

## ***Bartonella henselae* IgG antibodies are prevalent in dogs from southeastern USA**

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**Abstract** – In contrast to the large body of literature regarding *Bartonella henselae* in humans and cats, there is little information about *B. henselae* as an infectious agent in dogs. Due to the paucity of information regarding the *B. henselae* serology in dogs, we performed a cross-sectional serosurvey using *B. henselae* antigen in order to compare the seroprevalence between sick and healthy dogs from the south-eastern USA. Ninety-nine sera were collected from clinically healthy dogs. Three hundred and one sera from sick dogs were submitted to North Carolina State University for serologic screening against a panel of arthropod-transmitted organisms. Serological tests were performed using *B. henselae* (*Bh*), *Rickettsia rickettsii* (*Rr*), *Ehrlichia canis* (*Ec*), *Bartonella vinsonii* subspecies *berkhoffii* (*Bvb*), *Babesia canis* (*Bc*) and *Borrelia burgdorferi* (*Bb*) antigens. Serum *B. henselae* IgG antibodies were detected in 10.1% of healthy dogs and in 27.2% of sick dogs. The difference in seroprevalence between the two groups was statistically significant. The majority of seroreactive dogs (80%) had low titers of 1:64 or 1:128. In healthy dogs, seroprevalence for *Rr* was 14.1% and for *Bvb* was 1%. In sick dogs, *Rr* seroprevalence was 29.7%, *Ec* 6.5%, *Bvb* 4.7%, *Bb* 1.7% and *Bc* was 0.85%. Of the sick dogs that were seroreactive to *B. henselae* antigens, 40.6% were also seroreactive to *Rr*, 15.0% reactive to *Bvb* antigens, 14.8% reactive to *Ec* antigens, 1.8% reactive to *Bc* antigens and 1.75% reactive to *Bb* antigens. Sera from dogs experimentally infected with *B. vinsonii* subsp. *berkhoffii*, *E. canis* or *R. rickettsii* did not cross react with *B. henselae* antigens, by IFA testing. This study indicates that *B. henselae* IgG antibodies are prevalent in healthy and sick dogs living in the south-eastern USA. Nevertheless, further studies are needed to evaluate the epidemiological, clinical and zoonotic relevance of *B. henselae* infection in dogs.

***Bartonella henselae* / dog / serology / vector-borne diseases**

### 1. INTRODUCTION

Members of the genus *Bartonella* are pleomorphic, gram-negative rods that are highly adapted to facilitate intracellular persistence in a wide variety of animals [7]. *Bar-*

*tonella* organisms can induce clinical disease in humans [35] and in other mammals such as the domestic cat and dog [7]. One of the most important *Bartonella* species that causes a broad spectrum of clinical conditions in humans is *Bartonella henselae*

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[17]. In immunocompetent patients, cat scratch disease (CSD) caused by *B. henselae* is mainly characterized by a benign regional lymphadenopathy, while a low proportion of CSD patients may develop atypical manifestations [12, 48]. *Bartonella henselae* is also a frequent cause of prolonged fever in children [33, 62]. In immunocompromised patients, bacillary angiomatosis [23] and bacillary peliosis hepatis or splenitis are the most common *B. henselae*-induced disease manifestations [47]. In people, the major risk factor associated with *B. henselae* infection is cat exposure, especially cat scratches [14, 64].

The cat flea (*Ctenocephalides felis*) is the main arthropod [16] vector of *B. henselae* and cats serve as the main vertebrate reservoir [15]. The most probable route for cat to cat transmission of *B. henselae* is via intradermal inoculation of infected feces from cat fleas [21, 22]. Several studies have found high seroprevalences in cats worldwide, ranging from 1% [5] to 81% [15] depending on the climate and presumably flea density of each geographical region studied. Seroprevalences of *B. henselae* antigens are much greater in cats that live in warm, humid regions of the world where flea infestation is expected [34]. The prevalence of bacteremia documented in different countries, although variable, is often high and ranges from 9% [49] to 90% [40], depending upon the study location and the cat population tested.

