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Preliminary Survey of Ectoparasites and Associated Pathogens from Norway Rats in New York City

M. J. FRYE,[1,2] C. FIRTH,[3,4] M. BHAT,[3,5] M. A. FIRTH,[6,7] X. CHE,[3] D. LEE,[3] S. H. WILLIAMS,[3] and W. I. LIPKIN[3]

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ABSTRACT The Norway rat (*Rattus norvegicus*) is a reservoir of many zoonotic pathogens and lives in close proximity to humans in urban environments. Human infection with rodent-borne disease occurs either directly through contact with a rat or its excreta, or indirectly via arthropod vectors such as fleas and ticks. Here, we report on the diversity and abundance of ectoparasitic arthropod species and associated pathogenic bacteria from 133 Norway rats trapped over a 10-mo period in Manhattan, New York, NY. Norway rats were host to the tropical rat mite [*Ornithonyssus bacoti* (Hirst)], the spiny rat mite (*Laelaps echidnina* Berlese), *Laelaps nuttalli* Hirst, the spined rat louse [*Polyplax spinulosa* (Burmeister)], and the Oriental rat flea [(*Xenopsylla cheopis*) (Rothschild)], with an average of 1.7 species per individual. A flea index of 4.1 *X. cheopis* was determined, whereas previous studies in New York City reported 0.22 fleas per rat. Multiple species of pathogenic *Bartonella* were identified from Oriental rat fleas that were related to *Bartonella tribocorum*, *Bartonella rochalimae*, and *Bartonella elizabethae*. However, no evidence of *Yersinia pestis* or *Rickettsia* spp. infection was detected in fleas. The identification of multiple medically important ectoparasite species in New York City underscores the need for future efforts to fully characterize the diversity and distribution of ectoparasites on Norway rats, and assess the risk to humans of vector-borne disease transmission.

KEY WORDS ectoparasite, pathogen, *Rattus norvegicus*, *Xenopsylla cheopis*, *Bartonella*

Introduction

The Norway rat [*Rattus norvegicus* (Berkenhout, 1769)] is a cosmopolitan pest species that exploits human resources to survive. Each year, rodents are responsible for billions of dollars in damage to food supplies (Pimentel et al. 2005), and can negatively impact human health in several ways. Frequent exposure to rodent hair, droppings, and urine in the home or workplace has been associated with an increased risk of both asthma and allergies, especially for children (Perry et al. 2003, Matsui 2009, Jeal and Jones 2010). These effects are greatest in urban environments (Simons et al. 2007) where abundant food, water, and shelter can support large rodent populations. Living in close proximity to rodents may lead to increased risk of zoonotic disease transmission for a range of bacterial, parasitic, protozoan, and viral pathogens (Meerburg et al. 2009). Humans can be exposed to rodent-borne pathogens either directly through bites (Childs et al. 1998) or indirectly via exposure to urine, feces (Hilton et al. 2002, Phan et al. 2011), or arthropod ectoparasites. In particular, fleas can vector between rodents and humans important pathogens such as *Bartonella* spp., *Yersinia pestis*, and *Rickettsia typhi*, the causative agents of bartonellosis, plague, and murine typhus, respectively (Meerburg et al. 2009, Eisen and Gage 2012).

To understand the risk of zoonotic disease transmission in an area, urban rodent surveys are used to obtain information on the distribution, population size, and pathogen diversity of rodents and their ectoparasites (Feng and Himsworth 2013). This information guides decisions about risk management and potential intervention strategies that include targeted rodent and ectoparasite control (Dennis et al. 1999). While these surveys were conducted during the mid-20th century to address outbreaks of arthropod-borne diseases such as murine typhus (Reeves et al. 2006), current risk assessments are often lacking for many urban centers. Only recently has there been a resurgence in rodent surveillance programs in North America to detect infectious microbes (Easterbrook et al. 2007, Billeter et al. 2011, Himsworth et al. 2014).

