Rickettsial Infections among Cats and Cat Fleas in Riverside County, California

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Abstract. Presently, few studies have investigated the role of domestic cats (*Felis catus*) in the recrudescence of flea-borne rickettsioses in California and the southern United States. In this study, we aimed to investigate the presence of *Rickettsia typhi* or *Rickettsia felis* in domestic cats (*F. catus*) and the fleas (primarily *Ctenocephalides felis*, the cat flea) associated with these cats in Riverside County, California. Thirty cats and 64 pools of fleas collected from these cats were investigated for rickettsial infections. Three cats and 17 flea pools (from 10 cats) tested positive for rickettsial infections. polymerase chain reaction and DNA sequencing indicated that one of the cats was positive for *R. felis* infections, whereas two were positive for *Candidatus Rickettsia senegalensis* infection. In addition, 12 of the flea pools were positive for *R. felis*, whereas five were positive for *Ca. R. senegalensis*. By contrast, no cats or their associated fleas tested positive for *R. typhi*. Finally, eight sera from these cats contained spotted fever group *Rickettsia* (SFGR) antibodies. The detection of *R. felis* and SFGR antibodies and the lack of *R. typhi* and TGR antibodies support *R. felis* as the main rickettsial species infecting cat fleas. The detection of *Ca. R. senegalensis* in both fleas and cats also provides additional evidence that cats and their associated fleas are infected with other *R. felis*-like organisms highlighting the potential risk for human infections with *R. felis* or *R. felis*-like organisms.

INTRODUCTION

Starting in 1915, cases of a sporadic type of typhus, different from the classic and more widely recognized epidemic typhus, were documented in California.^{1–3} It was quickly established that many of these cases referred to as endemic typhus or murine typhus were due to a flea-borne *Rickettsia* carried by rats.⁴ Murine typhus continued to be considered a major health concern through the 1940s in California and parts of the southern United States, with 16,332 cases of murine typhus documented in the United States between 1922 and 1939 ranging from 24 in 1922 to 2,942 cases in 1939.² An estimated 505 cases of murine typhus were diagnosed between 1916 and 1948 in California alone, with most cases of murine typhus reported from the southern counties.^{1,2} Vector control techniques, which mainly included dichlorodiphenyltrichloroethane dusting, were implemented during this time period, and the number of cases of murine typhus dropped significantly from its peak in the 1940s.⁵

More recently, cases of murine typhus began to resurface and identification of cases increased in the 1980s and 1990s in parts of southern California and the southern United States.^{6–10} However, these cases were not attributed to the classical (urban) cycle of murine typhus of the past. Traditionally, transmission of *Rickettsia typhi* (causative agent of murine typhus) involved rats (*Rattus rattus* and *Rattus norvegicus*) and rat fleas (*Xenopsylla cheopis*), but many of these more recent cases have been attributed to a suburban cycle involving cats (*Felis catus*), opossums (*Didelphis virginiana*), and the cat flea (*Ctenocephalides felis*).^{7,8,10–12} The cat flea has been shown to harbor *R. typhi*, and is thought to be the principal vector responsible for the increase in murine typhus cases in the southern United States and California.^{8,11–15}

However, the role of cats as a reservoir for infecting cat fleas with *R. typhi* in the environment is not well known.

Ctenocephalides felis fleas are arguably the most important ectoparasite of domesticated cats and dogs worldwide.¹⁶ In southern California, in addition to parasitizing cats, *C. felis* fleas are found in abundance on a wide variety of mammals, including opossums collected in areas adjacent to the residences of human typhus cases.^{13,16} From 2001 to 2016, 738 human cases of flea-borne typhus were reported to the California Department of Public Health, with most cases occurring in Los Angeles and Orange counties; the etiologic agent of these flea-borne rickettsioses is presumed to be caused by infections with *R. typhi*.^{17,18}

In addition to *R. typhi*, *C. felis* fleas have also been shown to harbor *Rickettsia felis*, another agent of flea-borne rickettsiosis (i.e., flea-borne spotted fever), which may be under-recognized as a cause of rickettsial disease in California.^{14,15,19,20} The presence of *R. felis* in *C. felis* fleas provides another possible etiology for flea-borne rickettsioses seen in the United States and in particular, California. *Rickettsia felis* was detected for the first time in 1991 in the blood of a Texas patient who had been diagnosed initially with murine typhus.²¹ Since then, *R. felis* has been found to be a common cause of rickettsiosis in sub-Saharan Africa and other regions of the world.^{22–25} Although human *R. felis* infections have been seen in Central and South America,^{23–25} *R. felis* is generally not considered a cause of flea-borne rickettsioses in the United States.^{22–28} However, reports from California indicate that *R. typhi*-endemic areas overlap (because of the common flea vectors) with areas where *R. felis* infections could be endemic.^{11,15,19,20,29,30} To further complicate matters, the clinical symptomology, consisting of high fever, headache, myalgia, and rash, of *R. typhi* and *R. felis* infections in humans is similar.²⁵

