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Serological Activity of White-Tail Deer against Several Species of *Brucella*

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ABSTRACT. In Mexico, brucellosis is a widely distributed disease of domesticated ruminants, but its frequency in wild ruminants has not been documented. Since northeast Mexico is the main distribution area of white-tailed deer and has been reported as an area positive for brucellosis in domesticated species, the present study was conducted in order to determine serological activity against several species of the genus *Brucella* in white-tailed deer. A total of 208 sera of white-tailed deer were collected during the springs of 1994 and 1995 in the north part of the states of Nuevo León and Coahuila. Each serum was analyzed for the detection of antibodies against two smooth (*B. abortus* and *B. melitensis*) and one rough (*B. ovis*) species of the genus *Brucella*. The serological tests used for the determination of the presence of antibodies against *Brucella* were card and plate agglutination for *B. abortus*, plate agglutination and rivanol precipitation for *B. melitensis*, and agar gel immunodiffusion for *B. ovis*. Each assay had positive and negative controls. None of the analyzed samples was found to be positive, and only two sera showed partial plate agglutination against *B. melitensis* at a dilution of 1:25; however, at higher dilutions and to the rivanol precipitation test the same samples were negative. Therefore, the percentage of positive sera was estimated at 0% (0/208). This result makes evident the absence of positive white-tailed deer against *Brucella* in the sampled area, despite that this disease is considered present in domesticated species. Therefore, white-tailed deer does not have, at the present time, an important role for the dispersion of the

RESUMEN. En México, la brucelosis es una enfermedad ampliamente distribuida de rumiantes domésticos, pero su frecuencia en rumiantes silvestres no ha sido bien documentada. Puesto que el noreste de México es la principal área de distribución del venado cola blanca y ha sido reportada como un área seropositiva para brucelosis en especies domésticas, se realizó el presente estudio para determinar actividad serológica contra varias especies del género *Brucella* en venado cola blanca. Un total de 208 sueros de venado cola blanca fueron colectados durante las primaveras de 1994 y 1995 en la parte norte de los estados de Nuevo León y Coahuila. Cada suero fue analizado para la detección de anticuerpos contra dos cepas lisas (*B. abortus* y *B. melitensis*) y una rugosa *(B. ovis)* del género *Brucella*. Las pruebas serológicas usadas para la determinación de la presencia de anticuerpos contra *Brucella* fueron aglutinación en tarjeta y en placa para *B. abortus*, aglutinación en placa y precipitación de rivanol para *B. melitensis* e inmunodifusión en gel de agar para *B. ovis*. Cada prueba tuvo controles positivos y negativos. Ninguna de las muestras analizadas se mostró positiva, y solo dos sueros mostraron aglutinación en placa parcial contra *B. melitensis* a una dilución de 1:25; sin embargo, a concentraciones mayores y en la prueba de precipitación de rivanol las mismas muestras resultaron negativas. Por lo tanto, el porcentaje de sueros positivos fue estimado en 0% (0/208). Este resultado hace evidente la ausencia de venados cola blanca positivos contra *Brucella* en el área muestreada, a pesar de que esta enfermedad se considera que está presente en las especies domésticas. Por lo tanto, el venado cola blanca no juega por el momento un papel importante en a dispersión de la enfermedad. Este mismo resultado ha sido reportado en otros países.

INTRODUCTION

Brucellosis is an infectious disease caused by bacteria of the genus *Brucella*. These pathogens have the ability of infecting humans and several other wild and domesticated mammals. In humans, brucellosis poses a health problem because it causes Malta fever when unpasteurized milk from goats infected with *B. melitensis* is con-

sumed.[2,3,9] Brucellosis diagnosis is accomplished by microbiology or serological means,[27] although recently new molecular biology techniques, such as restriction endonuclease analysis and DNA amplification by the polymerase chain reaction, are being evaluated with promising results.[4,6,16,18] Infection with *Brucella abortus* can be considered a occupational disease of veterinarians, cowboys and shepherds, due to the contact and handling of infected animals.[12] Brucellosis has become epizootic in some wild species populations, such as bison and wapiti,[5,16,19] caribou,[12] russian antelope, desert rat, and probably moose[13,15] and hippo.[24] The most frequent clinical sign of infection in domesticated animals is abortion, and although this alteration has been observed in some wild ruminants, its effects in some of these species remains unknown.[3] On the other hand, moose appear to be a final host for *B. abortus*, causing death.[8]

