Rodent-Borne *Bartonella* Infection Varies According to Host Species Within and Among Cities

Anna C. Peterson, Bruno M. Ghersi, Fernando Alda, Cadhla Firth, Matthew J. Frye, Ying Bai, Lynn M. Osikowicz, Claudia Riegel, W. Ian Lipkin, Michael Y. Kosoy, and Michael J. Blum

1Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA
2Museum of Natural Science, Louisiana State University, Baton Rouge, LA
3School of BioSciences, The University of Melbourne, Parkville, VIC, Australia
4New York State IPM Program, Cornell University, Geneva, NY
5Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO
6City of New Orleans Mosquito, Termite and Rodent Control Board, New Orleans, LA
7Center for Infection and Immunity, Mailman School of Public Health, Columbia University, New York, NY
8Bywater Institute, Tulane University, New Orleans, LA

Abstract: It is becoming increasingly likely that rodents will drive future disease epidemics with the continued expansion of cities worldwide. Though transmission risk is a growing concern, relatively little is known about pathogens carried by urban rats. Here, we assess whether the diversity and prevalence of *Bartonella* bacteria differ according to the (co)occurrence of rat hosts across New Orleans, LA (NO), where both Norway (*Rattus norvegicus*) and roof rats (*Rattus rattus*) are found, relative to New York City (NYC) which only harbors Norway rats. We detected human pathogenic *Bartonella* species in both NYC and New Orleans rodents. We found that Norway rats in New Orleans harbored a more diverse assemblage of *Bartonella* than Norway rats in NYC and that Norway rats harbored a more diverse and distinct assemblage of *Bartonella* compared to roof rats in New Orleans. Additionally, Norway rats were more likely to be infected with *Bartonella* than roof rats in New Orleans. Flea infestation appears to be an important predictor of *Bartonella* infection in Norway rats across both cities. These findings illustrate that pathogen infections can be heterogeneous in urban rodents and indicate that further study of host species interactions could clarify variation in spillover risk across cities.

Keywords: Fleas, *Rattus norvegicus*, *Rattus rattus*, Rodent-borne pathogens, Zoonoses

INTRODUCTION

Zoonotic pathogens are an emerging threat to human health and well-being (Jones et al. 2008), especially in areas where humans and wildlife frequently come in contact (Despommier et al. 2007; Jones et al. 2008; Lloyd-Smith et al. 2009). Rodent-borne pathogen transmission is of
particular concern in cities, where rodents can be widely distributed and hyper-abundant (Bradley and Altizer 2007; Rael et al. 2016). Commensal rodents like Norway rats (*Rattus norvegicus*) and roof rats (*Rattus rattus*) can carry bacterial and viral assemblages, including pathogens of concern (Ellis et al. 1999, Himsworth et al. 2013a, b; Firth et al. 2014). With rodents likely to drive future epidemics as cities continue to expand worldwide (Bordes et al. 2013; Han et al. 2015), determining the diversity and prevalence of rodent-borne pathogens in cities represents a vital step toward understanding how disease risk will progress with global demographic trends.

Many bacteria within the genus *Bartonella* are rodent-borne pathogens of concern (Anderson and Neuman 1997). *Bartonella* are gram-negative bacteria that can infect erythrocytes and endothelial cells in mammals (Anderson and Neuman 1997). At present, over 40 *Bartonella* species have been described, with most having been detected in bats and rodents (Jiyipong et al. 2012). Though *Bartonella* infections are thought to be relatively benign in rodents, several rodent-borne *Bartonella* species cause disease in humans, including febrile illness and endocarditis (Buffet et al. 2013). Humans can indirectly acquire pathogenic *Bartonella* from blood-feeding arthropods such as fleas (Bai et al. 2009; Billeter et al. 2011; Morick et al. 2011; Gutiérrez et al. 2015), or through biting or scratching by an infected mammalian host (Tsai et al. 2010; Billeter et al. 2011; Harms and Dehio 2012, Kosoy et al. 2012).

