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Nilgai Antelope in Northern Mexico as a Possible Carrier for Cattle Fever Ticks and *Babesia bovis* and *Babesia bigemina*

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ABSTRACT: Of 20 blood samples from nilgais from México, five were polymerase chain reaction-positive for *Babesia bigemina* and one for *Babesia bovis*. Positive samples had the expected 170 (*B. bigemina*) and 291 (*B. bovis*) base pairs and were identical to GenBank *B. bigemina* accession S45366 and *B. bovis* M38218.

Understanding the impact of wildlife diseases on wildlife populations has become a recent issue of global priority. Knowledge of such impacts is critical when disease risks involve not only wild populations but also human health and livestock industries. Such is the case with bovine babesiosis in the border region of México and the USA. Bovine babesiosis is a common and widely distributed arthropod-transmitted disease (Homer et al., 2000) and is considered the most economically important disease of livestock (Bock et al., 2004). The disease is caused by the protozoan parasites *Babesia bigemina* and *Babesia bovis*, transmitted to the host by the tick vectors *Rhipicephalus annulatus* and *Rhipicephalus microplus* (Bock et al., 2004), known as cattle fever ticks (CFTs). Although cattle are the main host for CFTs, other ungulates such as white-tailed deer (*Odocoileus virginianus*; Hagan and Bruner, 1951; Cantú et al., 2007), sheep (*Ovis aries*; Mungall and Sheffield, 1994), and nilgai antelope (*Boselaphus tragocamelus*; Sheffield et al., 1983) have been found with CFTs.

Nilgai antelope are an exotic species introduced into Texas, USA, in the 1940s (Sheffield et al., 1983). They successfully adapted to this range, and their numbers have been increasing. Approximately 30,000 nilgai are found along the Texas-México border in the quarantine zone (APHIS, 2006). Because nilgai can interchange freely across the border, the likelihood of nilgai acquiring bovine babesiosis is high.

The role that nilgai antelope may play in bovine babesiosis is unknown. No study has investigated whether nilgai are susceptible to *Babesia* spp. Because other wildlife species are known to become infected with *Babesia* spp., determining whether nilgai antelope may serve as a carrier for the parasite is critical, particularly in areas where cattle and nilgai coexist. Our objective was to look for evidence of *B. bovis* and *B. bigemina* and CFTs in nilgai antelope in México.

The study was conducted on a private ranch in the state of Coahuila, México (approximately 67°33′N, 43°78′E). Our study area was a 2,765-ha high-fenced area within the 4,218-ha ranch. In addition to nilgai, other free-ranging native and exotic species were present, including white-tailed deer, black buck (*Antilope cervicapra*), blue wildebeests (*Connochaetes taurinus*), zebras (*Equus zebra*), and giraffe (*Giraffa camelopardalis*). Cattle had been removed from the area for approximately 10 yr. Nilgai of all ages and both sexes were harvested ($n=20$ during the day by waiting for animals at watering points and at night by spot-lighting along roads. Research was performed under a scientific collecting permit issued by the Subsecretaria de Recursos Naturales, Dirección de Recursos Forestales y Vida Silvestre del Estado de Coahuila. Two

TABLE 1. Nucleotide sequence of primers used for PCR and nested PCR to detect *Babesia* spp. in México (Figueroa et al., 1993).

Organism	Primer sequence[a]	Product length (base pairs)
Babesia bigemina		
Simple PCR	F: 5′-CCTCGGCTTCAACTCTGATGCCAAAG-3′ R: 5′-CATCTAATTTCTCTCCATACCCCTCC-3′	278
Nested PCR	F: 5′-CGCAAGCCCAGCACGCCCCGGTGC-3′ R: 5′-CCGACCTGGATAGGCTGTGATG-3′	170
Babesia bovis		
Simple PCR	F: 5′-CACGAGGAAGGAACTACCGATGTTGA-3′ R: 5′-CCAAGGAGCTTCAACGTACGAGGTCA-3′	350
Nested PCR	F: 5′-TCAACAAGGTACTCTATATGGCTACC-3′ R: 5′-CTACCGAGCAGAACCTTCTTCACCAT-3′	291

[a] F = forward; R = reverse.

blood samples, one sample for whole blood and second sample for serum, were collected from the jugular vein or the heart of each animal, generally within 15 min after harvest. Animals were thoroughly inspected (Pelzel, 2005) in searching for ticks. Blood was stored at 4 C until further transportation to the National Center of Disciplinary Research in Animal Parasitology (CENID-PAVET), Jiutepec, Morelos, México.

Simple PCR was performed on blood samples, followed by a nested PCR (nPCR) from the product to increase the detection of the expected fragments. Before DNA extraction, blood samples were pretreated with saponin to facilitate lysis (Figueroa et al., 1992). The protocols for PCR and nPCR amplification and primer sequences identifying the gene *Rap-1* for *B. bovis* and *B. bigemina* (Table 1) followed Figueroa et al. (1993). The protocol followed for the nPCR was identical to the simple PCR. We used positive controls derived from an in vitro culture for both *Babesia* spp. and DNA from a cow free of *Babesia* spp. as a negative control. For the simple PCR protocol, amplicon expected sizes were 278 and 350 base pairs (bp) for *B. bigemina* and *B. bovis*, respectively. The resulting amplicons from positive samples had the expected sizes of 170 bp (*B. bigemina*) and 291 bp (*B. bovis*) and were identical to published sequences of both species (*B. bigemina* GenBank accession S45366; *B. bovis* accession M38218). Amplified products were visualized using gel electrophoresis on a 2% agarose-ethidium bromide gel (Promega, Madison, Wisconsin, USA) by using a 123-bp ruler (Invitrogen, Carlsbad, California, USA).

We documented five samples positive for *B. bigemina* based on amplicon size (170 bp), of which one sample also was positive for *B. bovis* (291 bp). The most important finding of this research is the presence of *B. bovis* and *B. bigemina* in free-ranging nilgai. No ticks were found on any of the harvested animals. Our PCR findings demonstrate the apparent presence of babesial parasite DNA in nilgai blood cells; however, these results do not address whether *B. bigemina* and *B. bovis* can complete their life cycles in nilgai. It also is unknown whether nilgai are susceptible to *Babesia* spp. infection, develop the clinical phase, and remain infective, thereby making them reservoirs. Further studies are needed to conclude that nilgai antelope act as a competent reservoir of *Babesia* spp.

Even though cattle were removed from the ranch, we did not disregard the possibility that CFTs were present on the study area maintained solely by wildlife. Kistner and Hayes (1970) reported white-tailed deer infested with CFTs in an area

where cattle were absent for >20 yr. The reason that no ticks were found on any *Babesia*-positive animals is unknown.

Evidence of *Babesia* spp. in nilgai has not been reported previously. The role of nilgai in bovine babesiosis cannot be determined from this study; however, nilgai cannot be disregarded as a potential reservoir of bovine babesiosis. Important modifications to CFT eradication strategies may need to be implemented if nilgai antelope are capable of disseminating CFTs and therefore maintaining bovine babesiosis.

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