Flea Abundance, Species Composition, and Prevalence of Rickettsioses from Urban Wildlife in Orange County, California, 2015-2019

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ABSTRACT: Fleas infesting urban wildlife have been epidemiologically linked to the transmission of flea-borne rickettsial pathogens in urban and suburban areas of Orange County, California. To understand the prevalence of flea-borne rickettsioses caused by either *Rickettsia felis* or *R. typhi*, a survey of fleas from wildlife was conducted to determine the flea species composition of host animals and prevalence of rickettsial pathogens in fleas on host animals. This study reports flea abundance, species composition, and infestation intensity on unowned domestic cats and wildlife (i.e., coyotes, opossums, rabbits, skunks, squirrels, raccoons, and commensal rodents) collected in urban neighborhoods of Orange County. The survey revealed presence of the northern rat flea on eastern fox squirrels, and widespread distribution of the human flea on skunks and coyotes in Orange County. The flea index and prevalence of flea-bone rickettsioses in fleas has been used by the Orange County Mosquito and Vector Control District to guide decisions regarding risk management and intervention strategies to reduce and prevent the transmission of flea-borne pathogens. The prevalence of *R. felis* and *R. typhi* in fleas in Orange County was 8.94% and 0.39%, respectively. Roof rats, eastern fox squirrels, and striped skunks had the highest diversity of flea species, while the Virginia opossum had the lowest, as determined by the Simpson's Diversity Index. The sticktight flea was found to have the highest diversity of mammal hosts. It is not known how flea species composition on hosts impacts the maintenance and persistence of rickettsial and other pathogens in fleas from urban wildlife in Orange County.

KEY WORDS: cat flea, *Ctenocephalides felis*, *Didelphis virginiana*, *Echidnophaga gallinacea*, ectoparasites, human flea, *Mephitis mephitis*, opossum, public health, *Pulex simulans*, rickettsia, skunk, sticktight flea, typhus, urban wildlife

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INTRODUCTION

Free-ranging urban wildlife in southern California, consisting primarily of California ground squirrels (*Otospermophilus beecheyi*), coyotes (*Canis latrans*), domestic cats *(Felis catus*), eastern fox squirrels (*Sciurus niger*), Virginia opossums (*Didelphis virginiana*), and striped skunks (*Mephitis mephitis*), are frequently parasitized by three important fleas of public health importance: the cat flea (*Ctenocephalides felis*), the sticktight flea (*Echidnophaga gallinacea*), and the false human flea (*Pulex simulans*). Detections of *Bartonella* and rickettsial human pathogens in these three flea species was reported previously in southern California (Probert et al. 2009, Billeter et al. 2011, Krueger et al. 2016, Osikowicz et al. 2016, Vasconcelos et al. 2018, Penicks et al. 2019, Rangel et al. 2019). Roof rats (*Rattus rattus*) and racoons (*Procyon lotor*) are common in urban neighborhoods of southern California, but infestations of fleas and detections of *Bartonella* and rickettsial infectious agents from these hosts are rare (Krueger et al. 2016, Penicks et al. 2019).

In Orange County, CA, studies of flea abundance on urban wildlife demonstrate that the average number of fleas per host animal, commonly referred to as the flea index, and widely regarded as a measure of flea-borne disease risk, has been steadily increasing since the 1960s (Penicks et al. 2019). Several studies have reported that the flea index rises during epizootic transmission of plague (Tripp et al. 2009) and flea-borne rickettsiosis (e.g., flea-borne typhus) (Krueger et al. 2016). During two outbreak years for flea-borne typhus in Orange County, 2012 and 2013, the flea index on opossums collected from human case exposure sites was 96 fleas per opossum, the highest known flea index reported from Orange County (Krueger et al. 2016, Penicks 2019). The county's high human population density (>12,000 persons /mi^2 in some cities; U.S. Census Bureau 2019) and semi-arid Mediterranean climate provide a favorable environment for urban wildlife and year-round flea development, especially for the cat flea (Rust and Dryden 1997).

During previous flea-borne rickettsioses studies, the Orange County Mosquito and Vector Control District (OCMVCD) did not analyze the flea species composition on urban wildlife. The composition of flea species on various host animals may play an important role in the maintenance of rickettsial pathogens at low levels in fleas and mammalian hosts (Azad et al. 1997, Azad and Beard 1998), in a similar way that has been hypothesized for the persistence of *Yersinia pestis* between fleas and host animals in enzootic plague transmission years (Friggens et al. 2010).