Although the prevalence of *B. henselae* infection can be high in apparently healthy cats, several studies suggest that cats may suffer clinicopathological consequences due to persistent *B. henselae* infection [38]. Cats experimentally infected with *B. henselae* developed various clinical signs such as fever, lethargy, transient anemia, lymphadenomegaly, neurological dysfunction, reproductive failure [28, 29, 39, 53]. Pathological abnormalities in experimentally infected cats included lymph node and splenic hyper-

plasia, splenic microabscesses, lymphocytic plasmacytic myocarditis, focal pyogranulomatous nephritis, lymphocytic interstitial nephritis and lymphocytic cholangiohepatitis [28, 39, 41]. Less information is available on clinical disease in naturally infected cats. However, based on serological studies, naturally infected cats are more likely to develop lymphadenitis, gingivitis, stomatitis and are predisposed to urological diseases [25, 63]. Additionally, uveitis associated with detection of *B. henselae* DNA and antibodies in aqueous humor has been reported in cats [44].

Since the isolation and characterization of *B. henselae* in 1992 [56], a large body of literature has been generated regarding bartonellosis in humans and cats. However, there is little information about *B. henselae* infection in dogs. Historically, dogs have been infrequently implicated in the transmission of *B. henselae* to humans [36, 61]. Recently, *B. henselae* DNA has been amplified and sequenced from the liver of a dog with peliosis hepatitis [37] and a dog with granulomatous hepatitis [24] and from the blood of three dogs with either fever, thrombocytopenia or neurologic dysfunction [51]. Three canine serosurveys carried out in Hawaii, Japan and the United Kingdom describe *B. henselae* seroprevalence of 6.5% [20], 7.7% [61] and 3% [4], respectively. In Japan, *B. henselae* PCR positive results were also reported from peripheral blood, nail clippings and oral swabs in 15% of the dogs studied [61]. To further characterize *B. henselae* seroprevalence in dogs, we performed a survey in a population of healthy and sick dogs from the south-eastern USA. To compare *B. henselae* seroprevalence with exposure to other vector-borne diseases, sera were also tested for *Rickettsia rickettsii*, *Ehrlichia canis*, *Bartonella vinsonii* subsp. *berkhoffii*, *Babesia canis* and *Borrelia burgdorferi* IgG antibodies in the same population of dogs.

## 2. MATERIALS AND METHODS

### 2.1. Dogs

Ninety-nine sera were collected between October 2002 and February 2003 at a private veterinary hospital located in Cary (North Carolina, USA). These sera represent a convenience sample of clinically healthy dogs that were screened for *Dirofilaria immitis* antigen, *E. canis* antibodies (P30 and P31 outer membrane proteins) and *B. burgdorferi* (C6 peptide) with a commercial assay kit (Canine SNAP<sup>®</sup> 3Dx<sup>™</sup> Test; IDEXX Laboratories, USA). Only sera from those dogs with normal physical examination findings and negative 3Dx test results were included in this study. Sixty-eight of the healthy dogs were females (67 spayed, 1 intact) and 31 dogs were male (25 neutered, 6 intact). The age was known for 95 dogs with a mean  $\pm$  standard deviation of  $5.1 \pm 2.9$  years. Ages ranged from 9 months to 13 years. Various breeds were represented and 11 dogs were mixed breed. Seventy-four dogs were treated with tick/flea control products. The tick/flea control status was unknown in 21 dogs and 4 dogs did not receive any tick/flea control treatment. The sera were tested by immunofluorescence assay (IFA) to determine *B. henselae* seroprevalence in a population of healthy dogs, with limited exposure to ticks and fleas. Sera from these dogs were also tested for *B. canis*, *B. vinsonii* subsp. *berkhoffii*, and *R. rickettsii* IgG antibodies by IFA.

Three hundred and one sera from sick dogs living in the southeastern USA (252 sera from North Carolina) that were submitted to the NCSU-Vector Borne Disease Diagnostic Laboratory for serologic screening for arthropod-transmitted diseases from October 2000 to April 2003 were also included in this study. Clinicopathological findings of sick dogs compatible with arthropod-transmitted diseases were categorized in neurological, ocular, cardiac, hematological, orthopedic or multisystemic disorders. Several purebred and mixed breed dogs were represented in the study population, but age, breed and sex was not provided for these diagnostic accessions. Sera from sick dogs

were tested for IgG antibodies to *B. henselae* ( $n = 301$ ), to *B. vinsonii* subsp. *berkhoffii* ( $n = 295$ ), to *R. rickettsii* ( $n = 232$ ), to *E. canis* ( $n = 231$ ), to *B. canis* ( $n = 233$ ) by an in house IFA and to *B. burgdorferi* ( $n = 230$ ) using the Canine SNAP<sup>®</sup> 3Dx<sup>™</sup> Test [46].

