



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

Authors: Nagamori, Yoko, Slovak, Jennifer E, and Reichard, Mason V

Source: Journal of Feline Medicine and Surgery Open Reports, 2(1)

Published By: SAGE Publishing

URL: <https://doi.org/10.1177/2055116916655174>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.



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

Journal of Feline Medicine and Surgery
Open Reports

1–4

© The Author(s) 2016

Reprints and permissions:

sagepub.co.uk/journalsPermissions.nav

DOI: 10.1177/2055116916655174

jfmsopenreports.com

This paper was handled and processed by the American Editorial Office (AAFP) for publication in JFMS Open Reports



Yoko Nagamori¹, Jennifer E Slovak² and Mason V Reichard¹

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.

Accepted: 21 May 2016

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

¹Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA

²Department of Veterinary Clinical Sciences, Washington State University, Pullman, WA, USA

Corresponding author:

Yoko Nagamori DVM, Clinical Assistant Professor, Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, 250 McElroy Hall, Stillwater, OK 74078, USA
Email: yokon@okstate.edu



Creative Commons Non Commercial CC-BY-NC: This article is distributed under the terms of the Creative Commons

Attribution-NonCommercial 3.0 License (<http://www.creativecommons.org/licenses/by-nc/3.0/>) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).

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 95°C for 5 mins, annealing at 54°C for 1 min and

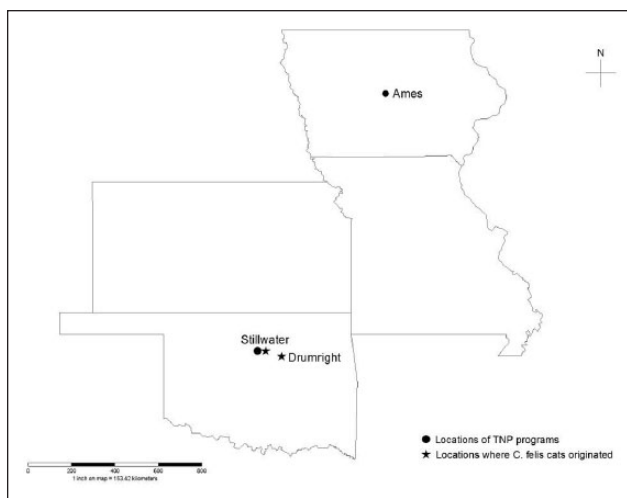


Figure 1 Trap–neuter–return (TNR) programs were conducted in Stillwater, OK, and Ames, IA. Cats infected with *Cytauxzoon felis* were found in Stillwater and Drumright, OK

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}

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.

Acknowledgements We would like to thank Dr Jennifer E Thomas, Jaime Goolsby and Zaria Vick for helping us to collect blood samples, and OSU Operation Catnip Stillwater and ISU Feral Cat Alliance for allowing us to collect blood samples from feral cats.

Funding The authors received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Reference

- 1 Reichard MV, Meinkoth JH, Edwards AC, et al. **Transmission of *Cytauxzoon felis* to a domestic cat by *Amblyomma americanum*.** *Vet Parasitol* 2009; 161: 110–115.
- 2 Reichard MV, Edwards AC, Meinkoth JH, et al. **Confirmation of *Amblyomma americanum* (Acari: Ixodidae) as a vector for *Cytauxzoon felis* (Piroplasmorida: Theileriidae) to domestic cats.** *J Med Entomol* 2010; 47: 890–896.
- 3 Blouin EF, Kocan AA, Glenn BL, et al. **Transmission of *Cytauxzoon felis* Kier, 1979 from bobcats, *Felis rufus* (Schreber), to domestic cats by *Dermacentor variabilis* (Say).** *J Wildl Dis* 1984; 20: 241–242.
- 4 Meinkoth JH and Kocan AA. **Feline cytauxzoonosis.** *Vet Clin North Am Small Anim Pract* 2005; 35: 89–101, vi.
- 5 Reichard MV, Baum KA, Cadenhead SC, et al. **Temporal occurrence and environmental risk factors associated with cytauxzoonosis in domestic cats.** *Vet Parasitol* 2008; 152: 314–320.
- 6 Rizzi TE, Reichard MV, Cohn LA, et al. **Prevalence of *Cytauxzoon felis* infection in healthy cats from enzootic areas in Arkansas, Missouri, and Oklahoma.** *Parasit Vectors* 2015; 8: 13.
- 7 Cohn LA, Birkenheuer AJ, Brunker JD, et al. **Efficacy of atovaquone and azithromycin or imidocarb dipropionate in cats with acute cytauxzoonosis.** *J Vet Intern Med* 2011; 25: 55–60.
- 8 The Humane Society of the United States. **How to determine a cat's or dog's age,** http://www.ruralareavet.org/PDF/Physical_Exam-How_to_Determine_Age.pdf (1996, accessed March 1st 2016).
- 9 Bondy PJ, Jr, Cohn LA, Tyler JW, et al. **Polymerase chain reaction detection of *Cytauxzoon felis* from field-collected ticks and sequence analysis of the small subunit and internal transcribed spacer 1 region of the ribosomal RNA gene.** *J Parasitol* 2005; 91: 458–461.
- 10 Bush AO, Lafferty KD, Lotz JM, et al. **Parasitology meets ecology on its own terms: Margolis et al. revisited.** *J Parasitol* 1997; 83: 575–583.
- 11 Reiczigel J. **Confidence intervals for the binomial parameter: some new considerations.** *Stat Med* 2003; 22: 611–621.
- 12 Rozsa L, Reiczigel J and Majoros G. **Quantifying parasites in samples of hosts.** *J Parasitol* 2000; 86: 228–232.
- 13 Brown HM, Berghaus RD, Latimer KS, et al. **Genetic variability of *Cytauxzoon felis* from 88 infected domestic cats in Arkansas and Georgia.** *J Vet Diagn Invest* 2009; 21: 59–63.

- 14 Raghavan RK, Almes K, Goodin DG, et al. **Spatially heterogeneous land cover/land use and climatic risk factors of tick-borne feline cytauxzoonosis.** *Vector Borne Zoonotic Dis* 2014; 14: 486–495.
- 15 Lingren M, Rowley WA, Thompson C, et al. **Geographic distribution of ticks (Acari: Ixodidae) in Iowa with emphasis on *Ixodes scapularis* and their infection with *Borrelia burgdorferi*.** *Vector Borne Zoonotic Dis* 2005; 5: 219–226.
- 16 Kier AB, Wagner JE and Morehouse LG. **Experimental transmission of *Cytauxzoon felis* from bobcats (*Lynx rufus*) to domestic cats (*Felis domesticus*).** *Am J Vet Res* 1982; 43: 97–101.
- 17 Birkenheuer AJ, Le JA, Valenzisi AM, et al. ***Cytauxzoon felis* infection in cats in the mid-Atlantic states: 34 cases (1998–2004).** *J Am Vet Med Assoc* 2006; 228: 568–571.
- 18 Ferris DH. **A progress report on the status of a new disease of American cats: cytauxzoonosis.** *Comp Immunol Microbiol Infect Dis* 1979; 1: 269–276.
- 19 Meinkoth J, Kocan AA, Whitworth L, et al. **Cats surviving natural infection with *Cytauxzoon felis*: 18 cases (1997–1998).** *J Vet Intern Med* 2000; 14: 521–525.
- 20 Haber MD, Tucker MD, Marr HS, et al. **The detection of *Cytauxzoon felis* in apparently healthy free-roaming cats in the USA.** *Vet Parasitol* 2007; 146: 316–320.