Evaluation of *Cytauxzoon felis* infection status in captive-born wild felids housed in an area endemic for the pathogen

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**Objective**—To determine whether apparently healthy captive-born wild felids that were not native to North America and were housed in an area endemic for *Cytauxzoon felis* harbored the pathogen.

**Design**—Prospective observational case series.

**Animals**—11 captive-born wild felids that were 1 bobcat (*Lynx rufus*) and 1 cougar (*Puma concolor*) or were not 1 lion (*Panthera leo*) and 8 tigers (*Panthera tigris*) native to North America and 6 domestic cats (6 pets and 1 feral).

**Procedures**—Blood was collected, and a PCR assay for *C. felis* was performed. The *C. felis* 18S rRNA gene sequence was characterized in samples that tested positive. Blood smears were evaluated microscopically for intraerythrocytic organisms consistent with *C. felis*. Blood smears from an additional 6 feral domestic cats found dead on the study premises were also evaluated.

**Results**—4 tigers and 6 domestic cats without clinical signs of disease tested positive for *C. felis* infection via PCR assay; intraerythrocytic organisms consistent with *C. felis* were identified in smears from 1 *C. felis*-infected tiger (which also had azotemia) and in smears from 11 of 12 domestic cats. Possible intraerythrocytic inclusions were identified in 1 tiger that tested negative for *C. felis*. Sequences of *C. felis* 18S rRNA amplicons from all infected tigers shared >99.8% identity with reported *C. felis* sequences from North American domestic cats and were identical to amplicons from domestic cats on the premises.

**Conclusions and Clinical Relevance**—Captive tigers without clinical signs of disease tested positive for *C. felis*. The PCR assay for *C. felis* appeared to be more reliable than cytologic detection of piroplasms in tigers. (J Am Vet Med Assoc 2012;241:1088–1092)

*Cytauxzoon felis* is a hemoprotozoan parasite of wild and domestic felids that is transmitted by the bite of a tick vector.1 Cytauxzoonosis develops due to the initial schizogonous phase of parasite replication in mononuclear cells, which causes widespread occlusion of blood vessels and a profound systemic inflammatory response.2–3 Mononuclear cells eventually become distended with organisms and rupture, releasing merzoites. Merzoites are then taken up by erythrocytes; the erythrocytic parasites are referred to as piroplasms.3 Felids that survive the schizogonous phase of infection generally remain healthy despite persistence of piroplasms for months to years.4–6 Bobcats (*Lynx rufus*), the primary reservoir hosts, are believed to only develop a brief illness prior to entering a persistent carrier state. In contrast, many domestic cats develop a profound illness that often results in death.6–7 Although related *Cytauxzoon* spp have been reported in Asia and Europe, infections with *C. felis* are largely limited to the Americas.12–19 In the United States, the disease in domestic cats is recognized predominantly in the south central, southeastern, and Atlantic states, although the pathogen has been reported to infect bobcats in states outside these regions, including North Dakota and Pennsylvania.16,20,21 Bobcats and possibly cougars (*Puma concolor*), which are native to the Americas, appear to be the predominant reservoir hosts in the United States.21–23 Whereas cytauxzoonosis is often mild in wild felids native to the Americas, severe morbidity (similar to that described in domestic cats) and death have been reported in felids that are not native to the Americas. Fatal cytauxzoonosis has been reported in 2 captive-reared Asian tigers (*Panthera tigris*) and an African lioness (*Panthera leo*) and her cub.23–25

Staff members at a wildlife refuge located in Eureka Springs, Ark, contacted the investigators after several tigers died over a period of years due to presumed or necropsy-confirmed cytauxzoonosis. The facility houses >120 captive wild felids, most of which are tigers, but also includes several lions, leopards, cougars, bobcats, and other species. All the wild felids housed at the facility were captive-born animals obtained after prior owners became unable or unwilling to care for them. The purpose of the study reported here was to determine whether apparently healthy captive-born...
Materials and Methods

