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Short Communication

First Detection of *Rickettsia typhi* and *Rickettsia felis* in Fleas Collected From Client-Owned Companion Animals in the Southern Great Plains

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Abstract

Flea-borne rickettsiosis occurs worldwide and includes a number of pathogens, namely, *Rickettsia typhi* and *Rickettsia felis*. Most studies in the United States have occurred in southern Texas and California where flea-borne rickettsiosis is endemic, resulting in a lack of information from other regions of the country. Between March and August 2016, 222 fleas were collected from 52 client-owned dogs and cats in two urban areas in Oklahoma. Fleas were identified using morphological characteristics then pooled and tested by polymerase chain reaction (PCR) using published primers for *gltA*, *ompB*, and 17-kDa. The majority (98.6%) of fleas collected were *Ctenocephalides felis* (Bouché) followed by *Pulex irritans* (L) (1.4%). Overall, fleas collected from 30.0% (6/20) cats and 43.8% (14/32) dogs were infected with *R. felis*. Three *C. felis*-pools collected from three dogs, two in the Enid area (central Oklahoma) and one in the Elk City area (western Oklahoma), were infected with *R. typhi* as well as *R. felis*. '*Candidatus* R. senegalensis' was detected in one pool of fleas taken from a cat in Oklahoma City. This is the first evidence that flea-borne *Rickettsia* species occur in fleas obtained from client-owned dogs and cats in the Great Plains region. The impact of these *Rickettsia* species on public health in the region needs further investigation.

Key words: *Rickettsia typhi*, *Rickettsia felis*, flea-borne rickettsiosis, Oklahoma

Flea-borne rickettsiosis occurs worldwide and includes a number of emerging pathogens (Azad 1990, Angelakis et al. 2016). *Rickettsia typhi* causes murine typhus, an acute undifferentiated febrile illness that mainly occurs in tropical and subtropical coastal regions. The classic transmission cycle involves rats (*Rattus* spp.) and rat fleas (*Xenopsylla cheopis* (Rothschild)) while an alternate cycle involves cat fleas (*Ctenocephalides felis* (Bouché)), opossums (*Didelphis virginiana* Kerr), and domestic cats (*Felis catus*; Azad et al. 1997, Adjemian et al. 2010, Eremeeva et al. 2012). *Rickettsia felis* has been linked with spotted fever group (SFG) rickettsia cases worldwide and is primarily transmitted by cat fleas (Azad et al. 1997, Angelakis et al. 2016, Brown and Macaluso 2016). In the United States, it has been mainly reported in regions with endemic *R. typhi* and is closely linked with opossums and domestic cats (Boostrom et al. 2002, Hawley et al. 2007, Eremeeva et al. 2008, Adjemian et al. 2010, Abramowicz et al. 2012, Eremeeva et al. 2012, Blanton et al. 2016). Recently, other *R. felis*-like organisms with unknown pathogenicity to humans or small companion animals, including '*Candidatus* Rickettsia senegalensis' and '*Candidatus* Rickettsia asemboensis' have been identified from cat fleas in the United States (Billeter et al. 2016, Blanton et al. 2016, Maina et al. 2016).

Within the United States, most ecological research on flea-borne rickettsiosis has occurred in southern Texas and California (Adjemian et al. 2010, Abramowicz et al. 2012, Eremeeva et al. 2012, Billeter et al. 2016, Blanton et al. 2016, Maina et al. 2016), the two regions where human cases have occurred along with infected opossums, domestic cats and dogs, and cat fleas. To date, nothing is known regarding these important flea-borne diseases in Oklahoma nor have the flea vectors been identified although historical data suggest both *R. typhi* and *R. felis* may be present. To begin addressing this knowledge gap, we identified the flea species collected from client-owned dogs and cats from two urban areas in Oklahoma and used molecular methods to test for the presence of flea-borne *Rickettsia* species.

