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A cross-sectional study of the impact of regular use of insecticides in dogs on Canine Leishmaniosis seroprevalence in southeast Spain

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ABSTRACT

The relationship between Canine Leishmaniosis (CanL) seroprevalence and regular use of topical insecticides was investigated in 800 pet dogs with no visible signs of CanL in Murcia, southeast Spain in 2011. Dogs were clients to 17 veterinary practices and were analyzed for *Leishmania infantum* antibodies in blood plasma using two commercial ELISAs (Ingezim, Ingenasa®, Spain; Leishcan, Hipra®, Spain). Owners were interviewed to gather data on dog related variables. They included date of birth, home address and frequency, duration and timing of insecticide treatments used to prevent ectoparasite infestations. The dog's residence was georeferenced and environmental data potentially associated with the dog's risk of *L. infantum* infection was obtained. A mixed logistic regression model was then developed to analyze the relationship between the dog's serological status and insecticidal treatment adjusted for demographic and environmental variables. Overall, CanL seroprevalence (95% confidence limits) was 18% (16–21%) including 11% in dogs not using insecticide treatments ($n = 60$) and 19% in those using them ($n = 740$) ($p > 0.05$). At least 16 different insecticide products were used and 73%, 26% and 1% of dogs received 1, 2 and 3 products a year. The most frequent commercial brands used and the only ones in the market claiming anti-sandfly activity, were Scalibor collars (deltametrin 40 mg/g; MSD®), Advantix pipettes (permethrin 500 mg/ml and imidacloprid 100 mg/ml; Bayer®) and Exspot spot-on pipettes (permethrin 715 mg/ml; MSD®). Seroprevalence was 9%, 16%, 20%, 22% and 25% for dogs with Scalibor collars plus Advantix pipettes, Scalibor collars plus ExSpot pipettes, Advantix pipettes alone, Scalibor collars alone and Exspot pipettes alone, respectively. The multivariable model confirmed a significant reduction in the risk of *Leishmania* spp. seropositivity in dogs using the Scalibor and Advantix combination compared to those using either product alone and provided evidence of greatly increased risk of CanL in rural areas situated at 300–500 m altitude and average March–July temperatures of 18.6–19 °C. The study highlights the difficulty in controlling CanL infection by means of insecticide use alone and that it could be improved by using the Scalibor and Advantix combination and identifying and targeting specific geographical areas.

1. Introduction

Canine Leishmaniosis (CanL) caused by *Leishmania infantum* is a major vector borne zoonotic infection in Mediterranean Spain causing mortality and a spectrum of severe clinical outcomes (Baneth et al., 2008). Its impact in endemic areas is further exacerbated by the difficulty in preventing infection (Otranto and Dantas-Torres, 2013). The efficacy of available vaccines is still under assessment and treatment of infected animals doesn't achieve parasitological cure. Moreover, it's not implemented on asymptomatically infected animals, which represent 50–80% of dogs living in some endemic areas in Spain (Solano-Gallego et al., 2001; Chitimia et al., 2011). In European countries CanL control efforts primarily focusses on preventing phlebotomine sandfly vector biting by using insecticides with residual activity in the dog (Otranto and Dantas-Torres, 2013).

When this study was carried out there were no leishmania vaccines in Spain and three commercial insecticide products specifically marketed to prevent phlebotomine sandfly vector

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infection were available: Scalibor insecticide-impregnated collars (deltametrin 40 mg/g; MSD®), Advantix topical (spot-on) pipettes (permethrin 500 mg/ml and imidacloprid 100 mg/ml; Bayer®) and Exspot spot-on pipettes (permethrin 715 mg/ml; MSD®). In laboratory conditions all three products have a sandfly repellent (blood feeding avoidance on a treated dog) efficacy greater than 90% and an insecticidal efficacy (additional mortality of fed and unfed sandflies exposed to a treated dog) of 30–99%, during the first three weeks after application (Killick-Kendrick et al., 1997; Mencke et al., 2003; Miró et al., 2007; Molina et al., 2012). Controlled field trials in pets and kenneled dogs in places with a high CanL prevalence corroborate that both Scalibor collars and Advantix and Exspot pipettes can be efficacious at preventing infection in dogs. However, the reduction in annual CanL seroconversión ranged from 50 to 100% depending on the year and the product used (Maroli et al., 2001; Foglia Manzillo et al., 2006; Otranto et al., 2007, 2010; Ferroglio et al., 2008). The residual activity of these products is time dependent, lasting 3 to 4 weeks for pipettes and around six months for the collar however, collar losses can be a major problem (Foglia Manzillo et al., 2006). Other factors conditioning the practicality of collars as a tool for controlling CanL is the percentage of the dog population collared and the infection incidence (the greater, the more useful collars are) (Maroli et al., 2001; Reithinger et al., 2004).