### 2.2. Serology

#### 2.2.1. Detection of IgG antibodies to *B. henselae*, *B. vinsonii* subsp. *berkhoffii*, *R. rickettsii*, *E. canis* and *B. canis*

*Bartonella henselae* [10], *B. vinsonii* subsp. *berkhoffii* NCSU 93CO1 [54] and *R. rickettsii* NCSU Domino strain [9] were cultivated in Vero cells and harvested when cells were more than 80% infected (2 to 9 days postinoculation). *Ehrlichia canis* (Florida strain) was grown as described previously by in vitro propagation in 030 cell line culture [60]. Antigen for IFA was prepared by pelleting and re-suspending microorganisms and cells in phosphate buffered saline (PBS). *Babesia canis* antigen slides were made from the blood of dogs experimentally inoculated with these piroplasms as previously described [45]. Antigens were applied to 30-well Teflon-coated slides (Celine Associates, Newfield, NJ, USA) in 3.0  $\mu$ L aliquots and air-dried. Slides were fixed in acetone for 10 min and frozen at  $-20^{\circ}\text{C}$  until use.

Three twofold serial dilutions of sera (1:16, 1:32, 1:64) in PBS 0.05% Tween 20 (T)-0.5% dried skim milk (M)-1% goat sera (G) were made in microtiter plates. Ten microliters of each dilution was applied per well, and slides were incubated at  $37^{\circ}\text{C}$  for 30 min, washed in PBS with agitation for 30 min and air-dried. Fluorescein conjugated goat anti-dog immunoglobulin (whole molecule immunoglobulin G; Cappel, Organon Teknika Corp., Durham, NC, USA) was diluted 1:100 in PBSTMG, filtered with 0.22  $\mu$ m filter to remove precipitants and applied to each well. Slides were incubated for 30 min at  $37^{\circ}\text{C}$  and washed again

in PBST with agitation for 30 min, rinsed with deionized water, air dried, cover slipped using mounting medium (90% glycerol and 10% PBS, pH 9.0) and viewed with a fluorescence microscope (magnification,  $\times 40$ ). *Ehrlichia canis* IFA was performed on each serum sample as described above; the only modification was that slides, after the last wash with PBST, were counter stained with Eriochrome black before the final rinse in deionized water. Samples with an IFA titer  $> 1:32$  were retested with serial dilutions from 1:16 to 1:8192. End-point titers were determined as the last dilution at which brightly stained organisms could be detected on a fluorescence microscope with exciter and barrier filters using a 50 watt light source.

For all antigens, a reactive serum was defined as a titer of  $\geq 1:64$ . Sera from dogs experimentally infected with *B. henselae* (titer 1:512) (kindly provided by Dr Bruno Chomel, University of California, Davis, USA, unpublished results), *B. vinsonii* subsp. *berkhoffii* (titer 1:1024), *R. rickettsii* (titer 1:2048), *E. canis* (titer 1:4096) and *B. canis* (1:1024) were used as positive controls, while a nonreactive serum from a specific pathogen free (SPF) dog was used as a negative control for all IFA testing.

### 2.2.2. Crossreactivity

Sera from dogs experimentally-infected with *R. rickettsii*, *E. canis* or *B. vinsonii* subsp.

*berkhoffii* were tested by *B. henselae* IFA to determine if there was crossreactivity among these organisms. These dogs were seronegative to respective antigens prior to the experimental infection. The median geometric *R. rickettsii* titer for six experimentally infected dogs was 1:512 at 21 days postinfection [9]. The median geometric *E. canis* titer for seven experimentally infected dogs was 1:1722 at 49 days postinfection [11]. The median geometric titer of nine dogs experimentally infected with *B. vinsonii* subsp. *berkhoffii* was 1:1755 at 31 days postinfection [55].