An understanding of the maintenance and persistence of rickettsial pathogens in fleas and on host animals is critical in order to design flea-borne rickettsioses control programs that can potentially eliminate the pathogens from fleas on urban wildlife in areas experiencing outbreaks of human cases. Historically, these programs have focused on control of fleas on urban rodents (i.e., roof rats and ground squirrels) and have not included control of fleas on opossums, skunks, coyotes, and tree squirrels (CDPH 1950). In the U.S., there are no topical or feed-through pesticide formulations for flea control on wildlife registered by the Environmental Protection Agency.

This paper focuses on fleas and hosts present in neighborhoods of Orange County where flea-borne rickettsioses are endemic. We report detailed flea species composition on host animals, including the recently introduced eastern fox squirrel (King 2004), and the identification of a new flea species, the northern rat flea (*Nosopsyllus fasciatus*), not reported in the area prior to 2017, on eastern fox squirrels (Hubbard 1947); the northern rat flea is a known vector of *Rickettsia* in the U.S. The impact on flea-borne rickettisial transmission in Orange County caused by the arrival of new flea species, mammal hosts, and composition changes of fleas on urban wildlife is further explored in this paper.

Flea-borne rickettsioses are zoonotic diseases found throughout the world and are caused by either of two related bacterial pathogens, *Rickettsia typhi* or *R. felis* (Azad et al. 1997, Perez-Osorio et al. 2008, Richards et al. 2010). *R. typhi* is the causative agent of the first recognized form of flea-borne rickettsiosis, murine typhus (Azad 1990); *R. felis*. The etiologic agent of flea-borne spotted fever was first identified in 1991 in a patient from Texas (Schriefer et al. 1994) and is now considered an emerging threat to human health worldwide; human cases of *R. felis* have been reported in fifteen countries (Legendre and Macaluso 2017).

Rickettsial transmission from infected fleas to other, non-infected fleas can be vertical (i.e., transovarial and transstadial), horizontal via rickettsemic amplification in a host, through mating, contact with contaminated feces (Azad 1990, Azad et al. 1997), or by co-feeding on a mammalian host with other fleas of the same or potentially different species (Hirunkanokpun et al. 2011, Brown and Macaluso 2016). Humans typically acquire either bacterial infection through a flea bite, contact with rickettsia-contaminated flea feces or crushed flea tissue rubbed into the bite wound, eyes, nose, or mouth. Among patients with severe clinical illness, symptoms for either pathogen are similar and typically appear within 6-14 days after exposure and include high fever, headache, myalgia, fatigue, confusion, and rash (Azad et al. 1997). Human infections with *R. typhi* and *R. felis* are diagnostically indistinguishable unless specific serologic assays or molecular methods are used. Mortality is less than 2% when patients receive appropriate antibiotic treatment, typically doxycycline (Azad and Beard 1998).

Of the 1,033 human cases of flea-borne rickettsial disease reported in California from 2010-2019, all reported exposure in southern California, with the majority of cases (>98%) occurring in Los Angeles and Orange counties (CDPH 2019). The reasons for the apparent regional endemicity of rickettsial disease in California are not known, but could be due to differences in the presence, infection rate, and/or species of *Rickettsia* in populations of various fleas and host animals from other areas of the state.

Despite its relative high infection rates in several species of fleas, especially in the ubiquitous cat flea (Abramowicz et al. 2010, Eremeeva et al. 2012, Maina et al. 2016, Rangel et al. 2019), some researchers question the role of *R. felis* as a human pathogen due to the lack of clinical evidence for human infections in the U.S. (Billeter and Metzger 2017, Blanton and Walker 2017). Although substantially less prevalent in flea populations, *R. typhi* is believed to be responsible for most of the hospitalized illnesses in California (CDPH 2019) and Texas (Blanton and Walker 2017). In the U.S., prospective patients infected with *R. felis* are likely to be diagnosed with murine typhus because of serologic cross-reactivity to the *R. typhi* antigen used in standard diagnostic tests (Schriefer et al. 1994). In contrast, studies in other parts of the world with confirmed human cases with *R. felis* have implicated *R. felis* as a human pathogen when discriminatory molecular methods or serologic testing have been used on patients (Perez-Arellano et al. 2005, Perez-Osorio et al. 2008, Richards et al. 2010, Parola 2011, Edouard et al. 2014, Teoh et al. 2018). The use of these or similar diagnostic tests would be helpful in detecting the correct etiologic agent responsible for flea-borne rickettsial disease cases in the U.S. (Eremeeva et al. 2012). The significance of recently discovered *Rickettsia felis*-like organisms (RFLOs), *Rickettsia asembonensis* and *Candidatus* R. senegalensis, in cat fleas in Orange County (Maina et al. 2016) also warrants investigation as to their capacity to cause human illness.

