Vector-Borne Diseases in Client-Owned and Stray Cats from Madrid, Spain

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Abstract
The role of various vector-borne pathogens as a cause of disease in cats has not been clearly determined. The current study evaluated risk factors, clinical and laboratory abnormalities associated with \textit{Ehrlichia} spp., \textit{Anaplasma} spp., \textit{Neorickettsia} spp., \textit{Leishmania} spp., and \textit{Bartonella} spp. infection or exposure in 680 client-owned and stray cats from Madrid, Spain. Our results indicate that a large portion (35.1\%) of the cat population of Madrid, Spain, is exposed to at least one of the five vector-borne pathogens tested. We found seroreactivity to \textit{Bartonella henselae} in 23.8\%, to \textit{Ehrlichia canis} in 9.9\%, to \textit{Anaplasma phagocytophilum} in 8.4\%, to \textit{Leishmania infantum} in 3.7\%, and to \textit{Neorickettsia risticii} in 1\% of the feline study population. About 9.9\% of cats had antibody reactivity to more than one agent. \textit{L. infantum} DNA was amplified from four cats (0.6\%), \textit{B. henselae} DNA from one cat (0.15\%), and \textit{B. clarridgeiae} DNA from another cat (0.15\%).

Key Words: Cats—PCR—Serology—Vector-borne diseases.

Introduction
Many vector-borne organisms are considered emerging or re-emerging pathogens, with increasing comparative biomedical importance throughout the world (Beugnet and Marié 2009). \textit{Ehrlichia canis} and \textit{Anaplasma phagocytophilum} are tick-transmitted rickettsiae, and both species cause disease in humans (Chen et al. 1994, Pérez et al. 1996), as well as cats, dogs, and other domestic animals (Barlough et al. 1996, Breitschwerdt et al. 2002, de la Fuente et al. 2005, Carrade et al. 2009). \textit{Neorickettsia risticii} is the agent of the equine monocytic ehrlichiosis (Potomac horse fever) (Holland et al. 1985) and has been described as a potential pathogen in cats and dogs (Dawson et al. 1988, Kakoma et al. 1994). Serological or molecular evidence of \textit{Anaplasma} and \textit{Ehrlichia} species have also been described in cats from different countries (Dawson et al. 1988, Beauflis et al. 2002, Breitschwerdt et al. 2002, Lappin et al. 2004, Tarello 2005). Leishmaniasis is a zoonotic disease of worldwide importance, caused by protozoa of the genus \textit{Leishmania}. Dogs are considered the primary reservoir host; however, other animal species can be infected, including cats (Maia et al. 2008). \textit{Bartonella henselae} is the primary cause of “cat-scratch disease” (CSD) in humans; however, other \textit{Bartonella} spp. have been implicated in CSD cases (Breitschwerdt et al. 2010). An increasing number of \textit{Bartonella} spp. is being implicated as a cause of disease in immunocompetent and immunosuppressed patient populations (Blanco et al. 1999, Breitschwerdt et al. 2007, 2008b, 2010). Due to transmission by cat fleas (\textit{Ctenocephalides felis}), \textit{B. henselae} has also been described in cats worldwide (Fabbri et al. 2004, Breitschwerdt 2008a). In addition, experimental infection of \textit{Bartonella} spp. in cats can cause severe disease and death (Bradbury and Lappin 2010).

In Spain, several serological studies have reported the presence of antibodies against \textit{A. phagocytophilum}, \textit{E. canis}, and \textit{N. risticii} antigens in dogs (Sainz et al. 1995, Solano-Gallego et al. 2006b, Amusategui et al. 2008). The presence of \textit{E. canis} in Spain was confirmed by isolation from a dog (Aguirre et al. 2004a). Leishmaniasis is an important and well-recognized endemic disease in dogs in Spain (Amusategui et al. 2004, Miró et al. 2007, Fernández-Bellon et al. 2008). Serological and DNA evidence of \textit{Bartonella} spp. infection has also been described in dogs and rodents in Spain (Solano-Gallego et al. 2006b, Maríquez et al. 2008). These organisms have potential public health significance, including human anaplasmosis, leishmaniasis, and bartonellosis, which have

