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Antibody detection against *Borrelia burgdorferi* in horses located in the suburban areas of Monterrey, Nuevo León

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ABSTRACT. The aim of the present study was to determine the presence of *Borrelia burgdorferi* antibodies in horses from the metropolitan area of Monterrey, Nuevo León, México. Blood serum was obtained from a total of 100 horses residing at different counties in the area. From each animal data was obtained on age, sex, county of residence, presence of ectoparasites and clinical signs. All sera samples were analyzed by indirect immunofluoresence and the sera that resulted positive to this test was analyzed by Western blot. The serological test yielded 34 positive sera at 1:64 dilution, and from them 6 were positive at 1:128 dilution, 3 at 1:256, and only one at 1:512. Confirmation of the infection by Western blot was obtained only in the sample positive at the 1:512 dilution. These results shown a low frequency of seropositivity to *B. burgdorferi* of the horses in the area, confirming previous studies indicating that in northeast Mexico Lyme disease is present in different animal species.

Key words: *Borrelia burgdorferi*, horses antibodies, Lyme disease.

RESUMEN. El objetivo del presente estudio fue determinar la presencia de anticuerpos contra *Borrelia burgdorferi* en caballos del área metropolitana de Monterrey, Nuevo León, México. Se obtuvo suero sanguíneo de un total de 100 caballos residentes en diversos municipios del área. De cada animal se obtuvieron datos de edad, sexo, municipio de residencia, presencia de ectoparásitos y signos clínicos. Todos los sueros fueron analizados por inmunofluorescencia indirecta y los sueros que resultaron positivos a esta prueba fueron analizados mediante Western blot. La prueba serológica dio 34 sueros positivos a la dilución 1:64, y de éstos 6 fueron positivos a la dilución 1:128, 3 a 1:256 y sólo uno a 1:512. La confirmación de la infección mediante Western blot sólo se obtuvo en la muestra positiva a la dilución 1:512. Estos resultados muestran una baja frecuencia de seropositividad a *B. burgdorferi* en los caballos del área, confirmando estudios previos que indican que en el noreste de México la enfermedad de Lyme existe en diferentes especies animales.

Palabras clave: *Borrelia burgdorferi*, caballos, anticuerpos, enfermedad de Lyme.

INTRODUCTION

In 1975, Lyme disease or Lyme borreliosis was first recognized as a epidemic of arthritis in humans of the town of Lyme in Connecticut, United States of America.[22] Lyme disease can develop into a complex multisystem disorder starting with flu-like signs and some times progressing into arthritis, neurologic symptoms, cardiac disorders, or chronic dermatitis of the extremities between 2 or 3 months after the skin lesion caused by the tick-bite. This borreliosis, according to the Center of Disease Control, is now the most common vector-borne infection in humans in the USA.[18] Actually Lyme borreliosis has been reported in more than 40 states of the United States of America, where the 80% of humans cases were in New York, Connecticut, Pennsylvania and Massachusetts.[18]

Borrelia burgdorferi, a Gram-negative, helix-shaped bacilli, is the etiologic agent of Lyme disease and can be transmitted by *Ixodes* ticks.[3]

In horses the clinical cases of Lyme borreliosis have been recently recognized in endemic areas, where the serological surveys have showed a 12-20% positive titers in asyntomatic horses.[14] Some researchers in 1985 reported 24% of positives of 50 randomly selected horses in Lyme disease endemic areas.[16] In 1988 were found 75% seropositive samples from clinically normal horses on IFA by researchers in Tuffs University.[10]

Diagnosis of Lyme borreliosis relies mainly on clinical and serological criteria. Because of the variety of clinical symptoms of this disease, it is often misdiagnosed. The clinical feature of Lyme disease in equines can be varied. In a study of 40 horses were found positive titers to *Borrelia burgdorferi* in 8 animals and only three with clinical signs.[14,19] Clinical disease in horses has been associated with arthritis, uveitis, neurologic signs and foal mortality.[6,7] Clinical signs of *B. burgdorferi* infection of horses are not completely known and diagnosis is frequently based on results of serologic test.[8,11]

Antibodies against the microorganism are demonstrable by indirect immunofluorescence (IFA), ELISA, and Western blotting.[9,15] The IFA has been used to identify *B. burgdorferi* positive equines in several studies.[7,10,14] The IFA has been popular because several different species

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can be tested with the same set up using a different labeled antibody.[18]

Although Lyme disease has been found in humans, dogs and deers from Nuevo Leon State in Mexico,[17,20,21] we think that the disease cab be developed in other animal species because the infection with *B. burgdorferi* has been recognized in wild and domestic animals around the world, including horses.[1,2,4,5,12,13] The purpose of the study reported here was to determinate the seroprevalence of antibodies to *B. burgdorferi*, etiologic agent of Lyme disease, in horses in the suburban areas of Monterrey, Mexico.