METHODS

Opossums, unowned domestic cats, striped skunks, eastern fox squirrels, California ground squirrels, roof rats and raccoons were obtained from the following sources: 1) carcasses submitted to the OCMVCD's dead bird, squirrel, and lagomorph surveillance system; 2) live-trapped in response to human cases of rickettsial disease or for routine plague surveillance by OCMVCD; 3) carcasses submitted by a local school district; and 4) carcasses submitted from local animal control agencies or wildlife rehabilitators from primarily moribund animals in Orange County. OCMVCD rarely collects fleas from domestic dogs because they are not free roaming for long periods of time and owners report flea control product use. All fleas from coyotes were collected and submitted by the University of California, Agriculture and Natural Resources, South Coast Research & Extension Center, via Dr. Niamh Quinn, Human-Wildlife Interactions Advisor. All animals collected by OCMVCD, school districts, animal care agencies, and wildlife rehabilitators were humanely trapped and euthanized in accordance with guidelines from the American Veterinary Medical Association (AVMA 2013).

Animal carcasses were placed in a plastic bag immediately following collection, labeled with collection number, and frozen for a minimum of 72 hours to ensure ectoparasite death. The carcasses were examined under a biological fume hood, and fleas were collected, counted, and placed in vials containing 70% diluted ethanol for identification (Campbell 2017) using a dissecting microscope. Male *Pulex* spp. fleas were identified to species and enumerated using a compound microscope following a published protocol for flea mounting (Campbell et al. 2018); all were identified as *P. simulans*, the false human flea. Flea species and counts were compiled and analyzed in Microsoft Excel®.

Simpson's Diversity Index (Simpson 1949) was used to compare the flea species composition on the urban mammal hosts (Simpson 1949). Flea species composition

Table 1. Flea infestations of urban wildlife from a flea-borne rickettsiosis endemic region of Orange County, California, 2015-2019.

		Total Number Fleas[2] by Species Collected per Animal Host (Avg. No. Fleas/Host)										
Host species (No. of Hosts)	**No. hosts infested with fleas (%)**	***Cf***	***Eg***	***Ps***	***Om***	***Ha***	***Nf***	***Oh***	***Ls***	***Cii***	***Osp***	**Total (%)**
Coyote[1] (215)	51 (23.7)	107 (0.5)	10 (0.05)	282 (1.31)	0	0	0	0	0	3 (0.01)	0	**402 (1.9)**
Roof rat (125)	6 (4.8)	13 (0.1)	23 (0.18)	0	13 (0.1)	0	0	0	4 (0.03)	0	0	**53 (0.4)**
Virginia opossum (115)	92 (80.0)	7,540 (65.6)	365 (3.17)	89 (0.77)	0	0	0	0	0	0	1 (0.01)	**7,995 (69.5)**
Striped skunk (70)	62 (88.6)	236 (3.4)	1,346 (19.23)	738 (10.54)	0	5 (0.07)	0	0	0	0	0	**2,325 (33.2)**
Eastern fox squirrel[1] (45)	9 (20.0)	0	63 (1.4)	7 (0.16)	0	0	31 (0.69)	18 (0.4)	0	0	0	**119 (2.6)**
California ground squirrel[1] (46)	31 (67.4)	1 (0.02)	327 (7.11)	0	32 (0.7)	53 (1.15)	0	0	0	0	0	**413 (9.0)**
Domestic cat (11)	8 (72.7)	90 (8.2)	5 (0.45)	1 (0.09)	0	0	0	0	0	0	0	**96 (8.7)**
Total (627)	**259 (41.3)**	**7,987 (12.7)**	**2,139 (3.4)**	**1,117 (1.8)**	**45 (0.07)**	**58 (0.09)**	**31 (0.05)**	**18 (0.03)**	**4 (0.01)**	**3 (0.005)**	**1 (0.002)**	**11,403**