Animals—A convenience sample of 17 felids was included in the study. Eleven captive-born wild felids comprised 1 cougar (a 15-year-old spayed female), housed on the study premises for 4 years), 1 bobcat (a 4-year-old castrated male, housed on the premises for 3.5 years), 1 lion (a 15-year-old spayed female, housed on the premises for 14 years), and 8 tigers (2 sexually intact males, 2 females, 2 sexually intact males, and 4 castrated males; age range, 11.5 to 15.5 years). One tiger had been housed on the premises for 2.8 years, and the remainder of these had been on the premises for 9 to 15.5 years. These animals were housed in groups of 2 or 3 in large (from 8,000 to 20,000 square feet) outdoor enclosures at a facility in a rural wooded area of northern Arkansas. Staff zoologists determined the animals' diets and general husbandry practices and observed each animal several times daily. The premises were regularly treated with carbaryl powder to minimize the animals' exposure to ectoparasites.

Domestic cats included 5 mixed-breed pet cats (3 castrated males and 2 spayed females of unknown age [all young to middle-aged adults]) that were kept on the premises for 1 to 3 years with indoor and outdoor access. One feral domestic cat, an adult sexually intact female that was trapped on the premises during the study period, was also included in the study after it was donated to the investigators in place of planned euthanasia. Owners of the pet cats provided informed consent prior to blood sample collection. Zoologists at the wildlife facility additionally obtained postmortem blood samples from 6 feral domestic cats that had been killed by captive wild felids after entering their enclosures and prepared unstained blood smears for cytologic evaluation.

Blood sample collection—All captive wild felids were anesthetized for veterinary examination and care at the time of sample collection. Six of the 8 tigers and the remaining 3 wild felids were apparently healthy (on the basis of appetite and activity) when samples were obtained; however, 2 tigers had been anorectic and lethargic for several days prior to examination and were anesthetized for diagnostic evaluation by the facility's regular veterinarian. Food was withheld from apparently healthy animals overnight (approx 15 hours) before anesthetic induction to facilitate safe handling. Anesthesia was induced via IM injection of ketamine hydrochloride and xylazine hydrochloride. Reported doses were 1.000 mg of ketamine and 100 mg of xylazine (tigers), 700 mg of ketamine and 70 mg of xylazine (lion), 350 mg of ketamine and 35 mg of xylazine (cougar), and 60 mg of ketamine and 6 mg of xylazine (bobcat); dose on a per-weight basis was estimated at 4.4 mg of ketamine/kg (2 mg/lb) and 0.44 mg of xylazine/kg (0.2 mg/lb) for all wild felids (these animals were not weighed). During the anesthetic episode, each animal underwent a complete physical examination and blood (16 mL) was collected via venipuncture of a medial saphenous vein into serum separator and EDTA-containing tubes.

Blood samples (3 mL) were collected from 5 unanesthetized pet cats via jugular venipuncture into EDTA-containing tubes. An identical sample was obtained from the feral cat after administration of ketamine (5.0 mg/kg [2.3 mg/lb]) and xylazine (0.5 mg/kg [0.23 mg/lb]) IM. The 5 pet cats and the feral cat that was captured alive were apparently healthy at the time of sample collection. Health status of the 6 feral cats that died on the study premises was unknown.

Blood sample analysis—Blood in serum separator tubes was used to prepare smears and to perform a CBC. Blood in serum separator tubes was used to collect serum for biochemical analysis for wild felids. Samples were stored at approximately 22°C until analyzed. The CBC and serum biochemical analysis were performed with in-house laboratory equipment within 12 hours after blood collection. Prepared blood smears were stained with Wright-Giemsa stain in a routine manner and were reviewed by a board-certified clinical pathologist (MSW) who was unaware of animal identification and results of PCR analysis.

