Prevalence and Risk Factors for *Borrelia burgdorferi* Infection in Mexicali, Baja California, a Mexico-US Border City

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ABSTRACT

Lyme borreliosis is a worldwide zoonotic disease caused by the spirochete *Borrelia burgdorferi*, which is transmitted by a tick bite, primarily from *Ixodes scapularis* and *Ixodes pacificus*. It is characterized by polysystemic disorders. In Mexico, native Lyme disease has been recently reported in humans. In dogs, *B burgdorferi* infection has been also reported in several areas of the country. In Monterrey, Nuevo Leon, Mexico, a seroprevalence of 16% was observed in dogs (136/850) and molecular evidence was found in dog synovial fluid. Moreover, a preliminary study performed in 2003 in Mexicali, Baja California, Mexico showed a prevalence of 7.4% (7/94) in dogs infested only by the tick *Rhipicephalus sanguineus*. The aim of this study was to estimate the seroprevalence of *B burgdorferi* in canine patients that were brought into private veterinary clinics in Mexicali. Out of a total of 98 active private clinics in Mexicali, 39 (40%) agreed to participate. Blood samples of 384 dogs were randomly selected from February 2005 to December 2006, and their sera were analyzed with 96% sensitivity and 95% specificity. An adjusted prevalence of 6.8% (95% CI 3.5%-8.9%) was obtained.

The seroprevalence obtained in this study was lower compared to those in Monterrey (16%) where the principal vector was *Ixodes scapularis*, and in Sao Paulo, Brazil (15.6%) where the principal vector was *Amblyomma cajennense*. Risk factors associated with *B burgdorferi* seropositivity were age (*Mantel-Haenszel* $\chi 2$, $P = 0.02$, OR = 2.7 [95% CI 1.2-6.1]), and the absence of a tick and Lyme disease preventive program (*Mantel-Haenszel* $\chi 2$, $P = 0.005$, OR= 4.9, [95% CI 1.4-16.8]). This study confirms the existence of *B burgdorferi* past/present infection in dogs in an area where the only identified tick is *R sanguineus*.

INTRODUCTION

Lyme borreliosis is a worldwide zoonotic disease caused by the spirochete *Borrelia burgdorferi*, which is transmitted by tick bite, primarily *Ixodes scapularis* and *Ixodes pacificus* in North America.[1-3] It is the most frequent tick-borne disease in Europe and the United States in human beings. The disease is characterized by arthritis, lameness, erythema migrans, fatigue, anorexia, general malaise, muscle pain, stiff neck, fever, heart block, kidney failure, and neurological changes such as seizures and aggressive behavior.[1,4-6]

Several animal species can be infected by the *B burgdorferi* spirochete, including rodents, deer, dogs, cats, cows, horses, reptiles, birds, and some other tick species.[5,6] Dogs are considered the most important reservoir for ticks in the home environment.[6,7]

In the city of Monterrey, Mexico, an indirect immunofluorescent assay (IFA) was used to detect antibodies against *B burgdorferi* in dogs with a resulting seroprevalence of 16%.[8] Molecular evidence of *B burgdorferi* was also found by amplification and DNA selected sequences from synovial fluid samples from dogs with arthritis, which suggests the presence of Lyme disease in the area.[9]

In a pilot study in the city of Mexicali, Mexico, seroprevalence to this spirochete was determined using the semiquantitative kit *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc., which resulted in 8.2% (95% CI 1.5%-13.3%) of the 94 dogs that were tested in autumn (September-November), with a sensitivity of 96% and a specificity of 95%.[10]

In Distrito Federal and the northeast of Mexico, a study of 2,346 human sera analyzed with ELISA and confirmed by Western blot found a prevalence of 3.43% and 6.2% respectively.[11] Also, 4 patients who resided in Distrito Federal and were bitten by ticks while visiting forestal parks (3 in Mexico City and 1 in Quintana Roo) were positive for *B burgdorferi* when their skin biopsies were tested by PCR using primers for the fla gene; 1 of the patients was also positive to OspA gene by sequencing.[12]

The aim of this study was to estimate the seroprevalence to *B burgdorferi* and associated risk factors in canine patients at veterinary clinics in the urban area of Mexicali, Baja California, a Mexico-U.S. border desert region.

