Serological Evidence of Exposure to *Rickettsia, Bartonella,* and *Ehrlichia* Species in Healthy or *Leishmania infantum*-Infected Dogs from Barcelona, Spain

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ABSTRACT

Fifty dogs from the area around Barcelona, Spain, were evaluated for serologic evidence of exposure to vector-borne pathogens. *Dirofilaria immitis*, *Ehrlichia canis*,*Borrelia burgdorferi*, *Leishmania infantum, Bartonella vinsonii* subspecies *berkhoffii,* and *Rickettsia rickettsii* antigens were used for testing purposes. Seroreactivity was determined in 3 different groups of dogs that were categorized based upon their *L infantum* infection status: uninfected healthy dogs (group 1), *L infantum*-infected healthy dogs (group 2), and *L infantum*-infected dogs with clinical manifestations consistent with leishmaniasis (group 3). Of the 50 dogs included in this study, 49 had serologic evidence of exposure to at least 1 organism for which testing was performed. Sera from 1 dog did not react to any test antigen. Seroreactivity was most frequently detected to *R rickettsii* antigens (41/50) and *L infantum* antigens (36/50), followed by *B vinsonii (berkhoffii)* antigens (14/50) and *E canis* antigens (2/50). No dog's sera reacted to *B burgdorferi* C-6 peptide and all *D immitis* antigen test results were negative. Sera from group 1 (n = 14) were reactive to *R rickettsii* (n = 13) and *B vinsonii (berkhoffii)* (n = 2). Sera from group 2 (n = 18) were reactive to *R rickettsii* (n = 16), *B vinsonii (berkhoffii)* (n = 5), and to *E canis* antigens (n = 1). Sera from group 3 dogs that had clinical manifestations of leishmaniasis (n = 18) were seroreactive to *R rickettsii* (n = 12), *B vinsonii (berkhoffii)* (n = 7), and *E canis* (n = 1). This study illustrates the potential for exposure to several vector-borne pathogens in dogs from the Barcelona area. It also indicates that exposure to *B burgdorferi* or *D immitus* is infrequent in this dog popula-

tion. The extent to which these dogs might serve as a reservoir or as a transport host for selected vector-borne pathogens is yet to be determined. Based upon these data, exposure to *Rickettsia* and *Bartonella* organisms is relatively common among dogs in the Barcelona area. Therefore, veterinarians should consider co-infection in this highly endemic region for leishmaniasis. Sequential or concurrent infection with vector-borne organisms could induce deleterious alterations in the dog's immune response, leading to atypical or unusual disease manifestations.

INTRODUCTION

Canine leishmaniasis (CL) is a severe systemic infectious disease of dogs that is caused by protozoan parasites of the genus *Leishmania*. The dog is considered the main peridomestic reservoir of the parasite, which results in zoonotic transmission to humans. When left untreated, leishmaniasis is usually fatal in people and in dogs.[1–5] CL is endemic in the Mediterranean basin, where the prevalence of infection in dogs can be as high as of 67% of selected populations.[5,6] Although CL is well recognized in highly endemic regions such as the Mediterranean basin, CL is occasionally diagnosed in nonendemic regions in dogs that have previously lived or have vacationed in endemic locations.[7,8] Clinical features of leishmaniasis can vary widely in sick dogs or in people as a consequence of organism- or host-specific factors. It is also possible that diversity of disease manifestations associated with leishmaniasis in some individuals is related to co-infection with other vector-borne organisms.[9] Classically described clinical signs of CL are nonspecific and include chronic wasting, weight loss, poor appetite, fever, anemia, non-pruritic alopecia and skin erosions or ulcerations, generalized lymphadenopathy, epistaxis, arthritis, and renal failure,[4,5,10,11]

In recent years an increasing number of publications have described simultaneous infection with another vector-borne pathogen in dogs that had a classical presentation for leishmaniasis. A few published examples include co-infections with *Leishmania* and *Neospora, Ehrlichia, Hepatozoon, Bartonella, Babesia,* or *Dirofilaria (Nochtiella).*[12-17] For some organisms, the association between the 2 infectious entities occurs with a greater frequency than would be expected based upon the incidence of each respective infection. For instance, an epidemiological study performed in the Campania region of Italy found *Neospora caninum* seroreactivity as a major risk factor for *Leishmania infantum* seroreactivity.[12] Co-infections should be expected in dogs living in areas that are highly endemic for several vector-borne organisms, in dogs that are maintained predominantly outdoors (enhanced vector transmission), and in dogs that are not routinely treated with acaracides or other ectoparacidacides. For example, serological evidence of exposure to multiple vector-borne organisms was recently reported in hunting dogs living outdoors on the island of Mallorca.[18] Although co-infections could occur merely as a function of lifestyle, it is also possible that one or more vector-borne organisms suppress the host immune response to other organisms that would typically be eliminated immunologically. As examples, infection with *L infantum* can induce suppression of the immune system or promote an abnormal response, and result in an imbalance between helper T-1 and helper T-2 cell responses.[19,20] Infection with *Bartonella vinsonii (berkhoffii)* can result in CD8+ lymphocytopenia, impaired monocytic phagocytosis, and impaired antigen presentation to helper T cells.[21]