[1]Mammals commonly submitted as roadkill.
[2]Fleas: *Cf: Ctenocephalides felis* (cat flea)*; Eg: Echinophaga gallinacea* (sticktight flea)*; Ps: Pulex simulans* (false human flea)*; Om: Oropsylla monatanus* (rock squirrel flea)*; Ha: Hoplopsyllus anomalous; Nf: Nosopsyllus fasciatus* (rat flea)*; Oh: Orchopeas howardii* (squirrel flea)*; Ls: Leptopsylla segnis; Cii: Cediopsylla inequalis interupta* (rabbit flea)*; Osp: Orchopeas* spp.

on each mammal host was estimated using the Simpson's Index of Diversity (D), described as:

$$D = 1 - \frac{\Sigma n(n-1)}{N(N-1)}$$

where n is the total number of hosts of a flea species, and N is the total number of all hosts of all species. Diversity of mammal hosts for cat fleas, sticktight fleas, and human fleas was estimated using the same equation. The Simpson's Diversity Index ranges from 0 to 1, with the larger value indicating greater diversity in the sample.

When possible, ten fleas from each flea species present on a host animal carcass were placed individually into vials containing 70% ethanol prior to rickettsial pathogen testing. A select number of fleas were tested for rickettsial pathogens using a duplex real-time PCR (qPCR) assay to detect *R. felis* and *R. typhi* (Rangel et al. 2019). The remaining fleas from each host were grouped by flea species, assigned a unique identifier in the OCMVCD Flea Bank Collection, and stored in a dark location in vials containing 96% ethanol.

RESULTS

Flea-host Associations within a Flea-borne Rickettsioses Endemic Area of Orange County, CA

During 2015-2019, a total of 11,403 fleas, comprising ten species, were removed and identified from 627 urban mammals (California ground squirrels, coyotes, fox squirrels, domestic cats, roof rats, striped skunks, and Virginia opossums) received by OCMVCD (Table 1). Of the 11,403 fleas identified to species, 2,682 fleas were tested for rickettsial agents (Table 2). The four most commonly collected hosts (coyotes, roof rats, Virginia opossums, and striped skunks) comprised 83% of all hosts examined (Table 1). Opossums, skunks, and cats were most commonly found infested with fleas. Less than 50% of coyotes, fox squirrels, and California ground squirrels were infested with fleas, as these hosts were commonly submitted as road-killed carcasses. Less than 5% of roof rats (6/125 animals) were found infested with fleas.

The cat flea, sticktight flea, and false human flea comprised 95% of all fleas removed from urban wildlife (Table 1). The sticktight flea was found on all seven mammal species, the cat flea on six mammal species (absent on fox squirrels), and the false human flea on five mammal species (absent on ground squirrels and roof rats) sampled during 2015-2019. The cat flea was the most abundant flea removed from urban wildlife, representing 70% of all collected fleas in Orange County during the study period, followed by sticktight fleas (18.8%), and false human fleas (9.8%). The remaining seven flea species comprised less than 1% of the total collection of fleas on free-ranging urban mammals. Simpson's Diversity Index showed that roof rats and fox squirrels are hosts to the most diverse assemblage of flea species, while California ground squirrels and Virginia opossums have the least diverse assemblage of flea species (Table 3). An analysis of the diversity of mammal hosts infested by cat fleas, false human fleas, and sticktight fleas found sticktight fleas with the highest diversity of hosts, followed by cat fleas, then false human fleas (Table 4).

The flea index, the average number of fleas found on a host animal, was highest on opossums (69.5 fleas/ animal), followed by striped skunks (33.2 fleas/animal), ground squirrels (9.0 fleas/animal), domestic cats (8.7 fleas/ animal), eastern fox squirrels (8.7 fleas/animal), coyotes

Table 2. Prevalence of *R. felis* and *R. typhi* in fleas removed from urban wildlife in Orange County, California, 2015-2019.