MATERIALS AND METHODS

Study Design and Population Characteristics

A descriptive study was designed, and 39 veterinary clinics agreed to participate. The data and blood collection started on February 2005 and ended on December 2006. A total of 384 serum samples were randomly taken from canine patients at veterinary clinics in the urban area of Mexicali. This city is situated along the state's northern border with California, and is the northernmost city in Latin America; it is located at 32°40'0"N, 115°28'0"W, with 855,962 inhabitants.[13] Climate is extreme, desert type and the average annual rainfall is 0.63 ± 0.43 cm. Climatic conditions data was collected from the United States National Weather Service of the National Oceanic and Atmospheric Administration (http://www.nws.noaa.gov/).

Data Collection

A questionnaire was designed to collect information of the tested dogs and included: 1) general information of dog: gender (female, male), age (< 1 year, ≥ 1 year), size (small,

medium, large), coat (short, medium, large); 2) dog handling: number of dogs in the house, antiparasitic treatments, dog mobilization between house and street, intensity of tick infestation: none, low (1-10 ticks), moderate (11-30 ticks), intense (> 30 ticks); and 3) living conditions of dogs: type of surface (ground or grass, or concrete), indoor/outdoor status of dog, origin of dog, and history of taking the dog outside the city limits. The outcomes of most of the questions were dichotomous. Questionnaires were administered by trained personnel at the clinics.

Blood Collection

Blood samples were collected by trained personnel. Briefly, 3 mL of blood were collected by punction of the cephalic vein after proper antisepsis of the area with isopropyl alcohol, and placed in Vacutainer® tubes. Each sample was properly labeled and centrifuged at 3,500 RPM for 10 minutes to separate the serum. The serum was transferred into 1-mL vials and were labeled and stored at -20°C until testing.

Serology

The *Borrelia burgdorferi* IgG Antibody ELISA Kit® Helica Biosystems, Inc is intended for the qualitative detection of canine IgG class serum antibody to *B burgdorferi* sensu lato that is allowed to react with a blend of *B burgdorferi* sensu lato antigens coated on specially treated micro-wells, guaranteeing a 95.8% sensitivity and a 94.7% specificity. The optical density (OD) at 450 nm was recorded, where an OD < 0.3 was considered negative and OD ≥ 0.3 as positive, according to the manufacturer.

Statistical Analysis

Seroprevalence values were calculated by dividing the number of positive sera obtained by the total number of samples analyzed (24/384). The adjusted prevalence and its 95% CI (confidence interval) were obtained using the Rogan-Gladen estimator.[14]

A *Mantel-Haenszel* $\chi 2$ test and odds ratio (OR) were used to determine differences in seroprevalence to *B burgdorferi* by groups and the association of risk factors. Statistical significance was considered at a *P*-value of < 0.05. Exact binomial confidence intervals were calculated individually for each proportion.[15]

All statistical analysis were performed using the Statistical Analysis System for Windows version 9.[1.3.16]

RESULTS AND DISCUSSION

The results of this study indicated an adjusted seroprevalence to *B burgdorferi* of 6.8% (95% CI 3.5%-8.9%) in canine patients from veterinary clinics of Mexicali. Since there is no vaccination against Lyme disease included in current immunization programs for dogs in Mexicali, the results of this research will not be affected by the presence of post-vaccination antibodies.

