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Detection of a *Babesia* Species in a Bobcat from Georgia

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Abstract - We describe the first detection of a *Babesia* sp. in a *Lynx rufus* (Bobcat). The Bobcat was from Georgia and was coinfected with *Cytauxzoon felis* and a *Sarcocystis* sp. The *Babesia* species was closely related to *Babesia* sp. "Coco", a parasite previously only detected in *Canis familiaris* (Domestic Dog). The only other *Babesia* sp. in North America that infects felids is a novel *Babesia* species in *Puma concolor coryi* (Florida Puma). The low prevalence of this *Babesia* (<1%) in Bobcats suggests that they are not the normal host or reservoir and this may have been an incidental infection.

Piroplasms (genera *Babesia, Theileria,* and *Cytauxzoon)* are tick-transmitted apicomplexan parasites which infect a wide range of mammals and birds worldwide (Criado-Fornelio et al. 2004). Species of all three genera infect erythrocytes, but in contrast to *Theileria* and *Cytauxzoon*, *Babesia* spp. do not have an extra-erythrocytic stage (Criado-Fornelio et al. 2004). Numerous species of piroplasms are important disease-causing agents for veterinary species, and *Babesia* are notable in that many species are zoonotic. Disease caused by *Babesia* is rare among wildlife, but disease can develop during stressful periods, after co-infection with immunosuppressive viruses, or when infections occur in aberrant hosts (e.g., *Panthera leo* L. [Lions] from Africa infected with a natural *Babesia* species but diseased when exposed to drought and coinfected with Canine Distemper Virus, or when exotic *Rangifer tarandus* L. [Reindeer] in the northeastern United States become infected with *Babesia* species native to the area; Bartlett et al. 2009, Munson et al. 2008).

Currently, only two piroplasms have been reported from felines in North America, *Cytauxzoon felis* Kier in *Felis catus* L. (Domestic Cat), *Lynx rufus* Schreber (Bobcat), and *Puma concolor* L. (Puma) from the eastern United States, and a novel *Babesia* species in *Puma concolor coryi* Bangs (Florida Puma) from southern Florida (Glenn et al. 1983, Yabsley et al. 2006). During a surveillance study (Shock et al. 2012) on wildlife reservoirs of *C. felis* involving Bobcats (n = 799) and Pumas (n = 49) from thirteen states (Florida, Georgia, Kansas, Kentucky, Louisiana, Missouri, North Carolina, North Dakota, Ohio, Oklahoma, Pennsylvania, South Carolina, and West Virginia), a *Babesia* species was detected in a single female Bobcat from Thomas County, GA (n = 143; 0.7%). This is the first report of a *Babesia* sp. infection in a Bobcat and is only the second felid-infecting *Babesia* species reported in North America.

The internal transcribed spacer (ITS)-1 region was amplified using a nested PCR that amplifies all known piroplasms (Bostrom et al. 2008). Briefly, for primary amplification, 5 ml of DNA was added to 20 ml of a master mix containing 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP (Promega, Madison, WI), 2.5 units GoTaq® Flexi DNA Polymerase (Promega), and 0.8 mM of primers ITS-15C (5'-CGATCGAGTGATCCGGTGAATTA) and ITS-13B (5'-GCTGCGTCCTTCATCGTTGTG). Cycling

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parameters were 94 °C for 1 min followed by 35 cycles of 94 °C for 30 sec, 52 °C for 30 sec, 72 °C for 1 min, and a final extension at 72 °C for 5 min. For the nested PCR, 1 ml of primary product was used as a template in a 25-ml reaction containing the same PCR components except inclusion of primers ITS-15D (5'-AAGGAAGGAGAAGTCGTAACAAGG) and ITS-13C (5'-TTGTGTGAGCCAAGACATCCA). The cycling parameters were the same as the primary reaction except the annealing temperature was 49 °C.

To prevent and detect contamination, primary and secondary amplification, and product analysis were done in separate dedicated areas. A negative water control was included in each set of DNA extraction, and one water control was included in each set of primary and secondary PCR reactions. The amplicon from the positive Bobcat was purified with a Qiagen gel extraction kit (Germantown, MD) and bi-directionally sequenced at the University of Georgia Integrated Biotechnology Laboratory (Athens, GA).