Total DNA was extracted from anticoagulated blood and analyzed via PCR assay according to a published method with minor modifications. Briefly, each reaction consisted of 12.5 µL of a PCR master mix, 7 µL of DNAase-free water, 50 pmol of each oligonucleotide primer (forward, 5′-GGCAATGGCATT-GCTTTATGCT-3′; reverse, 5′-CAATTGATACCTCCGG-GAAAGAG-3′), and 5 µL of the DNA sample. Thermal cycling conditions were as follows: 98°C for 30 seconds, followed by 45 cycles at 95°C for 5 seconds and 68°C for 5 seconds. Melting curve analysis was initiated at 75°C, and data were captured at increasing increments of 0.5°C for 30 time points. For each PCR assay, positive (DNA extracted from whole blood samples from domestic cats naturally infected with *C. felis*) and negative (no DNA template) controls were used. Standard precautions were used to prevent amplicon carryover among samples. Samples were considered to be positive for the presence of *C. felis* DNA if the melting temperature of the amplicon matched that of the positive control sample.

To further characterize samples that tested positive for *C. felis*, a PCR assay that amplified a nearly full-length portion of the 18S rRNA gene sequence in multiple Apicomplexan species was used to characterize the 18S sequences from *C. felis*-infected blood samples. Selected sequences were 5′-GGTGAATCCGAG-3′ and 5′-AACCTGTAGATGCT-3′ for the forward and reverse primers, respectively. Each 50-µL reaction contained 1 µL of DNA template, 50 pmol of each primer, 10 nmol of deoxynucleotide triphosphates, 75 nmol of MgCl₂, 3.75 µL of DNA polymerase, and a 1X concentration of PCR buffer. Optimized thermal cycling conditions consisted of an initial denaturation at 94°C for 5 minutes, followed by 50 amplification cycles (94°C for 20 seconds, 56°C for 30 seconds, and 68°C for 3.5 minutes) and a final extension at 72°C for 7 minutes. Positive controls consisted of DNA extracted from whole blood from dogs naturally infected with *C. felis*.
A nearly full-length 18S rRNA amplicon of the expected 1,741-base pairs size was amplified from all 4 *C. felis*-positive samples from tigers and from all 6 domestic cats. The DNA sequences (excluding unreadable sequence at ends of amplicons) of these 4 amplicons shared > 99.8% identity (1,665 to 1,668 bp) with reported 18S rRNA gene sequences from North American felids infected with *C. felis*. Comparison with 4 published full-length 18S rRNA sequences of organisms isolated from domestic cats in GenBank (accession Nos. AY679105, AF399930, AY331524, and L19080) revealed secondary peaks at position 256 (position number determined on the basis of sequence AY679105) in samples from all 4 infected tigers and all 6 domestic cats, with both cytosine and thymine detected in that position, whereas the published sequences indicated thymine in this position. In some samples, cytosine appeared dominant in the chromatogram, and in others, thymine appeared dominant. One of the 4 reported sequences (AY331524) had cytosine at position 1,585, compared with thymine in this position for other reported sequences and for all positive samples from tigers and domestic cats in the present study. For another reported sequence (L19080), 2 nucleotides at positions 109 and 1,638 were unresolved, but both positions held thymine in the other reported sequences and in all wild and domestic felids of the present study.

Results of physical examination were considered normal for all live domestic cats. The only abnormality detected via CBC from any domestic cat was neutropenia in 1 pet cat (1.73 × 10^9 neutrophils/µL; reference interval, 2.5 × 10^9 neutrophils/µL to 12.5 × 10^9 neutrophils/µL). Evidence of *C. felis* infection was found in most of the domestic cats evaluated. Piroplasms were identified during microscopic evaluation of blood smears from the feral domestic cat trapped on the premises, from 5 of 5 pet cats, and from 5 of the 6 feral domestic cats that died on the premises. Results of the PCR assay were positive for *C. felis* in blood samples collected from the 5 pet cats and the live feral cat, and *C. felis* 18S rRNA gene sequencing results were identical to those reported for *C. felis*-infected tigers in the study. The donated feral cat remained healthy for the next 23 months, until euthanasia for reasons not related to *C. felis* infection. All pet cats were healthy and persistently parasitemic 32 months after original testing.

