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Variation in the ITS-1 and ITS-2 rRNA genomic regions of *Cytauxzoon felis* from bobcats and pumas in the eastern United States and comparison with sequences from domestic cats

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ABSTRACT

Cytauxzoon felis, a tick-borne protozoan parasite, is the causative agent of cytauxzoonosis in domestic cats in the United States. The natural reservoir for this parasite is the bobcat (*Lynx rufus*), which typically does not develop clinical signs. Although not likely important reservoirs, *C. felis* has also been detected in pumas (*Puma concolor*) in Florida and Louisiana. Recent studies suggest that specific genotypes of *C. felis* that circulate in domestic cats may be associated with variable clinical outcomes and specific spatial locations. In the current study, we investigated the intraspecific variation of the *C. felis* internal transcribed spacer (ITS)-1 and ITS-2 rRNA regions from 145 wild felids (139 bobcats and six pumas) from 11 states (Florida, Georgia, Kansas, Kentucky, Louisiana, Missouri, North Carolina, North Dakota, South Carolina, Oklahoma, and Pennsylvania). Unambiguous ITS-1 and ITS-2 data were obtained for 144 and 112 samples, respectively, and both ITS-1 and ITS-2 sequences were obtained for 111 (77%) samples. For the ITS-1 region, sequences from 65 samples collected from wild felids were identical to those previously reported in domestic cats, while the other 79 sequences were unique. *C. felis* from 45 bobcats and one puma had ITS-1 sequences identical to the most common sequence reported from domestic cats. Within the ITS-2 region, sequences from 49 bobcats were identical to those previously reported in domestic cats and 63 sequences were unique (with some occurring in more than one

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bobcat). The most common ITS-2 sequence from domestic cats was also common in wild felids (31 bobcats and a puma). Samples from three pumas from Florida and two bobcats from Missouri had a 40- or 41-bp insert in the ITS-2 similar to one described previously in a domestic cat from Arkansas. Additionally, a previously undescribed 198- or 199-bp insert was detected in the ITS-2 sequence from four bobcats. Collectively, based on combined ITS-1 and ITS-2 sequences, five different genotypes were detected in the wild felids. Genotype ITSa was the most common genotype (11 bobcats and one puma) and fewer numbers of ITSb, ITSe, ITSg, and ITSi were detected in bobcats. These data indicate that, based on ITS-1 and ITS-2 sequences, numerous *C. felis* strains may circulate in wild felids.

1. Introduction

Cytauxzoon felis, a tick-borne protozoan parasite, is the causative agent of cytauxzoonosis in domestic cats in the United States. First identified in domestic cats (*Felis silvestris catus*) from Missouri, Texas, and Arkansas in the 1970s (Bendele et al., 1976; Wagner, 1976; Wightman et al., 1977), *C. felis* has subsequently been identified in domestic cats from numerous southeastern, midwestern, and mid-Atlantic states (Wagner, 1976; Ferris, 1979; Kier et al., 1982b; Birkenheuer et al., 2006). In addition to domestic cats, infections of *C. felis* have been determined in bobcats (*Lynx rufus*) and pumas (*Puma concolor*).

In domestic cats, *C. felis* can cause erythrocyte hemolysis and occlusion of the lumen of blood vessels by large schizont-laden mononuclear phagocytes in the lungs, liver, lymph nodes, and spleen (Simpson et al., 1985; Kier et al., 1987; Kocan and Kocan, 1991; Kocan et al., 1992). Historically, infection with this parasite was believed to be nearly uniformly fatal (Ferris, 1979); however, recent studies have discovered an increasing number of domestic cats that have subclinical chronic infections (Haber et al., 2007; Birkenheuer et al., 2006). Both *Amblyomma americanum* (lone star tick) and *Dermacentor variabilis* (American dog tick) are confirmed vectors, and both tick species are common in the southeastern and midwestern United States where the majority of *C. felis* infections have been identified (Blouin et al., 1984; Kocan et al., 1992; Reichard et al., 2008, 2010; Shock et al., 2011).

The bobcat (*L. rufus*) is considered to be the primary wildlife reservoir. In bobcats, prevalences of >30% have been documented in populations from several states: Arkansas, Florida, Kansas, Kentucky, Missouri, Oklahoma, North Carolina, and South Carolina (Wagner, 1976; Glenn et al., 1983; Birkenheuer et al., 2008; Brown et al., 2010; Shock et al., 2011), while lower prevalences were observed in bobcats sampled from Pennsylvania and North Dakota (Birkenheuer et al., 2008; Shock et al., 2011). Experimental and field-based studies indicate that the majority of infected bobcats have subclinical infections; however, rare cases of mortality have been observed in experimentally and naturally infected bobcats (Kier et al., 1982a, 1982b; Glenn et al., 1983; Blouin et al., 1984, 1987; Nietfeld and Pollock, 2002). The life stage of the parasite used in the inoculum during experimental trials seems to be important for disease outcome. Bobcats inoculated with schizogenous stages of the parasite died of acute cytauxzoonosis, while bobcats experimentally infected by a natural route (i.e., tick transmission) developed a limited schizogenous phase, which led to long-term subclinical parasitemia (Kier et al., 1982b; Blouin et al., 1987).

Natural infections have been reported from a puma from Louisiana and Florida pumas (*P. concolor coryi*) (Butt et al., 1991; Yabsley et al., 2006; Shock et al., 2011). In contrast to the bobcat, *C. felis* appears to cause a mild, likely transient, hemolytic anemia in Florida pumas (Harvey et al., 2007). Worldwide, other *Cytauxzoon* spp. that are distinct from *C. felis* have been identified in numerous wild felid species (Butt et al., 1991; Luaces et al., 2005; Peixoto et al., 2007; Ketz-Riley et al., 2003).

Several treatment options have been investigated for domestic cats and a combination treatment with atovaquone and azithromycin increased survival compared with treatment with imidocarb dipropionate alone (Cohn et al., 2011). However, increasing reports of subclinical infections that appear to be unrelated to treatment could be due to (1) emergence of different strains of *C. felis* that may differ in their virulence for domestic cats, (2) some selection of nonpathogenic strains by domestic cat survival which may be maintained in subsequent generations or (3) use of more sensitive diagnostic tests (e.g. PCR) which has led to increased detection (Birkenheuer et al., 2006; Haber et al., 2007; Brown et al., 2008).

Currently, there is no data on virulence genes for *C. felis*, but four recent studies have examined genetic variation within the internal transcribed spacer (ITS) regions of the ribosomal RNA genes (Brown et al., 2009a, 2009b, 2010; Cohn et al., 2011). In the initial characterization paper, significant spatial correlations and associations with clinical outcome were associated with specific genotypes (Brown et al., 2009a); however, subsequent studies failed to find an association (Brown et al., 2009b, 2010; Cohn et al., 2011). Only a single study has genetically characterized *C. felis* strains from bobcats, but this study was limited to 25 *C. felis* samples from bobcats from Arkansas, Florida, and Georgia (Brown et al., 2010). Of the 11 *C. felis* genotypes detected in bobcats, only three had been previously identified in domestic cats. The current study aims to further understand the sylvatic cycle of *C. felis* and to more robustly characterize the strains of *C. felis* circulating in wild felids. Because bobcats are the natural reservoir, we hypothesize that additional genetic variability will be identified in *C. felis* strains from wild felids and that spatial correlations with genotype will be more easily identified because of the high prevalence of *C. felis* infections in these reservoirs and the lack of movement of wild felids to new geographic regions compared to domestic cat human-associated movement.