Materials and Methods

Flea Collection

Client-owned dogs and cats coming for routine procedures in two veterinary clinics in Enid and Oklahoma City were examined for ectoparasitic fleas. These clinics were selected because they have a

wide clientele from diverse socio-economic backgrounds. The collection protocol was approved by the Institutional Animal Care and Use Committee at Oklahoma State University. If fleas were encountered on a dog or cat during the initial examination, the owner or client was informed concerning the project after which their approval was requested for the removal of fleas for research purposes. With verbal agreement, the zip code of the owner was noted and further investigation occurred. Depending on time and visibility of the fleas, up to 30 fleas (most cases involved 1 to 5 fleas) were removed and placed into 1.5-ml tubes containing 70% ethanol. As part of the service, the technician or attending vet discussed flea treatment options with the client. All collected fleas were identified in the laboratory using standard keys (Furman and Catts 1982, CDC 2006).

Molecular Analyses

Pools of collected fleas were tested for *R. felis* and *R. typhi* using modified end-point polymerase chain reactions (PCR) protocols (Salazar 2015, Noden et al. 2017). Fleas from each animal were grouped into pools of one to five fleas. Only fleas of the same species were processed together. To limit DNA contamination, all flea DNA extractions were conducted using site-specific reagents and equipment in a biosafety cabinet in a different laboratory from where the PCR assays were run. After washing in deionized water and 70% ethanol, pools of adult fleas were heated at 80–90 °C for 15 min in 2-ml vials (Sarstedt, Bartlesville, OK) with 100 μl of DNAzol Direct (Molecular Research Center, Inc., Cincinnati, OH) sample processing reagent. After heating, zirconia or silica beads (BioSpec Products, Bartlesville, OK) were added and the tubes were placed in a Mini-Beadbeater-16 (BioSpec Products) for 3 min. After bead-beating, resulting supernatant was collected and stored at −20°C until DNA testing.

Preliminary screening for SFG *Rickettsia* DNA in pooled flea samples utilized an end-point PCR assay developed for the *gltA* gene (primer pairs CS-78/CS-323; Labruna et al. 2004) as well as the PCR primers for an assay that targets a 122-bp fragment of the *ompB* gene (Henry et al. 2007). One microliter of flea-derived DNA was used in each 25-μl PCR reaction. All positives from the *gltA* assay were further confirmed by a nested PCR assay (outer primers (R17-122/R17-500); inner primers (*R. felis* (TZ15/TZ16))) that target the 17-kDa protein gene (Tzianabos et al. 1989, Massung et al. 2001) as well as a PCR assay targeting the *ompB* gene (Roux and Raoult 2000). Selected positive samples of the *gltA*, 17kDa TZ15/TZ16, and *ompB* assays were sequenced to confirm *R. felis* and other flea-borne *Rickettsia* species. Only pools that were positive for the two genes were considered infected. All positive samples for the *R. typhi*-specific *ompB* assay were confirmed using the nested PCR assay described above with inner primers (RPID/RP2) (Tzianabos et al. 1989, Massung et al. 2001) accompanied by sequencing of positives to confirm the amplification of *R. typhi*. Controls for the end-point PCR reactions included a negative control (DNAse-free water) and a positive control (1 μl *R. felis* or *R. typhi* genomic DNA). Bidirectional sequencing of selected positive samples occurred in the Oklahoma State University Core Facility. Resulting sequences were searched in the nucleotide BLAST database to verify that the primers amplified the targets.

Results

Between March and August 2016, 222 fleas were collected from 11 client-owned dogs in the Enid region, 37 cats ($n = 18$ including 1 stray) and dogs ($n = 19$ including 1 stray) in the Oklahoma City region, and 4 cats ($n = 2$) and dogs ($n = 2$) from communities in other areas of Oklahoma (Fig. 1; Table 1). The sampling area represented 11 households in Enid (4 zip codes), 35 households plus 2 strays from Oklahoma City (18 zip codes), and 4 households from communities outside urban areas (4 zip codes). The majority (98.6%) of fleas collected were *C. felis* followed by *P. irritans* (1.4%). The *P. irritans* were recovered from one stray cat, one stray dog, and one client-owned dog from Oklahoma City.