Studies conducted in California's Los Angeles and Orange counties found *R. felis* to be the predominant *Rickettsia* spp. detected in pooled *C. felis* fleas (ca. 28–53%), whereas *R. typhi* infections were relatively few (ca. 1.3–3%).^{14,18,20} Rickettsial disease investigations in southern California have

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shown that opossums carry the highest *C. felis* flea load of any host animal.^{11,14,15,19,20} By contrast, *C. felis* fleas collected off cats from case households in Los Angeles and Orange counties were comparatively fewer in number than those collected from opossums but exhibited similar or higher *R. felis* infection rates.^{14,20}

Cats are readily parasitized by *C. felis*, and previous investigations have implicated cats as sources of flea-borne rickettsial disease outbreaks.^{10,12,31} *Rickettsia typhi*-seropositive cats have been found in close association with human cases,^{13,31} and experimental infection of cats with *R. felis* has been demonstrated.³² However, infection rates of *R. felis* and *R. typhi* in cats are largely unknown in southern California. To this end, we herein report the results from a study undertaken in Riverside County, California, which borders Los Angeles and Orange counties (Figure 1), to assess the presence of rickettsial agents and antibodies against both spotted fever group *Rickettsia* (SFGR) and typhus group *Rickettsia* (TGR) in cats and the presence of rickettsial agents in *C. felis* and sticktight fleas (*Echidnophaga gallinacea*) fleas removed from these cats.

MATERIALS AND METHODS

Sample collection. Groups of fleas, serum samples, and tissue samples were collected from 30 randomly chosen unsocialized (also referred to as unowned, feral, stray, or community cats) cats impounded by the Riverside County Department of Animal Services, Riverside, California (33°7827739°N, 116°976688°W), from July 2014 through

January 2015 that were scheduled for euthanasia (independent of this study) by shelter staff. Each submitted cat represented a unique geographical location in the county (Figure 1). Cats were euthanized following American Veterinary Medical Association guidelines with sodium pentobarbital.³³ Fleas, blood samples, and sections of the ear, liver, lymph node, rump muscle, spleen, lungs, heart, and kidneys were collected and stored at -80°C for testing.

DNA extraction. Fleas were washed in molecular-grade water and mechanically disrupted using disposable pellet pestles (Fisher Scientific, Pittsburgh, PA). Genomic DNA was extracted using the Prepman Ultra sample preparation kits (Applied Biosystems, Foster City, CA). Genomic DNA from cat tissue was extracted using the DNeasy blood and tissue kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions and using a final elution volume of 100 µL.

qPCR assays. Flea and cat tissue nucleic acid preparations were initially screened for rickettsial DNA using a genus-specific qPCR assay (Rick17b) targeting the 17-kDa antigen gene.³⁴ DNA from flea and cat samples that tested positive by the Rick17b assay were subsequently tested using a species-specific *R. felis* assay (Rfel_phosp_MB), targeting the membrane phosphatase gene from *R. felis* and the *R. typhi* species-specific qPCR assay (Rtyph), which targets a fragment of the *R. typhi* OmpB gene.^{35,36} In addition, flea DNA that tested positive for Rick17b were tested with a *Rickettsia asembonensis*-specific qPCR assay, which targets a fragment of *R. asembonensis* ompB.³⁷

Sequencing. PCR amplification and sequencing of *ompB* was attempted for a subset of flea DNA preparations. This

FIGURE 1. Map showing sites where cats and cat fleas with rickettsial infections and cats with antibodies to TGR and/or SFGR were found in Riverside County, California.

