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Prevalence of *Cytauxzoon felis* infection in healthy free-roaming cats in north-central Oklahoma and central Iowa

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Abstract

Case summary Cytauxzoonosis is a tick-borne disease of cats, and Oklahoma (OK), USA, is considered an enzootic state. To determine the prevalence of *Cytauxzoon felis*, blood was collected from free-roaming cats, as they are frequently exposed to tick vectors. Our objective was to determine the prevalence of *C felis* infection in free-roaming cats in north-central Oklahoma and central Iowa (IA). Infection with *C felis* was determined using DNA extracted from blood and PCR amplification. Blood was collected from 380 free-roaming cats between January and April in 2014 in OK. DNA from *C felis* was detected in 3/380 (0.8%; 95% confidential interval [CI] 0.22–2.3%). In IA, 292 blood samples were collected between 2012 and 2014. No *C felis*-infected cats were detected (0; 95% CI 0–0%).

Relevance and novel information The prevalence of *C felis* (0.8%) in north-central OK reported herein was lower than the previously reported 3.4% in domestic cats in OK. Our study supports that the prevalence in a given enzootic area can vary by location and from the pool of cats sampled. None of 291 (0%) cats were infected with *C felis* in central IA. To date, only one case of cytauxzoonosis in a domestic cat has been reported in IA. It is important to monitor cats for *C felis* infections in northern US states, as geographic distribution of *Amblyomma americanum* expands northward. As free-roaming cats have more contact with the tick vectors of *C felis*, this population allows us to monitor the expansion of *C felis* distribution.

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Introduction

Cytauxzoon felis is a tick-transmitted protozoan parasite that can cause cytauxzoonosis in wild and domestic felids. Transmission of *C felis* has been demonstrated with *Amblyomma americanum* and *Dermacentor variabilis*.[1–3] Cytauxzoonosis in domestic cats has been reported throughout central, south-eastern and south-central USA.[4,5] Oklahoma is considered enzootic for *C felis* and Iowa (IA) is a non-enzootic state but borders other enzootic states.[6] Domestic cats infected with *C felis* often show severe, acute clinical signs characterized by fever, inappetence, anorexia, dyspnea and icterus. Onset of disease typically occurs 10–14 days after *C felis*-infected ticks feed on naive cats and progresses quickly, with fatalities reported 1–7 days after onset of clinical signs. A recent study demonstrated 60% survival in *C felis*-infected cats that received a combination therapy of azithromycin and atovaquone with supportive care.[7]

A free-roaming cat is a domestic cat that has been born and raised without or limited contact to humans and is unsocialized. As free-roaming cats live outdoors

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and are exposed to ticks, they are favorable populations in which to examine and monitor the distribution and range of cytauxzoonosis. Our objective was to determine the prevalence of *C felis* infections in free-roaming cats in enzootic north-central Oklahoma (OK) and non-enzootic central IA.

Materials and methods

Blood samples were collected from free-roaming cats in Stillwater, OK, and Ames, IA, as part of community trap–neuter–return programs. Cats were trapped mainly in north-central OK and in central IA. In OK, all cats were sedated with a mixture of tiletamine hydrochloride and zolazepam hydrochloride (Telazol; Zoetis), ketamine hydrochloride (Ketamine; Putney) and xylazine (AnaSed; Akorn). In IA, a mixture of ketamine hydrochloride (Ketamine; Putney), dexmedetomidine hydrochloride (Precedex; Orion Pharma), buprenorphine (Simbadol; Zoetis) and butorphanol tartrate (Torbugesic-SA; Zoetis) was used for sedation in 2012–2013. In 2014, butorphanol tartrate (Torbugesic-SA; Zoetis) was added to the mixture but only for fractious cats. The approximate ages of cats were determined based on dentition,[8] and cats that were 4–6 months or older were selected for blood collection to increase the chance of finding *C felis*-infected cats. Cats were placed in dorsal recumbency, fur around the neck was clipped and 70% isopropyl was sprayed in the area. Approximately 1 ml blood was collected from the jugular vein and was immediately placed in an EDTA collection tube.

Genomic DNA was extracted from peripheral whole blood samples using GeneJET Whole Blood DNA Purification Mini Kit (Thermo Scientific). Briefly, 200 µl whole blood was mixed with 20 µl proteinase K solution and 400 µl lysis solution. After incubating the sample at 56°C for 10 mins, 200 µl ethanol (96–100%) was added into the sample. The prepared mixture was transferred to the spin column and centrifuged for 1 min at 6000 × *g* (~8000 rpm). The column was then washed with 500 µl Wash Buffer I and centrifuged again for 1 min at 8000 × *g* (~10,000 rpm). The column was then washed with Wash Buffer II and centrifuged for 3 mins at 20,000 × *g* (14,000 rpm). DNA was extracted with 200 µl preheated (approximately 56°C) PCR-quality water added to the center of the column membrane to elute genomic DNA. The sample was incubated for 2 mins at room temperature and centrifuged for 1 min at 8000 × *g* (~10,000 rpm).