Currently, there is limited information regarding the prevalence of many vector-borne infections in dogs residing in specific geographical areas of Europe where leishmaniasis is an endemic infectious disease. Some of the manifestations of leishmaniasis are similar or identical to clinical signs, and hematological and biochemical abnormalities reported in dogs with other vector-borne

infections. Therefore, the primary purpose of this study was to determine if seroreactivity using *Erlichia canis, Borrelia burgdorferi, Rickettsia rickettsii, B vinsonii (berkhoffii),* or *Dirofilaria immitus* antigens could be detected in 3 different groups of dogs that varied in their respective *L infantum* status (uninfected healthy dogs, *L infantum*-infected healthy dogs, and *L infantum*-infected dogs with clinical manifestations consistent with leishmaniasis) from the area surrounding Barcelona, Spain.

MATERIALS AND METHODS

Animals

Dogs enrolled into this study were from Barcelona, Spain, or the immediate surrounding area, an area endemic for canine leishmaniasis. Fifty dogs of varying breeds and ages, which were examined at the Veterinary Teaching Hospital of the Autonomous University of Barcelona, were selected for study. All blood collections were performed in January (n = 5), February (n = 12), March (n = 15), April (n = 14), and May (n = 4) of 2000.

With the permission of the owners and prior to obtaining blood samples, all dogs received a complete physical examination, with specific care to detect clinical signs that are compatible with canine leishmaniasis. Regardless of the clinical status of the dog, all dogs were tested for the presence of *L infantum* antibodies. Thirty-two healthy dogs were being investigated to eliminate the possibility of *L infantum* infection. In endemic areas of Spain, an annual serological screen for *L infantum* antibodies is recommended to facilitate early detection and treatment of leishmaniasis. When CL was suspected based upon clinical or laboratory abnormalities, the diagnosis was confirmed by concurrent detection of *L infantum* antibodies and the direct cytological observation of the parasite and/or by identification of *Leishmania* DNA, using a polymerase chain reaction (PCR) test. Based upon the physical examination status (healthy or unhealthy) and the results of cytology, serology, and PCR testing, the dogs in this study were assigned to 1 of 3 groups. Group 1 included 14 healthy dogs that lacked clinical, hematological, biochemical, or serological evidence of *L infantum* infection, group 2 consisted of 18 healthy dogs that lacked clinical, hematological, or biochemical abnormalities but had been exposed to and infected with *L infantum* based upon serological criteria, and group 3 consisted of 18 *L infantum*-infected dogs with disease manifestations compatible with leishmaniasis in conjunction with *L infantum* seroreactivity and direct cytological observation of parasite or positive *Leishmania* PCR test results.

Sampling

Blood was collected by cephalic or jugular venipuncture. Serum samples were stored at –20˚ C until used for the detection and quantification of organism-specific antibodies. For some dogs, both ethylenediaminetetraacetic acid (EDTA)-anti-coagulated blood and bone marrow samples were submitted for *L infantum* PCR testing. Following sedation, bone marrow aspiration samples were obtained from the costochondral junction using a 22-gauge needle.

Serology

All sera were screened for *D immitis* antigen, *E canis* antibodies (P30 and P31 outer membrane proteins), *B burgdorferi* (C6 peptide), and *L infantum* with 2 commercial assay kits (Canine SNAP 3DX Test and SNAP *Leishmania* Test, IDEXX Laboratories, Westbrook, Maine, USA).