### 2.3. Statistical analysis

Chi-square was used to test for associations between groups. Differences were considered significant if the *P*-value was  $< 0.05$ .

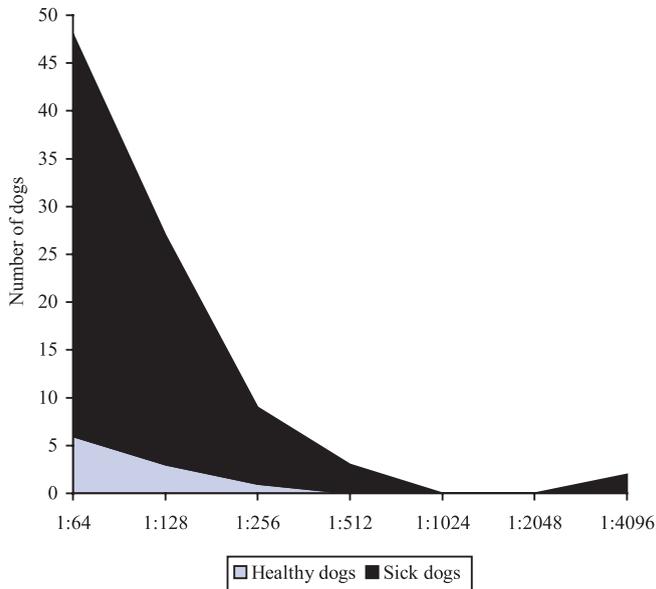
## 3. RESULTS

The results of *B. henselae*, *R. rickettsii*, *E. canis*, *B. vinsonii* subsp. *berkhoffii*, *B. burgdorferi*, *B. canis* seroprevalences in healthy and sick dogs are shown in Table I. The differences in *B. henselae* and *R. rickettsii* seroprevalences between the healthy and sick dog populations were statistically significant (Chi-square = 12.36, *P* = 0.00043; Chi-square = 8.99, *P* = 0.0027; respectively). The difference in *B. vinsonii* subsp. *berkhoffii*

**Table I.** Seroprevalences of different arthropod-transmitted organisms in clinically healthy and sick dogs.

Arthropod-transmitted organisms	Healthy dogs*	Sick dogs*	All dogs*
<i>R. rickettsii</i>	14/99 (14.1)	69/232 (29.7)	83/331 (25.0)
<i>B. henselae</i>	10/99 (10.1)	82/301 (27.2)	92/400 (23.0)
<i>E. canis</i>	n.d.	15/231 (6.5)	15/231 (6.5)
<i>B. vinsonii</i> subsp. <i>berkhoffii</i>	1/99 (1)	14/295 (4.7)	15/310 (4.8)
<i>B. burgdorferi</i>	n.d.	4/230 (1.7)	4/230 (1.7)
<i>B. canis</i>	n.d.	2/233 (0.85)	2/233 (0.85)

\* Number of seroreactive dogs/total number of dogs (% of seroreactive dogs).  
n.d.: not determined with the same technique.



**Figure 1.** Frequency of *B. henselae* seroreactive titers in healthy and sick dogs from the southeast region of USA.

seroprevalence between the two groups was not statistically significant (Chi-square = 2.765,  $P = 0.096$ ; Yates' Chi-square = 1.897,  $P = 0.168$ ).

In clinically healthy dogs, six dogs had a *B. henselae* titer of 1:64, three dogs 1:128 and one dog 1:256, with a geometric median titer of 1:90. *Rickettsia rickettsii* titers ranged from 1:64 to 1:2048 with a geometric median titer of 1:210. The only dog seroreactive to *B. vinsonii* subsp. *berkhoffii* antigens had a titer of 1:64. None of the healthy dogs that were seroreactive to *R. rickettsii* or *B. vinsonii* subsp. *berkhoffii* antigens were seroreactive to *B. henselae* antigens.

In sick dogs, *B. henselae* positive titers ranged from 1:64 to 1:4096 with a geometric median titer of 1:118; however, the majority of seroreactive dogs (80.4%, 66 of 82) had low titers (1:64 or 1:128) (Fig. 1). *Rickettsia rickettsii* titers ranged from 1:64 to 1:2048 with a geometric median titer of 1:146. *Ehrlichia canis* titers ranged from 1:64 to 1:4096 with a geometric median titer of 1:308. *Bartonella vinsonii* subsp. *berkhof-*

*fii* titers ranged from 1:64 to 1:512 with a geometric median titer of 1:136. *Babesia canis* titers were 1:64.

