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Molecular detection of *Babesia bovis* and *Babesia bigemina* in white-tailed deer (*Odocoileus virginianus*) from Tom Green County in central Texas

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ABSTRACT

Serologic and molecular evidence suggest that white-tailed deer in South Texas and North Mexico carry the agents of bovine babesiosis, *Babesia bovis* and *Babesia bigemina*. To determine if white-tailed deer in central Texas, which is outside the known occurrence of the vector tick at this time, harbor these parasites, blood samples from free-ranging and captive white-tailed deer (*Odocoileus virginianus*) in Tom Green County were tested by polymerase chain reaction (PCR) assays for *B. bovis* and *B. bigemina* 18S rDNA. Of the 25 samples tested, three (12%) were positive by nested PCR for *B. bovis*. This identity was confirmed by sequence analysis of the cloned 18S rDNA PCR product. Further confirmation was made by sequence analysis of the rRNA internal transcribed spacer (ITS) 1, 5.8S rRNA gene, and ITS 2 genomic region in two (representing samples from two different ranches) of the *B. bovis* positive samples. Three samples were positive by *B. bigemina* nested PCR, but sequencing of the cloned products confirmed only one animal positive for *B. bigemina*; *Theileria* spp. DNA was amplified from the other two animal samples. In addition to *Theileria* spp., two genotypically unique *Babesia* species sequences were identified among the cloned sequences produced by the *B. bigemina* primers in one sample. Phylogenetic analysis showed no separation of the deer *B. bovis* or *B. bigemina* 18S rDNA, or deer *B. bovis* ITS region sequences from those of bovine origin. Clarification of the possible role of white-tailed deer as reservoir hosts in maintaining these important pathogens of cattle is critical to understanding whether or not deer contribute to the epidemiology of bovine babesiosis.

1. Introduction

Babesia bovis and *Babesia bigemina* are causative agents of bovine babesiosis transmitted by Cattle Fever ticks, *Rhipicephalus* (*Boophilus*) spp. An intensive 36-year campaign by the Cattle Fever Tick Eradication Program (CFTEP) cleared the vector ticks from the U.S. nearly 60 years ago. Since then a permanent Fever Tick quarantine buffer zone has been maintained in Texas along the Rio Grande to prevent re-establishment of the vector tick and concomitant outbreaks of bovine babesiosis in the U.S. (Graham and Hourrigan, 1977; Bram et al., 2002).

Fever ticks are one-host ticks that preferentially feed on cattle but will feed on alternate ungulate hosts, such as white-tailed deer (Pound et al., 2010). The role of white-tailed deer in the recent resurgence of Fever Tick outbreaks in Texas is of major concern. As traditional cattle ranches turn increasingly to recreational ventures, the white-tailed

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Table 1
Details for white-tailed deer blood samples.

ID	Collection date	Ranch	Giemsa-stained blood film		18S rDNA PCR	
			Theileria	*Babesia*	*B. bigemina*	*B. bovis*
W1	5-20-08	N	**Positive**	Negative	Negative	Negative
W2	5-20-08	N	**Positive**	Negative	Negative	Negative
W3	5-20-08	N	**Positive**	Negative	Negative	Negative
H1	5-20-08	N	**Positive**	Negative	Negative	Negative
H2	5-20-08	N	**Positive**	Negative	Negative	Negative
H3	5-20-08	N	**Positive**	Negative	Negative	Negative
N3	7-7-08	S	**Positive**	Negative	Negative	Negative
N4	7-7-08	S	**Positive**	Negative	Negative	Negative
H4	7-7-08	S	Negative	Negative	Negative	Negative
H5	7-7-08	S	**Positive**	Negative	Negative	Negative
D1	7-7-08	W	Negative	Negative	Negative	Negative
D2	7-7-08	W	**Positive**	Negative	Negative	Negative
H6	7-28-08	N	**Positive**	Negative	Negative	Negative
H7	7-28-08	N	**Positive**	Negative	**Positive**	Negative
H8	7-28-08	N	**Positive**	Negative	**Positive**[a]	**Positive**
NR6	7-28-08	S	Negative	Negative	Negative	**Positive**
NR7	7-28-08	S	Negative	Negative	Negative	Negative
NR8	7-28-08	S	**Positive**	Negative	Negative	**Positive**
N9	9-9-08	S	Negative	Negative	Negative	Negative
N10	9-9-08	S	**Positive**	Negative	Negative	Negative
N11	9-9-08	S	**Positive**	Negative	Negative	Negative
N12	9-9-08	S	Negative	Negative	Negative	Negative
H9	9-9-08	N	**Positive**	Negative	Negative	Negative
H10	9-9-08	N	**Positive**	Negative	**Positive**[b]	Negative
H11	9-9-08	N	**Positive**	Negative	Negative	Negative