Immunofluorescence. *B vinsonii* (*berkhoffii)* 93-CO-1 and *R rickettsii* North Carolina State University (NCSU) Domino strain were cultivated in Vero cells and harvested when cells were more than 80% infected (2 to 9 days postinoculation). The decision to use *R rickettsii* was based upon antigen availability in the US laboratory in which testing was performed rather than a more appropriate endemic antigen such as *R conori.* Cross-reactivity between the 2 spotted fever group antigens has long been recognized.[22,23] Antigen for immunofluorescent

antibody (IFA) was prepared by pelleting and resuspending microorganisms and cells in phosphate buffered solution (PBS). Antigen was applied to 30-well Teflon-coated slides (Cell-line Associates, Newfield, NJ, USA) in 3.0-µL aliquots and air dried. Slides were fixed in acetone for 10 minutes and frozen at -20˚C until use.

Three 2-fold serial dilutions of sera (1:16, 1:32, 1:64) in PBS-0.05% Tween 20 (T)/0.5% dried skimmed milk(M)/1% goat serum(G) (TMG) were made in microtiter plates. Ten microliters of each dilution was applied per well, and slides were incubated at 37℃ for 30 minutes, washed in PBS with agitation for 30 minutes, and air dried. Fluorescein-conjugated goat anti-dog immunoglobulin (ICN Pharmaceuticals, Costa Mesa, CA, USA) was diluted 1:100 in PBSTMG, filtered with a 0.22-µm filter and applied to each well. Slides were incubated for 30 minutes at 37˚C and washed again in PBS-0.05% Tween 20 (PBST) with agitation for 30 minutes, rinsed with distilled water, air dried, cover-slipped using mounting medium (90% glycerol and 10% PBS, pH 9.0) and viewed with a fluorescence microscope (magnification, X40). Samples with an IFA titer > 1:32 were retested with serial dilutions from 1:16 to 1:8192. Endpoint titers were determined as the last dilution at which brightly stained organisms could be detected on a fluorescence microscope with exciter and barrier filters. A reactive serum was defined as a titer of ≥ 1:64.[23,24] Reactive sera (titers 1:1024) from dogs experimentally infected with *B vinsonii (berkhoffii)* or *R rickettsii* and a nonreactive serum from a specific pathogen-free (SPF) dog were used as positive and negative controls for all IFA testing.

L infantum *Enzyme-linked Immunosorbent Assay.* An enzyme-linked immunosorbent assay (ELISA) was performed as previously described.[25] Briefly, microtiter plates were coated with a 20-µg mL^{-1} concentration of *L infantum* antigen in 0.1 mL of coating buffer (0.1 M carbonate-bicarbonate, pH 9.6) and incubated overnight at 4˚C. One hundred microliters of dog sera per well was diluted 1:400 in PBST-1% dried skim milk and was incubated for 1 hour at 37˚ C. After washing 3 times with PBST and once with PBS, 100 µL of anti-dog immunoglobulin G (IgG) (1:20,000) conjugated horseradish peroxidase (ortho-phenylene-diamine solution, Sigma-Aldrich, St. Louis, MO, USA) was added. This conjugate was incubated for 1 hour at 37˚C, and the plates were rewashed. The substrate solution (0.4 mg/mL) and H_2O_2 (0.4 µL/mL) in 0.1 M of phosphate-citrate buffer (pH 5.0) was added at 200 µL/well and developed for 20 minutes at 24˚C. The reaction was stopped with 50 µL of H_2SO_4 3M. Absorbance was read at 490 nm in an automatic microELISA reader (EL 312 microplate, Bio-tek Instruments, Winooski, Vermont, USA).

Sera from 28 dogs not infected with *L infantum* that were living in a region where the disease is endemic were tested to determine a cut-off for IgG-specific ELISA determinations. The cut-off absorbance was established as the mean plus 3 standard deviations (SD), resulting in 0.236 for IgG (mean 0.099, SD 0.0456). All determinations included the serum from a sick dog with confirmed *L infantum* infection as positive control and the serum from a healthy dog as a negative control.

DNA Isolation

DNA was obtained from 0.5 mL of bone marrow aspirate or peripheral blood. Samples were washed in Tris(hydroxymethyl)-aminomethane-EDTA buffer (pH 8.0) to disrupt the erythrocyte membrane until the leukocyte pellet was white. Leukocytes were then lysed by incubation of the pellet in 0.1 mL of Proteinase K buffer [50 mM KCl, 10 mM Tris (pH 8.0), 0.5% Tween-20] at 56˚C for 5 hours. Before running the PCR, proteinase K was inactivated by incubation of the samples at 90˚C for 10 minutes. DNA was diluted 1/5 in milliQ water and 5 µL were used for PCR.

