Cytauxzoon felis infections are present in bobcats (Lynx rufus) in a region where cytauxzoonosis is not recognized in domestic cats

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Abstract

This study was performed to determine the prevalence of Cytauxzoon felis (C. felis) infections in bobcats (Lynx rufus) from a region where C. felis is recognized in domestic cats, North Carolina (NC), and a region where C. felis is not recognized in domestic cats, Pennsylvania (PA). Samples from NC (n = 32) were obtained post-mortem via cardiac puncture from legally trapped bobcats. Samples from PA (n = 70) were collected post-mortem onto Nobuto blood collecting strips by the PA Game Commission. Each sample was tested using a C. felis specific PCR assay as well as a PCR assay targeting host DNA to rule out the presence of PCR inhibitors. Three samples were excluded due to the presence of PCR inhibitors. Thirty-three percent (10/30) of the samples from NC and 7% (5/69) of the samples from PA tested positive for the presence of C. felis. The proportion of C. felis positive bobcats from NC was significantly different than that from PA (P < 0.005). Despite the lower prevalence of C. felis infections in bobcats from PA this finding is unique and indicates the potential for C. felis infections in domestic cats in the northeastern USA if the appropriate tick vectors are present. Veterinary practitioners in PA should be on alert for cytauxzoonosis in domestic cats. Further studies about the epidemiology and transmission of C. felis infections among both domestic cats and bobcats are needed.

Keywords: Cytauxzoonosis; Piroplasmosis; Cytauxzoon felis; Bobcats; PCR; Tick

1. Introduction

Cytauxzoonosis is an emerging infectious disease of domestic cats in North America caused by the protozoan parasite Cytauxzoon felis (C. felis) (Birkenheuer et al., 2006a). Domestic cats that become infected with C. felis develop a rapidly progressive febrile illness and succumb to multi-organ failure secondary to schizont-laden macrophages occluding small blood vessels. Death occurred in the majority of infected cats reported in the literature (Birkenheuer et al., 2006a; Ferris, 1979; Hoover et al., 1994; Wagner, 1976). Because of this, domestic cats are typically considered to be an accidental host for C. felis. They are presumed to become naturally infected with C. felis via tick vectors and bobcats (Lynx rufus) are considered to be the reservoir host. The American dog tick (Dermacentor variabilis) has been
shown to transmit *C. felis* to domestic cats from bobcats under laboratory conditions and is assumed to be the primary tick vector in nature (Blouin et al., 1984). Unlike in domestic cats, *C. felis* appears to undergo a limited schizogenous phase in bobcats and therefore bobcats exhibit minimal morbidity and mortality as a result of the infection (Blouin et al., 1987; Glenn et al., 1983).

Cytauxzoonosis was first reported in Missouri in 1979 and the disease was only recognized in the south central and southeastern United States for the past 20 years. The geographic distribution of cytauxzoonosis in domestic cats has been recently documented to extend east and northeast of its previously recognized range (Birkenheuer et al., 2006a; Jackson and Fisher, 2006). Since cytauxzoonosis is not a difficult disease to diagnose, it seems more likely that these infections represent a true expansion of the disease distribution rather than a new recognition of a disease that has been historically present in these regions at low levels.

Even with this expanded distribution, cytauxzoonosis in domestic cats does not appear to correlate with the natural distribution of the presumed reservoir host, *L. rufus*, which includes most of North America and extends as far south as southern Mexico (Long, 2003). The cause or causes for this discrepancy are unknown. Some possibilities include a lack of *C. felis* in bobcats throughout their entire range or the absence of competent vectors that can transmit *C. felis* from bobcats to domestic cats in areas where cytauxzoonosis is not recognized. Studies investigating the prevalence of *C. felis* in bobcats are limited to the southcentral region of the US. These studies found that *C. felis* could be detected by examination of stained blood smears in 30–50% of wild-trapped bobcats (Glenn et al., 1982; Kocan et al., 1985). To the authors’ knowledge, studies examining the prevalence of *C. felis* infections in bobcats from areas of the US where cytauxzoonosis is not recognized in domestic cats have not been performed. The purpose of this study was to determine and compare the proportion of bobcats that were infected with *C. felis* from a region where cytauxzoonosis is recognized in domestic cats to that where it is not recognized.