[1] New York State IPM Program, 630W. North St., Geneva, NY 14456
[2] Corresponding author, e-mail: mjf267@cornell.edu
[3] Center for Infection and Immunity, Mailman School of Public Health, Columbia University, 722W. 168th St., New York, NY 10032
[4] Current affiliation: CSIRO Biosecurity Flagship, Australian Animal Health Laboratory, Geelong, Victoria, Australia
[5] Current affiliation: The Nature Conservancy, North America Region, New York, NY
[6] Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065
[7] Current affiliation: Walter and Eliza Hall Institute of Medical Research, 1 G Royal Parade, Parkville, Victoria, 3052, Australia

The following study was undertaken as part of an effort to survey Norway rat populations in Manhattan, New York, NY, for the presence and prevalence of medically important ectoparasite species and the bacterial pathogens they may carry. Rats were live-trapped in areas with a high probability of human–rodent contact, focusing on indoor environments. We report here on the ectoparasite fauna collected and associated flea-borne zoonotic bacterial species commonly associated with rodents.

Materials and Methods

Rodent Collection. Norway rats were collected from five sites: three residential buildings (sites A, B, and C; March–April 2013), one outdoor location (site D; June 2013), and one indoor mixed-use location (site E; October–December 2012). Rats were live-captured using Tomahawk Pro-Series Traps (Tomahawk Live Trap LLC, Hazelhurst, WI), which were placed in areas of high rat activity, baited and left open for 7–10 d at each location to allow the rats to acclimate to their presence. Fresh bait was applied 2 d prior to a trapping event using one or more pieces of chicken, cucumber, or apple. Trapping occurred overnight, with each trap set for not >12 h, in accordance with Columbia University IACUC Protocol No. AC-AAAE6805. Animals were euthanized with isoflurane followed by bilateral thoracotomy. Each rat was weighed, sexed, and assigned to one of three age categories based on body weight: juvenile (<80 g), subadult (80–180 g for females, 80–200 g for males), or adult (>180 g for females, >200 g for males; McGuire et al. 2006).

Ectoparasite Collection and Identification. Rat carcasses were fumigated using ethyl acetate for ~5 min to kill ectoparasites, and were combed using a fine-toothed flea comb over dry ice. Carcasses were inspected using forceps to lift fur and abrade skin. Ectoparasites were sorted visually and maintained on dry ice. A representative of each ectoparasite type was placed in ethyl alcohol and identified using standard taxonomic keys and techniques. Insect (IDL 13046; IDL 13047) and mite voucher specimens were deposited at the Cornell University Insect Collection. The flea index was calculated as the total number of fleas divided by the total number of rats collected (Dennis et al. 1999).

Molecular and Phylogenetic Analyses. Oriental rat fleas were pooled from each animal for DNA extraction using the DNeasy Blood and Tissue Kit (Qiagen Inc., Alameda, CA). DNA was quantified and diluted to a working concentration of ≤400 ng/µl. Pooled samples were tested by polymerase chain reaction (PCR) for the presence of several flea-borne zoonotic pathogens using published primers for *Bartonella*, *Rickettsia*, and *Y. pestis* (Norman et al. 1995, Loïez et al. 2003, Stewart et al. 2008, Karpathy et al. 2009). Positive PCR products were confirmed by bidirectional dideoxy sequencing, revealing the presence of many sequences with ambiguous nucleotides that may indicate mixed infections. As a result, the PCR products from six pools were subcloned into pGEM-T Easy vectors (Promega), and five subclones from each pool were sequenced.

Products (nucleotide sequences) representing a conserved 327-nucleotide region (nucleotide positions 801–1127) of the *gltA* gene commonly used for taxonomic classification of *Bartonella* sp. were aligned along with representative sequences from other members of the genus using CLUSTALW in Geneious v.7 (Biomatters, Auckland, New Zealand; Thompson et al. 1994). Duplicate sequences were removed from the alignment, and a maximum likelihood phylogenetic tree was constructed in the program PhyML using the best-fit TPM3uf + gamma model of nucleotide substitution, as determined by jmodeltest and the SPR + NNI method of branch swapping (Guindon and Gascuel 2003, Darriba et al. 2012). Five hundred bootstrap replicates were performed using the best-fit model and NNI branch swapping. The GenBank accession numbers for the sequences obtained in this study are KM266586–KM266615.