A conventional PCR was performed to amplify the *C felis* small subunit rRNA (ssu-rRNA). A nested PCR amplification was performed using primers (CfnestF. 5′-TCGCATTGCTTTATGCTGGCGATC-3′ and CfnestR. 5′-GCCCTCCAATTGATACTCCGGAAA-3′) that amplify 289 bp of the 18S rRNA gene of *C felis*.[2] Cycling conditions of nested PCR were as follows: denaturation at 95ºC for 5 mins, annealing at 54°C for 1 min and extension at 72°C for 1 min. The cycle was repeated 34 times. To determine success of genomic DNA extraction, PCR was performed for the gene encoding the ssu-rRNA.[9] PCR products were separated on 1.75% agarose gel and viewed with ultraviolet light. Positive control templates consisted of DNA extracted from whole blood of a cat that died from an infection of *C felis*, whereas negative control reactions used DNA isolated from purified water. PCR products were purified using A QIAquick PCR purification kit (QIAGEN) and amplicons were sequenced by Eurofin Genomics (Huntsville, AL). Sequences from infected cats were compared using BLAST in GenBank.

The prevalence of *C felis* was calculated according to Bush et al.[10] Ninety-five percent confidence intervals (CIs) were calculated according to Sterne's exact method, using Quantitative Parasitology 3.0.[11,12]

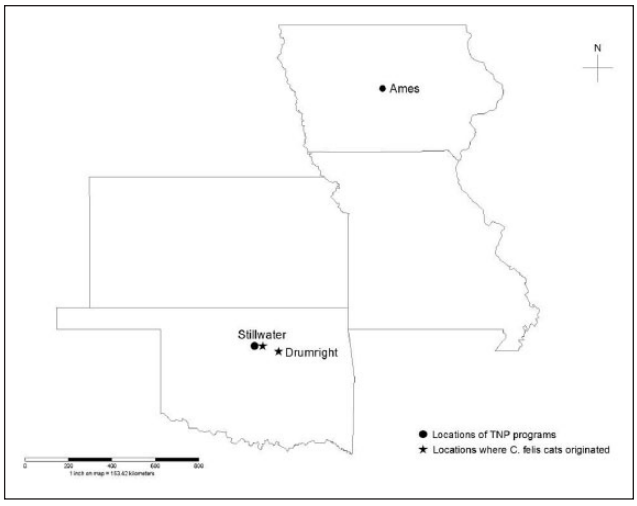

Figure 1 Trap–neuter–return (TNR) programs were conducted in Stillwater, OK, and Ames, IA. Cats infected with *Cytauxzoon felis* were found in Stillwater and Drumright, OK

Results

A total of 380 blood samples were collected from January to May 2014 in OK. Three of 380 (0.8%; 95% CI 0.22–2.3%) samples showed approximately 250 bp bands, which were targeted product size for *C felis*. BLAST comparison of these three sequences showed 100% identity to *C felis* (eg, L19080, AY531524, AY679105, AF399930, GU903911). All *C felis*-infected cats were male and older than 12 months of age. Two infected cats were from Stillwater, OK, and caught separately in March and April respectively. One *C felis*-infected cat originated from Drumright, OK, in March (Figure 1). A total of 292 blood samples were collected from August 2012 to April 2014 in IA. Genomic DNA extraction from 1/292 blood samples was not successful. There were no positive samples (0%; 95% CI 0–0%).

Discussion

The prevalence of *C felis* (0.8%) in north-central OK found in the current study was lower than the previously reported 3.4% in domestic cats in OK.[6] Rizzi et al reported a difference in prevalence of *C felis* infection depending on geographic locations within OK; 13/77 (16.9%) cats were infected with *C felis* in eastern OK, while 10/602 (1.7%) cats were infected with *C felis* in north-central OK.[6] Rizzi et al postulated differences in the prevalence of *C felis* within OK could include strain variation in virulence of *C felis*, differences in immunologic responses of cats to infection with *C felis* and differences in *C felis* inoculation from ticks. Three different genotypes of *C felis*, ribosomal internal transcribed spacer regions (ITS) A, B and C, have been demonstrated, and ITSB and ITSC seem to be more pathogenic with a higher mortality rate than ITSA.[13] Although it has not been established which genotype is more prevalent or pathogenic than others in OK, genotypic variations could influence prevalence because cats infected with the more pathogenic genotype are likely to succumb to infection. As cats become infected with *C felis* through tick bites, prevalence of *C felis* infection in domestic cats is likely affected by geographic variation of the abundance and activity of ticks and reservoir hosts. Our study supports the report of Rizzi et al,[6] which demonstrated that the prevalence of *C felis* in a given enzootic area can vary from location to location and from the population of cats sampled. DNA of *C felis* was not detected in 291 blood samples collected from free-roaming cats in central IA, a non-enzootic state that borders enzootic states. One case of *C felis* infection in a domestic cat has been reported in south-western IA along the Missouri river.[14]

Unfortunately, this report did not provide details regarding travel history, age, or sex of the infected cat. In our study, blood samples were collected from relatively young cats, and that might have influenced the results as those cats had experienced less time being exposed to tick vectors. As the geographic distribution and range of *A americanum* expands northwards,[15] it is important to keep monitoring free-roaming cat populations where cytauxzoonosis has not been considered enzootic.

Conclusions

Epizootiology of *C felis* has not been completely elucidated. Historically, only bobcats were thought to be reservoirs for *C felis*; however, reports have indicated domestic cats that survive acute cytauxzoonosis become chronically infected with *C felis* and can also serve as a source of *C felis* infection.[1,2,6,16–20] As free-roaming outdoor cats encounter ticks, the source of *C felis* infections, they are an excellent population to monitor the expansion of *C felis* distribution in the USA.

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