subset included all preparations that were positive for rickettsial DNA using the Rick17b qPCR assay but negative for *R. felis* DNA based on the Rfel_phosp_MB qPCR assay. This also included three flea DNA samples positive with both the Rick17b and the Rfel_phosp_MB qPCR assays to confirm the specificity of Rfel_phosp_MB.³⁷ PCR amplification and sequencing of *ompB*, *gltA*, and 17-kDa antigen gene fragments were attempted for all cat tissue DNA samples that tested positive for *Rickettsia* DNA by qPCR, as previously described.³⁷ Sequencing reactions were performed in the forward and reverse directions using the BigDye Terminator v3.1 Reaction Cycle sequencing kit (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions using an ABI 3500 genetic analyzer (Applied Biosystems). Sequence assembly was performed using CodonCode Aligner version 5.0.1 (CodonCode Corporation, Centerville, MA), and sequences obtained were compared with sequences available through NCBI using MEGA 6 software.³⁸

Serology. Evidence of previous infection in cats by SFGR and TGR was assessed using group-specific immunoglobulin G (IgG) enzyme-linked immunosorbent assays (ELISAs) based on whole cell antigens *Rickettsia conorii* str. Morocco (SFGR) and *R. typhi* str. Wilmington (TGR), as previously described.^{20,39} Serum samples were diluted 1:100 and screened for antibodies against rickettsiae using both the SFGR and TGR ELISAs. Screen-positive samples (those with net absorbance of ≥ 0.5) were titrated by 4-fold serial dilution (100–6,400). Commercial anti-cat IgG (KPL, Gaithersburg, MD) antibodies labeled with horseradish peroxidase were used in both ELISAs.

RESULTS

Thirty sera and 231 tissue samples were collected from 30 cats in Riverside County. Liver, lung, spleen, and heart samples were obtained from all 30 cats, whereas ear, lymph node, rump muscle, and kidney samples were collected from 29, 29, 28, and 25 cats, respectively. Of the 30 cats, 17 harbored fleas with a range of 1–14 fleas per cat. A total of 77 fleas (*C. felis* and *E. gallinacea*) were removed from 17 cats ($N = 17$ of 30 cats, 56.7%) (Figure 1). The fleas were separated into 64 samples, including 55 single *C. felis* fleas, one pool of 14 *C. felis* fleas, and eight single *E. gallinacea* fleas.

Flea survey results for *Rickettsia*. A total of 17 of 64 flea samples (26.6%) were positive for rickettsial DNA by the Rick17b qPCR assay with a minimum infection rate of 22.1% (95% exact binomial confidence interval = 13.4–33%). All 17 positive samples were derived from *C. felis* flea samples ($N = 17$ of 56 samples [30.4%]), whereas none of the *E. gallinacea* fleas ($N = 8$ samples from 3 cats) were positive for rickettsial DNA by the Rick17b qPCR assay. In addition, the 17 flea samples positive for rickettsial DNA were obtained from 10 of the 17 cats (58.8%) harboring fleas (Table 1).

Flea samples positive by the genus-specific Rick17b assay were further tested using species-specific qPCR assays. Twelve of the 17 flea nucleic acid preparations that tested positive for rickettsial DNA using the Rick17b qPCR assay also tested positive for *R. felis* DNA using the Rfel_phosp_MB qPCR assay, indicating that 18.8% of all the flea DNA samples and 21.4% of the *C. felis* flea DNA samples were positive for *R. felis*. These 12 samples were obtained from eight of 17 individual cats (47.0%) included in this study or 8 of 16 cats (50%) that harbored *C. felis* fleas. No flea DNA tested positive using the Rtyph qPCR assay (Figure 1, Table 1). The results from the genus- and species-specific qPCR assays were confirmed by PCR amplification and sequencing of *ompB*. The 518-bp *ompB* sequences were generated for eight flea DNA samples. Five single flea samples from two cats that were positive for rickettsial DNA using the Rick17b assay but negative for all other assays generated amplicons that were 100% identical to *Candidatus Rickettsia senegalensis* PU01-02 KF666470 (Figure 1). The remaining three fleas, which had tested positive by the Rick17b and the Rfel_phosp_MB qPCR assay, generated amplicons that were 100% identical to *R. felis* CP000053.

Cat tissue results. A total of six of 231 DNA preparations (2.6%) from tissue samples (one ear, two spleen, and three muscle) obtained from three of 30 cats (10%) were positive using Rick17b genus-specific qPCR assay (Table 2). Samples positive by the Rick17b assay were further tested using species-specific qPCR assays. Two of these samples (muscle and spleen from one cat (#3)) also tested positive for *R. felis* DNA using the Rfel_phosp_MB qPCR assay. No DNA samples were positive using the Rtyph qPCR assay (Table 2). Interestingly, two of the three cats that were positive for rickettsial infection also harbored fleas positive for rickettsial species (Figure 1).