Statistical Analyses. The distribution of ectoparasites was assessed in Microsoft Excel (v. 14.4.4, 2011, (Microsoft, Redmond, WA) with a multinomial test of goodness of fit, with the expected distribution derived from the number of rodents captured per site.

Results

Rodent Collection. In total, 133 Norway rats were trapped, comprising 72 males (29 juveniles, 24 subadults, and 19 adults) and 61 females (26 juveniles, 16 subadults, and 19 adults). Fifty-seven rats were collected from residential buildings (sites A, B, and C), 26 from the sole outdoor location (site D), and 50 from the sole indoor mixed-use location (site E). Rats ranged in size from 29 to 495 g, with a mean weight of 159 ± 11 g ($\overline{X}$ ± SEM). No other rodent species were caught.

Ectoparasite Collection and Identification. Ectoparasites were found on 132 of the 133 rats from the five collection sites (Table 1). On average, rats hosted 1.7 ± 0.06 ($\overline{X}$ ± SEM) species, and ectoparasite distribution varied by collection site (Table 1). The tropical rat mite, *Ornithonyssus bacoti*, (Hirst), was the most abundant species collected (Table 1), but 99.96% of mites were from the sole indoor mixed-use location (site E). The spiny rat mite, which reflects both *Laelaps echidnina* and *Laelaps nuttalli*, were recovered from all sites (Table 1) and it infested 70.7% of all rats (Table 2). The Oriental rat flea, *Xenopsylla cheopis* (Rothschild), was the only flea species collected, and it infested 30.1% of rats (Table 2). For all rats captured, there was an average of 4.1 fleas per animal. Rats trapped indoors had an average of 5.1 fleas, whereas site C rats had an average of 25.7 fleas each. Finally, the spined rat louse, *Polyplax spinulosa* (Burmeister), infested 34.6% of rats (Table 2), but was only present at four of the five sites (Table 1).

Molecular and Phylogenetic Analyses. None of the pooled flea samples were positive for *Y. pestis* or

Table 1. Numbers of ectoparasites collected per site and goodness-of-fit test to assess distribution between sites

Site	No. of Rats	*O. bacoti*	*L. echidnina*, *L. nuttalli*	*X. cheopis*	*P. spinulosa*
A	23	2 (0–2)[a]	250 (0–63)	12 (0–4)	75 (0–16)
B	14	0	159 (0–73)	7 (0–3)	4 (0–2)
C	20	0	419 (0–88)	513 (0–83)	24 (0–9)
D	26	0	309 (0–36)	1 (0–1)	113 (0–30)
E	50	4,638 (0–366)	32 (0–5)	12 (0–2)	0
Total	133	4,640 $\chi^2_4 = 7689.795$[b] $P < 0.0001$	1,169 $\chi^2_4 = 766.292$ $P < 0.0001$	545 $\chi^2_4 = 2677.288$ $P < 0.0001$	216 $\chi^2_4 = 254.624$ $P < 0.0001$

Rodents were collected from three residential buildings (sites A, B, and C; March–April 2013), one outdoor location (site D; June 2013), and one indoor mixed-use location (site E; October–December 2012).

[a] Total number of ectoparasites (range of ectoparasites collected per host).

[b] Multinominal test of goodness-of-fit compares observed ectoparasite numbers to expected distribution based on host numbers collected per site. A significant result indicates that ectoparasites are not evenly distributed with their rat hosts.

