MEDWELL PUBLISHING
Journal of Animal and Veterinary Advances 8 (2): 251-254, 2009
ISSN: 1680-5593

Prevalence and Risk Factors for *Borrelia burgdorferi* Infection in Dogs of Animal Control Centers from Mexicali, Baja California: A Mexico-US Border City

[1,2]Luis Tinoco-Gracia, [2]Héctor Quiroz-Romero, [2]María Teresa Quintero-Martínez,
[1]Tomás Benjamín Rentería-Evangelista, [1]Alberto Barreras-Serrano, [1]Sawako Hori-Oshima,
[1]Gilberto López-Valencia, [1]Alma Rossana Tamayo-Sosa, [1]Gerardo Medina-Basulto,
[1]Paulina Haro-Alvarez, [3]Manuel Moro and [4]Javier Vinasco
[1]Instituto de Investigaciones en Ciencias Veterinarias,
Universidad Autónoma de Baja California, México
[2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México
[3]Division of Comparative Medicine, National Center for Research Resources,
National Institutes of Health, USA
[4]College of Veterinary Medicine, Kansas State University, USA

Abstract: Lyme borreliosis, a worldwide zoonotic disease and characterized by polisystemic disorders, is caused by the spirochete *B. burgdorferi*, which is transmitted by a tick bite, primarily from *Ixodes scapularis* and *I. pacificus*. In Mexico, native Lyme disease has been recently reported in humans, while canine *B. burgdorferi* infection has been also reported in several areas of the country. In Monterrey, Mexico, a seroprevalence of 16% was observed in dogs (160/850) and molecular evidence was found in canine synovial fluid. Moreover, a preliminar study performed in 2003 in Mexicali, 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 dogs captured by personnel from the animal control centers in the city of Mexicali. Blood samples from 384 dogs were randomly selected from February 2005 to December 2006 and their sera were analized by the semiquantitative kit *Borrelia burgdorferi* ELISA® Helica Biosystems, Inc., with 96% sensitivity and 95% specificity. An adjusted prevalence of 12% (95% IC 7.5-14.3%) was obtained using the Rogan-Gladen estimator. 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 main vector was *Amblyomma cajennense*. No risk factors were associated with *B. burgdorferi* seropositivity. This study confirms the existence of *B. burgdorferi* past/present infection in dogs in an area where the only identified tick is *R. sanguineus*.

Key words: *Borrelia burgdorferi*, tick, lyme disease, borreliosis

INTRODUCTION

Lyme borreliosis is a worldwide zoonotic disease caused by the spirochete *Borrelia burgdorferi*, which is transmitted by tick bite, primarily *Ixodes scapularis* and *I. pacificus* in North America (Barbour and Hayes, 1986; Magnarelli *et al*., 1987; Greene *et al*., 2000). In Europe and the United States, it is the most frequent tick-borne disease in human beings. Among its sypmtoms: 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 (Burgess, 1986; Magnarelli *et al*., 1987; Faul *et al*., 1999; Straubinger, 2000).

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 (Faul *et al*., 1999; Straubinger, 2000). Dogs are considered the most important reservoir for ticks in the home environment (Straubinger, 2000; De Lacerda *et al*., 2004).

An indirect Immunofluorescent Assay (IFA) was used to detect antibodies against *B. burgdorferi* in dogs in Monterrey, Mexico, with a resulting seroprevalence of 16% (Salinas-Melendez *et al*., 1999). Also, molecular evidence of *B. burgdorferi* was found by amplification and DNA selected sequences from canine synovial fluid samples from dogs with arthritis, which suggests the presence of Lyme disease in the area (Salinas-Melendez *et al*., 1995).

In a pilot study in the city of Mexicali, Mexico, seroprevalence to this spirochete was determined using

Corresponding Author: Luis Tinoco-Gracia, Av. Michoacán 2101, Fracc. Orizaba, Mexicali, B.C. México, CP 21130

B. burgdorferi ELISA® Helica Biosystems, Inc., which resulted in 8.2% (95% CI 1.5-13.3%) out of the 94 dogs that were tested in autumn (September-November) with a sensitivity of 96% and a specifity of 95% (Tinoco-Gracia *et al*., 2007).

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 (Gordillo-Perez *et al*., 2003). Also, 4 patients who reside in Distrito Federal and were bitten by ticks while visiting forestal parks (3 in Mexico City and 1 in Quintana Roo) were positive to *B. burgdorferi* when their skin biopsies were tested by PCR using primers for the *fla* gene; one of the patients was also positive to OspA gene by sequencing. (Gordillo-Perez *et al*., 2007).