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all been reported in Spain (Oteo et al. 2000, Martínez-Osorio et al. 2005, Valcárcel et al. 2008). There have also been studies that have addressed the serological and molecular prevalence of these pathogens in cats in Spain, with most studies analyzing client-owned cats (Aguirre et al. 2004b, Ortúño et al. 2005, Fons et al. 2005, Solano-Gallego et al. 2006a, 2007, Martín-Sánchez et al. 2007, Aylloñ et al. 2008, Tabar et al. 2008). To date, *A. phagocytophilum*, *B. henselae*, *E. canis*, or *N. risticii* isolates from cats from the Madrid area have not been genetically characterized. Recently, *Leishmania infantum* was amplified and sequenced from a cat that resided in Madrid (Aylloñ et al. 2008).

The aim of the current study was to determine the serological and/or molecular prevalences of *A. phagocytophilum*, *Bartonella* species, *E. canis*, *L. infantum*, and *N. risticii* in stray and client-owned cats from Madrid (Spain) and to identify risk factors and clinical signs associated with the exposure to and/or the infection with these organisms in cats.

**Materials and Methods**

**Animals**

Between September 2005 and August 2008, blood samples were collected from 680 cats in Madrid, Spain. Five hundred thirty-nine (79.3% of total) client-owned cats were presented at the Complutense Veterinary Teaching Hospital (CVTH), College of Veterinary Medicine, or at three different private veterinary clinics in Madrid due to medical or surgical reasons or annual check-up. One hundred forty-one (20.7% of total) stray cats were enrolled at five humane societies in the Madrid area.

**Inclusion criteria**

Written consent for patient enrolment was obtained for every case from owners or humane societies. No specific inclusion or exclusion criteria were established; therefore, any cat seen at the CVTH or private clinics from which blood samples were collected for routine laboratory testing was enrolled in the study. Cats at the humane societies were randomly selected among those that were neutered/spayed. No clinical or epidemiological information was available from stray cats.

**Clinical evaluation and data collection**

For client-owned cats, the following data was obtained from each cat: month of evaluation, season, breed (European or other breeds), sex, age (young, < 1 year; adult, between 1 and 10 years; old, > 10 years), neutered/spayed, environment (urban, periurban or rural), area (Center, North, South, East, or West of Madrid, Spain), housing (flat, house, outdoors, and humane society), outdoor access, contact with other animals, arthropod-exposure history (ticks and fleas) contact with other animals (rodents, birds, and others), ectoparasiticide treatments, previous anti-rickettsial treatments (in the last 60 days), blood transfusions, travel history, FeLV/FIV status, clinical signs (general signs, digestive signs, cardiovascular/respiratory signs, ocular signs, musculoskeletal abnormalities, renal signs, and neurological signs), and hematological/biochemical data (erythrocyte count, hemoglobin, hematocrite, platelets, leukocyte count, urea, creatinine, total proteins, glucose, and serum transaminases). Cats were classified as healthy or unhealthy depending on the clinical history and presence of clinical signs. For cats coming from shelters, the only available data were month of evaluation, season, origin, breed, sex, age, environment, and presence of clinical signs.

**Sample collection**

Blood was collected in heparin and EDTA-anticoagulant tubes by jugular venipuncture for complete blood count, serum biochemical profile, serology, and polymerase chain reaction (PCR) testing. All samples used for serology and PCR were stored at −20°C and −80°C, respectively, until use.

**Serology**

The presence of IgG antibodies against *A. phagocytophilum*, *B. henselae*, *E. canis*, *L. infantum*, and *N. risticii* was analyzed by using immunofluorescent antibody (IFA) testing, as previously described (Sainz et al. 1995, Amusategui et al. 2003). Cut-off titers were established at 1:40 for *A. phagocytophilum*, *E. canis*, and *N. risticii*; at 1:50 for *L. infantum*; and at 1: 64 for *B. henselae*. Positive and negative controls were included for each IFA assay.