***L infantum* PCR Amplification**

PCR was performed as previously described.[26] Briefly, *Leishmania*-specific

oligonucleotide primers SP176 (5'-TCTTGCGGGGAGGGGGTG-3') and SP177 (5'-TTGACCCCCAACCACATTT-TA-3') were used to amplify a 120-base-pair fragment of *Leishmania* kinetoplast DNA minicircles. PCR was conducted in a 50-μL final reaction mixture under standard conditions. The thermal cycling profile was as follows: 31 cycles at 94˚C during (30") and 50˚C during (2'). Amplified fragments were analyzed by 2.5% agarose gel electrophoresis. After running the gels, they were then stained in a 0.5 μg/mL ethidium bromide solution for 20 minutes.

Statistical Analysis

For statistical analysis the SPSS V12.0 software for Windows was used (SAS Corporation, Cary, SC, USA). Contingency table analysis was performed and statistical significance was set at $P \leq 0.05$.

RESULTS

Characteristics of Dogs

Of the 50 dogs included in this study, 30 were males and 20 were females. Dogs ranged in age from 1 to 11 years and in weight from 6.5 kg to 51 kg. The study was composed of mixed breeds (n = 15), German Shepherd (n = 7), Rottweiler (n = 5), Breton spaniel (n = 3), Boxer (n = 3), Giant Schnauzer (n = 2), Siberian Husky (n = 2), Doberman (n = 2), Collie (n = 1), Irish Setter (n = 1), Pyrenees Mastiff (n = 1), Golden Retriever (n = 1), Great Dane (n = 1), Fox Terrier (n = 1), Alaskan Malamute (n = 1), Basset Hound (n = 1), Cocker Spaniel (n = 1), Belgian Shepherd (n = 1) and Catalan Shepherd (n = 1). Heavy tick exposure was not reported by the owner of any dog.

Historical abnormalities for dogs in group 3 (n = 18) included apathy (n = 10), anorexia (n = 4), epistaxis (n = 2), hematuria (n = 1), weight loss (n = 15), muscular atrophy (n = 2), alopecia (n = 12), desquamation (n = 6), diarrhea (n = 6), vomiting (n = 1), skin ulceration (n = 13), lameness (n = 6), polyuria/polydipsia (n = 4), and onychogryphosis (n = 1). Abnormalities on physical examination in group 3 dogs included fever (n = 4), pale mucous membranes (n = 4), splenomegaly (n = 3), uveitis (n = 5), keratoconjunctivitis (n = 4), and lymphoadenopathy (n = 14). Hematological and biochemical abnormalities among group 3 dogs included anemia [packed cell volume (PCV) < 35%] (n = 9), thrombocytopenia (platelets < 150,000/μL) (n = 15), leukopenia [white blood cells (WBC) < 6000/μL] (n = 3), leukocytosis (WBC > 17000/μL) (n = 3), hypoalbuminemia (albumin < 2.6 g/dL) (n = 13), hypergammaglobulinemia (gammaglobulin > 0.8 g/dL) (n = 16), azotemia (creatinine > 1.5 mg/dL) (n = 3), and proteinuria (protein/creatinine ratio > 1) (n = 5). On microscopic examination of peripheral blood smears *Anaplasma platys* morulae were observed in the platelets of 1 dog.

Between 8 and 11 months before blood collection, 16 dogs were treated. Seven dogs were treated with doxycycline (3 from group 1, 1 from group 2, and 3 from group 3) for 3 weeks due to thrombocytopenia. Three dogs were treated with cephalexin (1 from group 1 and 2 from group 2) for 2 weeks due to bacterial dermatitis. One dog in group 1 was treated with metronidazole for 1 week due to acute colitis. Two dogs were treated with enrofloxacin (1 each from groups 1 and 2) for 4 weeks due to chronic prostatitis. One dog in group 1 was treated with meloxicam for 5 days due to acute lameness. Two dogs in group 1 were treated with prednisone for 2 weeks due to acute moist dermatitis. The 18 dogs in group 3 in which leishmaniasis was diagnosed as a function of this study were treated with meglumine antimoniate (Glucantime, Sanofi-Aventis, Paris, France) and allopurinol (Zyloric, GlaxoSmithKline, Middlesex, England).