The 222 fleas collected during this study were grouped into 72 pools by individual animal (25 pools from cats and 47 pools from dogs) and species of flea. Of the 72 flea pools tested for the presence of rickettsial pathogens, 29 (40.3%) were positive for *R. felis* (Table 1). No pathogens were found in the pools of *P. irritans*. Nine of 25 cat flea pools collected from cats (36.0%) and 20 of 47 cat flea pools collected from dogs (42.6%) were infected with *R. felis* (Table 1). A total of 63.3% (7/11) of the dogs sampled in Enid had *R. felis*-infected fleas compared with 38.9% (7/18) of dogs in the Oklahoma City. Of the cats sampled in Oklahoma City and surrounding areas, 27.8% (5/18) had *R. felis*-infected fleas. Of those sampled from outside areas in Oklahoma, 100% (1/1) of the cats and 50% (1/2) of the dogs had *R. felis*-infected fleas.

BLAST analysis of the sequences from positive *R. felis* pools showed 97–99% similarity with known sequences of *R. felis* ($n = 29$; 99% similarity *gltA*: accession numbers JQ674484.1 and AF210692.1; 97–98% similarity 17kDa: KR709306.1 and AF210693.1; 98–99% similarity *ompB*: KF056801.1 and AF210695.1). One positive pool was 98–99% identical to known sequences of 'Candidatus R. senegalensis' ($n = 1$; 99% similarity *gltA*: KU499847.1 and KF666472.1; 98% similarity 17kDa: KU167052.1).

Of the pooled fleas from 52 animals tested for presence of rickettsial pathogens, there was an overall *R. typhi* prevalence of 4.2% (3/72; Table 1). BLAST analysis of the sequences from the three positive *R. typhi* pools showed similarity with known sequences of *R. typhi* (97% similarity *ompB*: KF241858.1; 98% similarity 17kDa: JX198507.1). The *R. typhi*-infected pools came from fleas obtained from one dog from Enid, Oklahoma, another from Lahoma, Oklahoma (just outside Enid), and one from Elk City, Oklahoma (Fig. 1). Pools of cat fleas collected only from dogs (6.4% (3/47)) were infected with *R. typhi* (Table 1). 18.2% (2/11) of the dogs sampled in Enid had *R. typhi*-infected fleas compared with 0.0% (0/18) dogs in the Oklahoma City. Of the dogs sampled in other areas of Oklahoma, 50.0% (1/2) had *R. typhi*-infected fleas. Three *C. felis*-pools collected from two dogs in central Oklahoma and one dog in western Oklahoma were infected with *R. typhi* as well as *R. felis*.

