



Research Article

Seroepidemiology of Selected Vector Borne Diseases in Urban and Rural Pet Dogs in Madrid, Spain

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Abstract

Vector-borne diseases (VBD) are an important cause of morbidity and mortality in dogs, and some of them are zoonotic. We evaluated 79 healthy and 117 sick dogs (n=196) in the Madrid region (Spain) for evidence of common vector borne diseases. The seroprevalence for the study group was 19.4% (38/196 dogs); of these, 15.8% (31/196 dogs) were seropositive for only one agent; there were 5 dogs positive for *Ec* (2.5%), 3 positive for *Ana* (1.6%), 0 positive for *Bb*, 2 positive for *Di* (1%), and 21 positive for *Li* (10.7%). Seven dogs were coinfecting; 5 dogs were seropositive for *Ec* and *Ana* (2.5%), and 2 for *Ec* and *Li* (1%). Twenty-six of the 38 (68.4%) seropositive dogs were sick. The seroprevalence was significantly higher in dogs that lived in a rural environment (28%), compared to those that resided in an urban environment (12.8%) (p=0.004). Nineteen of 87 (21.8%) seropositive dogs from rural environment were sick, and 5/87 (5.5%) were healthy; whereas 7/109 (6.4%) seropositive urban dogs were sick and 7/109 (6.4%) were healthy. Both sick and seropositive dogs had clinically relevant clinicopathologic abnormalities. Although the seroprevalence of these selected VBDs is similar to those previously reported, we found a high percentage of seropositive urban dogs. Interestingly, the seroprevalence of LI was similar between rural and city dogs.

Keywords

Ehrlichiosis; Leishmaniasis; Anaplasmosis; Anemia; Thrombocytopenia; Hyperglobulinemia

Abbreviations: Ana: *Anaplasma* spp; Aph: *Anaplasma phagocytophylum*; Apl: *Anaplasma platys*; Bb: *Borrelia burgdorferi*; Bh: *Bartonella henselae*; Bvb: *Bartonella vinsonii* subsp *berkhoffii*; CBC: Complete Blood Count; Di: *Dirofilaria immitis*; Ec: *Ehrlichia canis*; HD: Healthy Dogs; IFA: Immunofluorescence Antibody Test; Li: *Leishmania infantum*; Nr: *Neorickettsia risticii*; Rc: *Rickettsia conori*; SD: Sick Dogs; VBD: Vector Borne Diseases

Introduction

Vector-borne diseases (VBD) are an important cause of morbidity and mortality in dogs worldwide; some of them are zoonotic [1,2]. Numerous studies have evaluated the seroprevalence of specific

VBDs in dogs and other species, including *Ehrlichia* spp, *Anaplasma* spp, *Rickettsia* spp, *Babesia* spp, *Borrelia* spp, *Bartonella* spp, and *Leishmania* spp [3-7]. Knowing the prevalence of VBDs in pet dogs in specific geographic regions may assist with disease prevention in humans, as dogs can be sentinels for human diseases [2].

Numerous studies on VBDs in pet dogs and cats from Spain have been reported, several of them focusing on leishmaniasis in endemic areas [3-6,8-15]. Those studies suggest a specific geographic distribution; however, most studies evaluated animals in Cataluña or Galicia, so there is a paucity of data regarding prevalence of VBDs in the Madrid area, located in central Spain [3,5,6,8,11-14,16]. Moreover, VBDs are an emerging human health concern in Spain, where both wild and domestic animals are likely reservoirs [16-22].

A recent study evaluated the seroprevalence of selected VBDs, including *Rickettsia conori* (Rc), *Ehrlichia canis* (Ec), *Anaplasma phagocytophylum* (Aph), *Bartonella henselae* (Bh), *Bartonella vinsonii* subsp *berkhoffii* (Bvb), *Leishmania infantum* (Li), and *Borrelia burgdorferi* (Bb); they also evaluated serum samples for the presence of *Dirofilaria immitis* (Di) antigen in three subpopulations of dogs in northeastern Spain [3]. These subpopulations included 466 dogs evaluated in a private veterinary hospital on the island of Mallorca, in a private veterinary hospital in Tarragona, and in a Veterinary Teaching Hospital in Barcelona, respectively [3]. Due to the cross reactivity between antibodies against Aph and *Anaplasma platys* (Apl) [23,24], it is unknown whether the positive samples were in dogs with antibodies against Aph or Apl. Interestingly, the molecular characterization of Apl, primarily transmitted by *Rhipicephalus* ticks, was reported in dogs in Spain [24], but Ixodes ticks, the vectors for Aph, have also been found in Northwestern Spain [23,25]. Therefore, in the rest of the manuscript we will refer to a positive antibody test for Aph as positive for *Anaplasma* spp (Ana).