To confirm the identities of the rickettsiae detected by the genus- and species-specific qPCR assays from cat tissues, PCR amplification and sequencing of the 17-kDa, *gltA*, and *ompB* genes were attempted for all positive samples. For two cats (#1 and #2), 416-bp sequences of the 17-kDa gene were obtained and found to be 100% identical to *Ca. R. senegalensis* (KU167052) and *Rickettsia* sp. cf15 (AY953285). For one of these cats (#2), a 1093-bp length sequence of *gltA* was also obtained and found to be 100% identical to *Ca. R. senegalensis* (KF666472). In addition, a 393-bp 17-kDa sequence and a 518-bp *ompB* sequence from one cat (#3) were found to be 100% identical to *R. felis* URRWXCal2 (Figure 1).

Serology. Sera collected from all 30 cats were tested for the presence of antibodies to both SFG and TG *Rickettsia*. Of the 30 cats, eight cats (26.7%) were positive for antibodies against SFG antigens, whereas only one cat (3.3%) was positive for antibodies against TG antigens. Of these eight cats, only three cats harbored fleas positive for rickettsial

TABLE 1
Summary of rickettsial qPCR assay results for fleas from 17 of 30 cats impounded in Riverside County, California

Flea species	No. of Flea DNA samples*	<i>Rickettsia</i> genus (%; 95 CI)	<i>Rickettsia felis</i> (%; 95 CI)	<i>Rickettsia typhi</i>	<i>Rickettsia assemonensis</i>	No. of cats	No. <i>Rickettsia</i> Pos cats (%; 95 CI)	No. of <i>Rickettsia felis</i> Pos cats (%; 95 CI)
<i>Ctenocephalides felis</i>	56	17 (30.4; 18.8–44.1)	12 (21.4; 11.6–34.4)	0	0	16	10 (62.5; 35.4–84.8)	8 (50; 23.0–72.2)
<i>Echinophaga gallinacea</i>	8	0	0	0	0	3	0	0
Totals	64	17 (26.6; 16.3–39.1)	12 (18.8; 10.1–30.5)	0	0	17	10 (58.8; 32.9–81.6)	8 (47; 24.7–75.3)

* Sixty-three individual fleas and one pooled sample that contained 14 individual *C. felis* fleas. Pool was positive for *R. felis*.

TABLE 2

Summary of rickettsial qPCR assay results for cat tissues (ear, liver, lymph node, rump muscle, spleen, lungs, heart, and kidneys) from cats in Riverside County, California

qPCR assay	Specificity	No. Pos tissue (%; 95 CI)	Total tissue	No. Pos cats (%; 95 CI)	Total cats	Tissue type Pos
Rick17b	<i>Rickettsia</i> spp.	6 (2.6; 1.0–5.6)	231	3 (10; 2.1–26.5)	30	Cat 1: Muscle, spleen; Cat 2: mar muscle; Cat 3: muscle, spleen
Rfel_Phos_MB	<i>Rickettsia felis</i>	2 (0.8; 0.1–3.1)	231	1 (3.3; 0.1–17.2)	30	Cat 3: muscle, spleen
Rtyph	<i>Rickettsia typhi</i>	0	231	0	30	N/A

species. The single cat that was positive for antibodies that reacted to the TG antigen was also positive for antibodies against the SFG antigen. Finally, none of the cats with tissue samples positive for rickettsial infection were positive for antibodies against SFG or TG antigens (Figure 1).

DISCUSSION

This study aimed to determine rickettsial infection rates in cats and their fleas in Riverside County, California. The investigation provided evidence that cats and their fleas are infected with two rickettsial species *Ca. R. senegalensis* and the human pathogen *R. felis*. Overall, the results indicate that 10% (3/30) of the cats had rickettsial infections, with rickettsial DNA detected in the ear, spleen, and rump muscle. Of the cats with fleas, 47% had at least one flea positive for *R. felis* and 11.8% of the cats with fleas had at least one flea positive for *Ca. R. senegalensis*. These results indicate that *R. felis* infection of *C. felis* fleas from cats in Riverside County is common and is the predominant *Rickettsia* detected in these fleas. The results support previous evidence showing *R. felis* to be the main *Rickettsia* spp. in *C. felis* fleas from cats and opossums in California^{14,15,18–20,40} and *C. felis* fleas from cats worldwide.³² Reports from South Carolina and California indicate the existence of *Ca. R. senegalensis* in the United States, and these results, herein further support this.^{20,41} However, to date, the pathogenicity of *Ca. R. senegalensis* in humans is unknown. Interestingly, *R. asembonensis* was not detected in this study. *R. asembonensis* is commonly found around the world in cat fleas^{37,42–44} and was recently found in cat fleas from Orange County, albeit at a very low prevalence.²⁰