Data obtained in several regions of the world have shown brucellosis as epizootic in some wild animal populations. Studies aimed at the determination of the role of deer as reservoirs have found a not significant participation in the dispersion of the disease.[10,11,14,17] However, most of these studies have used as antigen *B. abortus*, but since the activity of this bacterial species is higher in domesticated cattle and the interaction in northeast Mexico between deer is mainly with ovine and caprine livestock, there is the possibility that deer in this region may be positive against *Brucella ovis* and *Brucella melitensis*, because of the positive serological results previously demonstrated in the small ruminant populations of that area.

Because brucellosis represents a zoo-sanitary problem for Mexico, there is currently under way an eradication program based in the detection and elimination of serological reactors from the domesticated ruminant herds, as has been done in other countries,[21] and studies have been conducted for the evaluation of vaccines against the disease.[7,20] That is why it is important to determine if the white-tailed deer can act as reservoir of *Brucella* species, since such a situation will represent an obstacle for the definitive eradication of brucellosis from a determined area. Therefore, the objective of this study is to determine the serological activity of white-tailed deer in northeast Mexico against several species of the genus *Brucella*.

MATERIAL AND METHODS.

White-tailed deer population and sampling. The white-tailed deer were captured and sampled from an area encompassing the dividing line of the states of Coahuila and Nuevo Leon, Mexico, including the counties of Ciudad Anahuac and Ciudad Acuña. Capture was conducted during early March of each year shooting net cannons from an helicopter.

A total of 208 blood samples were obtained, 165 in 1994 and 43 in 1995. After collection, the samples were refrigerated and send to the Veterinary Diagnostic Central Laboratory of the College of Veterinary Medicine, Autonomous University of Nuevo León, were the blood serum was collected by centrifugation and frozen until testing.

Serological diagnosis of *Brucella* spp. infection.

A. Plate agglutination test. Antibody detection against *B. abortus* was made with a commercial antigen (PRONABIVE, S.A. de C.V.), which is prepared from *B. abortus* strain 1119-3, with a final bacterial concentration of 11%, pH 6.4-7.0, and stained with brilliant blue and crystal violet. In order to demonstrate antibodies against *B. melitensis*, a strain of this bacteria isolated from a natural source containing unstained and inactivated microorganisms was used, with a final concentration of 11-12%. Reaction mixtures were made with 30 µl antigen and 5-80 µl serum (5, 10, 20, 40, and 80 µl), for concentrations of 1:200 to 1:25, respectively. Mixtures were placed on a glass plate, with a incubation of 8 min, with mixing at times 0 and 4 min. Results reading and interpretation were made according to Alton.[1]

B. Card agglutination test. For this test dead *B. abortus* cells of strain 1119-3 were used as antigen, but the final bacterial concentration was 8%, stained with Bengal pink and pH 3.65 (PRONABIVE, S.A. de C.V.). Reaction mixtures were made of 30 µl serum and 30 µl antigen, incubated for 4 min and with continuous rotation. Results were interpreted according to the following criteria: Negative (-): uniform pink coloration, without agglutination or "brow" formation. Positive (+): Barely perceptible agglutination and/or "brow" formation. Positive (++): Slight agglutination, defined "brow" and some clearing. Positive (+++): Well defined agglutination and clearing.