The presence of serum *B. henselae* antibodies was not associated with seroreactivity to *B. burgdorferi* or *B. canis* antigens. In contrast, of the samples that were reactive with *R. rickettsii*, *E. canis* and *B. vinsonii* subsp. *berkhoffii* antigens, 34%, 53% and 85% ( $P = 0.035$ ,  $P = 0.011$  and  $P = 0.0000029$ ; respectively) were seroreactive to *B. henselae* antigens (Tab. II).

The presence of *E. canis* or *R. rickettsii* antibodies was not associated with seroreactivity to *B. vinsonii* subsp. *berkhoffii* antigens. In contrast, the detection of *E. canis* antibodies was associated with seroreactivity to *R. rickettsii* antigens (Chi-square = 14.28,  $P = 0.00015$ ; Yates' Chi-Square = 12.16,  $P = 0.00048$ ).

Table III summarizes the results of sera that were reactive to both *B. vinsonii* subsp. *berkhoffii* and *B. henselae* antigens. Six out of 12 dogs had a higher *B. henselae* titer

**Table II.** Measure of association between *Bartonella henselae* seroreactivity and *Rickettsia rickettsii*, *Ehrlichia canis*, and *Bartonella vinsonii* subspecies *berkhoffii*, but not *Borrelia burgdorferi* or *Babesia canis* seroreactivity in sick dogs from the south-eastern USA.

Serology (IFA or C6 peptide)	<i>R. rickettsii</i>		<i>E. canis</i>		<i>B. vinsonii</i> subsp. <i>berkhoffii</i>		<i>B. burgdorferi</i>		<i>B. canis</i>		
	+	-	+	-	+	-	+	-	+	-	
<i>B. henselae</i>	+	24 <sup>a</sup>	35	8 <sup>b</sup>	46	12 <sup>c</sup>	68	1 <sup>d</sup>	56	1 <sup>e</sup>	54
	-	45	128	7	170	2	213	3	170	1	177

<sup>a</sup> Chi-square = 4.418; *P* = 0.035.

<sup>b</sup> Chi-square = 8.037; *P* = 0.0045; Yates' chi-square = 6.348; *P* = 0.011.

<sup>c</sup> Chi-square = 25.5; *P* = 4.4e<sup>-7</sup>; Yates' chi-square = 22.5; *P* = 0.00000209.

<sup>d</sup> Chi-square = 0; *P* = 1; Yates' chi-square = 0.329; *P* = 0.566.

<sup>e</sup> Chi-square = 0.779; *P* = 0.377; Yates' chi-square = 0.002; *P* = 0.96.

**Table III.** Comparative IFA titers to *B. henselae* and *B. vinsonii* subsp. *berkhoffii* (*Bvb*) antigens among 12 dogs seroreactive to both antigens.

Dog ID	IFA titers for <i>Bvb</i>	IFA titers for <i>B. henselae</i>
1	1:256	1:64
2	1:128	1:128
3	1:64	1:64
4	1:64	1:64
5	1:64	1:512
6	1:128	1:64
7	1:512	1:1024
8	1:512	1:512
9	1:128	1:1024
10	1:512	1:4096
11	1:64	1:256
12	1:128	1:256

than *B. vinsonii* subsp. *berkhoffii* titer. Four out of 12 dogs had the same titer to both organisms and two out of 12 dogs had a higher titer to *B. vinsonii* subsp. *berkhoffii* antigens than for *B. henselae* antigens.

Sera from the dogs experimentally infected with *R. rickettsii* or *E. canis* were not seroreactive to *B. henselae* antigens (all *B. henselae* titers were less than 1:16). One out of nine sera from the dogs experimentally infected with *B. vinsonii* subsp. *berkhoffii* was reactive to *B. henselae* anti-

gens with a titer of 1:64, two dogs had titers of 1:32 and one dog had a titer of 1:16. *Bartonella henselae* antibodies were not detectable in the remaining 5 samples (titers were less than the 1:16 screening dilution).