Discussion

Flea-borne rickettsiosis in the United States, particularly *R. typhi*, is normally associated with endemic areas characterized by occasional outbreaks in southern Texas and California (Adjemian et al. 2010, Abramowicz et al. 2012, Eremeeva et al. 2012, Billeter et al. 2016, Blanton et al. 2016, Maina et al. 2016). In this study, we report *R. typhi*, *R. felis*, and 'Candidatus R. senegalensis' in pools of fleas obtained from client-owned dogs and cats in urban areas in Oklahoma, an area of the United States not normally associated with murine typhus. While the first to focus on client-owned companion animals, Wilcomb et al. (1952) reported 28 cases of murine typhus in Oklahoma between 1939 and 1943 and Marshall and The Tick-Borne Infections in Children Study (TICKS) Group (2000) recorded *R. typhi* antibody titers in three sick children from Oklahoma City. These historical studies, together with the data
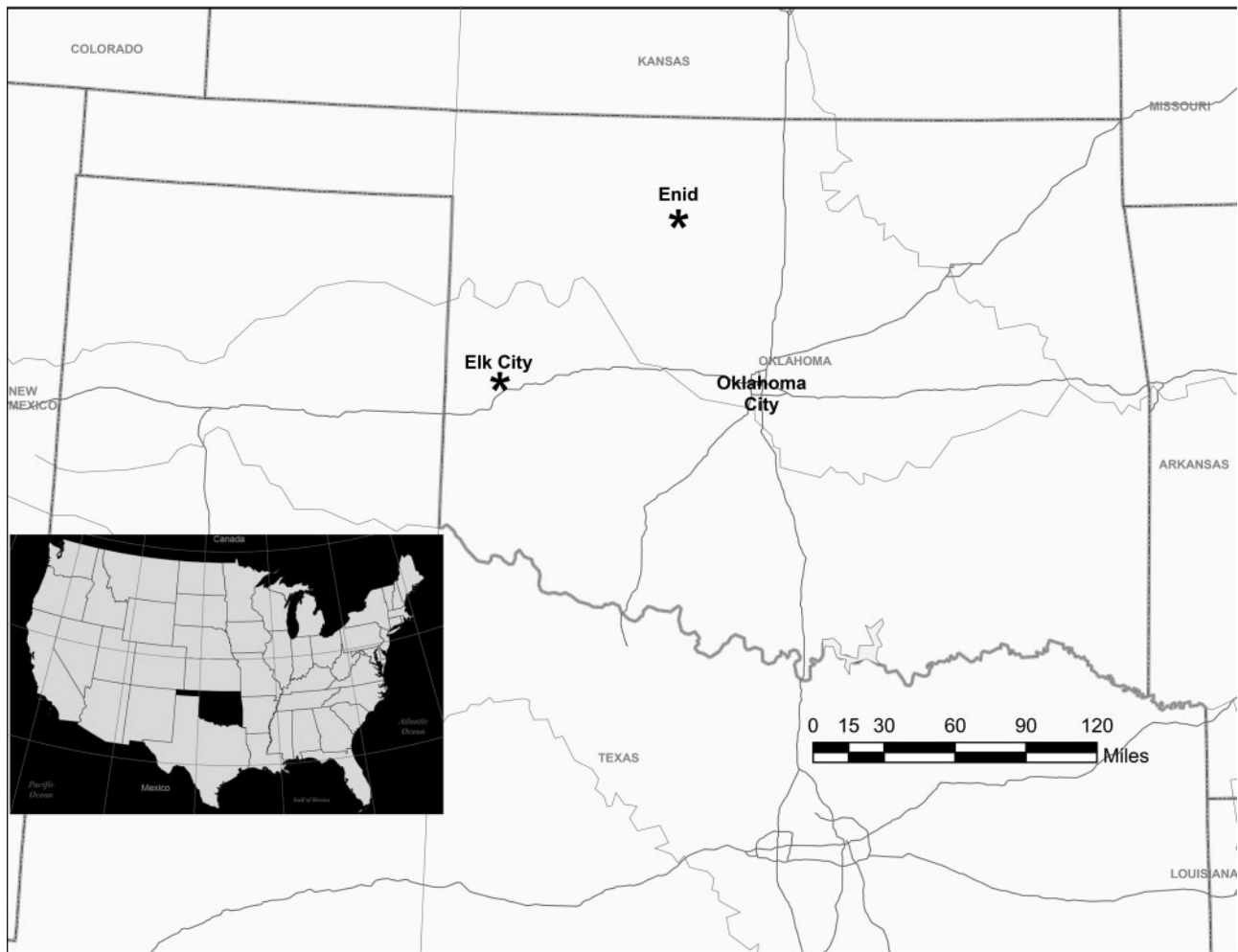
Fig. 1. Sites in Oklahoma where fleas infected with *Rickettsia* species were collected from client-owned dogs and cats. Names include all sites where collections were carried out in 2016. The sites marked with * are the sites where fleas infected with *R. typhi* were collected.

Table 1. Detection of *R. typhi* and *R. felis* DNA in pooled fleas collected from client-owned dogs and cats in two urban areas in Oklahoma between March and August 2016

Location	Animal sampled	Flea species	No. of flea pools tested (total fleas)	No. of positive pools (%)		
				R. felis	*R. typhi*	Dual positive
Enid	Dog	*C. felis*	21 (90)	12 (57.1)	2 (9.5)	2 (9.5)
		P. irritans	0 (0)	0 (0)	0	0
Oklahoma City	Dog	*C. felis*	22 (67)	7 (31.8)	0	0
		P. irritans	2 (2)	0 (0)	0	0
	Cat	*C. felis*	22 (56)	8 (36.4)	0	0
		P. irritans	1 (1)	0 (0)	0	0
Outside	Dog	*C. felis*	2 (4)	1 (50.0)	1 (50.0)	1 (50.0)
	Cat	*C. felis*	2 (2)	1 (50.0)	0	0
		Total	72 (222)	29 (40.3)	3 (4.2)	3 (4.2)

from this current study, indicate that flea-borne rickettsiosis may occur in humans and wildlife surrounding periurban or exurban areas in Oklahoma.