The purpose of this study was to estimate the seroprevalence to *B. burgdorferi* and associated risk factors in canines captured by personnel from both animal control centers in Mexicali, Mexico, a Mexico-U.S. border desert region.

MATERIALS AND METHODS

Study design and characteristics of the population: A descriptive study was designed in both animal control centers in Mexicali, Mexico, Centro Antirrábico Veterinario de Mexicali and Centro Municipal de Control Animal de Mexicali. The data and blood collection started on February 2005 and ended on December 2006. A total of 384 serum samples were randomly taken. Mexicali 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. Its 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.

Data collection: A questionnaire was designed to collect information of the tested dogs and it included: general information of dog, such as gender (female, male) age (<1 year, = 1 year) size (small, medium, large) and coat (short, medium, large) and intensity of tick infestation: none, low (1-10 ticks) moderate (11-30 ticks) intense (>30 ticks). The outcomes of most of the questions were dichotomous.

Blood collection: Blood samples were collected by trained personnel. Three 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 3500 RPM for 10 min to separate the serum. The serum was transferred into 1 mL vials, labeled and stored at -20°C until testing.

Serology: Antibodies against *B. burgdorferi* were measured with the semiquantitative kit *B. burgdorferi* ELISA® Helica Biosystems, Inc. for the detection of canine IgG class which guarantees a sensitivity of 95.8% and a specificity of 94.7%. 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 (Greiner and Gardner, 2000).

A Mantel-Haenszel χ^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 $p<0.05$. Exact binomial confidence intervals were calculated individually for each proportion (Walker, 1997).

All statistical analysis were performed using the Statistical Analysis System for Windows, version 9.1.3 (SAS, 2004).

RESULTS AND DISCUSSION

The results of this study indicated an adjusted seroprevalence to *B. burgdorferi* of 12% (95% CI 7.5-14.3%) in dogs from both animal control centers in Mexicali, Mexico (Greiner and Gardner, 2000) and it was slightly higher than the one obtained from a pilot study carried out in the same city, 8.2% (95% CI 1.5-13.3%) (Tinoco-Gracia *et al*., 2007). Also, the prevalence in this study was lower to that found in Monterrey, Mexico, with a prevalence value of 16% (160/850) in canines tested by an indirect immunofluorescent assay (Salinas-Melendez *et al*., 1999). 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 (Magnarelli and Anderson, 1988; Lane, 1996; Adelson *et al*., 2004) have not been found in this region. The only specie 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 of 15.6% (31/199) and the main tick vector was *Amblyomma cajennense* (O'Dwyer *et al*., 2004).

None of the risk factors considered for this study-age, gender, size and coat of the canines in the animal control centers-seemed to be related to *B. burgdorferi* seropositivity. This could mean that these dogs have the same chances to get borreliosis, despite the reasons they were considered for this study: Age, since borreliosis is a chronic disease; gender, in case there could be some affinity of the infected ticks to sexual hormones in the host; size, since the larger the host, the larger the area available for the vectors, even though it has been demonstrated that it only takes one bite from an infected tick for the host to get infected with Lyme disease and coat, since the seroprevalence could be closely related to the length of the hair of the host, because long hair would protect the infected ticks. An important finding was that the only tick reported so far in Mexicali was *R. sanguineus* (in press) which is not considered as vector for borreliosis. It is also necessary to determine the vector involved in the transmission of *B. burgdorferi* in this area. Also, since borreliosis is a zoonotic disease, it is imperative to know the prevalence in humans, particularly in places like Mexicali, where serological evidence has been obtained.

CONCLUSION

A seroprevalence value of 12% (95% CI 7.5-14.3%) to *Borrelia burgdorferi* was observed in dogs captured by personnel from the animal control centers in Mexicali, Mexico, a region where the only tick that has been taxonomically identified in canines is *Rhipicephalus sanguineus*. And none of the factors considered for this study showed any association to seropositivity to *B. burgdorferi*.

ACKNOWLEDGEMENT

The research was supported by the Instituto de Investigaciones en Ciencias Veterinarias, Universidad Autónoma de Baja California, in México and the Colegio de Médicos Veterinarios en Pequeñas Especies de Mexicali, A. C. (COMVEPE MEXICALI, A. C.).

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