Table 2. Percent infestation of rodents by site and overall

Site	No. of Rats	*O. bacoti*	*L. echidnina*, *L. nuttalli*	*X. cheopis*	*P. spinulosa*
A	23	4.3%	95.7%	21.7%	73.9%
B	14	0	85.7%	28.6%	14.3%
C	20	0	100.0%	100.0%	40.0%
D	26	0	96.2%	3.8%	73.1%
E	50	98.0%	30.0%	20.0%	0
Total	133	37.6%	70.7%	30.1%	34.6%

Rodents were collected from three residential buildings (sites A, B, and C; March–April 2013), one outdoor location (site D; June 2013), and one indoor mixed-use location (site E; October–December 2012).

any *Rickettsia* species; however, all pools were positive for at least one species of *Bartonella*. Phylogenetic analysis of the *gltA* gene sequences recovered from infected Oriental rat fleas indicated the presence of multiple lineages of *Bartonella* (Fig. 1). The most common of these clustered closely with *B. tribocorum*, whereas sequences related to *B. rochalimae* and *B. elizabethae* were also recovered from pooled samples of fleas. Sequences obtained in this study were considered to be similar to those of validated *Bartonella* spp. if the percent pairwise nucleotide identity between sequences was >96% (La Scola et al. 2003).

Discussion

To our knowledge, this is the first report of medically important rodent ectoparasite species from Manhattan, New York, NY, since the 1920s (Fox and Sullivan 1925), and the first analysis of their capacity to harbor pathogenic microorganisms. Norway rats were host to the Oriental rat flea, the tropical rat mite, the spiny rat mite, and the spined rat louse. Arthropod ectoparasites were recovered from all habitats and sites, but were not distributed evenly with their rodent hosts. Molecular analysis of pooled flea samples revealed the presence of at least one species of pathogenic bacteria in the genus *Bartonella*, which are known to cause a wide range of clinical syndromes in mammalian hosts (Kamani et al. 2013). These findings may have important implications for disease transmission and human health.

Of the ectoparasite species collected, the Oriental rat flea is the most important vector of human disease, as it is capable of transmitting the etiologic agents of plague (*Y. pestis*; Eisen et al. 2007) and murine typhus (*R. typhi*; Traub et al. 1978). In addition, this organism is thought to play a role in the transmission of *Bartonella* spp. and the agent of tularemia (*Francisella tularensis*; Eisen and Gage 2012). Disease transmission occurs when an infected ectoparasite leaves its rodent host and feeds on a human (Pollitzer 1954). The incidence of accidental feeding is highest when humans reside in close contact with rodent populations that are large and harbor high ectoparasite loads (Engel et al. 1998), or when rodent populations suffer mortality from disease (Pollitzer 1954). Because rodent populations can vary over short geographical distances, such as between city blocks, the risk of human exposure to ectoparasites (and thus rodent-borne diseases) may also be unequally distributed across urban environments (Himsworth et al. 2014). Indeed, 94.1% of Oriental rat fleas in our survey were recovered from a single residential location, highlighting the importance of surveillance efforts to identify areas at risk of vector-borne disease transmission.

The World Health Organization recommends implementation of vector surveillance programs to determine the regional quantity and diversity of rodent ectoparasites (Dennis et al. 1999). For plague and murine typhus, one measurement of risk is the flea index, which assesses the average number of fleas per rat (Grubbs 1927). This metric is calculated at the population level, as ectoparasites are often unevenly distributed among hosts (Krasnov et al. 2007, Khokhlova et al. 2009) based on differences in host grooming (Bordes et al. 2007; Hawlena et al. 2007), host sex, and host age (Khokhlova et al. 2011). Previous evaluations of the flea index have determined that values of less than one flea per rat represent minimal risk of epidemic disease spread to humans, even in the presence of pathogens (Pollitzer 1954, Dennis et al. 1999). In New York City, early calculations of the flea index by Fox and Sullivan (1925) produced values of 0.22 for *X. cheopis*. The results of this survey, however, yielded a flea index of 4.1 *X. cheopis* when all sites were included. Moreover, rats captured from residential locations had an index value of 5.1, and one residential site had an average of 25.7 fleas per rat. With new information available about the number of on-host versus nest fleas (Krasnov et al. 2004), the efficiency of