In that study, the seroprevalence rates for all 3 subpopulations of dogs in decreasing order were: Rc: 56.4%, Li: 30%, Bh: 16.7%, Ec: 16.7%, Ana: 11.5%, Bvb: 1.1%, Di: 0.6%, and Bb: 0.6%. There was no association between the clinical status (i.e.; sick vs healthy), gender, time of the year when samples were collected, lifestyle or exposure to ticks or fleas, and a positive test result for Ec, Bh, Bv, or Di. Li seroreactivity was associated with illness and living outdoors; Rc seroreactivity was associated with the male gender, and Ana seroreactivity was associated with living outdoors [3].

In another study, Amusatogui et al. [5] evaluated 649 dogs from northwestern Spain (Galicia) for serologic evidence of exposure to the following vector-borne diseases using immunofluorescence antibody (IFA) tests: Ec, Ana, *Neorickettsia risticii* (Nr), Rc, and Bb [5]. The study evaluated two cohorts of dogs; cohort I (n=479) included dogs presented to small animal clinics in two provinces (Ourense and Pontevedra), whereas cohort II (n=170) consisted of randomly chosen dogs in a public shelter in Ourense. The prevalence of exposure in dogs in cohort I was as follows, Rc (24.6%), Bb (6.26%), Ec (3.13%), Ana (5.01%), and Nr (1.04%); in contrast, in dogs in cohort II the prevalence of exposure was, Rc (50%), Bb (8.8%), Ec (54.7%), Ana (45.3%), and Nr (4.7%). The seroprevalence of Ec and Ana were significantly higher in dogs in a shelter environment, where there was also a significant association for coexposure to Ec and Aph [4].

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We recently reported the seroprevalence of selected VBDs in 131 dogs in an animal shelter in central Spain using point-of-care assays (SNAP 4DX and SNAP Leishmania, IDEXX Laboratories, Westbrook, ME). The SNAP 4DX detects *Di* antigen and antibodies against *Ec*, *Bb*, and *Aph*; the SNAP Leishmania kit detects antibodies against *Li*. Dogs were classified as healthy or sick based on physical examination, complete blood counts, and serum chemistry profiles [6]. The prevalence of positive test results was as follows: *Ec*, 5.3% (n=7); *Ana*, 19.0% (n=25); *Bb*, 0%; *Di*, 0%; *Li*, 5.3% (n=7). Four dogs (3%) were coexposed to *Ec* and *Ana*, and 3 dogs (2.3%) were coexposed to *Ana* and *Li*. There was no significant correlation between positive serology and clinical status (sick vs healthy) or hematologic/biochemical abnormalities [6].

Recently, Miró et al [15] evaluated 1,100 dogs serologically throughout several geographic regions in Spain. Of those, 187 were from the Central Spain area that includes Madrid and suburbs. The overall seropositivity rates were: *Li* (15.7%), *Ec* (5%), *An* (3.1%), *Di* (1.25%) and *Bb* (0.4%). For the dogs in Central Spain, the seroprevalence rates were: *Li* (6.4%), *Ec* (5.1%), *An* (5.0%), *Di* (2.3%) and *Bb* (0%). There was a significantly higher seropositivity to *Ec* (40%) and *Bb* (6.7%) in dogs under one year of age, when compared with adults (p<0.05); and a higher seropositivity to *An* and *Li* in dogs that lived outdoors versus indoors (p=0.01; p<0.001, respectively). The seropositivity rates in asymptomatic dogs (n=556) were as follows: *An* (2.1%), *Bb* (0%), *Ec* (1.7%), *Di* (0.5%) and *Li* (4.2%), compared to 3.8%, 0.6%, 7.5%, 1.8% and 25.9%, respectively, for those with clinical signs suggestive of a VBD (n=507)(15). In that study, the authors did not evaluate the seroprevalence in dogs that lived in urban versus rural environments, nor did they provide details of clinicopathologic evaluation. .