Despite potential public health risks, little work has been done to assess the diversity and prevalence of *Bartonella* in urban rodents. So far, studies have primarily surveyed Norway rats at small geographical scales, such as in a neighborhood within a city (Easterbrook et al. 2007, Gundi et al. 2012; Himsworth et al. 2013a, 2015). Yet infection in rodents appears to be heterogeneous, suggesting that ecological factors like host population size and movement might determine the diversity and prevalence of *Bartonella* in cities (Firth et al. 2014; Himsworth et al. 2015). Thus, it is possible that patterns of *Bartonella* infection may vary across and among cities, especially cities that harbor different rodent assemblages (Kosoy et al. 2015).

In this study, we examined the incidence of *Bartonella* in rats from two cities: New Orleans, Louisiana (NO), and New York City, New York (NYC). Several species of rats, including Norway rats and roof rats, occur in NO (Rael et al. 2016), whereas only Norway rats occur in NYC (Childs et al. 1998). Prior surveys of rats in NO have detected *Bartonella* (Ellis et al. 1999) among a suite of other zoonotic pathogens (Campbell and Little 1988; Cross et al. 2014). A recent survey in NYC also found that *Bartonella* was the most prevalent bacterial agent infecting Norway rats (Firth et al. 2014). We characterized the diversity and distribution of *Bartonella* in NO and NYC to assess whether the prevalence of *Bartonella* differs according to the (co)occurrence of host species within and among cities (Keesing et al. 2006, 2010). This enabled us to identify factors that might influence spillover risk (i.e., transmission from wildlife hosts to humans) and thus provide practical guidance for improving pathogen surveillance programs.

**METHODS**

**Sample Collection**

In NO, we collected a total of 342 rats from May 2014 to March 2015 (Table 1) following Tulane University IACUC-approved protocol #0451. A subset of 272 rats was collected during a quantitative population survey across 78 residential city blocks in eight neighborhoods (Fig. 1) (Gulachenkski et al. 2016; Rael et al. 2016). Each block was visited twice, once during May–August 2014, and a second time during November 2014–February 2015. During each trapping period, we set 30 Tomahawk traps (Tomahawk Live Trap Company, Tomahawk, WI) in areas with potential or evident rodent activity for a minimum of three consecutive nights. Trapping efforts were sustained at each site until no additional rodents were captured. We trapped the remaining 70 rats opportunistically as part of control efforts conducted by the City of New Orleans Mosquito, Termite, Rodent Control Board (NOMTRB) between May 2014 and March 2015. Rats were collected using the same methods reported above, but the number of trapping days varied by location.

We necropsied all NO rats at NOMTRB’s facility following a standard protocol. We euthanized NO rats using isoflurane anesthesia followed by cardiac puncture. Blood samples were spun down to separate serum from coagulates. We took standard weight and length measurements and determined the species, sex, and sexual maturity as well as parity in females. We combed each individual for ectoparasites, which we later identified using standard keys (Furman and Catts 1970). We also collected lung, liver, spleen, kidney, urine, and tail tissue samples, which we archived in −80°C freezers.
In NYC, we collected 133 Norway rats (*R. norvegicus*) from five locations in midtown and lower Manhattan between September 2012 and June 2013 (Fig. 1; Table 1) (Firth et al. 2014). Trapping sites included high-density housing complexes, a mixed-use indoor public space and an urban park (Firth et al. 2014; Frye et al. 2015). We trapped all rodents using Tomahawk traps that were baited and left open for 7–10 days to allow for acclimation by the rodents, followed by up to 10 nights of trapping. All captured individuals were euthanized with an over-anesthetization of isoflurane according to Columbia University IACUC-approved protocol #AC-AAAE6805. Following euthanasia, we fumigated carcasses with ethyl acetate and combed carcasses over dry ice for ectoparasite collection. Ectoparasites were identified using standard keys (Frye et al. 2015). Data on rat weight and sex were obtained, and following a standardized necropsy protocol (described in Firth et al. 2014), serum, liver, spleen, kidney, and heart tissue samples were collected and stored at −80°C. 

