

# A Pilot Survey of Vector-Transmitted Diseases in Cartagena and Barranquilla, Colombia

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## ABSTRACT

The tick-borne bacteria *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Anaplasma platys*, and *Borrelia burgdorferi* are widespread and known to infect numerous wild animals as well as many domestic species. On October 2017, 150 pet dogs and 49 dogs in from shelters in Barranquilla, the capital city of the Atlantico Department in the northern part of Colombia, and 200 dogs from Cartagena, located on the northern coast in the Caribbean Coastal Region of Colombia, were surveyed using a rapid in-clinic ELISA test to detect adult *D. immitis* antigens and anti-*E. canis*, *A. platys*, and *B. burgdorferi* antibodies. The majority of dogs primarily lived indoors (44.9%), 138 dogs lived outdoors (34.6%), and 13 had access

to indoors and outdoors (3.3%). The overall canine vector borne disease prevalence was 67.2% (268/399), including some dogs that were infected with multiple species. *D. immitis* infection rate was 20.8% (83/399) and antibodies were detected in 18.3% (*A. platys*), and 62.7% (*E. canis*) of the dogs sampled. No dog was positive for antibodies to *B. burgdorferi*. *E. canis* was the most prevalent parasite in all three groups of dogs. Since several dogs, particularly those from shelters, were positive for both anti-*E. canis* and anti-*A. platys* antibodies, and *R. sanguineus* is the known vector for *E. canis*, it is highly likely that *R. sanguineus* is the vector for both pathogens in Colombia. Veterinarians should provide owners with information about the risks of exposure of their dogs to ticks and mosquitoes that are vectors for these infections, and that off-label use and incorrect dosing of macrocyclic lactones

must be avoided in order to control ticks and preserve the long-term efficacy of these products. Veterinarians must also stress to owners that all dogs need and deserve to receive proper preventive health care.

## INTRODUCTION

Arthropod-transmitted pathogens are a major concern in veterinary medicine, especially due to expansion of arthropod habitats into northern latitudes and to higher altitudes as a consequence of climate changes occurring throughout the world.<sup>1,2</sup> Among those pathogens, *Dirofilaria immitis* is known to infect high numbers of domestic and some wild canids. Cats and ferrets may also occasionally harbor low and transient levels of microfilariae. *Dirofilaria immitis* is vectored by various species of mosquitoes,<sup>3</sup> but the tick-borne pathogens generally demand specific species.

The tick-borne bacteria *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Anaplasma platys*, and *Borrelia burgdorferi* are widespread and known to infect numerous wild animals as well as many domestic species.<sup>4-6</sup> These species also infect humans rarely (*E. canis*), occasionally (*A. phagocytophilum*), or commonly in some areas (*B. burgdorferi*).<sup>6</sup> The principal vector of *E. canis* and most likely *A. platys* worldwide is *Rhipicephalus sanguineus*, the brown dog tick.<sup>7,8</sup> Therefore, *A. platys* is likely the primary organism responsible for cases of anaplasmosis in dogs in South America. Vectors of *A. phagocytophilum* and *B. burgdorferi* in the Northern Hemisphere are of the *Ixodes ricinus* complex, a group of ticks poorly represented in South America, although *Ixodes pararicinus* has been found infected by *Borrelia burgdorferi* sensu lato in Uruguay.<sup>9</sup>

The distribution of dogs infected by these arthropod-borne parasites depends on the cohabitation of vectors, infected hosts, and unprotected dogs. As vectors feed on blood from infected hosts, they become infected, and subsequent exposure of unprotected dogs to the infected vectors enhances the risk for the unprotected dog to become ill and/or become a new source for infection

of other unprotected animals.

*Dirofilaria immitis*, an important parasite in canine medicine worldwide, was initially detected in 0.25% to 7.4% of dogs surveyed in Colombia.<sup>10</sup> As interest in *D. immitis* grew, especially when the animal health industry sponsored surveys prior to launching specific chemoprophylactic drugs, updates on canine heartworm infection prevalence in South America were published.

