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Serological Survey of Canine Borreliosis

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ABSTRACT. Lyme disease or Borreliosis, a tick-borne disease caused by *Borrelia burgdorferi*, has been described recently in dogs. A total of 850 blood samples were obtained from dogs in the metropolitan area of Monterrey, Mexico. An indirect immunofluorescent assay (IFA) was used to detect antibodies against *Borrelia burgdorferi*, the etiologic agent of Lyme disease in human beings. The 16% (136) of these dogs had positive results. These findings suggest that exposition to this microorganism is common in dogs in this area and that this disease is of importance to veterinarians.
Key words: Canine Borreliosis, Serology

RESUMEN. La enfermedad de Lyme o Borreliosis, la cual ha sido descrita recientemente en perros, es una enfermedad trasmitida por garrapatas y es causada por *Borrelia burgdorferi*. Un total de 850 muestras de sangre fueron obtenidas a partir de perros del área metropolitana de Monterrey, México, para un ensayo de detección de anticuerpos utilizando una prueba de inmunofluorescencia indirecta (IFA) contra *Borrelia burgdorferi*, el agente etiológico de la enfermedad de Lyme en seres humanos. Los análisis se realizaron durante Febrero a Mayo de 1995; el 64% de las muestras analizadas se obtuvieron de clínicas veterinarias y el 36% de centros de salud. El 16% (136) de los perros obtuvo resultados positivos, y entre éstos los mayores títulos de anticuerpos variaron entre 1:64 y 1:1024. Estos hallazgos sugieren que la exposición a este microorganismo es común en perros el área, y que asimismo la enfermedad es de importancia para los veterinarios. La relativamente baja prevalencia de *B. burgdorferi* encontrada en el presente estudio es similar a la reportada en los estados sureños de los E.U.A. y difiere de la alta prevalencia encontrada en los estados norteños de ese país. Este trabajo constituye, hasta donde sabemos, el primer estudio epidemiológico sobre *B. burgdorferi* en México.
Palabras clave: Borreliosis canina, Serología

INTRODUCTION

Lyme disease, caused by the spirochete *Borrelia burgdorferi* is the most frequent tick-borne disease in Europe and the United States.[3,13] Borreliosis was first recognized in human beings in Lyme, Connecticut, United States and was reported for the first time in 1977.[24] Twenty years after its discovery, this disease has emerged as a multi-complex disease of significance in the world, and has been reported in England, other countries of Europe, China, Australia and Mexico.[14,23]

Dogs are frequently exposed to tick bites and develop antibodies to *B. burgdorferi*.[7] In the U.S.A., there have been canine clinical reports from New York, Connecticut and recently from Wisconsin and Texas.[10] Diagnosis of Borreliosis is not straightforward, primarily because of several characteristics of the *B. burgdorferi* infection, and delayed treatment may lead to serious sequelae.[7] However, prior to any prevention strategy, areas presenting human risk must be accurately identified.[10] Diagnosis of Lyme disease relies mainly on clinical and serological criteria. Antibodies against this microorganism are demonstrable by indirect immunofluorescence (IFA), ELISA, and western blotting.[12,19] IFA staining methods for detecting antibody to this *Borrelia* in dogs are highly sensitive and their use does not result in cross-reactivity with *Leptospira interrogans*.[5]

The specificity of IFA is greater than 90%.[25] Isolation of *B. burgdorferi* from patient samples is difficult, and detection of the microorganism is not very sensitive.[2] Therefore, serology is the most widely used diagnostic procedure for this disease.[6,18]

The purpose of this study was to measure the prevalence of canine exposure to this spirochete within the city of Monterrey. The study was conducted at the Immunology Diagnostic Laboratory of the Veterinary College, Nuevo Leon

State University (U.A.N.L.), between February and May of 1995.

MATERIAL AND METHODS

From February to May 1995, 850 dogs were located at diverse Health Centers (Subsecretaría de Salud) and veterinary clinics of Monterrey, N. L., México. Sex distribution was 53% males and 47% females. Age data was not available.