**Bartonella Screening**

Both NO and NYC samples were screened for *Bartonella* following PCR-based protocols. With the exception of one Norway rat (*R. norvegicus*) and one roof rat (*R. rattus*), we extracted and screened DNA from all NO samples (Table 2). We homogenized 10–20 mg of spleen tissue from each NO individual in a bead vial with 100 µL of brain–heart infusion medium (BHI), which was then lysed overnight at 55°C. All DNA extractions were subsequently completed using a QIAxtractor® (Qiagen, Valencia, CA) following manufacturer instructions. We used the resulting DNAs in a multiplex qPCR of the tmRNA region to screen for *Bartonella* as well as other pathogens (Bai et al. 2013). We also screened for *Bartonella* through conventional PCR of the ITS (325, 1100) region (Table 2) (Diniz et al. 2007). For all individuals that tested positive for *Bartonella* through either method, we confirmed infection by sequencing both strands of the citrate synthase gene *gltA* region using forward and reverse primers BhCS781.p and BhCS1137.n (Norman et al. 1995). Only animals from which we were able to sequence the *gltA* region were considered positive for *Bartonella* infection (Table 2). Similarly, for all NYC rodents, DNA extracted from fecal, liver, serum, and spleen tissue was screened separately for *Bartonella* using a PCR assay targeting the *gltA* region (Table 2), which was then sequenced to confirm infection (Firth et al. 2014).

We also screened for *Bartonella* by culturing from blood sampled from all NO rodents (Table 2) and by culturing from heart tissue of NYC rodents that tested positive according to *gltA* PCR screening from any tissue (Table 2). For NO rodents, we plated ~10 µL of blood, while for NYC rodents we homogenized heart tissue in 400 µL of BHI medium and plated 100 µL of the homogenate. All cultures were plated on BHI agar supplemented with 10% rabbit blood and incubated at 5% CO₂ and 35°C for 4 weeks. We checked all plates once weekly to screen for the presence of colonies exhibiting a morphology consistent with *Bartonella* (e.g., round, opaque, white-to-cream in

### Table 1. Sex, Age Categories and Percent of All Captured Rodent Individuals Infested with Fleas (*C. felis* and *X. cheopis*) from New Orleans, LA (NO), and New York City, NY (NYC).

<table>
<thead>
<tr>
<th>Age class</th>
<th>Total infested with *C. felis (%)</th>
<th>Total infested with *X. cheopis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R. norvegicus (NO)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juvenile</td>
<td>Subadult</td>
<td>Adult</td>
</tr>
<tr>
<td>12 F</td>
<td>18 F</td>
<td>53 F</td>
</tr>
<tr>
<td>7 M</td>
<td>13 M</td>
<td>59 M</td>
</tr>
<tr>
<td><strong>R. rattus (NO)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 F</td>
<td>62 F</td>
<td>11 F</td>
</tr>
<tr>
<td>19 M</td>
<td>42 M</td>
<td>21 M</td>
</tr>
<tr>
<td><strong>S. hispidis (NO)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 F</td>
<td>0 F</td>
<td>1 F</td>
</tr>
<tr>
<td>0 M</td>
<td>0 M</td>
<td>1 M</td>
</tr>
<tr>
<td><strong>R. norvegicus (NYC)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 F</td>
<td>16 F</td>
<td>19 F</td>
</tr>
<tr>
<td>29 M</td>
<td>24 M</td>
<td>19 M</td>
</tr>
</tbody>
</table>

*Number of female rodents captured.