The first update reported 4.8% of 864 dogs tested had microfilariae in the blood, including 2.3% of the dogs tested in Bogota despite the city's high elevation above sea level.<sup>11</sup> In a second survey, which included microfilaria and antigen testing of dogs from 32 cities in 30 departments in Colombia, the overall prevalence of heartworm-infected dogs was 8.4%, including 75 dogs (3.8%) positive for circulating microfilariae and 92 (4.6%) that had occult infections.<sup>12</sup> The highest infection rates were in Atlantico (22%), Risaralda (21%), and Magdalena (16%). Among the 30 departments surveyed, only Quindio, located in the western central region of the county, had no heartworm-infected dogs detected.<sup>12</sup>

During the past decade, the only published update of the prevalence of *D. immitis* in Colombia included a study of dogs from Barranquilla (n=223), Medellin (n=175), and Cartagena (n=100) for *D. immitis* antigens using rapid in-clinic ELISA test (Snap<sup>®</sup> 4Dx; IDEXX Laboratories, Inc., Westbrook, Maine, USA).<sup>13</sup> In that survey, *D. immitis* was not detected in any dogs in Medellin. However, 2.2% of the dogs tested in Barranquilla and 3% of those tested in Cartagena were positive for *D. immitis* antigens.

*Ehrlichia canis* has been known to be present in Colombia for more than 30 years.<sup>14,15</sup> Seroreactivity of dogs to *Ehrlichia* spp. was 31.8% in a rural area of the country<sup>16</sup> and 31.7% of dogs from an urban area.<sup>17</sup> In a survey conducted in Barranquilla to identify canine infections with tick-borne pathogens, seroprevalence of *E. canis* was 74% and *A. platys* seroprevalence was 53%.<sup>18</sup> Evaluation of these samples by PCR

detected DNA of *E. canis* in 21.1%, DNA of *A. platys* in 8.7%, and DNA of both pathogens in 7.3% of the dogs tested. Another recent survey identified 28% seroprevalence for *E. canis* and 6% for *Anaplasma* sp when symptomatic dogs from Barranquilla were tested.<sup>19</sup>

## MATERIALS AND METHODS

In October 2017, 199 dogs from Barranquilla, the capital city of the Atlantico Department in the northern part of Colombia, and 200 dogs from Cartagena, located on the northern coast in the Caribbean Coastal Region of Colombia, were surveyed using a rapid in-clinic ELISA test (SNAP® 4Dx® Plus; IDEXX Laboratories, Inc., Westbrook, Maine, USA). The sample size of the canine population in each area was calculated using EPI INFO 3.5.2 for a confidence level of 95%.<sup>20</sup> The sample size considered an expected *D. immitis* infection frequency of 5% in Barranquilla, with the lowest acceptable limit of 2%, and an expected *D. immitis* infection frequency of 8% for Cartagena, with the lowest acceptable limit of 4%.

In Barranquilla, 49 animals were from 3 different shelters and 150 were pet dogs. In Cartagena, all 200 dogs were pets. Therefore, 3 different groups were formed, pet dogs from Barranquilla; pet dogs from Cartagena, and shelter dogs from Barranquilla.

After owners' consent for testing was obtained, a blood sample was collected from each dog and serum was obtained and kept at 4°C until processing by the in-clinic ELISA to detect adult *D. immitis* antigens and anti-*E. canis*, *A. platys*, and *B. burgdorferi* antibodies. Assays were performed according to the manufacturer's instructions. Briefly, the SNAP 4Dx<sup>4</sup> Plus assay was performed by mixing 3 drops of test sample (whole blood, serum, or plasma) with 4 drops of conjugate and applied to the flow matrix. If present in the test sample, peptide-specific antibody (*Anaplasma*, *E. canis*, or *B. burgdorferi*) or *D. immitis* antigen would bind to the peptide-horseradish peroxidase (HRP) conjugate. Immune complexes that

formed would bind to the peptide-bovine serum albumin conjugates on the flow matrix. The ELISA test was then exposed to wash solution and substrate reagents. The appearance of blue spots on designated areas of the device after 8 minutes indicated a positive result for one or more of the analytes included in the ELISA.