Sequence analysis of the ITS-1 region (601 bp) indicated that the greatest similarity (92%) was with a novel large *Babesia* sp. "Coco'" that was first identified in a *Canis familiaris* L. (Domestic Dog) from North Carolina in 2002 (GenBank accession number: AY618928; Fig. 1; Birkenheuer et al. 2004). The phylogenetic relationship based on ITS1 between this *Babesia* and other *Babesia* spp. is similar to the relationship between *Babesia* sp. "Coco" and other *Babesia* spp. based on analysis of the 18S rRNA gene (Birkenheuer et al. 2004). The only difference between the Bobcat *Babesia* sequence and *Babesia* sp. "Coco" was the presence of a 45-bp insert in the Bobcat *Babesia* sp. at nucleotide site 434. Outside the insert region, bases 1–434 and 435 to 557 of *Babesia* sp. "Coco" (EU109720) were 100% similar to our Bobcat *Babesia*. Thus, we believe that this

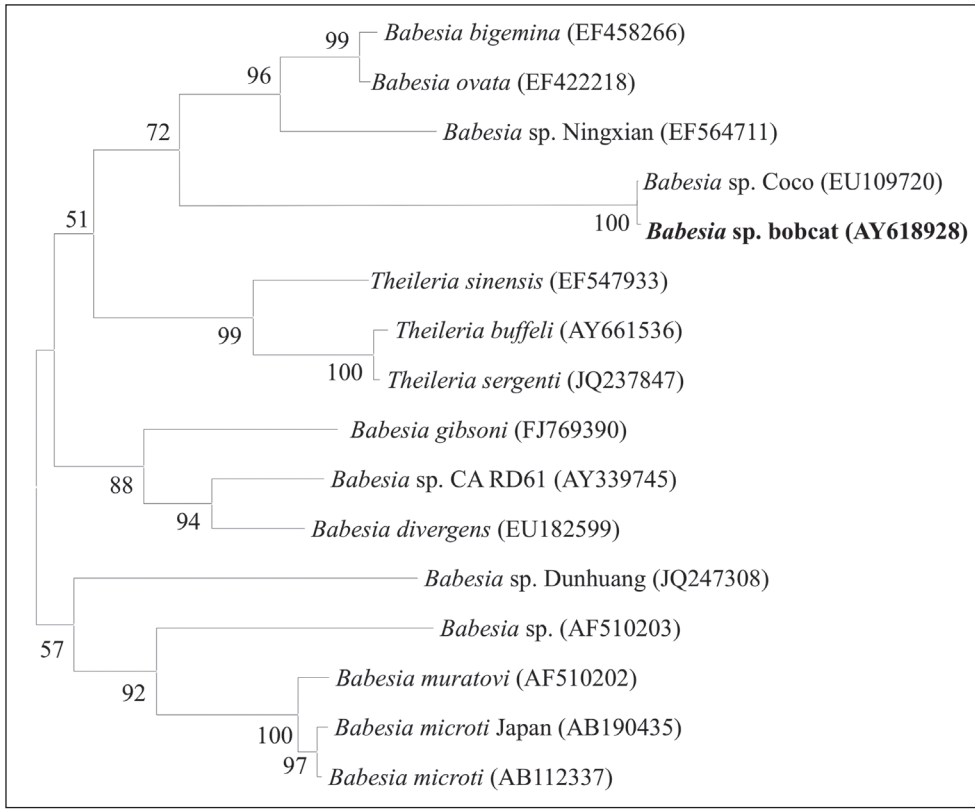

Figure 1. Phylogenetic relationships between *Babesia* spp. inferred from internal transcribed spacer (ITS)-1 rRNA region sequences.