*Number of male rodents captured.
color) as well as the presence of other bacterial colonies. For all instances of putative *Bartonella* growth, a single colony was collected from each plate and placed in glycerol and heated for 10 min at 95°C for lysis and DNA extraction. The lysate was used for PCR and sequencing of a partial region of the *gltA* gene using forward and reverse primers BhCS781.p and BhCS1137.n (Norman et al. 1995). If a plate showed evidence of morphologically dissimilar *Bartonella* colonies, we extracted and sequenced a separate isolate from each *Bartonella* morphotype. NO samples (n = 28) that exhibited overgrowth of putative non-*Bartonella* bacterial contamination were excluded from all analyses.

**Phylogenetic Analyses**

We edited and trimmed all sequences of the *gltA* gene to a 327-bp fragment overlapping the most extensive archive of reference sequence data available in GenBank. In addition to retrieving all available *Bartonella gltA* reference sequences for comparison to NO and NYC isolates, we also retrieved *Rickettsia gltA* sequences to serve as outgroups.
We constructed phylogenetic hypotheses using Bayesian Inference in MrBayes 3.2.6 (Ronquist et al. 2012). Using the GTR + G model, we ran two simultaneous Markov chain Monte Carlo analyses with four chains for $4 \times 10^6$ generations. Trees were sampled every 1000 generations, and the first 1000 trees (25%) were discarded as burn-in. Convergence was established when the final deviation of split frequencies fell below 0.005. All analyses were performed in the CIPRES Science Gateway 3.1 Portal (Miller et al. 2010). Sequences of all variants encountered in NO and NYC were deposited in GenBank (accession numbers MG027916–MG027998).

Species were identified according to percent sequence similarity and coverage of gltA amplicons in comparison with archived sequences using the Basic Local Alignment Search Tool for nucleotides (BLAST), and through phylogenetic analysis of sequence variation.

### Ecological Analyses

We developed generalized linear models (GLMs) with a binomial error distribution to determine the relationship between Bartonella infection and attributes of individual rodents. For NO, we ran a single GLM model to determine if species was a significant predictor of Bartonella detection (i.e., 0 vs. 1) and then ran two separate GLM analyses to determine whether age class, sex, or flea infestation status (i.e., 0 vs. 1) were significantly related to Bartonella infection in Norway rats and roof rats (Table 2), respectively. Similarly, we ran a GLM analysis to determine whether age class, sex, or flea infestation status was significantly related to Bartonella infection in Norway rats from NYC. All individuals for both NYC and NO were placed into an age class (juvenile, subadult, adult) based on body weight (Table 1) (Mcguire et al. 2006; King et al. 2011).

<table>
<thead>
<tr>
<th>Method</th>
<th>R. norvegicus (NO)</th>
<th>R. rattus (NO)</th>
<th>S. hispidus (NO)</th>
<th>R. norvegicus (NYC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>163  29$^a$  19  10  5  0  0</td>
<td>177  5$^a$  0  0  0  5  0</td>
<td>2  0  0  0  0  0  0</td>
<td>31  25$^a$  26  3  0  0  0</td>
</tr>
<tr>
<td>Direct PCR (tmRNA)$^b$</td>
<td>162  17$^c$</td>
<td>176  3$^c$</td>
<td>2  0  0  0  0  0  0</td>
<td>31  25$^a$  26  3  0  0  0</td>
</tr>
<tr>
<td>Direct PCR (ITS)$^b$</td>
<td>125  40$^c$</td>
<td>79  6$^c$</td>
<td>2  0  0  0  0  0  0</td>
<td>31  25$^a$  26  3  0  0  0</td>
</tr>
<tr>
<td>Direct PCR (gltA)$^b$</td>
<td>86  19$^a$  4  1  0  0  0</td>
<td>66  0  0  0  0  0  0</td>
<td>2  0  0  0  0  0  0</td>
<td>133  31$^a$  24  1  0  0  0</td>
</tr>
</tbody>
</table>

Individuals that were successfully PCR-amplified for tmRNA and ITS were not all necessarily confirmed positive. Confirmation of Bartonella infection in both culture and tissue provided only through sequencing of the gltA gene.