The detection of *R. typhi* in pools of fleas taken from two client-owned dogs in the Enid and Oklahoma City area is notable. In the United States, *R. typhi* has been reported in fleas collected from domestic cats and opossums, but there is no recent reference to detecting *R. typhi* in fleas from client-owned dogs. The main question revolves

around the origin of the *R. typhi*-infected fleas. While dogs can serve as reservoirs for *R. typhi* (Nogueras et al. 2013), only one of the two pools of fleas obtained from the same dog in Lahoma in the current study was positive for both *R. typhi* and *R. felis* while no rickettsial DNA was amplified from the second pool of five fleas from the same dog. If the fleas had been infected by the dog, one would have expected to find infected fleas in both pools from the same animal. An alternative possibility would be exposure to *R. typhi*- and *R. felis*-infected cat

fleas from stray cats and opossums around the peridomestic environment where the dog lives. More follow-up is needed to establish the origin and risks involved with the infected fleas in the local regions.

The principal flea species collected from client-owned dogs and cats in periurban Oklahoma was the cat flea (*C. felis*) while *P. irritans* was limited mainly to stray animals. This finding agrees with another Oklahoma-based study that collected *C. felis* from a large cohort of free-roaming domestic cats (Thomas et al. 2016). Only two previous studies have described flea populations in Oklahoma: One involving *Oropsylla hirsuta* (Baker) from prairie dogs (Reeves et al. 2007) and the other describing four species from swift foxes (Criffield et al. 2009).

In this study, both pools containing *R. typhi*-infected fleas also contained fleas infected with *R. felis*. This was not surprising, as both pathogens are known to cocirculate within the same environments (Eremeeva et al. 2008, Karpathy et al. 2009, Eremeeva et al. 2012). While only one person in the United States has been identified with an active *R. felis* infection (Schriefer et al.1994), human infections with *R. felis* are being identified worldwide and have been linked with fatal outcomes (Angelakis et al. 2016, Brown and Macaluso 2016). This is not the first time *R. felis* has been reported in fleas collected in Oklahoma. A study testing an Oklahoma-based commercial cat flea colony derived from wild-caught fleas found *R. felis* (ELB agent at that time) at an 83% prevalence rate (Higgins et al. 1994). While most amplicons from the current study aligned with known sequences of *R. felis*, the DNA from one pool of fleas aligned with known sequences of 'Candidatus R. senegalensis'. This species was originally isolated from *C. felis* fleas removed from cats from Senegal (Mediannikov et al. 2014) and found in opossum and cat fleas from southern California (Maina et al. 2016) and Texas (Blanton et al. 2016). The pathogenicity of 'Candidatus R. senegalensis' to humans and its role in the epidemiology of flea-borne rickettsiosis remains unknown.

In conclusion, this is the first detection of *R. typhi* and *R. felis* in fleas collected from client-owned dogs and cats in urban areas in the Great Plains region. While not possible to know whether these fleas pose a risk to the owners or persons in the local area, it is indicative that these pathogens are occurring in a wider area in the United States than just in areas of known endemicity. Further clarification is necessary to establish how these pathogens in fleas in periurban areas on the Great Plains are impacting the wider public health within the region. As causes of undifferentiated febrile illness around the world (Azad 1990, Angelakis et al. 2016), these flea-borne rickettsioses could potentially be involved in the high incidence of symptomatically treated tick-borne SFG rickettsiosis and ehrlichiosis cases in the region (Biggs et al. 2016).

Acknowledgments

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