**Polymerase chain reaction**

Two hundred microliters of whole blood were used for DNA extraction with an UltraClean™ DNA Blood Spin Kit (Mo Bio Laboratories, Inc.) following the manufacturer’s instructions. The final eluted volume of DNA extracted was 200 µL per sample. DNA was then quantified by spectrophotometry (NanoDrop™; Thermo Scientific). Presence of PCR inhibitors was assessed by the amplification of a fragment of the constitutive gene for the glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) protein (Birkenheuer et al. 2003). *Anaplasma* spp., *Ehrlichia* spp., and *Neorickettsia* spp. DNA was amplified by a conventional PCR targeting the 16S rRNA gene and groEL heat shock operon, using primers GEP-s (5′-CTGCGCGCAAGCTAAACACATGCAAATCGAAACGGA) and GEP-as (5′-CTTTTTRGTTACCCCGTATTA TCTTCYYAYTG) for the 16S rRNA gene (Eddlestone 2007), and GroEL-643s (5′-ACTGATGTTATGACCCCTGCT) and GroEL-1236as (5′-CTTTTRCCTTTTCYMCTCAC TTCC) for the groEL heat shock operon (Barber et al. 2010). Primers LSH1 (5′-CATTTCCTGTGCCCGGGA) and LSH2 (5′-CCACCTGCTATTTACCAA) were used for the amplification of *Leishmania* spp. kinetoplast DNA by using a conventional PCR (Lachaud et al. 2002). *Bartonella* spp. DNA was also amplified by conventional PCR targeting the intergenic transcribed spacer (ITS) region, using the primer pair ITS 325-s (5′-CTTCAGATGATGATCCTCCCAACGCTTGTTCG) and ITS 1100-as (5′-GAACCCAGACCGCCTGCTGTC) (Maggi and Breitschwerdt 2005) and also the RpoB gene, using the primers pair RpoB-1615s (5′-ATAYCAATAARCGYCGTCTTCTGCTCTTGGG) and RpoB-2267as (5′-GATCTAATATCCTYTGACAGCAATACAGC) (Diniz et al. 2007). To prevent PCR amplicon contamination, sample extraction, reaction setup, PCR amplification, and amplicon detection were performed in separated areas. Negative controls were included with each run.

The PCR products were cloned into the plasmid pGEM®-T Easy Vector System I (Promega). When inserts had been obtained, amplicons were sequenced by the automatic DNA
sequencer ABI 3100 by using the Big Dye sequencer kit version 3.1 (Applied Biosystems) by Davis Sequencing. Interpretation of results was made by using Contig-Express and AlignX softwares (Vector NTI Suite 10.1; Invitrogen Corporation). Species definition was made based on similarities with other bacteria in GenBank by using the Basic Local Alignment Search Tool (BLAST).

Data analysis

Age was considered a parametric variable for univariate analysis and was transformed into categorical data as previously described for multivariate analysis. All hematological and blood chemistry data were transformed into categorical data and classified in three groups: normal range, over the normal range, or under the normal range.

Univariate analysis of categorical data was performed by using chi-square test or Fisher’s exact test. Associations were described by using the statistical significance and adjusted odds ratio (OR). Normal distribution of parametric variables was evaluated by using the Kolmogorov-Smirnov test. Multivariate analysis of categorical data was evaluated by using a decision tree model based in the classification and regression tree analysis. Statistical analysis was performed by using the computer program SPSS 17.0.

Results

Cats ranging in age from 1 month to 21 years were enrolled in the study, of which 75% (463/617) were European breeds and 25% (154/617) were non-European breeds. Male cats comprised 46.5% (289/622), and female cats comprised 53.5% (333/622) of the study population, with 56.3% (325/577) of the total cat population, 38.6% (154/399) had a travel history to other regions. Based on ELISA testing, 7.1% (36/504) of the cat population was infected with FeLV, and 5.1% (26/506) was FIV seroreactive. Clinical signs of disease and/or laboratory abnormalities were found in 71.3% (409/574) of the cats in this study. Of the total cat population, 33.2% (191/574) had general clinical signs, 32.1% (184/574) had gastrointestinal signs, 23.5% (135/574) had cardiovascular/respiratory signs, 22.3% (128/574) had renal signs, 10.1% (58/574) had ocular signs, 8% (46/574) had musculoskeletal abnormalities, and 3.3% (19/574) had neurological signs. Frequency of laboratory abnormalities in infected/exposed cats and negative cats is provided at Table 1.