#### 4. DISCUSSION

This study indicates that *B. henselae* IgG antibodies are prevalent in healthy and sick dogs living in the southeastern USA. The total *B. henselae* seroprevalence (23.5%) is greater than previous serosurveys, that described a *B. henselae* seroprevalence of 6.5% in Hawaii [20], 7.7% in Japan [61] and 3% in the United Kingdom [4]. The differences between seroprevalences could be explained by several factors such as differences in IFA technique, differences in the dog populations sampled, differences in climate, the timing of sample collection, or differences in the mode or modes of transmission among the different geographic regions.

Cat to cat transmission of *B. henselae* occurs via intradermal inoculation of infected flea feces [22]. Transmission of *B. henselae* from cats to people occurs most frequently via cat scratches, presumably contaminated with flea excrement [27]. Our hypothesis is that the transmission of *B. henselae* to dogs occurs via flea excrements, ticks and scratches.

Self-inoculation with flea excrement may result in direct transmission to a flea-infested dog. Dogs and cats are commonly infested with the same flea (*Ctenophalides felis*) [58] that is known to transmit *B. henselae* from infected to SPF cats [16]. Both *C. felis* from cats and dogs and *C. canis* from dogs were reported to be positive for *B. henselae* DNA [32]. Recently, DNA from several *Bartonella* spp., including *B. henselae* DNA, was detected by PCR in questing *Ixodes pacificus* ticks in California [13] and in *Ixodes ricinus* removed from people in Italy [59]. Consequently, vectors, such as fleas and ticks, may be implicated in *B. henselae* transmission to dogs. It is also possible that cats could infect dogs via a scratch or bite, as occurs with human cat scratch disease. Transmission of *B. henselae* from cat to cat and from cats to people is very well established. However, future studies are needed to define the route or routes of *B. henselae* transmission to dogs.

In this study, the total *B. henselae* seroprevalence (23.5%) in dogs was much lower than the seroprevalence (50%) found in cats from the same geographical region [3, 34]. In humans, *B. henselae* seroprevalence ranges between 5.7% in healthy human blood donors [52] and up to 87% in human patients with suspected cat scratch disease [57]. Cats appear to be the main reservoir of *B. henselae* infection as indicated by the high seroprevalences found in cats worldwide [7] as well as documentation of persistent bacteremia in naturally and experimentally infected cats [1, 40, 43]. Bacteremia has been infrequently documented in dogs, and only by PCR amplification [6, 51]. The role of dogs as a reservoir for *B. henselae* infection is unclear and needs further investigations.

This report describes statistically significant differences in *B. henselae* infection between clinically healthy dogs selected for lack of exposure to ticks or fleas and dogs with clinical signs compatible with other vector-borne diseases for which veterinarians sought testing. This difference could be

related to selection bias; however, *B. henselae* has been detected in several tissues from sick dogs with a variety of clinical presentations [24, 37, 51]. Further studies, such as case-control studies, are needed to elucidate the clinical relevance of *B. henselae* antibodies in dogs.

In humans, serological cross-reactions between *B. henselae* and *Coxiella burnetii* [42] and between *B. quintana* and *Chlamydia pneumoniae* [50] have been reported. There are no studies in cats or dogs that assess the possibility of serological cross-reactions between these or other bacterial antigens. In the current study, based upon testing of sera obtained from experimentally infected dogs, there was no cross-reactivity between *R. rickettsii*, *E. canis* and *B. henselae*, and minimal cross-reactivity between *B. vinsonii* subsp. *berkhoffii* and *B. henselae*. However, the data provided on cross-reactivity is not conclusive due to the small sample size and the fact that the dogs were experimentally inoculated and tested in the acute phase of the infection. Due to the limitations of this study, future investigations should address the question of cross-reactivity to ensure that the *B. henselae* seroprevalence found in cats and dogs are truly reflective of *B. henselae* exposure.