The number of positive or negative samples for each parasite was recorded. Also, the number of dogs positive for more than one parasite was noted and recorded. Test results were compiled by dog characteristics (age groups, sex, lifestyle) or group (Barranquilla pet, Cartagena pet, or shelter) for determination of statistical significance by chi square, using Yates correction when needed, using EPI INFO 3.5.2.<sup>20</sup>

## RESULTS

Of the 399 dogs evaluated, 34 breeds were represented, although the majority were mixed breed (n=148) or mongrel (n=41). Ages ranged from 6 months to 14 years. Breed was not recorded for 28 dogs, and age was not recorded for 115 dogs. The sex distribution was 1.3 females (n=211) to 1 male (n=167), and sex was not recorded for 21 dogs. The majority of dogs primarily lived indoors (44.9%), 138 dogs lived outdoors (34.6%), and 13 had access to indoors and outdoors (3.3%). Lifestyle was not recorded for 69 dogs. The majority of the animals were pets (n=350) and 49 were shelter animals.

Among shelter dogs, the majority were female (33/49) and mixed bred (43/49), while 5 were French bulldogs and 1 was a Doberman pinscher. Ages varied from 6 months to 10 years. Most dogs were adult (>2 to 6 years) (33/49), and only 2 were older than 6 years. All shelter dogs lived outdoors.

Considering all locations (groups), the overall canine vector borne disease (CVBD) prevalence was 67.2% (268/399), including some dogs that were infected with multiple species (Tables 1 & 2). Among the total population of dogs sampled, *D. immitis* infection rate was 20.8% (83/399) and

**Table 1.** Results of evaluations by an in-clinic rapid ELISA test\* for dogs surveyed for *Ehrlichia canis* and *Anaplasma platys* antibodies and *Dirofilaria immitis* antigens in Colombia according to different categories

Category	<i>E. canis</i>		<i>A. platys</i>		<i>D. immitis</i>	
	No. positive/ category total	%	No. positive/ category total	%	No. positive/ category total	%
Age (y)						
< 2	47/76	61.8	10/76	13.2	8/76	10.5
>2 - 4	63/81	77.8	23/81	28.4	19/81	23.5
>4 - 6	38/57	66.7	14/57	24.6	9/57	15.8
>6	45 /70	64.3	11/70	15.7	19/70	27.1
		NS		NS		P<0.05
Not recorded	57/115		15/115		27/115	
Sex						
Male	108/167	64.7	24/167	14.4	47/167	28.1
Female	24/211	11.4	43/211	20.4	33/211	15.6
		P<0.01		NS		P<0.01
Not recorded	13/21		6/21		3/21	
Life style						
Outdoor	123/138	89.1	47/138	34.1	31/138	22.5
Indoor	89/179	49.7	72/179	40.2	49 /179	27.4
Both	9/13	69.2	1/13	7.7	0/13	0
		P<0.01		P<0.01		NS
Not recorded	29/69		3/69		3/69	
Group						
Barranquilla pets	102/150	68.0	36/150	24.0	36/150	24.0
Cartagena pets	102/200	51.0	17/200	8.5	43/200	21.5
Shelter	46/49	93.9	20/49	40.8	4/49	8.2
		P<0.05		P<0.05		P<0.05
Total	250/399	62.7	73/399	18.3	83/399	20.8

NS, not significant

\*SNAP® 4Dx® Plus; IDEXX Laboratories, Inc., Westbrook, Maine, USA.

antibodies against *A. platys* were detected in 18.3% and against *E. canis* in 62.7% of the dogs sampled (Table 1). No dog included in the survey was positive for antibodies against *B. burgdorferi*. *E. canis* was the most prevalent tick-transmitted pathogen in all 3 groups of dogs: Barranquilla pet dogs ( $\chi^2=81.63$ ;  $P=0.00001$ ;  $df=2$ ), Cartagena pet dogs ( $\chi^2=81.58$ ;  $P=0.00001$ ;  $df=2$ ), and shelter dogs ( $\chi^2=51.74$ ;  $P=0.00001$ ;  $df=2$ ).