Based on serology, some cats included in this study were exposed to at least one of each of the five agents that were analyzed. Seroprevalence to A. phagocytophilum, B. henselae, L. infantum, and N. risticii is presented in Table 2. Stray and client-owned cats had a similar seroprevalence for A. phagocytophilum (p = 0.131), B. henselae (p = 0.928), E. canis (p = 0.120), L. infantum (p = 0.055), and N. risticii (p = 0.147). Specifically, stray cats had a seroprevalence of 9.1% (n = 8) for A. phagocytophilum, 24.1% (n = 31) for B. henselae, 6.4% (n = 9) for E. canis, 6.4% (n = 9) for L. infantum and 21% (n = 3) for N. risticii, whereas client-owned cats had a seroprevalence of 5.7% (n = 9) for A. phagocytophilum, 23.7% (n = 128) for B. henselae, 10.8% (n = 58) for E. canis, 3% (n = 16) for L. infantum, and 0.7% (n = 4) for N. risticii.

Older age was statistically associated with E. canis seroreactivity (x = 7.43) when compared with seronegative cats (x = 5.88) (p = 0.019). Seropositivity to E. canis antigens was higher for samples collected in May and November (p = 0.022,

### Table 1. Frequency of Laboratory Abnormalities in Cats from Madrid Infected/Exposed to *Ehrlichia* spp., *Anaplasma* spp., *Neorickettsia* spp., *Leishmania* spp., and/or *Bartonella* spp., When Compared with Negative Cats

<table>
<thead>
<tr>
<th>Laboratory abnormality</th>
<th>Infected and/or exposed cats</th>
<th>Negative cats</th>
<th>Univariate p-value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Odds ratio (5%–95% CI)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>13.80 (33/240)</td>
<td>12.6 (56/447)</td>
<td>0.744</td>
<td>1.01 (0.67–1.74)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>30.96 (74/240)</td>
<td>9.07 (40/447)</td>
<td>0.026</td>
<td>2.08 (1.08–4.01)</td>
</tr>
<tr>
<td>Leukocytosis</td>
<td>10.98 (19/240)</td>
<td>23.40 (73/447)</td>
<td>0.0008</td>
<td>0.40 (0.23–0.7)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>16.76 (29/240)</td>
<td>12.18 (38/447)</td>
<td>0.1611</td>
<td>1.45 (0.86–2.46)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>12.20 (20/240)</td>
<td>7.36 (22/447)</td>
<td>0.0830</td>
<td>1.74 (0.92–3.31)</td>
</tr>
<tr>
<td>Neutrophilia</td>
<td>6.71 (11/240)</td>
<td>17.73 (53/447)</td>
<td>0.0010</td>
<td>0.33 (0.16–0.66)</td>
</tr>
<tr>
<td>Lymphocytosis</td>
<td>1.22 (2/240)</td>
<td>2.67 (8/447)</td>
<td>0.3048</td>
<td>0.45 (0.09–2.15)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>30.49 (50/240)</td>
<td>31.00 (93/447)</td>
<td>0.9091</td>
<td>0.97 (0.65–1.48)</td>
</tr>
<tr>
<td>Azotemia</td>
<td>22.98 (37/240)</td>
<td>24.38 (69/447)</td>
<td>0.7395</td>
<td>0.92 (0.59–1.46)</td>
</tr>
<tr>
<td>Hyperproteinemia</td>
<td>40.51 (64/240)</td>
<td>18.12 (79/447)</td>
<td>0.0097</td>
<td>1.71 (1.14–2.59)</td>
</tr>
<tr>
<td>Hypoproteinemia</td>
<td>4.43 (7)</td>
<td>11.15 (31)</td>
<td>0.0168</td>
<td>0.36 (4.65)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Cats in this group were exposed to or infected with at least one organism evaluated in this study.

<sup>b</sup>p-Value and odds ratio were calculated for the occurrence of each laboratory abnormality in the group of infected/exposed cats by using chi-square or Fisher’s exact test.