Positive results are shown in bold type; positive PCR results are in bold type and underlined.

[a] Identified as *Theileria* sp. by sequence analysis.

[b] Identified as *Theileria* sp. and *Babesia* spp. by sequence analysis.

deer population is burgeoning. It is feared that these large numbers of deer not only may serve as hosts for the tick, but also as reservoirs for bovine *Babesia* spp. (Perez de Leon et al., 2010).

Molecular and serologic evidence of bovine *Babesia* spp. in white-tailed deer in northern Mexico states bordering Texas and in South Texas counties (LaSalle and Webb) was recently documented (Cantu et al., 2007, 2009; Ramos et al., 2010). However, the white-tailed deer in Webb and LaSalle counties in South Texas were infected with *B. bovis*-like organisms with single nucleotide polymorphisms in 18S rRNA gene that differentiated them from bovine *B. bovis* isolates (Ramos et al., 2010). In the current study, white-tailed deer in central Texas (Tom Green County) were surveyed molecularly for the presence of *B. bovis* and *B. bigemina* using a polymerase chain reaction (PCR) targeting the parasite 18S ribosomal RNA gene. Further molecular and sequence analyses were conducted on the genomic DNA region comprising the rRNA intervening transcribed spacers and the 5.8S rRNA gene in *B. bovis* PCR-positive samples.

2. Materials and methods

Twenty-five white-tailed deer blood samples in ethylenediamine tetraacetic acid–K_3 (EDTA) anticoagulant were obtained as shared samples in May, July, and September 2008 from collections in Tom Green County, Texas (Texas Parks and Wildlife Department, Scientific Permit Research, SPR 0807-1416) for another ongoing study on epizootic hemorrhagic fever in deer. The deer were located on 3 different ranches: one in the northern part of the county (N), one in the southern part (S), and one to the west of San Angelo (W) (Table 1). The deer on ranches N and S were free-ranging, whereas the deer on ranch W were captive. Bovine blood samples (USDA and Merida) from *B. bovis* natural infections were included as regional isolate controls for 18S rDNA sequences due to the variation found in *B. bovis* rDNA sequences available in the GenBank database.

A Giemsa-stained blood smear from each white-tailed deer sample was examined microscopically under oil immersion at 1000× for the presence of hemoparasites.

Genomic DNA was extracted (FlexiGene DNA Kit, Qiagen, Valencia, CA) from the blood samples and the concentration of each was determined by spectrophotometry (NanoDrop ND-1000 Spectrophotometer, NanoDrop Technologies, Wilmington, DE). Nested polymerase chain reactions were used to assay the samples for the presence of *B. bovis* and *B. bigemina* 18S rRNA genes. The primary reaction using primers A and B (Sogin, 1990) targeted the hemoparasite full-length 18S rRNA gene as previously described (Ramos et al., 2010) (Table 2). The reaction mixtures, excluding DNA, were composed following manufacturer's instructions (Platinum High Fidelity Taq, Invitrogen) in a designated DNA-free hood with PCR-dedicated equipment and materials. Approximately 50 ng DNA was then added to each reaction tube under a laminar flow hood in another room. Water was included as a negative control and a plasmid containing *B. bigemina* 18S rRNA gene served as a positive control for the primary PCR. The primary reactions (25 μl reaction volume) were cycled