Among the 3 groups, shelter dogs pre-

sented the highest *E. canis* prevalence rate (93.9%), followed by those from Barranquilla (68%) and Cartagena (51%) ( $\chi^2=33.86$ ;  $P=0.0001$ ;  $df=2$ ) (Table 1). Similarly, dogs from the shelters had the highest prevalence of *A. platys* (40.8%), followed by those from Barranquilla (24.0%).

The lowest prevalence was in pet dogs in Cartagena (8.5%) ( $\chi^2=32.73$ ;  $P=0.0001$ ;  $df=2$ ). *Dirofilaria immitis* was detected in similar rates in pet dogs (24.0%

**Table 2.** Number and percentage of pet and shelter dogs tested for antibodies to *Ehrlichia canis* and *Anaplasma platys* antibodies and *Dirofilaria immitis* antigen by a commercial in-clinic rapid ELISA test\* for dogs in Colombia

Groups	N	Number and percentage of dogs positive for one or multiple infections					
		<i>E. canis</i>	<i>A. platys</i>	<i>D. immitis</i>	<i>E. canis</i> + <i>A. platys</i>	<i>E. canis</i> + <i>D. immitis</i>	<i>E. canis</i> + <i>A. Platys</i> + <i>D. immitis</i>
Barranquilla (pets)	150	49 (32.7%)	3 (2.0%)	8 (5.3%)	25 (16.7%) <sup>a</sup>	20 (13.3%)	8 (5.3%)
Cartagena (pets)	200	49 (24.5%) <sup>a</sup>	3 (1.5%)	4 (2.0%)	14 (7.0%) <sup>b</sup>	39 (19.5%)	0 (0)
Shelter	49	22 (44.9%) <sup>b</sup>	0 (0)	0 (0)	20 (40.8%) <sup>c</sup>	4 (8.23%)	0 (0)
Total	399	120 (31.1%)	6 (1.5%)	12 (3.0%)	59 (14.8%)	63 (15.8%)	8 (2.0%)

Different superscript letters within columns indicate significant difference ( $P < 0.05$ ).

\*SNAP® 4Dx® Plus; IDEXX Laboratories, Inc., Westbrook, Maine, USA.

in Barranquilla and 21.5% in Cartagena). However, the prevalence of *D. immitis* in Barranquilla pet dogs was significantly higher than that for shelter dogs (8.2%;  $\chi^2=4.82$ ;  $P=0.0280$ ).

Age and sex influenced *D. immitis* infection rates (Table 1). Younger dogs presented the lowest prevalence (10.5%;  $\chi^2=7.85$ ;  $P=0.0492$ ;  $df=3$ ) and females were also the less infected (15.6%;  $\chi^2=8.00$ ;  $p=0.0046$ ). Sex and life style influenced *E. canis* seroprevalence. Males had a higher prevalence of *E. canis* than did female dogs (64.7%;  $\chi^2=114.18$ ;  $P=0.0001$ ) and dogs that lived outdoors had the highest prevalence among the 3 life styles (89.1%;  $\chi^2=54.74$ ;  $P=0.0001$ ;  $df=2$ ). Paradoxically, *A. platys* was more prevalent among indoors animals (40.2%;  $\chi^2=6.09$ ;  $P=0.0476$ ;  $df=2$ ) than in outdoor or indoor/outdoor dogs (Table 1).

When comparing the 3 groups of dogs, considering each dog according to single or multiple infections, there was no difference among most multiple infections (Table 2). *Ehrlichia canis* single infection was shown to be more prevalent among shelter dogs (44.9%) than Cartagena pet dogs ( $\chi^2=7.06$ ;  $P=0.0078$ ), but no difference was observed when comparing pet dogs from Barranquilla with either of the other groups. On the other hand, combined *E. canis* and *A. platys* seroprevalence was higher in the shelter group (40.8%) than in Barranquilla (16.7%) or

Cartagena (7%) ( $\chi^2=8.55$ ;  $P=0.0138$ ) (Table 2).