Blood samples were taken from the radial vein of 850 dogs using Vacutainer tubes. All blood samples were collected in 4 ml vacuum tubes containing separator gel and were processed within 4 h of collection. Blood were centrifuged at 3,000 rpm for 10 min and the serum collected and stored at –20°C for serology. Serum was inactivated at 60° C for 30 min. Procedures for the detection of antibodies to *B. burgdorferi* through the IFA test have been previously reported.[18] The culture[b] was supplied for Texas Veterinary Medical Diagnostic Laboratory, Texas A&M University (Texas A&M University System, College Station, Texas 77843-4461). The microorganism was grown in fortified Kelley medium for at least one-week.[10] The culture was centrifuged at 9,000 x g for 20 min and washed twice in phosphate-buffered saline solution, pH 7.2. The spirochetes were suspended in phosphate buffered saline and diluted so that 10 µl of suspension contained approximately 50 organisms. One drop of antigen was applied to each glass slide, which was air dried and fixed in acetone at –20°C for 10 min. To minimize non-specific staining reactions, serum samples were diluted 1:64 in 1% yolk sac in PBS, pH 7.2.[5] The indirect immunofluorescent (IFA) test antibody used fluorescein isothiocyanate (FITC) labeled rabbit antidog IgG immunoglobulin (Antibody Incorporated California, Woodland, Calif.) in a dilution 1:50.[17]

On the basis of IFA test results for 850 dogs from areas where Lyme disease has been not reported, reaction 1:64 were considered positive for prior *B. burgdorferi* infection.[17] Known IFA positive and negative control serums were tested simultaneously with the sample dog serum.

RESULTS

During the period February to May 1995, 850 serum samples obtained from dogs of the metropolitan area of Monterrey were tested for the presence of antibodies *to B. burgdorferi*. The total population was sampled once. Males and females were approximately equally represented (53% *VS* 47%, respectively). The breed distribution of the dogs studied were 66% mixed breeds and 34% working dogs. Of the 850 dogs tested, 136 (16%) were seropositive for the microorganism by results of IFA (Table 1). For the 136 seropositive dogs, the highest antibody titer in serum specimen ranged from 1:64 to 1:1024. Of these 136 dogs, 87 (64%) were from veterinary practices, the remaining 49 (36%) dogs were from diverse Health Centers.

IFA test (%)		Sex (%)	
Positive	Negative	Male	Female
16	84	53	47

Table 1. Prevalence of infection and sex distribution in dogs from Monterrey, Mexico tested for Borrelia burgdorferi antibodies.

DISCUSSION

Previous epidemiological studies have shown low prevalence of antibodies against *B. burgdorferi* (1.7% in Alabama, 2.7% in North Carolina, 5.5% in Texas & 4.3% in Maine), as well as high prevalence (26.2% in Connecticut, 57.8% in New York, 53% in Wisconsin) in dogs from the United States.[4,5,8,10,16,17,22]

In the present study, the relatively low presence of *B. burgdorferi* (16%) is similar to the southern states of United States of America previously mentioned (Alabama, North Carolina and Texas), which are geographically closer to Mexico, and differs from the high prevalence found in the northern states (Connecticut, New York and Wisconsin).

Case studies have also shown the existence of canine Lyme borreliosis in Israel,[11] Japan,[1] Croatia,[9] Spain,[8] the Netherlands[21] and Belgium.[20] In northern Mexico, a case study showing the existence of *B. burgdorferi* in a dog by the polymerase chain reaction (PCR) method suggested the existence of the disease in this country.[23] The present study gives further evidence of the presence in dogs of Lyme disease in the northern Mexico. However, to our knowledge no previous epidemiological studies of *B. burgdorferi* in Mexico exist.

The seroprevalence of antibodies to *B. burgdorferi* can be related to human cases of Lyme disease.[15] The PCR study realized in Mexico [23] also detected *B. burgdorferi* in humans with acrodermatitis, therefore, the present study indicates that Lyme disease can be a human health problem in northern Mexico and epidemiological studies should be conducted in other areas and animal species of the country to asses this risk.

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