* infections in the canine and human populations of La Rioja (Spain) and confirm that the existence of irrigated areas is an important factor for the development of dirofilariasis in dry zones of the Iberian Peninsula, because they provide the humidity necessary for vector breeding during the hot season of the year. Moreover, human *Dirofilaria* infections exist where canine dirofilariasis is present. More research must be carried out in other non-studied areas to complete the epidemiological picture of heartworm and human infections in Spain.

ACKNOWLEDGEMENTS

This work has been partly supported by Agencia de Desarrollo Económico de Castilla y León (co-financed with FEDER funds).

DECLARATION OF INTEREST

None.

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