Antibody and Antigen Testing

Of the 50 dogs included in this study, 49 had serologic evidence of exposure to at least 1 organism for which testing was per-

Table 1. Seroreactivity to *Ehrlichia canis, Rickettsia rickettsii,* and *Bartonella vinsonii (berkhoffii)* antigens in dogs from Barcelona*

	Group 1 (n = 14)	Group 2 (n = 18)	Group 3 (n = 18)
E canis	0	1	1
R rickettsii	13	16	12
B vinsonii (berkhoffii)	2	5	7

*Serum from group 1 (healthy dogs lacking clinical and serological evidence of *L infantum* infection) was reactive to *R rickettsii* and *B vinsonii (berkhoffii)*. Serum from group 2 (healthy dogs infected by *L infantum*) was reactive to *R rickettsii, B vinsonii (berkhoffii)*, and to *E canis* antigens. Serum from group 3 dogs that had clinical manifestations of leishmaniasis was seroreactive to *R rickettsii, B vinsonii (berkhoffii)*, and *E canis*.

Table 3. Reciprocal Immunofluorescence Antigen Testing (IFA) of *Bartonella vinsonii (berkhoffii)* Antibody Titers in Healthy or Sick Dogs from Barcelona, Stratified by *Leishmania* Infection Status at Time of Examination*

IFA Antibody Titers	Group 1 (n = 14)	Group 2 (n = 18)	Group 3 (n = 18)
<64	12	13	11
64	0	3	3
128	1	1	4
512	1	1	0

*Serum from group 1 (healthy dogs lacking clinical and serological evidence of *L infantum* infection) was reactive to *R rickettsii* and *B vinsonii (berkhoffii)*. Serum from group 2 (healthy dogs infected by *L infantum)* was reactive to *R rickettsii, B vinsonii (berkhoffii)*, and to *E canis* antigens. Serum from group 3 dogs that had clinical manifestations of leishmaniasis was seroreactive to *R rickettsii, B vinsonii (berkhoffii)*, and *E canis*.

formed (Table 1). Serum from 1 dog did not react to any test antigen. Seroreactivity was most frequently detected to *R rickettsii* antigens (41/50) and *L infantum* antigens (36/50), followed by *B vinsonii (berkhoffii)* antigens (14/50) and *E canis* antigens (2/50). No dog's serum reacted to *B burgdorferi* C-6 peptide and all *D immitis* antigen test results were negative. Serum from group 1 [healthy dogs lacking clinical and serological evidence of *L infantum* infection (n = 14)] were reactive to *R rickettsii* (n = 13) and *B vinsonii (berkhoffii)* (n = 2). Serum from group 2 [healthy dogs

Table 2. Reciprocal Immunofluorescence Antigen (IFA) Testing of *Rickettsia rickettsii* Antibody Titers in Healthy or Sick Dogs from Barcelona Stratified by *Leishmania* Infection Status at Time of Examination

IFA Test	Group 1 (n = 14)	Group 2 (n = 18)	Group 3 (n = 18)
<64	1	2	6
64	4	2	1
128	6	4	6
256	2	6	2
512	0	4	1
1024	1	0	2

*Serum from group 1 (healthy dogs lacking clinical and serological evidence of *L infantum* infection) was reactive to *R rickettsii* and *B vinsonii (berkhoffii)*. Serum from group 2 (healthy dogs infected by *L infantum*) was reactive to *R rickettsii, B vinsonii (berkhoffii)*, and to *E canis* antigens. Serum from group 3 dogs that had clinical manifestations of leishmaniasis was seroreactive to *R rickettsii, B vinsonii (berkhoffii)*, and *E canis*.

infected by *L infantum* (n = 18)] were reactive to *R rickettsii* (n = 16), *B vinsonii (berkhoffii)* (n = 5), and to *E canis* antigens (n = 1). Serum from group 3 dogs that had clinical manifestations of leishmaniasis (n = 18) was seroreactive to *R rickettsii* (n = 12), *B vinsonii (berkhoffii)* (n = 7), and *E canis* (n = 1). Antibody titers were highly variable (Tables 2 and 3). Seroreactivity to both *E canis* and *R rickettsii* antigens was found in 2 samples (1 each from group 2 and 3), and reactivity to *R rickettsii* and *B vinsonii (berkhoffii)* was found in 10 samples (2 from group 1, 5 from group 2, and 3 from group 3). When reciprocal antibody titers to the various organisms were stratified according to gender, age, breed, and date of blood collection, no serological associations were noted (Table 4).