The goals of the current study were to evaluate the seroprevalence of selected VBDs (*Ec*, *Ana*, *Bb*, *Di*, *Li*) using point-of-care assays in asymptomatic and sick dogs presented to a large veterinary center in urban Madrid. In addition, we evaluated whether the seroprevalence was different in dogs that lived in urban versus rural environments; we also evaluated the prevalence of clinicopathologic abnormalities in seropositive and seronegative dogs.

Materials and Methods

We evaluated blood samples from 196 dogs that presented to the Hospital Veterinario Mediterráneo (Madrid, Spain) (Latitude: 40° 24' N Longitude:3° 40' W) between May 16, 2010 and November 16, 2010 for routine health care (n=79), designated as “healthy dogs” (HDs) or for evaluation of “clinical signs” of illness or health concerns of the owners (n=117), designated as sick dogs (SDs). “Healthy dogs” had presented for routine wellness evaluation, and had no clinical signs according to their owners. Jugular or cephalic venous blood samples (3ml) were collected in tubes containing lithium-heparin (for VBD testing and chemistry profile) and sodium EDTA (for complete blood count-CBC). In 156 dogs we performed a CBC (LaserCyte Dx Hematology Analyzer, Idexx Laboratories, Westbrook, ME), a SNAP 4DX (IDEXX Laboratories, Westbrook, ME), an ELISA test for *Li* (Ingezim Leishmania, Laboratorio Ingenasa, Madrid, Spain), and a chemistry profile (Catalyst Dx Chemistry Analyzer, IDEXX Laboratories, Westbrook, ME). In the remaining 40 dogs we only performed tests for VBDs, as described above.

Statistical analysis was performed using Graph-Pad Prism v.6.0 (San Diego, California, US); a normality test (D’Agostino and Pearson omnibus test) and descriptive statistics were performed

for all clinicopathologic parameters. Not all parameters passed the normality test, so results are presented as median ± SE. Comparison of clinicopathologic findings between HD and SD, and between seronegative and seropositive dogs was done using the Mann-Whitney U test. Statistical significance was set at P<0.05.

For statistical comparison of independent proportions, an unconditional Barnard’s test was performed by using open software specifically designed for it: TMP: <http://www.ugr.es/~bioest/software.htm> (A.Martin Andrew, Department of Statistics and I.O., University of Granada, Spain). A P-value<0.05 was considered statistically significant.

Results

The study population included 98 females and 98 males, with a mean age of 6.2 ± 3.8 years (range 7 months to 14 years). There were 53 mixed breed dogs, and 143 purebred dogs of various breeds; 87 dogs lived in a rural environment and 109 in an urban environment (city dogs). Clinical signs/health problems in SDs included unspecified renal problems or protein losing nephropathy (n=15), anemia (n=12), neurologic signs (n=12), skin problems (n=10), fever (n=8), bleeding disorders (n=14; including 7 dogs with epistaxis), respiratory signs (n=6), liver disease (n=6), polymyositis (n=6), tick infestation (n=5), gastrointestinal problems (n=4), splenomegaly (n=3), lameness (n=3), peritonitis (n=1), uveitis (n=1), leukopenia (n=3), erythrocytosis (n=1), thrombocytopenia (n=1), heart disease (n=1), myoglobinuria (n=1), and pyometra (n=1).

The seroprevalence for the study group was 19.4% (38/196 dogs were positive for one or more agents); of these, 15.8% (31/196) were seropositive for only one agent; there were 5 dogs positive for *Ec* (2.5%), 3 positive for *Ana* (1.6%), 0 positive for *Bb*, 2 positive for *Di* (1%), and 21 positive for *Li* (10.7%). Seven dogs (3.7% of all dogs; 18.4% of the seropositive dogs) were coinfectd; 5 dogs were seropositive for *Ec* and *Ana* (2.5%), and 2 for *Ec* and *Li* (1%).