## DISCUSSION

Considering that vectors are of paramount importance for CVBD transmission, it may be inferred that mosquitoes and ticks are prevalent in the environment for all 3 groups of dogs sampled in this study. *Dirofilaria immitis* was detected less frequently among shelter dogs than in pet dogs, even though the shelter dogs all lived outdoors, and many pet dogs from both locations lived mostly indoors. Pet owners were not questioned regarding their use of heartworm preventatives for their dogs; therefore, no conclusions can be drawn regarding the effectiveness of heartworm preventive programs in these areas.

Although data regarding the use of macrocyclic lactones for control of ticks was not included in the survey of these animals, anecdotal reports indicate the misuse of off-label injectable macrocyclic lactones to control tick population is widespread among shelters worldwide. The misuse of these drugs, according to the results of CVBD detection in shelter dogs in Barranquilla, indicates that such treatments administered to these dogs were ineffective for control of ticks in that 93.9% of the shelter dogs were positive for antibodies to *E. canis*. Treatments also did not prevent heartworm infec-

tion, although it did reduce its infection rate compared with findings in pet dogs in both locations. Pet dogs in Cartagena seemed to be the less affected by mosquitoes and ticks than dogs in Barranquilla, although pet dogs in both locations lived in similar conditions.

Since several dogs, particularly those from shelters, were positive for both anti-*E. canis* and anti-*A. platys* antibodies, and *R. sanguineus* is the known vector for *E. canis*, it is highly likely that *R. sanguineus* is the vector for both pathogens in Colombia. A previous study in Santiago, Chile, where *R. sanguineus* is the only tick species found on dogs, identified *A. platys* DNA in the blood of 6 dogs with clinical signs of ehrlichiosis.<sup>8,21</sup> Outdoor dogs were more frequently positive for antibodies against *E. canis* antibodies relative to indoor dogs. Conversely, anti-*A. platys* antibodies were detected in greater frequency among indoor animals, which are likely to be less frequently exposed to ticks. This suggests that there may be cross-reaction among *Ehrlichia* spp and *Anaplasma* spp,<sup>22</sup> or that indoor conditions may somehow facilitate *A. platys* perpetuation in an isolated tick population.

The prevalence of anti-*E. canis* antibodies was significantly higher in males (67.3%) than in females (11.4%), despite the fact that females comprised the majority of shelter animals, which were maintained outdoors at all times. Besides having a higher prevalence of anti-*E. canis* antibodies in males, *D. immitis* antigens also were more prevalent in male dogs, suggesting that male dogs may be less cared for than females. Older dogs were more frequently infected by *D. immitis* than dogs in the younger age groups, suggesting that exposure to mosquitoes over a longer time provides more opportunities for an infected mosquito to transmit the parasite to the dog. However, paradoxically, outdoor lifestyle did not increase the prevalence of *D. immitis* infection in the population of outdoor dogs surveyed, suggesting that a number of outdoor animals may be receiving heartworm preventive treatment. It is

expected that dogs living outdoors with constant exposure to vectors would have had the higher prevalence of *D. immitis*.

The results of the present survey strongly suggest that dogs living in Colombia should be tested for CVBD on a routine basis. The SNAP 4Dx Plus ELISA is a rapid in-clinic test with high sensitivity and specificity for detection of antibodies to *E. canis* and *A. platys* and *D. immitis* antigen as well as antibodies to other pathogenic species that may not be present in Colombia at this time, including *Ehrlichia ewingii*, *Anaplasma phagocytophilum*, and *Borrelia burgdorferi*.<sup>23</sup> Veterinarians should provide owners with information about the risks of exposure of their dogs to ticks and mosquitoes that are vectors for these infections and that off-label use and incorrect dosing of macrocyclic lactones must be avoided in order to control ticks and preserve the long-term efficacy of these products. Veterinarians must also stress to owners that all dogs need and deserve to receive proper preventive health care including a yearlong heartworm preventive treatment.

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