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Authors: Henriquez, Brandon E., Featherston, Kyah, and Noden, Bruce H

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Detection of *Bartonella henselae* and *Rickettsia felis* in Fleas from Client-Owned Pets in Oklahoma

Brandon E. Henriquez, Kyah Featherston, and Bruce H Noden*

Department of Entomology and Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, OK 74078

Abstract. *Bartonella* sp. and *Rickettsia* sp. DNA were detected in cat fleas, *Ctenocephalides felis* (Bouché), collected from urban, client-owned cats (*Felis catus* L.) and dogs (*Canis lupus familiaris* L.) at Tulsa, OK, during 2017. *Bartonella henselae* (Regnery et al. 1992) was detected in four of six fleas (67%) from one cat, while *Rickettsia felis* (Bouyer et al. 2001) was detected in eight fleas from five animals.

Flea-borne pathogens, including *Bartonella henselae* (Regnery et al. 1992) and *Rickettsia felis* (Bouyer et al. 2001) affect humans and companion animals worldwide. Infections with *B. henselae,* commonly known as cat-scratch disease, are usually associated with infected cats and the cat flea, *Ctenocephalides felis* (Bouché) (Cheslock and Embers 2019). *Rickettsia felis,* an intracellular bacterium in *C. felis*, produced adverse effects in humans in central Africa but has never been isolated from humans in the United States (Brown and Macaluso 2016). Both pathogens are not notifiable conditions in the United States, so regional epidemiology is limited (Brown and Macaluso 2016, Nelson et al. 2016) as are flea-focused studies. The purpose of this study was to determine whether the pathogens were in client-owned dogs and cats at Tulsa, OK.

We collected fleas from client-owned cats and dogs in the Tulsa metropolitan area between May and August 2017. The study was designed as a one-semester undergraduate research project. The clinic was selected because it serves a variety of socioeconomic backgrounds and rural and urban pet ownership practices. The collection protocol was approved by the Institutional Animal Care and Use Committee at Oklahoma State University. After examination of the animals upon arrival, the owner was informed about the project if fleas were encountered, and approval was requested for removal of the fleas for research purposes. The species of host animal and zip code were documented. Fleas were put into 1.5-ml vials containing 70% EtOH until identified using established keys (CDC 2006).

Individual fleas were processed and tested for pathogen DNA using published polymerase chain reaction (PCR) protocols (Noden et al. 2017). To limit contamination, all DNA extractions used site-specific reagents in a different laboratory than where PCR assays were done. Fleas were evaluated for the citrate synthase (*gltA*) gene for *Bartonella* sp. (primer pair BhCS781.p / BhCS1137.n; (Norman et al. 1995)) and *Rickettsia* sp. (primer pair CS-78 / CS-323; (Labruna et al.

*Corresponding author: bruce.noden@okstate.edu

2004)). All positive amplicons were bidirectionally sequenced at the Oklahoma State University Core Facility. Each resulting sequence was verified using BioEdit 7.2 (Ibis Therapeutics, https://bioedit.software.informer.com/7.2/) and aligned bidirectional sequences to create individual consensus sequences using Clustal Omega (EMBL-EBI, https://www.ebi.ac.uk/Tools/msa/clustalo/). Resulting sequences were compared with Genbank submissions using default conditions on NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) where the greatest percent sequence identity was used to determine similarity of species.

Forty *C. felis* were collected, with 22 (55%) from seven cats and 18 (45%) from six dogs from 13 houses in the Tulsa metropolitan area (six zip codes). Of the fleas collected, 26 (65%) were female.

Evaluation of the 40 fleas detected 12 (30%) positives (four *Bartonella* sp. and eight *Rickettsia* sp.), with flea prevalence of 10% (*Bartonella*) and 20% (*Rickettsia*) from six (46%) animals. No co-infected fleas were detected. Consensus sequences demonstrating 100% identity were determined with the 305-bp portion of the *gltA* gene of a *B. henselae* isolate (GenBank accession no. MN107415) and the 353-bp portion of the gltA gene of a *R. felis* isolate (GenBank accession no. MG952935). All four fleas infected with *B. henselae* were from one cat/house (four of six (67%) fleas infected, three of four (75%) females). The eight fleas infected with *R. felis* were from five animals (two dogs and three cats) from five houses in four zip codes, with most being female fleas (five of eight - 62.5%). The cat with the *B. henselae*-infected fleas and two (40%) animals with *R. felis*-infected fleas were from a high socio-economic neighborhood in south-central Tulsa.

Fleas infected with *B. henselae* and *R. felis* were identified from client-owned dogs and cats at Tulsa, OK. The west-south central region of the United States, in which Oklahoma is located, has the most incidence of cat scratch fever (Nelson et al. 2016), yet little is known of the ecology of the pathogen at the community level. As a zoonotic pathogen, it is especially dangerous for immuno-compromised people, but the greatest incidence occurs in children and the elderly (Nelson et al. 2016). While *B. henselae* is named after Diane Hensel who worked at an Oklahoma City laboratory (etymologia 2008), this is the first published record of *B. henselae* in fleas in Oklahoma. Detection of the pathogen in a relatively small random sample of fleas collected at a centrally-located veterinary clinic in one of the largest metropolitan areas in the state was surprising and led to questions regarding the general prevalence of the pathogen in fleas in the region. While *B. henselae* has never been documented in fleas in Oklahoma, *Bartonella* spp. were detected in *Oropsylla hisuta* (Baker) fleas collected in 2004 from prairie dogs (*Cynomys* spp.) near Oklahoma City (Reeves et al. 2007).

While potentially interesting as a pathogen of public health importance, Noden et al. already reported *R. felis* in fleas on Oklahoma-based pets in 2017. The potential presence of the two human pathogens in a large metropolitan area in Oklahoma necessitates the need for more focus on flea-borne pathogens in this region of the US.

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