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# Immunologic and Molecular Identification of *Babesia bovis* and *Babesia bigemina* in Free-Ranging White-Tailed Deer in Northern Mexico

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ABSTRACT: The suitability of white-tailed deer (*Odocoileus virginianus*) as hosts for the cattle ticks *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus* (*Boophilus*) *annulatus,* has been well documented. These ticks have a wide host range, and both transmit *Babesia bovis* and *Babesia bigemina,* the agents responsible for bovine babesiosis. Although this disease and its vectors have been eradicated from the United States and some states in northern Mexico, it still is a problem in other Mexican states. It is not known if wild cervids like white-tailed deer can act as reservoirs for bovine babesiosis. The purpose of this study was to determine if *B. bovis* and *B. bigemina* or antibodies against them occur in white-tailed deer in the states of Nuevo Leon and Tamaulipas, Mexico. Twenty blood samples from white-tailed deer from two ranches were collected and tested with a nested polymerase chain reaction (nested PCR) and indirect immunofluorescence antibody test (IFAT) for *B. bovis* and *B. bigemina.* Eleven samples were positive for *B. bigemina* and four for *B. bovis* by nested PCR; amplicon sequences were identical to those reported in GenBank for *B. bovis* (*Rap 1*) and *B. bigemina.* Results of the IFA test showed the presence of specific antibodies in serum samples. This is the first report of the presence of *B. bovis* and *B. bigemina* in white-tailed deer using these techniques and underscores the importance of cervids as possible reservoirs for bovine babesiosis.

*Key words: Babesia bigemina*, *Babesia bovis*, immunoflourescence, Mexico, nested PCR, white-tailed deer.

During the past two decades the population of white-tailed deer (*Odocoileus virginianus*) in northeastern Mexico has increased considerably. Because deer and domestic ruminants occupy a sympatric range, they may share infectious disease agents and their associated vectors. These infectious agents may be transmitted from wildlife to livestock or from livestock to wildlife and back to livestock with serious implications for humans. In this context the question is not whether deer and livestock can coexist, but rather what management strategies can be used to ensure maintenance and expansion of wildlife populations, while maintaining livestock stocking rates and preventing disease outbreaks and spread. The suitability of white-tailed deer and other cervids as hosts for cattle fever ticks is well documented (Owen, 1977; Cooksey, 1989; Szabo, 2003). *Rhipicephalus* (*Boophilus*) *microplus* and *Rhipicephalus* (*Boophilus*) *annulatus,* cattle fever ticks, have a limited host range, and both transmit *Babesia bovis* and *Babesia bigemina,* the agents responsible for bovine babesiosis. This disease and its vectors have been eradicated from the United States and from some states in northern Mexico, but still are a problem in other Mexican states. The success of the eradication program is based on preventing the movement of infected ticks into disease-free areas by monitoring imported cattle and eradicating tick infestations.

It is not known if wild cervids like white-tailed deer can serve as reservoirs for bovine babesiosis; however, *B. bovis* has been demonstrated to infect erythrocytes from a wide range of mammals (Gaffar, 2003) and can be cultured in white-tailed deer erythrocytes in the presence of bovine serum (Holman, 1993). This contrasts with *B. bigemina,*

which has been reported to be host-specific (Kania, 1995). To date, there is no evidence that deer can be experimentally or naturally infected with either of these two *Babesia* species. Because white-tailed deer coexist with cattle on many ranches in the northern part of Mexico that are infested by both *R.* (*B.*) *microplus* and *R.* (*B.*) *annulatus,* there is the possibility that white-tailed deer could be infected. The objective of this study was to determine the presence of *B. bovis* and *B. bigemina* and antibodies against those disease agents in white-tailed deer captured from two ranches located in two states in Mexico.

The study was conducted in the states of Nuevo Leon and Tamaulipas, Mexico (approximately 27–28°N and 99°W). Deer were live-captured from helicopter using a net gun, and blood samples were collected from 10 white-tailed deer on each ranch. Collections were done during the spring of 2004, and all the work was performed under a scientific collecting permit issued by the Mexican Division Animal Wildlife Health (Dirección de Salud Animal de Vida Silvestre, SEMARNAT). Two blood samples were collected per deer; one blood sample was collected with anticoagulant (acid citrate dextrose), and another sample was used to obtain serum. Samples were stored on wet ice and transported to the laboratory. Adult engorged and semi-engorged ticks from deer were collected as they were available, and held at 24 C with a relative humidity of 80%. Replete females were allowed to oviposit. Partially engorged ticks and eggs from engorged females from each deer were macerated and used for polymerase chain reaction (PCR) analysis. Blood samples and ticks collected from the deer were analyzed by nested PCR using the protocol previously reported by Figueroa (1993) and specific primers for *B. bovis* that identified the gene *Rap-1* and *B. bigemina.* The amplified PCR products were electrophoresed on agarose gels, stained with ethidium bromide, visualized, and photographed under ultraviolet light. To further substantiate the PCR results, amplicons from two *B. bigemina*–positive and one *B. bovis*–positive blood samples were sequenced. For the immunologic analysis, serum collected from each deer was tested for evidence of exposure to *B. bovis* and *B. bigemina* using an indirect immunofluorescence antibody test (IFAT). Briefly, *B. bovis*– and *B. bigemina*–infected bovine erythrocytes were obtained from in vitro culture as previously described by Levy (1980) and Vega (1985) and were smeared and kept at −70 C until used. The IFA slides were fixed with acetone and incubated with each of the serum samples at a screening dilution of 1:100 for 30 minutes at 37 C. This dilution was chosen because cross-reaction between *B. bovis* and *B. bigemina* does not occur in cattle. The reaction was detected with protein G conjugated with Alexa 488 (Molecular Probes, Eugene, Oregon, USA) at a 1:100 dilution followed by an incubation time of 30 min at 37 C. As positive controls, bovine sera from cattle experimentally infected with *B. bovis* or *B. bigemina* were used at the same dilution. Sera from uninfected deer were used as negative controls.