There is however a lack of information on the performance of dog insecticides to prevent CanL infection in the wide dog population in endemic areas in uncontrolled field conditions. Such studies maybe hampered by the difficulty in comparing the risk of infection in heterogeneous dog populations with widely variable exposure to infected sandflies. In the present study we employed multivariable regression modeling to investigate the relationship between regular use of anti-sandfly insecticide products and dogs CanL serological antibody status in endemic Murcia Region, to control for critical dogís demographic and residential environmental variables. CanL seroprevalence in Murcia was recently estimated at 7% for dogs from periurban residential areas (Chitimia et al., 2011).

2. Materials and methods

2.1. Study population and data collection

The study was carried out on up to 800 pet dogs aged 6 months-old or older, with no visible clinical signs of CanL, clients to 17 veterinary practices across Murcia Region, in November 2011. This is a traditionally endemic southern Mediterranean area (de Ybáñez et al., 2009) situated 38°N and 1°50′W, ranging 11300 km^2, with approximately 1470000 inhabitants. There is no recent information on sandfly abundance in the region. Studies in the 1980s showed that *Phlebotomus perniciosus* was the main *L. infantum* vector in Murcia with peaks of maximum abundance in June and September and no activity recorded from mid-November until April (Martínez Ortega and Conesa Gallego, 1987).

Dogs selected attended the veterinary practice to participate in the study as they were offered a CanL serological analysis free of charge, or for a consultation unrelated to CanL. Upon selection, dogs were clinically examined to discard animals with visible CanL signs (Baneth et al., 2008) and were then blood sampled and weighed. Owners were asked to answer a brief questionnaire about the dogís date of birth, gender, breed, residential home address and type, presence of people and animals in their home, sleeping place and insecticide usage in the animal (Tables 1–3). The specific questions concerning insecticide use were: (i) do you use insecticides in the dog on a regular basis to protect it against insects, mites and ticks? if you do, (ii) which insectide brand or brands do you use on your dog on a regular basis? and (iii) how many months and when in the year is your dog under insecticide treatment?

Table 1
Canine Leishmaniosis seroprevalence (95% confidence intervals) according to dogís signalment and body weight. A cross-sectional study in dogs from Murcia, southeast Spain in autumn 2011.

Variable	Level	*N*	ELISA			*P* value
			% Positive	95−	95+	
Gender	Female	426	15	11	18	0.0041
	Male	375	23	18	27	
Age (years)	0.5–1	172	9	5	14	0.0484
	2	112	16	9	23	
	3	103	16	9	23	
	4	72	21	11	30	
	5	57	19	9	30	
	6	69	22	12	31	
	7	44	25	12	38	
	8	25	24	7	41	
	9	33	27	12	42	
	10	27	30	12	47	
	>10	78	23	14	32	
Breed	Mixed	306	18	14	22	0.3659
	Spanish	37	27	13	41	
	Other countries	457	18	14	21	
Body weight	1.5–7.0	210	9	5	13	<0.0001
	7.1–15.0	189	18	13	23	
	15.2–27.0	177	19	13	25	
	27.5–73.0	216	27	21	33	

2.2. Blood sampling and L. infantum ELISA antibody analysis

Blood samples were taken from the cephalic vein into EDTA vacuum tubes, plasma recovered by centrifugation and used to detect *L. infantum* IgG antibodies with two commercial antibody tests: Ingezim Leishmania (Ingensa®, Spain) and Civtest Canis Leishmania (Hipra®, Spain, later commercialized by Esteve®, Spain as Leiscan). Both tests use crude immunodominant *L. infantum* antigens and conjugates include a specific canine IgG monoclonal in the Ingezim test and a generic protein A/HRPO in the Civtest. The validity of these tests has been assessed by manufacturers using the indirect immunoflorescence test (IFI) as the reference test. The Ingezim showed a 95% and 80% results agreement for 1/100 and 1/160 IFI cut-offs, respectively, and the estimated Civtestís sensitivity (Se) and specificity (Sp) was 98% and 96%, respectively. Moreover, the performance of both tests was recently assessed in experimentally infected dogs by Rodríguez-Cortés et al. (2013). Sp was 100% for both tests whilst Se were 98% for Leiscan and 78% for Ingezim.

Samples were done in duplicate and antibody optical densities (OD) were read in a spectrophotometer. The mean of both readings were used to classify samples as positive,-negative or- inconclusive, following kit instructions.

2.3. Spatial characterization of the dogís residential area

The dogís residential environment was characterized using a geographical information system (ArcGIS v.10, ESRI, Redlands, USA), and used with other dog variables, as covariates in the analysis of the relationship between the dogs CanL serological status and insecticide use. The approach taken was described in detail in a recent human Leishmaniosis study in Murcia (Pérez-Cutillas et al., 2015). Briefly, the dogís home address was used to obtain the geographical coordinates (http://iderm.imida.es/) and define a 500 m radius circular area around the point location from which environmental data was extracted (Tables 4 and 5). Ecological variables considered were the average monthly mean temperature, rainfall and relative humidity, obtained from an interpolation using point data from 45 regional weather stations (http://siam.