MATERIAL AND METHODS

Study population and serum samples. Samples of serum were obtained from horses located in the suburban areas of Monterrey, N. L., Mexico, between March and September 1997. Blood samples were taken from the jugular vein of 100 horses using 4 ml Vacutainer® tubes. A total of 100 blood samples were taken. All samples were sent to the Immunology Diagnostic Laboratory of the Veterinary College, UANL to make the analysis. Records were kept on the date of sampling, the place of origin and when possible, clinical signs.

Blood were centrifuged and stored for serology as previously described.[21] Complete procedures for the detection of antibodies to *B. burgdorferi* through the IFA test have been previously described.[21] Serum from the horses were tested by the IFA test for IgG antibodies to *B. burgdorferi*.

The indirect immunofluorescent antibody (IFA) test labeled antibody used fluorescein isothiocyanate (FITC) labeled goat antihorse IgG immunoglobulin (VMRD, Inc. Pullman, WA) in a dilution 1:50.[13] On the basis of IFA test results, 100 horses from areas where Lyme disease has been not reported, a reaction 1:32 were considered positive for *B. burgdorferi* infections.[4] Positive and negative control serums were tested simultaneously with all samples horse serums.

The IFA for detection of antibodies in horses sera was performed as previously described, using goat anti-horse ready to use (VMRD, Inc. Pullman, WA, USA.), according to the method described by the manufacturer. Positive, negative and saline controls were included on each slide. Titers were expressed as the reciprocal of the highest serum dilution showing distinct fluorescence of at least 50% of the spirochetes in each fold. As has been used in other studies, a reciprocal titer less than 1:64 was considered negative.

Distinct fluorescence of spirochetes in a dilutions major o equal a 1:64 was considered evidence of prior *B. burgdorferi* infections.[15] All sera were aseptically collected, coded and sent to the laboratory, where they were heat inactivated at 56ºC for 30 min and evaluated by an IFA test. The assay were performed according to the method described previously[15] using goat-produced fluorescein isothiocyanate conjugated anti-horse IgG.

For the Western blot test, a bacterial protein suspension was prepared and a *Borrelia* protein profile was made by its electrophoretic separation and transfer to nitrocellulose membranes (Trans-blot Electroforetic Transfer Cell, Bio-Rad), according to standard laboratory procedures. Horse sera diluted at 1:50 were added to the membranes, followed by the addition of peroxidase-labelled horse anti-IgG (diluted 1:100).

Animals. The horses included a wide variety of breeds and ages. Animals were male and female. All blood samples were collected in 1997 from 100 horses living in the metropolitan area of Monterrey, N.L. (counties of Apodaca, Escobedo, Guadalupe, Monterrey, San Nicolas de los Garza and Santiago), where cases of Borreliosis has been reported.[21]

RESULTS

Indirect immunofluorescent antibody (IFA) test. All the 100 horse serum samples were initially diluted to a 1:64 concentration. At this dilution, 34 samples were positive. At increased dilutions the number of positive samples decreased, having 6 positive samples at 1:128, 3 at 1:256, 1 at the 1:512 and at the 1:1024 dilutions.

Of the 34 animals that had a positive result in the immunofluorescence test at a dilution of 1:64, 14 were from Cd. Guadalupe, 7 from Monterrey, 6 from San Nicolás de los Garza and the rest from Apodaca, Escobedo, and Santiago. Although most positive animals were from Guadalupe, no one of these were positive at dilutions higher than 1:128. However, in Monterrey one animal reacted positively at a dilution of 1:512 (Table 1).

Regarding the animals health at the moment of sampling, of the 34 positive animals at a dilution of 1:64, 22 did not show any clinical signs, 3 showed claudication, 8 mycosis and 1 uveitis. At higher dilutions only one animal showed claudication, whereas 3 had mycosis at 1:128 dilution but only one at 1:256 dilution. No animals showed papilloma at any dilution and only one had uveitis at the 1:64 dilution (Table 2).

According to the age, at the dilution of 1:64 positive animals were found at all the age ranges. Two animals were positive at 1-5 years of age and 4 at 10 years or more. At the dilution of 1:128, 2 animals were positive at 1-5 years of age and 4 at 10 years or more. At higher dilutions only 3 animals of 10 or more years of age were positive (Table 3).

All IFA-positive animals were screened with Western blot and only one reacted against *B. burgdorferi* proteins,

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showing a positive reaction for the proteins of 60, 41, 34 and 20 kilodaltons (Fig. 1). This animal was also the only one with positive IFA results at the 1:512 dilution.