In addition to providing DNA evidence of rickettsial infections in cats and cat fleas, our study found that antibodies against SFGR were present in 26.7% of cats. Antibodies to TGR were seen in only one of 30 cats (3.3%), but this might be because of cross-reactivity of SFG antibodies to TGR antigens. However, data from previous studies indicate that when fleas infected with *R. felis* are fed on cats, these cats produced antibodies that reacted to SFGR (*R. conorii* str. Morocco) and not TGR (*R. typhi* str. Wilmington) ELISA antigens.^{20,45} Therefore, the results suggest that the high prevalence of anti-SFGR antibodies is due to infection with SFGR such as *R. felis*, whereas *R. typhi* infections in cats appear to be much less common. The high seroprevalence of antibodies against SFGR and low prevalence of antibodies against TGR agree with our findings that *C. felis* fleas have substantial infection rates with *R. felis* and *Ca. R. senegalensis*.

Interestingly, no cats or flea pools were positive for *R. typhi*, which is consistent with the historically small number of flea-borne rickettsiosis cases reported from Riverside County ($N = 2$, 2001–2016).^{1,2,18} Investigations in nearby Los Angeles and Orange counties, where cases of flea-borne

rickettsiosis occur annually, show high prevalence of TGR antibodies in cats and opossums, suggesting that flea-borne rickettsial infection in southern California could be due to *R. typhi* alone^{13,20,26} and that the high prevalence of *R. felis* in cat fleas does not predispose people to increased risk of infection.^{40,46}

This, however, does not rule out the possibility that some flea-borne rickettsial infections in southern California might be due to *R. felis*, or *R. felis*-like organisms. Eremeeva et al. reported cases where *C. felis* fleas collected off cats and opossums from households in Los Angeles and Orange counties demonstrated the presence of *R. felis* but not *R. typhi*,¹⁴ and associations of rickettsial disease cases with cats and their *C. felis* fleas infected only with *R. felis* have also been observed in Australia.⁴⁷ Whereas most cases in Texas have been attributed to *R. typhi* infection, some flea-borne rickettsiosis victims have tested positive for *R. felis* antibodies,²⁷ as has been reported elsewhere in the world.^{22–25,48}

Our results provide additional evidence that cats become infected with *R. felis* and *R. felis*-like organisms. Both the prevalence of antibodies to SFGR antigens and the detection of rickettsial DNA in multiple cat tissues indicate that cats in Riverside County are infected with *R. felis* and/or *R. felis*-like organisms, and the lack of *R. typhi* DNA in tissue, along with the low prevalence of antibodies to TGR antigens, suggests that cats could be acting as important vertebrate reservoirs of *R. felis* and *R. felis*-like organisms and under-recognized contributors of flea-borne rickettsiosis. Opossums and cats (especially free-roaming ones) in southern California share ecological niches and *C. felis* fleas, making it imperative for cats to be monitored regularly and treated for fleas to minimize health risks to humans. Municipal animal shelters that engage in Trap-Neuter-Release (TNR) programs, where healthy unsocialized cats are trapped, neutered, and released to neighborhood cat colonies (groups of cats that live together in a territory) cared for by humans to lessen shelter euthanasia rates, should incorporate zoonotic disease reduction practices and prohibit placing TNR cats in areas endemic for flea-borne rickettsiosis or other zoonotic diseases.^{49–52} Cat colonies have been documented with flea infestation rates as high as 92.5% because flea control products are not routinely applied to these cats.⁵³ Hotspot analysis of flea-borne rickettsial disease cases in Orange County demonstrated significant clustering of rickettsial disease occurrence (0.7–1.5 cases/km²) in areas with high TNR cat density compared with other, less affected areas of the county (Cummings et al., unpublished data). Several flea-borne rickettsiosis outbreaks in Los Angeles and Orange counties have been very focal geographically, occurring in case clusters in families or mobile home communities with high cat densities.^{54,55} Although exposure to flea-borne rickettsial disease agents is recognized as being highly variable and ecologically complex,¹² further research in the eco-epidemiology of flea-borne rickettsiosis

is needed to provide a better understanding of the causes of these outbreaks.

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