	***R. felis*-positive**					***R. typhi*-positive**				
Host animal (No. collected)	**% Host with Pos. Flea Pools (No.)**	**% Pos. *C. felis* (No.)**	**% Pos *E. gallinacea* (No.)**	**% Pos. *P. irritans* (No.)**	**% Pos. *Hoplopsyllus* spp. (No.)**	**% Host with Pos. Flea Pools (No.)**	**% Pos *C. felis* (No.)**	**% Pos. *E. gallinacea* (No.)**	**% Pos. *P. irritans* (No.)**	**% Pos. *Hoplopsyllus* spp. (No.)**
Coyote (51)	7.8% (4/51)	12.0% (9/75)	0	0/232	0	1.96% (1/51)	1.33% (1/75)	0	0/232	0
Opossum (92)	67.4% (62/92)	12.9% (147/1140)	0/146	0/47	0	7.61% (7/92)	1.40% (16/1140)	0/146	0/47	0
Striped Skunk (62)	22.6% (14/62)	11.2% (18/161)	0.8% (2/248)	0.29% (1/349)	0	3.23% (2/62)	0.62 % (1/161)	0.40% (1/248)	0/349	0
Human (16)	37.5% (6/16)	8.5% (13/153)	0/1	0/1	0	0/16	0/153	0	0	0
Domestic cat (8)	87.5% (7/8)	17.3 (9/52)	11.1% (1/9)	0	0	9.09% (1/11)	1.92% (1/52)	0/9	0	0
Domestic rabbit (2)	50.0% (1/2)	12.5% (1/8)	0/1	0	50.0% (1/2)	0/2	0/8	0/1	0	0/2
Desert cottontail rabbit (2)	50.0% (1/2)	0	0/8	0/1	50.0% (1/2)	0/2	0/2	0/8	0/1	0/2
Roof rat (6)	0/6	0	0/12	0	0	0/6	0	0/12	0	0
Total (239)	**39.7% (95/239)**	**12.4% (197/1,589)**	**0.01% (3/432)**	**0.15% (1/658)**	**50.0% (2/4)**	**4.60% (11/239)**	**1.19% (19/1,590)**	**0.23% (1/432)**	**0 (0/658)**	**0 (0/4)**

Table 3. Simpsons Diversity Index: Diversity of flea communities on urban wildlife in Orange County, CA.

Host	**Simpson's Diversity Index Value**
Roof rat	0.71
Eastern fox squirrel	0.63
Striped skunk	0.55
Coyote	0.54
Domestic Cat	0.53
California Ground Squirrel	0.35
Virginia Opossum	0.11

Table 4. Simpsons Diversity Index: Comparison of host animal diversity of three flea species of public health concern, Orange County, CA.

Host	**Simpson's Diversity Index Value**
Sticktight flea (*E. gallinacean*)	0.73
Human flea (*P. simulans*)	0.64
Cat flea (*C. felis*)	0.63

(1.9 fleas/animal), and roof rats (0.4 fleas/ animal) (Table 1). The false human flea (*P. simulans*) was the most prevalent flea on coyotes, followed by cat fleas (*C. felis*), sticktight fleas (*E. gallinacean*), and rabbit fleas (*C. inequalis interupta*). On eastern fox squirrels, sticktight fleas were the most abundant, followed by rat flea (*N. fasciatus*) and squirrel flea (*Orchopeas howardi*).

Flea Test Results for Rickettsial Pathogens

Of the 2,683 individual fleas tested for *R. felis* and *R. typhi* from 2015 to 2019, 203 were positive for *R. felis*, and 20 were positive for *R. typhi* (Table 2). Sixty-two opossums (62/92), 14 striped skunks (14/62), seven domestic cats (7/8), four coyotes (4/51), and two rabbits (1 European and one desert cottontail) were hosts to *R. felis*-positive fleas; while seven opossums (7/92), two striped skunks (2/62), one coyote (1/51), and one domestic cat (1/11) were hosts to *R. typhi*-positive cat fleas. *Rickettsia typhi* was also detected in a sticktight flea from a striped skunk, and *R. felis* was detected in three sticktight fleas removed from two striped skunks, and a domestic cat (Table 2). Of the 658 false human fleas tested for rickettsia pathogens, a single flea removed from a striped skunk tested positive for *R. felis*, and there were no detections of *R. typhi*. Interestingly, 50% of *Hoplopsyllus* spp. fleas removed from two rabbits tested positive for *R. felis*.