$^a$Confirmed Bartonella positive through sequencing of the gltA gene, some individuals infected with more than one Bartonella variant.

$^b$Direct PCR of spleen tissue.

$^c$Successfully amplified (produced a PCR band), considered putatively positive.

$^d$Direct PCR of spleen and heart tissue, considered positive if either heart or spleen was positive.
We used several approaches to assess the (co)occurrence and distribution of Bartonella variants. We first constructed median-joining networks of variants in Network (Fluxus Technology Ltd., http://www.fluxus-engineering.com) according to screening method, species, and city. Following Firth et al. (2014), we also explored patterns of coinfection within individuals using the Fortran software PAIRS v 1.1, which implements a Bayesian approach to detect non-random associations between pairs of taxa. This was done only for Norway rats, as no roof rats exhibited coinfection with more than one Bartonella species. We considered coinfection in individuals from both NO and NYC together, as well as from each city individually. Additionally, we compared Bartonella diversity between cities, accounting for variation in the scale and intensity of sampling efforts. Using the package rareMNtests in R (Cayuela et al. 2015; R Core Team 2013), we employed biogeographic and ecological null model comparisons of sample-based rarefaction curves of Bartonella variant diversity in NO and NYC.

Results

Bartonella Infection Prevalence

Collections of rats and fleas differed between cities. We collected three species of rat in NO: hispid cotton rats (Sigmodon hispidus; n = 2), Norway rats (R. norvegicus, n = 163), and roof rats (R. rattus, n = 177). Only Norway rats (n = 133) were collected in NYC (Table 1). We detected Xenopsylla cheopis and Ctenocephalides felis fleas on NO rats, but only X. cheopis was detected on NYC rats (Frye et al. 2015) (Table 1).

We confirmed Bartonella infection in 13.5% of rats from NO and 23% of rats from NYC. Bartonella infection was confirmed in 40 Norway rats from NO and in 31 Norway rats from NYC (Table 2), whereas it was only confirmed in 5 roof rats from NO. We did not detect Bartonella in either of the NO cotton rats; thus, no further consideration was given to cotton rats in this study. For the NO rats, direct PCR of the ITS region for NO rats identified a greater number of individuals as putatively positive than the number confirmed to be infected through sequencing of the gItA region (Table 2). We also confirmed infection in a greater number of individuals through a combination of culture and sequencing than through direct PCR and sequencing in NO (Table 2). Direct PCR and sequencing yielded a slightly higher number of confirmed infections in rats from NYC (Table 2).

The prevalence of Bartonella infection was heterogeneous in both cities. In NO, within-site prevalence ranged from 0 to 97% of individuals infected, with 85% of all Bartonella positive individuals captured within a single city block. All Bartonella positive Norway rats were captured at two locations, where no roof rats were present. We captured Bartonella infected roof rats from five locations. Both roof rats and Norway rats were captured at four of these locations, though none of the Norway rats were Bartonella positive at the locations. We detected Bartonella positive Norway rats at all five trapping locations in NYC, though within-site prevalence ranged from 10 to 85%.

Species, flea infestation, and age class were significant predictors of Bartonella infection. Species identity was a predictor of Bartonella infection in NO rats (P < 0.01, coef. = −2.05, d.f. = 329). When considering Norway and roof rats separately, flea infestation was a significant predictor of infection in Norway rats from both NO and NYC, whereas it was not a predictor of infection in roof rats from NO (Table 3). Bartonella infection corresponded to age class in Norway rats from NYC, with juvenile individuals less likely to harbor Bartonella relative to subadults and adults (Table 3). Both male and female rats had an equal likelihood of infection in both NO and NYC (Table 3).