CI, confidence interval.
Organisms

<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. of positive/tested</th>
<th>Percentage (5%–95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplasma + Neorickettsia risticii</td>
<td>5/680</td>
<td>0.73 (0.4–1.1)</td>
</tr>
<tr>
<td>Ehrlichia canis + L. infantum</td>
<td>5/680</td>
<td>0.73 (0.4–1.1)</td>
</tr>
<tr>
<td>Neorickettsia risticii + Leishmania infantum</td>
<td>5/680</td>
<td>0.73 (0.4–1.1)</td>
</tr>
<tr>
<td>Bartonella henselae + E. canis</td>
<td>5/680</td>
<td>0.73 (0.4–1.1)</td>
</tr>
<tr>
<td>Bartonella henselae + L. infantum</td>
<td>5/680</td>
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</tr>
<tr>
<td>Bartonella henselae + Leishmania infantum</td>
<td>5/680</td>
<td>0.73 (0.4–1.1)</td>
</tr>
</tbody>
</table>

Seroreactivity to more than one of the tested organisms included in the study was found in 9.9% of the cats. Thus, 55 cats (8%) were seroreactive to two organisms, and 12 cats (1.8%) were seroreactive to three organisms (Tables 3 and 4). Association between seropositivity to E. canis, with A. phagocytophilum, B. henselae, and N. risticii was found (p=0.001, p<0.001, and p<0.001, respectively).

The serological results obtained in this study indicate that a large portion (35.1%) of the cat population of Madrid, Spain, are exposed to at least one of five vector-borne pathogens, including A. phagocytophilum, B. henselae, E. canis, L. infantum, and N. risticii. As reported in another study (Luria et al. 2004), there was no statistical differences in seroprevalence for any organism between stray and client-owned cats. However, other studies are discordant with our results, thereby finding a higher E. canis and A. phagocytophilum seropositivity in cats with outdoors access (Stubb et al. 2000). This result would suggest that a large portion of the pet cat population in this study did not receive routine applications of acaracides. Besides, our data should be cautiously interpreted, because in our study population, many owners were unaware of the origin of their cats, and most cats were rescued from an outdoor environment, or had the possibility of outdoor access before adoption.

The seroprevalence for E. canis (9.9%±1.1%) in this study was similar to rates previously described in cats from Spain.

Discussion

The seroprevalence for E. canis, A. phagocytophilum, N. risticii, L. infantum, and B. henselae in cats from Madrid was found (p=0.001, p<0.001, and p<0.001, respectively).

A. phagocytophilum antibodies were more often detected in autumn months (September–November), which was also supported by the multivariate analysis (p=0.002, OR=2.44) and in cats with musculoskeletal abnormalities (p=0.035, OR=2.37).

Only female cats were seroreactive to N. risticii. Statistical differences for this variable were found by applying both univariate and multivariate analysis (p=0.013).

Older age was statistically associated with L. infantum (x²=9.25) seroreactivity when compared with seronegative cats (x²=5.94) (p=0.032). Seroreactivity to L. infantum was also more frequent in FIV seroreactors (p=0.024, OR=4.04) and in cats with oral disease (p=0.006, OR=3.67). However, when multivariate analysis was performed, no association was found.

Older age was also associated with B. henselae seroreactivity (x²=6.83) as compared with seronegative cats (x²=5.82) (p=0.017). B. henselae antibodies were more often detected in cats infested with fleas (p=0.011, OR=1.99). Total serum protein value (p=0.026, OR=1.74) was higher in B. henselae seroreactive cats. Multivariate analysis supports the association between antibody positivity to B. henselae and the presence of fleas (p<0.001, OR=2.44).
FELINE VECTOR-BORNE DISEASES IN SPAIN

Table 4. Frequency of Cats from Madrid Exposed to Multiple Pathogens

<table>
<thead>
<tr>
<th>Organisms with concomitant exposure</th>
<th>Exposed/ tested</th>
<th>Percentage (5%-95% CI)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. henselae + E. canis + A. phagocytophilum</td>
<td>8/680</td>
<td>1.2 (0.1–2.2)</td>
</tr>
<tr>
<td>B. henselae + E. canis + N. risticii</td>
<td>3/680</td>
<td>0.45 (0–4.2)</td>
</tr>
<tr>
<td>B. henselae + A. phagocytophilum + L. infantum</td>
<td>1/680</td>
<td>0.15 (0–2.8)</td>
</tr>
</tbody>
</table>

a In the current study, some of the cats were seroreactive simultaneously to three agents and seronegative to the others.