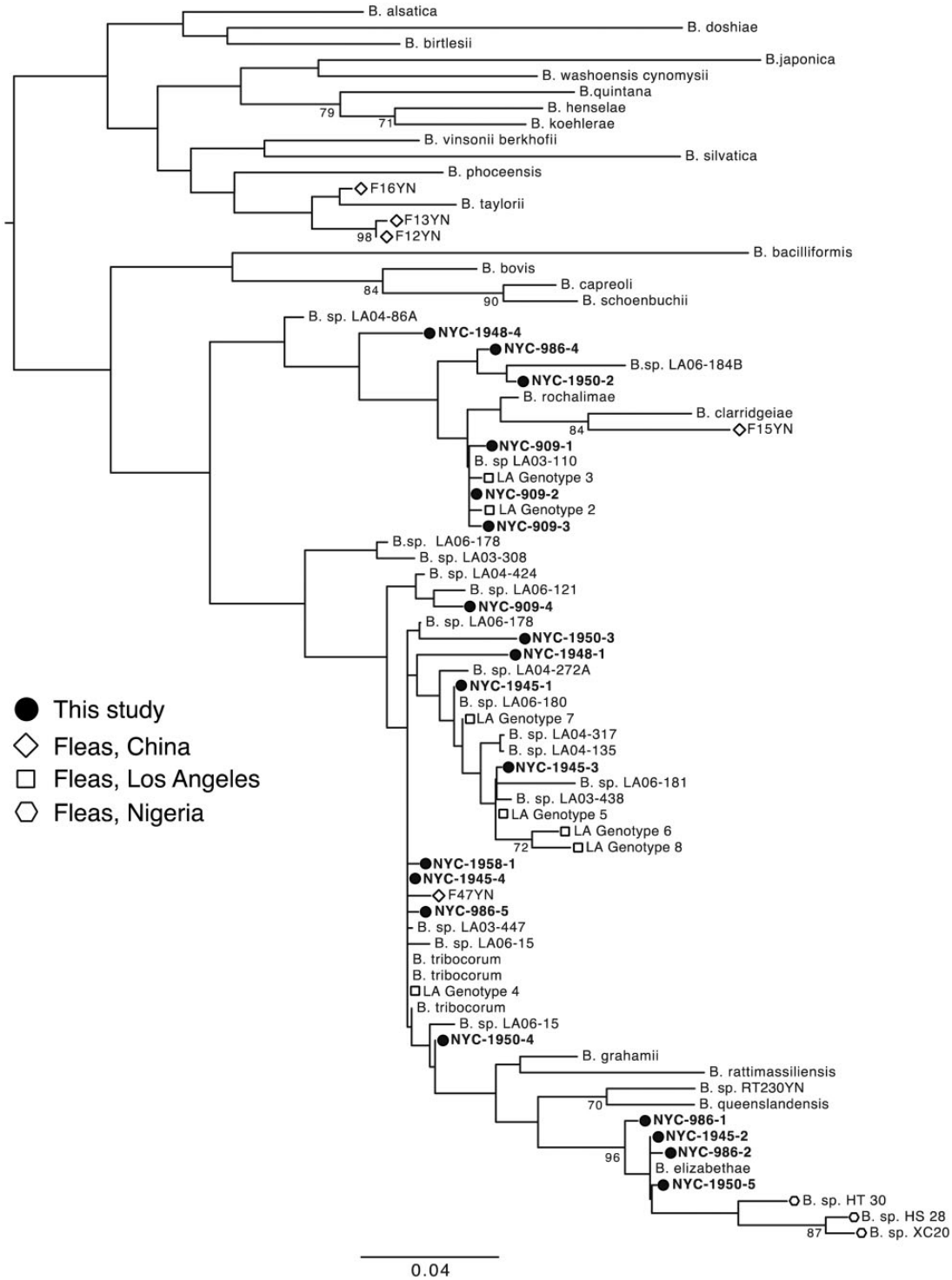

Fig. 1. Maximum likelihood phylogeny of a 327- nucleotide region of the *gltA* gene of *Bartonella* aligned along with representative sequences from other members of the genus available from GenBank. The sequences derived from this study are indicated by a circle, and those recovered from fleas in China, Los Angeles, and Nigeria are given by diamonds, squares, and triangles, respectively. The scale bar is in units of nucleotide substitutions per site.

X. cheopis as a vector (Eisen et al. 2007), and the factors influencing plague distribution (Maher et al. 2010), flea indices reported here suggest the potential for epidemic spread of plague in New York City if *Y. pestis* were introduced (Pollitzer 1954).