Bartonella Diversity

There were significant differences in Bartonella diversity among host species within the same city and in the same host species between cities (Table 2; Figs. 2, 3). Sequences from NO rats aligned with B. cooperisplainsensis (100% similarity, 100% coverage), B. rochalimae (98–99% similarity, 100% coverage), B. elizabethae (99–100% similarity, 100% coverage), B. tribocorum (99–100% similarity, 99–100% coverage), and B. queenslandensis (100% similarity, 100% coverage). Direct PCR and sequencing recovered variants of B. rochalimae, B. elizabethae, and B. tribocorum, whereas culture and sequencing recovered variants of B. elizabethae, B. tribocorum, and B. queenslandensis (Table 2, Fig. 3) from Norway rats in NO. A variant of B. cooperisplainsensis was only detected in roof rats from NO via culture and sequencing (Table 2, Fig. 3). Sequences from NYC Norway rats aligned with B. elizabethae (100% similarity, 100% coverage) and B. tribocorum (99–100% similarity, 99–100% coverage). Provisional identifications of
variants agreed with the recovery of aligned sequences in well-supported clades (Fig. 2).

Results of the rarefaction null model comparisons indicate that *Bartonella* variant richness and species assemblages (as detected through culture) significantly differed between NO and NYC. The ecological null model was rejected ($P < 0.05$), indicating that differences in variant richness observed between NO and NYC are greater than would be expected from a random sampling from a single assemblage (Cayuela et al. 2015). The biogeographical null model also was rejected ($P < 0.05$), further indicating that there are significant differences in variant richness between NO and NYC, regardless of host species composition (Cayuela et al. 2015).

**Bartonella Coinfection and Co-occurrence**

Norway rats from NO and NYC were infected by more than one species of *Bartonella*, but we did not detect a significant association between any particular species pair (PAIRS analysis, $P > 0.05$). In NO, 20% of Norway rats harbored more than one *Bartonella* species, while only 9% of Norway rats in NYC harbored more than one species. We detected up to three different *Bartonella* species in individuals sampled from NO and up to two different *Bartonella* species in individuals sampled from NYC. Coinfections were observed between all combinations in Norway rats from NO and NYC. Additionally, we detected different *Bartonella* species in spleen versus blood from the same individual from NO.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictor</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. norvegicus</em> (NYC)<em>a</em> infection</td>
<td>Flea infestation</td>
<td>2.23</td>
<td>0.55</td>
<td>$&lt; 0.05$</td>
</tr>
<tr>
<td></td>
<td>Age class (juv.)</td>
<td>-2.65</td>
<td>0.73</td>
<td>$&lt; 0.05$</td>
</tr>
<tr>
<td></td>
<td>Sex (M)</td>
<td>0.25</td>
<td>0.51</td>
<td>0.63</td>
</tr>
<tr>
<td><em>R. norvegicus</em> (NO)<em>b</em> infection</td>
<td>Flea infestation</td>
<td>5.40</td>
<td>0.75</td>
<td>$&lt; 0.05$</td>
</tr>
<tr>
<td></td>
<td>Age class (juv.)</td>
<td>-0.56</td>
<td>1.31</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>-0.95</td>
<td>0.76</td>
<td>0.21</td>
</tr>
<tr>
<td><em>R. rattus</em> (NO)<em>c</em> infection</td>
<td>Flea infestation</td>
<td>-16.16</td>
<td>10754.01</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Age class</td>
<td>-17.41</td>
<td>1788.03</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Sex</td>
<td>-0.39</td>
<td>0.81</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Statistically significant predictors are in bold.

*a* $d.f. = 158$.

*b* $d.f. = 166$.

*c* $d.f. = 132$.