2. Materials and methods

Bobcats were sampled from a state where cytauxzoonosis is recognized in domestic cats, North Carolina (NC) and another state where cytauxzoonosis in domestic cats has never been reported, Pennsylvania (PA). All bobcats were legally trapped by licensed personnel. All samples were obtained post-mortem and the carcasses were either fresh or had been frozen at −20 °C and thawed immediately prior to sample collection. Blood samples from NC (*n* = 32) were obtained via cardiac puncture. All samples were frozen at −20 °C until processing. Total deoxyribonucleic acid (DNA) was extracted from 100 μg of clotted whole blood samples using a commercially available kit (QIAamp DNA blood mini kit, Qiagen, Valencia, CA). Blood samples from bobcats from PA (*n* = 70) were collected directly onto Nobuto blood collecting strips (Advantec, Toyo Roshi Kaisha, Japan) as previously described (Mucker et al., 2006). Samples from PA were collected between October and February of 2002. Samples from NC were collected in January and February during 2004, 2005 and 2006. Total DNA was extracted from dried blood strips using a commercially available kit (DNeasy tissue kit, Qiagen, Valencia, CA).

Each sample was tested using a *C. felis* specific polymerase chain reaction assay designed to amplify a 284 base pair fragment of the 18S rRNA gene that is able to detect as few as ten copies of the target DNA per microliter of sample with 100% sensitivity (Birkenheuer et al., 2006b). The assay did not produce amplicons when feline DNA, Babesia canis vogeli, *B. c. canis*, *B. c. rossi*, *B. gibsoni* (Asian genotype), *B. gibsoni* (CA genotype), *B. annae*, *Ehrlichia chani*, *E. ewingii*, *E. chafeensis*, Neorickettsia risticii, Anaplasma phagocytophilum, *A. platys*, Mycoplasma haemofelis, *M. haemominutum*, Rickettsia rickettsii, Leishmania infantum, Bartonella henselae or Bartonella vinsonii ssp. berkoffii were used as templates (Birkenheuer et al., 2006b). Positive controls consisted of DNA extracted from the blood of *C. felis* infected domestic cats. The *C. felis* 18S rRNA genes from these positive control cats have been characterized previously (Birkenheuer et al., 2006b). Negative (i.e. no DNA) water controls were included with each amplification run. To demonstrate that the extracted DNA was amenable to PCR testing, each sample was tested using an assay designed to amplify a fragment of a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) pseudogene from most mammals as previously described (Birkenheuer et al., 2003). Amplicons were visualized by ethidium bromide staining and ultraviolet trans-illumination after electrophoresis in a 2% agarose gel. Randomly selected amplicons (*n* = 5) were purified using a commercially available kit (Qiagreen PCR purification kit, Qiagen, Valencia, CA) and sequenced directly (Davis Sequencing, Davis, CA). These sequences were aligned with *C. felis* 18S rRNA sequences obtained from domestic cats with cytauxzoonosis (ClustalW, http://www.mbio.ncsu.edu/BioE-
dit/bioedit.html). The proportions of *C. felis* infected bobcats from each region were compared using a Fisher’s exact test.