C. Card rivanol precipitation test. This test was used with two sera showing slight agglutination at 1:25 dilution in the card agglutination test. Antigens were *B. melitensis* and *B. abortus* strain 1119-3 (PRONABIVE S.A. de C.V.), with rivanol concentration of 1%. Interpretation of results were according to Alton.[1] Reaction mixtures were 40 µl serum and 40 µl. rivanol, with incubation for 30 min in Ependorff tubes. Tubes were spinned at 3000 rpm for 5 min, and the supernatant was deposited in amounts of 5-80 µl on a glass plate. To each mixture 30 µl. of rivanol antigen was added. Incubation and result interpretation were similar than for the plate test.

D. Gel diffusion test. This test was performed using soluble antigen produced in the laboratory from *B. ovis*, according to the methodology described in Velázquez.[26] For this test, 0.8% agar was dissolved in borate buffer (0.03 M, pH 8.3) and 5% saline solution. This mixture was heated in a water bath until clarification, and 1 ml. of 1% sodium azide was added before placing it on Petri dishes. After solidification, 6 peripheric and one central holes were made, containing soluble saline antigen (central hole), suspected sera, and positive and negative controls (peripheric holes).

Petri dishes were then incubated in a humid chamber for 72 h. Reading of results was done on a transiluminator, recording the precipitation lines between the central and the peripheric holes, indicating a positive result.

RESULTS AND DISCUSSION

Brucellosis in white-tailed deer has received attention in some countries, with early serologic results revealing that of 12,706 animals sampled in 24 states of the U. S. A., only 20 deer (0.16%) had titers considered positive for domesticated animals. An additional 0.13% had suspected titers.[12] No other reports altering this findings had been made public since 1961. It must be noted that such small amount of positive animals does not indicate that brucellosis represent a health problem for with-tailed deer, but that some animals had been exposed to *Brucella* spp. or antigenically related microorganisms. A positive exam can not be considered as proof of active infection.

Of all sera analyzed in the present study only two reacted against *B. melitensis* in the plate agglutination test at 1:25 dilution, but the same samples were negative for the rivanol precipitation test (with *B. abortus* and *B. melitensis* antigen, separately). Therefore, serologic prevalence was estimated at 0% (0/208). In a previous study by Siller,[23] goats from Zaragoza, Coahuila (a county next to the area sampled in this study) showed 6.0% and 21.86% serologic positives against *B. abortus* and *B. melitensis*, respectively. Considering both the behavioral activity of white-tailed deer and the goat management system at the area (open range), it seems that the potential risk of deer for acquiring brucellosis is low or is not evident with the serological methodology used in the present study. It may seem possible that deer is in risk of infection since goats travel great distances daily for feeding, and if a goat infected with *B. melitensis* have an abortion on the field it can disperse the bacteria on the vegetation or other vehicles for dispersion of the disease (soil, water, etc.), which will lead to an exposure of deer. However, the deer population studied had no serologic evidence of having been in contact with *B. melitensis* or other species of *Brucella*.

In regard to cattle reports in the studied area, a paper by Teclaw[25] using ELISA, antibody activity against *B. abortus* was found in the states of Coahuila, Nuevo Leon and Tamaulipas. The prevalence was estimated at 2.4% and 35% of the studies herds were affected. Therefore, the low level of brucellosis found in deer may reflect the presence of the disease in the herds of domesticated ruminants of the area. For all of the above mentioned, it seems very unlikely that brucellosis will become a health problem for captive or wild herds of deer.

CONCLUSIONS

Although at the studied area there is evidence of the presence of brucellosis in domesticated ruminants, white-tailed deer at the zone did not presented antibodies against *Brucella* species. This result is in agreement with previous findings of deer living in contact with infected cattle, in which the conclusion was that presence of brucellosis in deer only reflects its presence in cattle.[12] Therefore, the role of white-tailed deer as a vector for the disease is null or very limited. Based on the results obtained in the present study, it can be concluded that mobilization of white-tailed deer to re-population areas will have no effect on the prevalence of brucellosis. Additionally, we can conclude that white-tailed deer will not represent an obstacle for the eradication of brucellosis form domesticated animals, and it will also not be an important factor for the transmission of the disease to humans.

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