Bobcat *Babesia* sp. represents a variant of *Babesia* sp. "Coco" and not a novel *Babesia* sp., although additional studies are needed to definitively determine the con-specificity of these two *Babesia* spp. Insertions and deletions are common in the ITS regions of other piroplasms (Aktas et al. 2007, Brown et al. 2009, Shock et al. 2012). Attempts to amplify other gene targets failed as the Bobcat was also co-infected with *C. felis*, and other targets (e.g., 18S rRNA gene and ITS-2) were positive but when sequenced were confirmed to be *C .felis*. These data highlight the need to utilize multiple gene targets when conducting pathogen surveillance. Unfortunately, a blood smear was not available from the *Babesia* sp.-infected Bobcat so no morphologic data is available.

Babesia sp. "Coco" has only previously been reported from immunosuppressed Domestic Dogs (Sikorski et al. 2010), so in an effort to better understand why this Bobcat was infected, we conducted additional pathogen screening using the limited samples available from this trapper-harvested animal. Serum from the Bobcat was negative for Feline Immunodeficiency Virus (FIV) antibodies and Feline Leukemia Virus (FeLV) antigens (IDEXX, Westbrook, ME). A low antibody titer (1:10) for Feline Panleukopenia Virus was detected (Animal Health Diagnostic Center, Cornell University, Ithaca, NY), which was not interpreted as an infection. PCR testing for other pathogens revealed that the Bobcat was positive for "*Candidatus* Bartonella volans" and was negative for hemoplasmas (Cadenas et al. 2008, Jensen et al. 2001). Histological examination of available tissues was unrewarding due to advanced autolysis, but *Sarcocystis* sp. cysts were observed in muscle tissue. All of these findings were considered incidental.

Currently, little is known about the natural history of *Babesia* sp. "Coco" and the *Babesia* sp. detected in the Bobcat from Georgia. *Babesia* sp. "Coco" was first reported from an immunosuppressed dog undergoing chemotherapy for lymphoma (Birkenheuer et al., 2004). Since the initial detection, eight additional canine infections have been reported from dogs, all with a travel history to Mid-Atlantic states. Six of these dogs were splenectomized, and two were immunosuppressed due to oncolytic drugs (Birkenheuer et al. 2004, Holman et al. 2009, Sikorski et al. 2010). At least 5 of the 9 dogs infected with *Babesia* sp. "Coco" had a history of tick exposure, and at least one sustained bites to the face, a risk factor for other *Babesia* sp., such as *B. gibsoni* Patton (Holman et al. 2009, Sikorski et al. 2010, Yeagley et al. 2009).

Worldwide, several *Babesia* spp. have been reported from felids including *B. herpailuri* Dennig and *B. pantherae* Dennig and Brocklesby from wild felids in Africa, *B. felis* Davis and *B. leo* Penzhorn from domestic cats and wild felids in Africa, *B. cati* Mudaliar in Domestic Cats from India, *B. canis canis* Uilenberg from domestic cats in Spain, *B. canis presentii* Baneth from Domestic Cats in Israel; a *Babesia* sp. from Domestic Cats in Portugal, (Baneth et al. 2004, Criado-Fornelio et al. 2004, Penzhorn et al. 2004). In the United States, the only previous report of a *Babesia* species in a felid is a novel *Babesia* sp. from Florida Pumas (Yabsley et al. 2006). The Puma *Babesia* sp. appears to be restricted to Florida Pumas because, in the current study, infections were not detected in 49 Pumas from Texas, Louisiana, Georgia, or North Dakota. Interestingly, the *Babesia* sp. from the Florida Puma is a small piroplasm morphologically and is indistinguishable from *C. felis*, whereas *Babesia* sp. "Coco" is a large *Babesia*. In addition, the two feline-infecting *Babesia* species from North America are easily distinguished based on sequence analysis of the ITS-1 region (B.C. Shock et al., unpublished data).

In summary, a *Babesia* sp. closely related to *Babesia* sp. "Coco" was detected in a single Bobcat from Georgia, which is the first report of *Babesia* infection of a Bobcat and the second report of *Babesia* in felids from North America. Bobcats likely do not

represent a natural host of this *Babesia* sp.; thus, additional surveillance studies are needed to understand the natural host of this parasite.

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