Twenty-six of the 38 (68.4%) seropositive dogs had clinical signs (Tables 1 and 2). The seroprevalence was significantly higher in dogs that lived in a rural environment (24/87 or 28%), compared to those that resided in an urban environment (14/109 or 12.8%) (p=0.004) (Table 2). Nineteen of 87 (21.8%) seropositive dogs from rural environment were sick, and 5/87 (5.5%) seropositive dogs were healthy; whereas 7/109 (6.4%) seropositive city dogs were sick and 7/109 (6.4%) were healthy. However there was not relationship between the health status and rural versus urban environments (p=0.07).

“Sick dogs” had significantly lower HCT (38.4% vs 46.3%; p<0.0001) and serum albumin concentration (2.7 G/dL vs 3.1 G/dL; p<0.0001), and higher monocyte counts (0.8×10⁹/L vs 0.6×10⁹/L;

Table 1: Seroprevalence of selected vector borne diseases in healthy and sick dogs in Madrid.

	Seropositive	Healthy (%-n)	Sick (%-n)
Ec	4	25% (1/4)	75% (3/4)
Ana	3	33% (1/3)	67% (2/3)
Bb	0	0%	0%
Di	2	0% (0/2)	100% (2/2)
Li	21	24% (5/21)	76% (16/21)
Ec + Ana	5	60% (3/5)	40% (2/5)
Ec + Li	2	50% (1/2)	50% (1/2)
TOTAL	37		

Table 2: Seroprevalence of selected vector borne diseases in dogs: urban versus rural environments, both symptomatic and asymptomatic dogs.

	<i>Ec</i>	<i>Ana</i>	<i>Bb</i>	<i>Di</i>	<i>Li</i>	<i>Ec + Ana</i>	<i>Ec + Li</i>	TOTAL
Rural, sick	3	2	0	2	9	1	2	19
Rural, healthy	0	1	0	0	3	1	0	5
Urban, sick	0	0	0	0	6	1	0	7
Urban, healthy	2	0	0	0	3	2	0	7
	5	3	0	2	21	5	2	38

p=0.045) and serum globulin concentrations (4.5 G/dL vs 3.4 G/dL; p=0.001) than “healthy dogs”. Seropositive dogs had significantly lower HCT (38.4% vs 44.8%, p=0.002), platelet counts ($174 \times 10^9/L$ vs $223 \times 10^9/L$; p 0.04), and albumin concentration (2.7 G/dL vs 3.1 G/dL; p=0.0001) and significantly higher total serum protein (7.5 G/dL vs 6.9 G/dL; p=0.03) and globulin concentrations (4.5 G/dL vs 3.6 G/dL; p=0.0004) than seronegative dogs.

Discussion

Most studies on canine VBDs in Spain were conducted in the northeastern or northwestern regions of the country [3,4]. A recent study included data from most regions of Spain [15]. In our previous publication, on VBDs in central Spain, dogs were in an animal shelter, and in most cases their previous medical history was unknown [6].

In the current study, all dogs enrolled were evaluated at a veterinary referral center in Madrid, and in most of them, the medical history was readily available. Not surprisingly, dogs from rural areas had significantly higher seroprevalence of VBDs (28% versus 12%, p=0.004). This is similar to the study by Amusatgeui et al (2008) conducted in northwestern Spain [5], who reported a higher prevalence of VBDs in shelter dogs when compared to pet dogs. This is likely the result of higher exposure to vectors in shelter dogs.

The prevalence of seropositivity for each organism in this study was: *Ec*: 2.5%, *Ana*: 1.6%, *Di*: 1%, *Bb*: 0%, and *Li*: 10.7%. These results are comparable to those reported by Miró et al in dogs from central Spain [15], in that *Li* had the highest seroprevalence (6.4%) and *Bb* the lowest (0%). We did not detect a single dog seropositive for *Bb* in our study. In previous studies, dogs from rural environments had higher seroprevalence of *Li*, likely due to higher exposure to the vector (phlebotomus) [26,27]. In contrast, in our study, the seroprevalence of *Li* was similar in dogs from rural (12/87) and urban environments (9/109); this is important, since the literature suggests [15] that city dogs are at lower risk for *Li* infection. We also found 2 dogs from urban environments seropositive for both *Li* and *Ec*.