Although we did not detect the presence of *Y. pestis* or *R. typhi* in our samples, we did identify multiple species of *Bartonella*. These gram-negative bacteria infect mammalian red blood cells (Eisen and Gage 2012), and are vectored by arthropod ectoparasites such as fleas, mites, and lice (Kamani et al. 2013). Pathogenic *Bartonella* spp. are common among rodent species (Ellis et al. 1999), and recent evidence suggests that Norway rats and black rats [*Rattus rattus* (L., 1758)] have played a key role in the evolution and dissemination of *Bartonella* lineages throughout the world (Hayman et al. 2013). In New York City, serological evidence of human infection with *B. elizabethae*, *Bartonella henselae*, and *Bartonella quintana* was found in intravenous drug users from central and east Harlem (Comer et al. 2001), while *B. elizabethae* was detected in rats in Baltimore, MD (Easterbrook et al. 2007), and both *B. tribocorum* and *B. rochalimae* have been detected in rats (Gundi et al. 2012) and fleas from Los Angeles, CA (Billeter et al. 2011). Our detection of *Bartonella* species, including a high incidence of *B. tribocorum* in Oriental rat fleas, is therefore not surprising. This pathogen has also been detected in Asia (Li et al. 2007) and Africa (Kamani et al. 2013) and it is suspected to be relatively host-specific to *R. norvegicus* (Kamani et al. 2013) and primarily vectored by the Oriental rat flea (Reeves et al. 2007, Billeter et al. 2011). Further investigation is required to understand the role, if any, that this organism plays in human disease, and whether vectors such as *X. cheopis* are competent in transmitting bacteria from rodent reservoirs to humans.

In addition to *X. cheopis*, we recovered four ectoparasite species from Norway rats that are globally distributed (Fox and Sullivan 1925, Williams et al. 1929, Paramasvaran et al. 2009, Yang et al. 2009) and can cause dermatitis when they feed on humans (Engel et al. 1998). Whereas the vector capacity of *L. nuttalli* remains unknown (Montasser 2013), *P. spinulosa* and *O. bacoti* have both been implicated in maintaining *R. typhi* within rodent communities (Traub et al. 1978, Reeves et al. 2006). Based on their medical importance, the collection of >6,500 individual ectoparasites from 133 Norway rats critically highlights the need for rodent control programs that also address ectoparasites. Previously, rodenticide baits containing insecticides have been investigated (Leirs et al. 2001), and might act to reduce the incidence of accidental feeding by ectoparasites when rodent hosts are killed. However, further research is needed to identify compounds that, when added to cereal-based rodenticide grain baits, will not significantly reduce palatability to rodents (Borchert et al. 2010). A more sustainable approach to preventing problems with rodent ectoparasites is to implement an integrated pest management program that reduces rodent access to food, water, and shelter within human-inhabited structures. Integrated pest management programs often combine exclusion techniques to keep rodents out of structures with population reduction methods.

This investigation represents a preliminary survey of ectoparasites from Norway rats in Manhattan, New York, NY. We identified five ectoparasite species with a cosmopolitan distribution, including the Oriental rat flea, which was first recorded in Manhattan in 1923 (Fox and Sullivan 1925), and from New Jersey in the 1950 s (Burbutis and Hansens 1955). Despite recent surveys in other major metropolitan areas (Easterbrook et al. 2007, Billeter et al. 2011, Himsworth et al. 2013), this is the first study of ectoparasite disease vectors in Manhattan in ~90 years. Future and ongoing surveillance of rodents, their ectoparasites, and associated pathogens will be needed to profile the distribution of the Oriental rat flea and other ectoparasite species, including demodectic mites (Izdebska and Rolbjecki 2012). Our identification of *Bartonella* spp. from fleas, and the detection of multiple human pathogens from Norway rats reported elsewhere (Firth et al. 2014), indicates a risk of disease exposure for humans (Nieto et al. 2007) that could be addressed with rodent management efforts (Mohr et al. 1953).

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