DISCUSSION

To our knowledge, this is only study since Adams et al. (1970) to examine flea composition and abundance on free-ranging urban mammals collected from densely populated urban neighborhoods in California. Flea indices, in conjunction with host abundance data and prevalence of infectious agents, provide indicators to the geographic localization of the vectors, their hosts, and risk of pathogen transmission to humans (Krasnov et al. 2004). This study shows that urban wildlife, except for roof rats, are commonly infested with fleas in Orange County. It is not known how the establishment of *S. niger* infested with *N. fasciatus* fleas in urban areas of Orange County will impact flea-borne disease transmission of *Rickettsia* to wildlife, people, and pets; such transmission is rare and has never been documented in California.

Coyotes, eastern fox squirrels, and California ground squirrels were commonly received by OCMVCD as road-killed animals. For "roadkill" animals, the fleas found on the carcass may not represent the flea composition of a living host animal because fleas may have jumped off the dead animal prior to collection. It is likely that the coyotes, eastern fox squirrels and California ground squirrels have a higher infestation prevalence of fleas than represented in this study. Although urban wildlife is commonly infested with fleas, the prevalence of *R. typhi* and *R. felis* in fleas removed from host animals varies, indicating some flea species may be better hosts for rickettsial agents. The prevalence of *R. felis* in cat fleas removed from opossums (12.9%) is lower than previously reported (32%), likely due to the testing of individual instead of pooled fleas (Eremeeva et al. 2012). Although the sample size was small, the 50% prevalence of *R. felis* in *Hoplopsyllus* spp. fleas removed from rabbits necessitates further investigation. *Rickettsia typhi* was most prevalent in cat fleas removed from opossums, cats, skunks, and coyotes. The prevalence of *R. typhi* in fleas from host animals in Orange County (4.6%) has been low compared to the prevalence of *R. typhi* in fleas from Norway rats from Los Angeles (7.8%) (Abramowicz et al. 2010). A single sticktight flea removed from a striped skunk was found positive for *R. typhi* in Orange County. A single false human flea, removed from a skunk, was found positive for *R. felis*. The role of the sticktight flea and the false human flea in the maintenance of rickettsial pathogens is not well understood, considering these species are commonly collected from urban wildlife.

Flea community composition varies by host animal and could influence prevalence and transmission of infectious agents within the community of fleas, as well as to humans and companion animals. Two specific mechanisms have been proposed: 1) the presence of multiple vector species that will infest multiple susceptible host species creates a more connected host network, and 2) increased flea diversity creates greater connections between zoonotic hosts and humans (Eisen et al. 2012). In many urban neighborhoods of Orange County, opossums, domestic cats, skunks, roof rats, tree squirrels, ground squirrels, coyotes, and rabbits are all present, suggesting a diversity and abundance of flea species. Sticktight fleas infest the most diverse assemblage of urban wildlife; more research is needed to understand this species' role in potential transmission of *Rickettsia* among wildlife species.

The diversity of flea hosts and flea species present in urban neighborhoods may impact rickettsial transmission to humans. Laboratory demonstrations of horizontal transmission of *Rickettsia* during co-feeding of fleas have broad implications for the ecology of rickettsiosis (Hirunkanokpun et al. 2011). Through co-feeding, rickettsial pathogens are exchanged from uninfected to infected fleas among fleas of the same or different species on mammalian hosts, regardless of rickettsial amplification in the host. In addition, the transmission mechanisms by which human infections occur through scarification or manual transfer of rickettsial pathogens into the bite wound or eyes, nose, or mouth, allows for transfer of rickettsial pathogens for all types of infected fleas, even less abundant species.

Having multiple species of fleas on a diverse assemblage of urban wildlife within a densely populated urban neighborhood makes control of fleas challenging. The presence of multiple flea species with *Rickettsia* allows pathogens to persist during seasons when host animals, or flea populations, decrease below levels where persistence would be possible if only one species was present. This study shows that in Orange County, urban wildlife are hosts to multiple species of fleas that can be infected with rickettsial pathogens, and that a flea-borne rickettsiosis control campaign will need to target control of fleas on all urban wildlife (especially those that utilize subterranean burrows) to prevent disease transmission.

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