## Discussion

**Bartonella Infection**

Public health threats from rodent-borne pathogens are expected to increase with global trends in urbanization (Han et al. 2015). Understanding the prevalence and distribution of rodent-borne bacteria can help mitigate transmission risk and spread of pathogenic species, especially in areas where humans and rodent reservoirs come into frequent contact. Notably, we detected pathogenic species (*B. tribocorum*, *B. elizabethae*, and *B. rochalimae*) in all but one of the Norway rats collect in NO, and in all of the Norway rats from NYC (Daly et al. 1993; Comer et al. 2001; Eremeeva et al. 2007). However, we found that the prevalence of *Bartonella* (including pathogenic species) is highly heterogeneous within and among the two cities. Consistent with patterns of prevalence in other temperate cities such as Vancouver (Himsworth et al. 2015), prevalence of *Bartonella* infection ranged from 0 to 97% among sites in NO and 10–85% among sites in NYC. This suggests that the potential risk of pathogen spillover is likely also asymmetrically distributed across urban landscapes and that there may be localized hot spots of risk in cities.

Though environmental or built features of the urban landscape may govern clustering of infection in rodent populations (i.e., by facilitating or impeding movement), our results indicate that clustering may instead reflect host–ectoparasite interactions. We found that *X. cheopis* flea infestation is a significant predictor of *Bartonella* infection.
in Norway rats (*R. norvegicus*) in both cities (Table 3). The relationship between flea infestation and *Bartonella* infection is evident even at very small spatial scales. In NO, 35 of the 38 Norway rats with detectable flea infestation were collected from a single location where 97% of individuals were also positive for *Bartonella* infection. Differences in ectoparasite communities may also account for differences found in *Bartonella* infection between co-occurring rat species. We found comparable levels of flea infestation in Norway rats from NO (~24%) and NYC (~30%), whereas flea infestation was much rarer in roof rats (*R. rattus*) from NO (~1%). Similarly, Norway rats were more likely to be infected with *Bartonella* than were roof rats in NO. *Bartonella* infection was rare in roof rats (~3% tested positive), and concordantly, flea infestation was not a significant predictor of *Bartonella* infection in roof rats (Table 3). Evidence that roof rats only carry *B. coopersplainensis* also suggests that the spread of *Bartonella* species differs according to host–ectoparasite interactions. Neither *X. cheopis* nor *C. felis* develop on their hosts, but rather develop in the nest, in organic debris, or in soil (e.g., Rothschild 1975). Differences in nesting behaviors may provide more (Norway rats) or less (roof rats) hospitable microclimates for flea development, as has been seen in other flea–rodent systems (Krasnov et al. 1997). Behavioral differences related to grooming may also be important (Bordes et al. 2007; Havlena et al. 2007). Additionally, ectoparasites other than fleas may spread *Bartonella* infection in roof rats, such as rat mites and rat lice (Tsai et al. 2010), which were found on all of the *Bartonella* positive roof rats in NO. Though this inference is consistent with prior surveys that have detected *B. tribocorum* and *B. elizabethae* where *X. cheopis* was more prevalent on roof rats (Morick et al. 2009), further comparisons will be necessary to clarify whether host–ectoparasite interactions mediate transmission of different *Bartonella* species in urban rats, including those known to cause human disease.

**Bartonella Diversity**

Results indicate that the diversity of *Bartonella* bacteria differs between hosts within a city and within a host between cities. Norway rats harbor a more diverse and distinct complement of *Bartonella* compared to roof rats in NO, and Norway rats in NO harbor a greater diversity of *Bartonella* than do Norway rats in NYC. Rejection of the biogeographical null model suggests that *Bartonella* diversity may reflect local conditions or historical events (Cayuela et al. 2015), while rejection of the ecological null model suggests that meta-community processes may play a role in structuring *Bartonella* diversity (Cayuela et al. 2015). This is consistent with our inference that *Bartonella* infection varies according to host–ectoparasite interactions. It is also consistent with prior work showing that arthropod vectors influence *Bartonella* diversity in rodent hosts (e.g., Buffet et al. 2013) and that the same host species harbors distinct ectoparasite assemblages in different cities. In NO, we detected *C. felis* and *X. cheopis* on rat hosts, whereas only *X. cheopis* was detected on rats in NYC. While we did not detect *C. felis* on infected rats in NO, experimental infections (Bouhsira et al. 2013) show that *C. felis* can carry *Bartonella*, which suggests that it can promote infection of rat hosts. This hypothesis could be tested by assessing *Bartonella* diversity within arthropod vectors and their associated rodent hosts.