Furthermore, the prevalence in this study was lower than that found in Monterrey, Mexico, with a prevalence value of 16% (136/850) in dogs tested by an indirect IFA.[8] The lower seroprevalence to *B burgdorferi* found in this study may be due to the fact that the known vectors for this spirochete, *I scapularis*, *I pacificus*, *Dermacentor variabilis*, and *Amblyomma americanum* in North America,[17-19] have not been found in this region. The only species of tick found in Mexicali has been *R sanguineus* (in press), which has not been considered a vector of borreliosis in other regions of the world. Meanwhile, in Sao Paulo, Brasil, the prevalence in dogs was 15.6% (31/199) and the main tick vector was *Amblyomma cajennense*.[20]

Risk factors found to be associated with *B burgdorferi* infection were age (*Mantel-Haenszel* $\chi 2$, $P = 0.02$) and the absence of a tick and Lyme disease preventive plan (*Mantel-Haenszel* $\chi 2$, $P = 0.005$). The study showed that the dogs that were more at risk were those of ≥ 1 year of age with an OR = 2.7 (95% CI 1.2-6.1), as well as the ones who did not have access to a tick control program, which consisted per year of at least 2 antiparasitic treatments (endo and ectoparasites), 2 tick treatment baths, and 2 home fumigations, with OR = 4.9 (95% CI 1.4-16.8).

The higher prevalence in dogs 0 ≥ 1 year of age, similar to other studies,[7,21,22] is justified by the nature of borreliosis, which is a chronic disease that may take several months to show its most notorious sign in dogs, lameness in one or more limbs.[1]

Gender in tested dogs showed no relation to seropositivity to *B burgdorferi*, similar to other studies,[7,21,23] indicating that apparently sexual pheromones specific to each sex do not contribute to vector attraction, as occurs in other arthropod animals.

Size and coat did not show an association to borreliosis either, compared with another study from Spain[21] where coat turned out to be important but size was not relevant; however, it was believed that the larger the body and the longer the hair, the more ticks the animal would get, since there would be broader body areas to be infested, and the longer hair would make it harder for the dog to shed the ticks. There is no evidence of any association since a bite of the vector is enough to start the Lyme infection.[24]

Although it was initially thought that the prevalence to borreliosis may be associated with the number of dogs in a house, the lack of concrete flooring throughout the residence, and to the transit of dogs into and out of the house, as it happened in the study conducted in Wisconsin and Illinois,[23] or tick infestation level,[7,21] this study did not show any statistical association with those factors.

The lack of a tick control system also was found to be associated to seropositivity to *B burgdorferi*, as it resulted in other studies,[21,23] meaning that seroprevalence could be reduced just by following a preventive plan, as previously mentioned, since it would interrupt the biological cycle of the vector.

Although the results of this study may suggest a relatively low prevalence for *B burgdorferi* infection in dogs, borreliosis is a zoonotic disease and it can be reduced following a minimum of preventive measures, like those analyzed in this study. Furthermore, it is necessary to determine the vector involved in the transmission of borreliosis in this area, considering that the only tick that has been observed in Mexicali dogs is *R sanguineus*. There were no statistical differences in the seroprevalence for dogs that have been kept within the city limits all of their lives and dogs that were either born in other geographic areas or taken outside the city limits where they might have been exposed to *Ixodes* ticks. It will be important to determine borreliosis prevalence in humans, specifically in places like Mexicali, where exposure to this agent is being demonstrated in the dog population and dogs may act as sentinels for *B burgdorferi* infection in humans.[22,23,25]

This study confirms the existence of *B burgdorferi* in dogs in an area where *R sanguineus* is the only tick that has been identified so far. Although this tick has not been considered an important vector for *B burgdorferi*, it can be brought into the homes and feed on humans,[26,27] considering that it only takes one bite from the vector to spread borreliosis.[24] Molecular evidence of *B burgdorferi* in dogs and ticks is currently being searched for, as well as the transmission mechanism.

CONCLUSIONS

A seroprevalence value of 6.8% (95% CI 3.5%-8.9%) to *B burgdorferi* was observed in dogs in Mexicali, B.C., an area where the only species of tick that has been observed in dogs is *R sanguineus*.

The evaluated risk factors were found to be related both to age in the dogs that were sampled (*Mantel-Haenszel* $\chi 2$, $P = 0.02$) and the absence of a tick and Lyme disease preventive plan (*Mantel-Haenszel* $\chi 2$, $P = 0.005$). The study showed that the dogs that were more at risk were those ≥ 1 year of age with an OR = 2.7 (95% CI 1.2-6.1), as well as the ones who did not have access to a tick control program.

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