DISCUSSION

Through blood smear examination and serologic testing, substantial evidence of vector-borne diseases was found in the dogs studied. It is notable that 98% (49 of 50) of the dogs in this study were seroreactive for at least 1 organism for which ELISA or IFA testing was performed. Although the entry

Table 4. Antibody Reactivity to Various Test Antigens Stratified According to Gender, Age, Breed, and Date of Blood Collection

Dogs	*Leishmania infantum*	*Rickettsia rickettsii*	*Bartonella vinsonii (berkhoffii)*	*Erlichia canis*
Total	36	41	14	2
Male	23	26	9	1
Female	13	15	5	1
Age (years)				
0–2	5	12	9	10
>2–4	2	15	8	16
>4–6	2	5	3	4
>6–11	1	0	1	0
Mixed breed	8	28	14	27
Pure breed	5	9	1	1
January	5	7	12	9
February	3	4	10	13
March	10	4	2	3
April	4	4	1	0
May	0	0	1	1

criteria for this study purposefully created a selection bias for the detection of vector-borne pathogens in dogs from a leishmaniasis-endemic area, the overall seroprevalence in this population was high, suggesting that canine exposure to vector-borne organisms in the Barcelona area is extensive.

The results of this study indicate that a seroprevalence study involving a random sample of dogs from this area should be performed in the future. Among dogs in group 3, clinicopathologic abnormalities were nonspecific and highly variable, which is consistent with canine leishmaniasis in an endemic area.[4,5]

In this study 82% (41 of 50) of dogs were seroreactive to *R rickettsii*, of which 34 had reciprocal titers ≥128 (16 were 128, 10 were 256, 5 were 512, and 3 were 1024). This finding presumably represents serologic evidence of canine exposure to a *Rickettsia* species endemic to the Barcelona area. Seroreactivity to *R rickettsii* antigens did not differ statistically among the 3 different study groups ($P = 0.107$). Cross-reactivity of varying degrees has been reported among spotted fever group and typhus group rickettsiae.[22,23,27] Therefore, *Rickettsia* species cannot be reliably determined through serological testing. Seroreactivity to *R conorii* has been documented in people in Barcelona area and more recently in dogs from the same area.[28,29,30] Detection of *Rickettsia* antibodies in otherwise healthy dogs is not completely unexpected, because many *Rickettsia* species induce acute, potentially self-limiting infection in dogs, and rickettsemia clears rapidly after treatment with a number of antibiotics.[31,32] In this study, there was a lack of a seasonal association with *Rickettsia* antibody titers during winter and spring months, as has been described in previous studies of dogs in the same geographic area.[28,29] This observation would argue against an acute infection as a cause of the *Rickettsia* antibodies detected in these studies.

Fourteen of 50 (38%) samples of dog sera were reactive at reciprocal titers ≥ 64 to *B vinsonii (berkhoffii)* antigens. This was an unexpected finding, since seroprevalence to this organism is usually much lower, in both sick and healthy dogs. Previous studies have detected lower *Bartonella* seroprevalences (3%) in dogs from the United Kingdom.[33,34] There was no statistical association between the presence of clinical signs and the detec-

tion of *B vinsonii (berkhoffii)* antibodies ($P = 0.187$). However, due to low number of both sick and healthy dogs included in this study, the potential pathogenic role of this organism should be investigated in future studies involving dogs from the Barcelona area.

Although seemingly variable results have been reported from different laboratories, there can be serological cross-reactivity between *E canis* and other *Ehrlichia* species and *Anaplasma* species antigens.[35] Therefore, dog sera that are reactive to *E canis* may in fact represent exposure to other *Ehrlichia* or *Anaplasma* species. In this study using a commercially available ELISA test, only 4% of the dogs surveyed were seroreactive to *E canis* antigens. Both of the *E canis* ELISA-positive dogs had been exposed to *L infantum.*

This study illustrates the potential for exposure to several vector-borne pathogens in dogs from the Barcelona area. It also indicates that exposure to *B burgdorferi* or *D immitus* is infrequent in this dog population. The extent to which these dogs might serve as a reservoir or as a transport host for the selected vector-borne pathogens is yet to be determined. Studies that incorporate pathogen isolation or PCR detection of organism-specific DNA will be necessary to clarify the extent to which antibody detection reflects prior or active infection. Based upon these data, exposure to *Rickettsia* and *Bartonella* organisms is relatively common among dogs in the Barcelona area. Therefore, regional veterinarians, who are very familiar with leishmaniasis, should consider the possibility of co-infection. Sequential or concurrent infection with vector-borne organisms could induce deleterious alterations in the dog's immune response, leading to atypical or unusual disease manifestations. Future seroepidemiological studies incorporating a larger population of dogs or molecular-based studies will be required to elucidate the immunopathogenic role of other vector-borne organisms in dogs living in *L infantum*-endemic regions.

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