The point-of-care assay used in this study (SNAP 4DX) did not detect antibodies against *E. ewingii* (*Ee*) in the *Ehrlichia* well, as the new version of this assay (SNAP 4DX PLUS) [28]. However, there is some cross-reactivity between *E. canis* and *E. chaffeensis* antibodies, so dogs positive for *Ec* may have had antibodies against *E. chaffeensis*. *Ee* is the most common *Ehrlichia* species in the Eastern United States [7], but to our knowledge, the seroprevalence of *Ee* and *E. chaffeensis* in Spain have not yet been reported. Given that the seroprevalence of *Ec* was similar to that previously reported, it is likely that *Ee* is not a common organism in this region.

The seroprevalence of *Ec* in this study was similar to those previously published by us (5.3%) and Miró et al. (5.1%) [6,15]. This is interesting, since the main vector for *Ec* is *Rhipicephalus sanguineus*, a tick likely less prevalent in city than in rural dogs. *R. sanguineus* is

prevalent in wild canidae that cohabitate with dogs in rural areas [27]. The prophylactic use of tick repellents in city dogs may potentially explain this discrepancy.

The prevalence of anaplasmosis in this study was significantly lower than that reported in our previous studies in shelter dogs. (1.6% versus 19%; p=0.0001). Again, this may reflect the higher prevalence of tick infestation in shelter dogs. As discussed above, the *Ap* well in the SNAP 4DX detects antibodies to both *A. platys* and *A. phagocytophylum* [29]; the former is primarily transmitted by *R. sanguineus* [30], whereas the latter is primarily transmitted by *Ixodes* ticks. Ideally, molecular diagnostic methods, such as PCR, should allow to distinguish between these 2 infectious agents [15]. Of the 8 dogs positive for anaplasmosis, 3 had normal platelets counts (range 175,000-500,000/ μ L), 1 had a mild decrease (172,000/ μ L), 2 had a moderate decrease (103,000/ μ L and 105,000/ μ L), and 2 had a severe decrease (53,000/ μ L and 20,000/ μ L).

Similar to previous studies, there were only 2 dogs positive for *Di* antigen, both from rural areas [6,15]. Although these dogs may have traveled to other areas with high prevalence of *Di*, such as the Canary Islands [31], we did not record travel history in our study. As previously reported, we did not find any dogs seropositive for *Bb* [6,15].

Interestingly, 7/38 dogs (18.4% of the seropositive dogs) were coinfecting; 5 dogs were seropositive for *Ec* and *Ana* (2.5%), and 2 for *Ec* and *Li* (1%). Four of these 7 dogs were symptomatic. As discussed above, it is likely that the *Ec/Ana* coinfecting dogs had *Apl* exposure, given the fact that both agents are transmitted by *Rhipicephalus* ticks [30].

Seventy percent (n=26) of the seropositive dogs had clinical signs of disease, that may have been related to the infectious agent in question; 11 seropositive dogs (30%) were asymptomatic. This is particularly important in the *Li* positive dogs (including both coinfecting with *Li* and *Ec*), where 17/21 (81%) dogs were sick; Miró et al proposed an association between coinfection with *Li* and *Ec* and clinical signs [15]. The 2 *Li* seropositive dogs in our study that were coinfecting with *Ec* were sick.

From a hematologic and serum biochemical standpoints, seropositive dogs had significantly lower hematocrit (p=0.002), platelet counts (p=0.04), and albumin concentration (p=0.001), and higher total protein (p=0.03) and globulin concentrations (p=0.0004) than the seronegative dogs. All these findings have been previously associated with several VBDs [32,33].

Conclusion

Although the seroprevalence of these selected VBDs is similar to those previously reported, we found a high percentage of seropositive urban dogs. Interestingly, the seroprevalence of *Li* was similar between rural and city dogs. Not surprisingly, seropositive dogs had a higher risk of concomitant clinicopathologic abnormalities. Therefore, symptomatic dogs with anemia, thrombocytopenia, hypoalbuminemia, and/or hyperglobulinemia should be tested for these VBDs, even when they live in an urban environment.

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