**Discussion**

*Cytauxzoon felis* infection has been reported in small numbers of captive tigers and lions that died of the disease.~23~25~ In the study reported here, 4 tigers without clinical signs of disease tested positive for *C. felis* infection via PCR assay. This finding indicates that tigers, similar to Felidae species that are native to the Americas, may harbor subclinical infection with this pathogen.

Until recently, only wild felids native to the Americas were believed to be capable of becoming reservoir hosts for *C. felis* because infection with this pathogen was reported to be fatal in domestic cats and in the rare instances of infection in tigers and lions.~6,3–5,26,29~ During the past decade, it has become clear that some domestic cats survive *C. felis* infection and can be competent pathogen reservoirs, although the importance of
domestic cats in the natural transmission of C felis has yet to be determined.1,30 Although the present study did not investigate the ability of tigers to become competent reservoir hosts, the finding that 4 of 8 tigers had been infected with no history of an acute febrile illness certainly suggests that this species can survive acute infection and develop a nonclinical carrier state similar to that described in bobcats. Although considered less likely, it is possible that the tigers evaluated in the present study may not have incurred a schizogonous phase of infection and instead may have been infected via direct blood inoculation, perhaps when infected domestic cats were killed by the tigers.

The tigers in the present study were housed in a heavily wooded rural area in northern Arkansas. It has been suggested that a strain of C felis less virulent to domestic cats may exist in this region because a number of cats from the geographic region have been reported as having chronic infection without a history of acute febrile disease.3,4 In the present study, 3 pet cats housed on the study premises and allowed access to the outdoors tested positive for C felis infection via blood smear examination and PCR assay, and none had any history of illness. Additionally, piroplasms were identified in postmortem blood smears prepared from 5 of 6 feral domestic cats that died on the premises. Although no history was available for these feral cats, it is unlikely that they were acutely ill, considering that they were apparently foraging for food when they were killed. Another feral cat caught in a live trap on the same premises was apparently healthy despite identification of piroplasms in a blood smear and a positive result for the C felis PCR assay.

It cannot be definitively determined whether the tigers, which came from various locations throughout the United States, acquired C felis infection at the wildlife facility where they were evaluated, but we consider this likely. The sanctuary is located in a heavily wooded area where cytauxzoonosis is considered common in domestic cats, and all of the tigers had been housed there for years prior to testing. Ticks are occasionally noticed on the tigers despite routine application of carbaryl to the enclosures. Although ticks were not detected at the time of examination and blood collection, most samples were collected from tigers during colder weather when the predominant tick vector, Amblyomma americanum, is less likely to be active.1 Additionally, ticks present on the tigers may simply have been overlooked because these animals are large and have thick coats.

The PCR analysis was more reliable for detection of C felis in the tigers than was routine microscopic examination of Wright-Giemsa–stained blood smears. Interestingly, the only tiger in which piroplasms were definitively identified had concurrent chronic renal disease. It is possible that concurrent illness permitted a greater degree of parasitemia, compared with other tigers that tested positive via PCR assay but had no piroplasms detected in blood smears, but it is also possible that this was simply coincidence. Rare inclusions were identified in the blood smear of the tiger that had poymera and tested negative for C felis infection via PCR assay. We believe it is more likely that these inclusions represented a staining artifact or a morphological RBC change than that the PCR result was falsely negative. Unlike the findings in tigers, piroplasms were seen in RBCs of blood smears from all 5 infected pet cats as well as 6 of 7 feral cats.