DISCUSSION

Previous studies on Lyme disease have shown the ability of *B.burgdorferi* to infect not only humans but also many wild and domesticated animals.[3,10] Among the later, *B. burgdorferi* in horses is of particular importance because people have direct physical contact with them, as well as because horses can be infested with the vectors carrying the bacteria. Lyme disease in horses have been reported in several countries, with seropositivity frequencies ranging from very low to 68%.[16,18] In the present study, according to the Western blot analysis only one animal had previous contact with *Borrelia.* This finding allows us to conclude that the IFA test can give many false positives, especially at low dilutions. Therefore, in order to use the serological diagnosis of *Borrelia* in horses, the serum samples must be diluted at least to 1:512, or confirm the suspected animals by means of Western blot. This results are in agreement with the findings of Lindenmayer et al.[10,18]

The serological frequency of *Borrelia* found in this study is low, and is similar to the results obtained in deer at Northeast México,[17] as well as in horses from Texas, U.S.A.[8] Other studies realized in the area have shown the presence of *Borrelia* also in humans and dogs;[20,21] therefore, we consider the area to be consider of importance for human health and suggest to continue future research in order to determine the possible development of Lyme disease in the area.

Arthritis is the clinical sign commonly associated with Lyme in domesticated animals.[13] In the present study the only animal positive to the Western blot analysis had claudication. However, no statistical association ($p < 0.01$) was found among clinical signs and seropositivity. Therefore, the serological results must be interpreted with caution, since seropositivity may reflect exposure to the agent but not necessarily the development of clinical signs. This means that horses from endemic zones (as the one studied here) showing clinical signs suggestive of Lyme, other causes must be ruled out before attaining a Lyme diagnosis.[18]

Table 1. Relationships between county of residence and IFA test results.

County of Residence	Dilutions 1:64	1:128	1:256	1:512	1:1024
Apodaca	3	0	0	0	0
Escobedo	2	0	0	0	0
Guadalupe	14	2	0	0	0
Monterrey	7	3	2	1	1
San Nicolás	6	1	1	0	0
Santiago	2	0	0	0	0
Total	34	6	3	1	1

Table 2. Relationships between clinical signs and IFA test results.

Clinical signs	Number of animal	Dilution 1:64	1:128	1:256	1:512	1:1024
Normal	64	22	2	1	0	0
Claudication	8	3	1	1	1	1
Papilloma	5	0	0	0	0	0
Mycosis	20	8	3	1	0	0
Uveitis	3	1	0	0	0	0
Total	100	34	6	3	1	1

Table 3. Relationships between age and IFA test results.

Age (years)	Dilution 1:64	1:128	1:256	1:512	1:1024
0-1	1	0	0	0	0
1-5	7	2	0	0	0
5-10	15	0	0	0	0
10 or more	10	4	3	1	1
Total	34	6	3	1	1

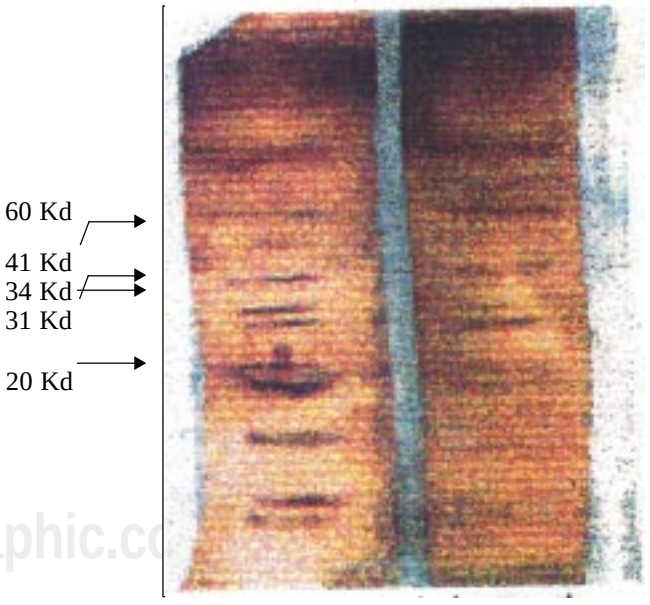

Figure 1. *Observed reaction against* Borrelia *proteins by Western blot with serum of one animal. The molecular weights in kilodaltons (Kd) are shown. A: serum of animal positive to IFA test at 1: 512 dilution. B: positive control.*

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Since the presence of vectors capable of transmitting the bacteria is an important factor for the maintenance of *Borrelia* in nature,[18] another possible cause of the low frequency of *Borrelia* seropositivity found in the present study is the low infestation with ticks in the horses from the studied area (data not reported).

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