**Bartonella Detection and Occurrence**

Despite differences in *Bartonella* variant assemblages, we detected identical *Bartonella* variants in Norway rats in both cities (Fig. 3). We recovered sequences from NO and NYC within clades of *B. tribocorum* and *B. elizabethae*, which are globally distributed species (Daly et al. 1993; Buffet et al. 2013). We found no overlap of *Bartonella* species or variants in Norway and roof rats in NO, even though nearly all roof rats were collected from locations that also harbored Norway rats (Fig. 1). This indicates that there is little-to-no transmission of *Bartonella* between Norway and roof rats in NO, which is consistent with evidence from wild sylvatic rodents that co-occurring host species can harbor unique assemblages of *Bartonella* (Kosoy et al. 1997). As with prevalence and diversity, patterns of *Bartonella* infection in urban rats could be attributable to differences in host–ectoparasite interactions.

Our results affirm that more than one method of testing can be necessary to detect and identify all *Bartonella* that...
may be present in urban rodents. Individual rodents can harbor several Bartonella species, which may not reside in the same tissue. Our findings also illustrate that direct PCR may not detect all Bartonella species or variants within an individual host or tissue (Harms and Dehio 2012). Culturing also has limitations; though the approach can be useful for detecting and sequencing morphologically dissimilar Bartonella isolates collected from a single individual host, some Bartonella species can be difficult to cultivate, including known human pathogens like B. rochalimae (Gundi et al. 2012). Consistent with this, we only detected B. rochalimae through direct PCR (Figs. 2, 3) (Firth et al. 2014).

Public Health Implications

Urban populations of commensal rats can support diverse and heterogeneous assemblages of Bartonella, including pathogenic species of concern. Variation in prevalence and diversity may give rise to hot spots of public health risk—even on very small spatial scales (e.g., we detected six unique Bartonella variants, including two species known to cause human disease, on a single city block in NO). Variation in prevalence and diversity may be a common phenomenon, as heterogeneous distributions of Bartonella have been detected in other cities (Himsworth et al. 2015). Similar patterns also have been observed with other rodent-borne pathogens such as hantaviruses and Rickettsia (Himsworth et al. 2015). Accordingly, additional cross-city comparisons could help constrain and reduce potential risk by informing disease surveillance programs. Further understanding of host–parasite interactions also could help reduce infection risk. Consideration should be given to factors that foster interaction diversity (Dyer et al. 2010), including conditions like mosaics of abandonment (Gulachenski et al. 2016; Rael et al. 2016) that can yield differences in ectoparasite communities on rodent hosts (Krasnov et al. 2007). Consideration should also be given to landscape management as an approach for reducing infection risk, particularly in cities where rodents are more abundant in disadvantaged neighborhoods (Gulachenski et al. 2016; Rael et al. 2016; Lewis et al. 2017).

Acknowledgements

We would like to thank T. Madere, F. Bauder, P. Smith, E. Guidry, A. Gulachenski, H. Rahn, A. Powell, J. Haydel, S. Triplett, S. Piper, and H. Patel for providing assistance with fieldwork and laboratory work. This study was supported by the National Science Foundation (BCS-1313703), the Louisiana Board of Regents LINK program and the National Institute of Environmental Health Sciences (5P30 ES009089) A. Peterson also was supported by a Louisiana Board of Regents graduate fellowship. The
funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

**References**


Krasnov BR, Shenbrot GI, Medvedev SV, Vatschenok VS, Khokhlova IS (1997) Host-habitat relations as an important determinant of spatial distribution of flea assemblages (Siphonaptera) on rodents in the Negev Desert. Parasitology 114:159–173. https://doi.org/10.1017/S0031182096008347