3. Results

Three samples, two from NC and one from PA, had to be excluded from the analyses because the GAPDH pseudogene fragment could not be amplified. *C. felis* specific amplicons were detected in 33% (10/30) of the bobcat samples from NC, the region where cytauxzoonosis is recognized in domestic cats. In contrast, *C. felis* DNA was detected by PCR from only 7% (5/69) of the bobcat samples from PA, the region where domestic cat cytauxzoonosis is not recognized. Amplicons were not detected in the negative controls (i.e. no DNA) throughout the study. The sequences (*n* = 5) of the randomly selected amplicons, three from PA and two

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Fig. 1. (A) The approximate distribution of bobcats (*Lynx rufus*) in the United States (shaded area) (Long, 2003). (B) The states in which cytauxzoonosis has been documented in domestic cats (shaded area) (Birkenheuer et al., 2006a; Greene, 2006; Jackson and Fisher, 2006). (C) The approximate distribution of *Dermacentor variabilis* in the United States (shaded area) (redrawn based on the distributions reported by the centers for disease control: http://www.cdc.gov). (D) The approximate distribution of *Amblyomma americanum* in the United States (shaded area) (redrawn based on the distributions reported by the centers for disease control: http://www.cdc.gov). (E) The approximate distribution of *Ixodes scapularis* in the United States (shaded area) (redrawn based on the distributions reported by the centers for disease control: http://www.cdc.gov).
from NC, were 100% identical to the *C. felis* 18S rRNA sequences from North America deposited in GenBank (Accession numbers L19080, AY679105, AY531524, and AF399930). The proportion of *C. felis* infected bobcats from PA was significantly lower ($P < 0.005$) than that of bobcats from NC.

4. Discussion

The geographic distribution of bobcats (Fig. 1A) does not correlate well with recognized distribution of cytauxzoonosis in domestic cats (Fig. 1B). The findings of this study suggest that the lack of recognition of cytauxzoonosis in domestic cats in PA is not due to the absence of *C. felis* infections in bobcats in that state. Assuming that the prevalence of *C. felis* infections in the PA bobcats tested in this study is representative of the prevalence of *C. felis* in all PA bobcats, this could contribute to a decreased reservoir capacity with a subsequent lower prevalence, or absence, of cytauxzoonosis in domestic cats. The possibility that *C. felis* infections in PA bobcats are a recent occurrence and that cytauxzoonosis in domestic cats may occur there in the future must also be considered. Ultimately, the presence of *C. felis* infections in PA bobcats mandates that veterinary clinicians in PA should be vigilant for cytauxzoonosis in domestic cats.

The presence of *C. felis* in PA bobcats along with the apparent absence of cytauxzoonosis in PA domestic cats may suggest that the routes of *C. felis* infection among bobcats and domestic cats differ. Perhaps similar to other piroplasms, *C. felis* infections can be transmitted transplacentally and can be perpetuated in bobcats in the absence of a tick vector. Alternatively intra-species and inter-species transmission of *C. felis* may occur via different tick species. While *D. variabilis* has been demonstrated to have vector competence for both inter- and intra-species transmission of *C. felis* from bobcats (*Blouin et al.*, 1984, 1987), the geographic distribution of *D. variabilis* (Fig. 1C) does not appear to correlate well with the recognized distribution of cytauxzoonosis in domestic cats. In fact, the distribution of cytauxzoonosis in domestic cats appears to correspond best with the distribution of the lonestar tick (*Amblyomma americanum*) (Fig. 1D) and the black-legged tick (*Ixodes scapularis*) (Fig. 1E). In North Carolina, *A. americanum* is frequently detected feeding on domestic cats that have access to outdoors (Birkenheuer, unpublished data). Potential vector competence of *A. americanum* is at least minimally supported by the detection of *C. felis* DNA by PCR from *A. americanum* ticks (*Bondy et al.*, 2005). Based on these findings, further studies are indicated evaluating the vector competence and vectorial capacity of *A. americanum* and perhaps *I. scapularis* for the transmission of *C. felis*.

This study represents the first to screen large numbers of bobcats for *C. felis* infections by PCR, and the first to screen bobcats from an area where cytauxzoonosis is not recognized in domestic cats. Very little is known about the epidemiology of *C. felis* infections and further studies are clearly indicated. Studies evaluating the reservoir competence and capacity of bobcats as well as the vector competence and capacity of several ixodid tick species for the maintenance and transmission of *C. felis* infections are needed.

References