(Aguirre et al. 2004b, Ortuño et al. 2005, Solano-Gallego et al. 2006a). A higher seroprevalence was found in older cats, which could be due to an increased opportunity for exposure to an *Ehrlichia* spp. with advancing age. This hypothesis could also explain the similar trend found when analyzing *B. henselae* and *L. infantum* seroreactivity in cats from this study. These results are similar to a previous *L. infantum* seroepidemiological study of cats from southern Italy (Pennisi 2002).

The higher rate of *E. canis* seropositivity in May and November, and to *A. phagocytophilum* in the autumn months, could be explained by vector dynamics, as *Rhipicephalus sanguineus* and *Ixodes* spp. populations are, respectively, increased during these seasons in Spain (Estrada-Peña 1994, Herrero and Beltrán 1994).

*Anaplasma* spp. seroprevalence (8.4% ± 1.1%) was higher than rates previously reported in cats from Spain (Aguirre et al. 2004b, Solano-Gallego et al. 2006a). Musculoskeletal abnormalities have been reported as a frequent occurrence in *A. phagocytophilum* infected dogs (Greig et al. 1996); however, a statistical association between *Anaplasma* spp. seroreactivity and musculoskeletal abnormalities, as found in this study, has not been previously described in cats. Some authors have described musculoskeletal abnormalities in *A. phagocytophilum* seroreactive cats; however, there were no statistical differences between healthy and sick cats in a study from the northeastern United States (Magnarelli et al. 2005). In addition, serological cross-reaction with other *Anaplasma* species cannot be ruled out, such as *Anaplasma platys* (Santos et al. 2009, Diniz et al. 2010), which is transmitted by the tick *R. sanguineus* (Hibler et al. 1986), and is prevalent in dogs in the same region of this study (Sainz et al. 1999, Aguirre et al. 2006).

Similar to a previous study from the same area (Aguirre et al. 2004b), a few cats in this study were seroreactive to *N. risticii* (1% ± 0.4%), thus suggesting a low level of exposure to this organism or potentially serological cross reactivity with a closely related bacteria. Similar seroprevalences were observed in dogs and cats in our country by other authors (Aguirre et al. 2004b, Amusategui et al. 2008).

Although *A. phagocytophilum* and *E. canis* DNA have been amplified from cat blood samples from different countries (Beaufils et al. 2002, Breitschwerdt et al. 2002, Lappin et al. 2004), no amplification of *Ehrlichia* spp., *Anaplasma* spp. or *Neorickettsia* spp. DNA was obtained in our cat population. Seroprevalence data can be used to infer exposure to these or related organisms, whereas isolation or PCR amplification of organism-specific DNA sequences is required to document infection with the organism. The lack of DNA amplification of these pathogens from cats that were seropositive could be related to immunological elimination after infection or the sequestration of the organism in other tissues in persistently infected cats, such as the spleen (Harrus et al. 1998). Sequestration or a low-level infection could result in false-negative PCR results due to a low concentration of DNA in the sample.

The *L. infantum* (3.7% ± 0.7%) seroprevalence in cats in our study is similar to seroprevalences described in other feline populations in Spain (Solano-Gallego et al. 2007), but is lower than the 60% seroprevalence reported in cats from the south of Spain (Martín-Sánchez et al. 2007). Differences among studies could be due to variability in the study populations, differences in the IFA technique, or the cut-off value used to define a seroreactor. In concordance with one study (Pennisi 2002) and contrary to the results of three other studies (Vita et al. 2005, Martín-Sánchez et al. 2007, Solano-Gallego et al. 2007), there was an association between *L. infantum* seropositivity and FIV infection in our study. Oral disease has been previously described in cats with leishmaniasis by different authors (Leiva et al. 2005). Other studies describe an increased prevalence of stomatitis in cats seropositive to *B. henselae* (Glaus et al. 1997). Seemingly, coinfection with multiple organisms including *B. henselae*, *L. infantum*, FIV virus, and in conjunction with changes in oral bacterial flora may be necessary to induce oral disease in an otherwise healthy cat.