The results obtained in the PCR assay showed 11 samples positive for *B. bigemina* and four for *B. bovis.* Seven of the samples positive for *B. bigemina* and all four positive for *B. bovis* were observed in deer collected on the ranch from Nuevo Leon (Table 1); the remaining four samples positive for *B. bigemina* were from the state of Tamaulipas (Table 1). None of the samples were positive for both *Babesia* species. The resulting amplicons from positive samples had the expected sizes of 170 base pairs (bp; *B. bigemina*) and 291 bp (*B. bovis*), and were identical to published sequences of both species (*B. bovis* accession number M38218; *B. bigemina* accession number S45366).

The results of the serologic testing showed six samples from the Nuevo Leon ranch positive for *B. bovis* antibodies,

TABLE 1. Results from 20 deer samples tested by nested-polymerase chain reaction (PCR) and indirect immunofluorescence antibody test (IFAT) to detect DNA and antibodies to *Babesia bovis* and *B. bigemina* from two ranches in the northern Mexico.

| | IFAT | | PCR | |
|---|---|---|---|---|
| Location | *B. bigemina* | *B. bovis* | *B. bigemina* | *B. bovis* |
| Nuevo Leon | 0 | 6 | 7 | 4 |
| Tamaulipas | 0 | 5 | 4 | 0 |
| Total | 0 | 11 | 11 | 4 |

while five samples were found positive for *B. bovis* in deer collected in Tamaulipas (Table 1). None of the samples were positive for antibodies against *B. bigemina* by the IFA test. Three out of the four Nuevo Leon samples positive for *B. bovis* by PCR were also positive on IFAT; none of the *B. bigemina*–positive samples by PCR were positive on IFAT (Table 2). All samples of *R.* (*B.*) *microplus,* analyzed for infection with *B. bovis* and *B. bigemina* by nested PCR, were negative.

We investigated the possible role of white-tailed deer in bovine babesiosis in two northern states of Mexico and have provided molecular evidence that the etiologic agents were identical to parasites previously characterized as *B. bovis and B. bigemina* (Figueroa, 1993). The organisms were reported previously in Mexico (Figueroa, 1998) and are well known as causal agents of bovine babesiosis in cattle but not in deer. At the time of sampling there were no obvious signs of illness observed in the deer. This is the first study in which molecular diagnostic techniques combined with immunologic screening were used to investigate the presence of *B. bovis* and *B. bigemina* in white-tailed deer and to assess simultaneous infection by both species. Serologic results provide information only that deer were exposed to *Babesia;* however, no implication can be made about the infection status of the disease. Five of 20 deer were infested with *R.* (*B.*) *microplus* ticks. Unfortunately we did not record at the time of sampling

TABLE 2. Results from white-tailed deer samples tested by nested PCR and indirect IFAT from two ranches in the northern states of Mexico 2004.

| | IFAT/PCR | | IFAT/PCR | |
|---|---|---|---|---|
| Location | *B. bigemina* | *B. bovis* | *B. bigemina* | *B. bovis* |
| Nuevo Leon | 0 | 3 | 0 | 3 |
| Tamaulipas | 0 | 2 | 0 | 0 |

from which animals the ticks were obtained or the number of ticks from each infested deer. Therefore, it is not possible to correlate if these ticks were sampled from positive or negative deer. All the ticks were negative for the presence of *Babesia.*

To our knowledge, this is the first report of molecular data for the two bovine *Babesia* species reported in Mexico or for *B. bovis* and *B. bigemina* in white-tailed deer. Even though *B. bigemina* and *B. bovis* infection in white-tailed deer were found by DNA amplification, we do not know if these PCR-positive animals were in the early phase or in the carrier stage of infection. Deer found to be positive for *B. bigemina* by nested PCR but negative by IFAT may have been in an early stage of infection. The detection of *Babesia* infection in animals in the early phase of infection or in carrier animals by DNA amplification is a powerful tool for epidemiologic investigation. Sequence analysis of these identified genes in white-tailed deer provides a precise means of *Babesia* species identification

Historical experiments to infect deer with *B. bovis* and *B. bigemina* have been negative (Kuttler, 1972). The data reported in this paper provide evidence for the presence of *B. bovis* and *B. bigemina* DNA in white-tailed deer. While these results are significant and intriguing, they do not confirm that white-tailed deer are or are likely to be competent hosts for these agents. Recently, two babesial parasites were reported in roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*).

The parasites were identified by nested PCR, and sequence analysis of the 18SrRNA showed 99.7% of identity of the roe deer *Babesia* EU1 with the human strain EU1. The *Babesia divergens* detected in cervids was 99.6% identical to bovine *B. divergens*, which suggests that the roe deer and red deer may serve as a reservoir for *B. divergens* (Duh et al., 2005). Therefore, our findings support that deer may be a reservoir of bovine *Babesia* species.

The only way to confirm that white-tailed deer in Mexico are hosts of the two *Babesia* species in question is to demonstrate transmission by infected cattle fever ticks to naive deer and subsequent transmission from infected deer to ticks whose offspring could then transmit to uninfected deer. Such an experiment involving deer and *R.* (*B.*) *microplus* has yet to be performed. The possibility of wildlife as reservoirs of bovine babesiosis should not be neglected and awaits further investigation.

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