Molecular techniques are necessary to distinguish between closely related Cytauxzoon spp because morphological observation of inclusions is inadequate to the task. In South America, pumas, ocelots (Leopardus pardinus), jaguars (Panthera onca), and oncillas (Leopar dus tigrinus) native to the continent as well as domestic cats have been reported to be chronic carriers of a Cytauxzoon-like parasite.3,9,31 Given that the 18S rRNA sequences from the South American wild felids share lower sequence identity (98%) with North American C felis strains, these organisms may be distinct from the North American strain of C felis.31 Further, Cytauxzoon manul and a Cytauxzoon sp organism isolated from Iberian lynx (Lynx pardinus) and from domestic cats in Spain were reported to appear morphologically identical to C felis in blood smear examinations.15,32,33 Babesia spp in felines found predominantly in Africa also appear morphologically similar to C felis.33 In the present study, results of PCR analysis of the 18S RNA gene from parasites obtained from infected tigers indicated that the agent was the North American strain of C felis.

The C felis 18S gene sequences amplified from tigers and domestic cats in the present study were nearly identical to the only 4 full-length C felis sequences in GenBank. We did identify secondary nucleotide peaks at 1 position (corresponding with position 256 on GenBank accession No. AY679103.1); both cytosome and thymine nucleotides were detected in this position. Otherwise, sequences of C felis from tigers and domestic cats in the present study were identical to each other and nearly identical to the 4 published sequences. The biologic importance of variation at this position is unknown, but this may represent microheterogeneity among rRNA operons in the C felis genome. Alternatively, this could represent a polymorphic site indicative of simple genetic diversity within the C felis population. The identical sequences of the 18S RNA gene in domestic cats and tigers on the same premises appear to support the idea of a common source of C felis infection at the facility.

Results of the present study showed that apparently healthy captive tigers can harbor C felis without clinical signs of cytauxzoonosis. Because only 1 lion was tested, we could not determine whether this species can also maintain nonclinical infection with this pathogen. However, chronic, subclinical C felis infections have been identified in domestic cats and in wild felids that are native or nonnative to the Americas.3,8,22,25,31 Screening via PCR assay of any captive wild felids coming from or being moved to a region in which C felis is endemic may be warranted. Identification of carriers could help prevent misdiagnosis of subsequent concurrent disease in these cats as cytauxzoonosis, a mistake that might otherwise be made on the basis of recognition of piroplasms in peripheral blood smears or after amplification of C felis DNA via PCR assay. Although captive wild felids would not be important reservoirs of disease for domestic cats in North America, they might
serve as reservoirs for other felids in zoos or in wildlife sanctuaries. It seems appropriate that the staff of such facilities should exercise precautions, including tick control measures, in an effort to minimize the risk of exposing captive wild felids to tick-borne diseases. Additionally, limiting contact with free-ranging wild felids and with domestic cats seems prudent.

a. Turpentine Creek Wildlife Refuge, Eureka Springs, Ariz.

b. Sevin Dust, GardenTech, Lexington, Ky.

c. IDEXX LaserCyte Hematology Analyzer, IDEXX Laboratories, Westbrook, Me.

d. IDEXX VetTest Chemistry Analyzer, IDEXX Laboratories, Westbrook, Me.

e. SsoFast, EvaGreen, Supermix Bio-Rad, Hercules, Calif.

f. Integrated DNA Technologies Inc, Coralville, Iowa.

g. North Carolina State University-Vector Borne Disease Diagnostic Laboratory, Raleigh, NC.

h. Applied Biosystems, Carlsbad, Calif.

i. Techne Inc, Burlington, NJ.


k. Seakem GTG agarose, Lonza Rockland Inc, Rockland, Me.

l. QIAquick PCR purification kit, Qiagen Inc, Valencia, Calif.

m. MCLAB, South San Francisco, Calif.

n. ClustalW (Full Multiple Alignment Default Settings) BioEdit Sequence Alignment Editor, North Carolina State University, Raleigh, NC.

References