In the current study, we detected *L. infantum* DNA in blood samples from four cats, a *Leishmania* species that has been previously reported to infect cats from the Mediterranean basin (Ozon et al. 1998, Vita et al. 2005, Maia et al. 2008), including Spain (Martín-Sánchez et al. 2007, Solano-Gallego et al. 2007, Ayllón et al. 2008, Tabar et al. 2008). The molecular prevalence of *Leishmania* spp. in cats in our area could be higher than observed in this study, as blood is not the optimal diagnostic sample for PCR detection of *Leishmania* spp. in dog or human samples (Vita et al. 2005). Future studies should utilize PCR from multiple cat tissues to better characterize the role of cats as persistently infected hosts.

The *B. henselae* seroprevalence (23.8% ± 1.6%) was similar to previous seroprevalence rates (29.6%) described in cats from Spain (Pons et al. 2005), but was considerably lower than the prevalence reported in cats from northeastern Spain (71.4%) (Solano-Gallego et al. 2006a). Differences in *B. henselae* seroprevalence could be due to inter-laboratory assay differences or due to climatic and environmental differences among the study areas, which result in more frequent flea infestation or a higher level of *B. henselae* infection among both cats and their fleas in different regions (Solano-Gallego et al. 2006a). Flea infestation in cats was associated with *B. henselae* seropositivity in this study, which is consistent with the results of experimental flea transmission studies (Chomel et al. 1996, Bradbury and Lappin 2010).

*Bartonella* spp. DNA was amplified from only two cats in this study, which represents a lower molecular prevalence than reported from other studies in Spain (Solano-Gallego et al. 2006a, Tabar et al. 2008). Although *B. henselae* and *B. clarridgeiae* have been previously detected by PCR in cats from Spain (Pons et al. 2005, Solano-Gallego et al. 2006a; Tabar et al. 2008), this is the first article to describe infection by these two agents in cats from the Madrid area.
With regard to laboratory findings, infection and/or exposure of cats from Madrid to these pathogens (and specifically, exposure to B. henselae and E. canis) was associated with thrombocytopenia and hyperproteinemia, laboratory abnormalities that have been classically associated with some of these infections, particularly in dogs (Woody and Hoskins 1991, Ciaramella et al. 2001).

Coinfections among ehrlichial species and other agents have been previously described in cats (Stubbs et al. 2000, Magnarelli et al. 2005) as they have been described in dogs (de Capraris et al. 2011). Previous exposure to multiple vector-borne pathogens could complicate the interpretation of seroepidemiological studies as well as the diagnosis and treatment of coinfections in clinical cases.

Further studies are needed to better define the clinical importance of these pathogenic organisms in cats; to determine the possible influences of coinfection on diagnosis, prognosis, and treatment; and to establish the epidemiological role of cats as a source of zoonotic infection.

Acknowledgments

The authors thank Julie Bradley for serological testing, Barbara Hegarty for preparation of Bartonella spp. antigens, Belen Cadenas for assistance with data analysis, and Ricardo Maggi for helpful comments and suggestions relative to PCR testing. They would also like to express their appreciation to The Asociación para la Liberación y Bienestar Integral de Acogida de Animales de la Comunidad de Madrid (CIAAM), and the Asociación para la Liberación y Bienestar Integral de Acogida de Animales de la Comunidad de Madrid (CIAAM), and the “Asociación para la Liberación y Bienestar Integral de Acogida de Animales de la Comunidad de Madrid” (CIAAM) and the “Asociación para la Liberación y Bienestar Animal” (ALBA), especially to Patricia Rodríguez, Daniel Aguirre, E, Sainz, A, Dunner, S, Amusategui, I, et al. First iso-

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