In this study, the presence of *B. henselae* antibodies was associated with being seroreactive to *R. rickettsii* antigens. This association may indicate that *R. rickettsii* seroreactivity is due to cross-reactivity with *R. felis*, *R. typhi*, other *Rickettsia* spp. or other bacteria. These results might support simultaneous transmission of both *Rickettsia* and *Bartonella* organisms to dogs by an insect vector. It is well known that canine *R. rickettsii* antibodies cross react with several *Rickettsia* spp. of unknown pathogenicity such as *R. rhipicephali* and *R. montana* [8]. In addition, flea-borne organisms (e.g., *Yersinia pestis*, *R. typhi*, *R. felis* and *B. henselae*) are widely distributed throughout the world in endemic disease foci. In the United States, *R. felis* and *R. typhi* DNA has been found in cat fleas [2] which supports the possibility

that fleas may co-transmit *B. henselae* and a *Rickettsia* spp. to dogs. Rocky Mountain spotted fever is an important tick-borne zoonosis that is especially prevalent in the southeastern United States [8]. In this study, the seroprevalence of *R. rickettsii* antigen in North Carolina was 29.7% in sick dogs. A similar seroprevalence (29.8%) was found previously in pet and stray dogs from North Carolina [8]. A lower *R. rickettsii* seroprevalence (14%) was found in the clinically healthy dogs in this study.

Based upon current evidence, *Bartonella vinsonii* subsp. *berkhoffii* has been considered the most frequent *Bartonella* species infecting dogs [17]. However, this conclusion may not be accurate, as sera from dogs have not been screened systematically against a large panel of *Bartonella* species antigens. *B. vinsonii* subsp. *berkhoffii* seroprevalence in this study (4.7%) was similar to three previous reports [30, 31, 54] and was much lower than the *B. henselae* seroprevalence (23.5%) found in dogs in the current study. Moreover, it is possible that an antibody cross-reaction occurs between *Bartonella* species as has been determined between *B. henselae* and *B. quintana* in cats [3]. Recently, *Bartonella washoensis* was isolated for the first time from the blood of a dog with endocarditis [18]. By IFA testing that dog was strongly seroreactive to several *Bartonella* antigens (*B. vinsonii* subsp. *berkhoffii*, *B. clarridgeiae* and *B. henselae*) suggesting exposure to several *Bartonella* species or cross-reactivity between species [18]. However, the antibody titer to *B. washoensis* was 1:8192 and to *B. vinsonii* subsp. *berkhoffii*, *B. clarridgeiae* and *B. henselae* 1:4096 [18]. In the present study, only two dogs had a *B. henselae* titer of 1:4096, and cross-reactivity might be less likely detected at lower antibody titers. In this study, all *B. vinsonii* subsp. *berkhoffii* seroreactive sick dogs were concurrently seroreactive to *B. henselae* antigens, but only 14.5% of *B. henselae* seroreactors were also seroreactive to *B. vinsonii* subsp. *berkhoffii*. This finding would tend to support unidirectional cross-reactivity or co-exposure to

*B. henselae* in dogs infected with *B. vinsonii* subsp. *berkhoffii*.

In this study, *E. canis* seroprevalence (6.5%) in sick dogs was slightly greater than from a previous report (2.4%) of sick dogs also living in North Carolina [60]. Detection of *B. henselae* antibodies was associated with seroreactivity to *E. canis* antigens. This association may support tick transmission of *B. henselae* in some cases, as *Ehrlichia* spp. are transmitted by ticks [19]. *Babesia canis* (0.85%) and *B. burgdorferi* (1.7%) antibodies were infrequently detected; further, C6 peptide seroprevalence was slightly lower than the *B. burgdorferi* IFA prevalence from the same area (2.5%) although these results are difficult to compare due to the fact that different serological tests were employed [26].

In conclusion, this study indicates that *B. henselae* IgG antibodies are prevalent in healthy and sick dogs living in the southeast region of the USA. *Bartonella henselae* seroprevalence seems greater in dogs with clinical signs compatible with arthropod vector-borne diseases than in healthy dogs, selected for infrequent exposure to ticks or fleas. Based upon testing sera from experimentally infected dogs, there does not appear to be cross-reactivity between *B. henselae* and *B. vinsonii* subsp. *berkhoffii*, *E. canis* or *R. rickettsii*. Currently, there is a significant, but unexplained, association between *B. henselae* and *R. rickettsii* antibodies in sick dogs from the southeastern USA. Further studies are needed to evaluate the epidemiological, clinical, and zoonotic relevance of *B. henselae* infection in dogs.

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