Bartonella spp. are small, pleomorphic, Gram-negative bacteria that can invade and replicate in erythrocytes and endothelial cells. Infection often leads to prolonged intracytoplasmic bacteremia within the reservoir host (4). Of the >20 Bartonella spp. characterized to date, more than half are suspected or known to be pathogenic to humans (3), and most are believed to be transmitted by arthropod vectors (1).

Fleas have been suspected vectors of Bartonella spp. for several decades. Bartonella DNA has been detected in flea species on all continents, excluding Antarctica (1). The Oriental rat flea, Xenopsylla cheopis, is believed to transmit several Bartonella spp., including Bartonella tribocorum, Bartonella elizabethae, Bartonella queenslandensis, and Bartonella rochali-mae (8), though experimental transmission studies have not been performed to verify this supposition. X. cheopis fleas are distributed worldwide and infest mainly rodents but will bite humans.

The aim of the current study was to determine the prevalence of Bartonella spp. in X. cheopis fleas removed from Rattus norvegicus rats in Los Angeles County, CA. In a separate survey, R. norvegicus rats, infested with fleas examined for this study, were also screened for the presence of Bartonella DNA. Ninety-one amplicons were sequenced: Bartonella rochalimae-like DNA was detected in 66 examined fleas, and Bartonella tribocorum-like DNA was identified in 25 fleas. The data obtained from this study demonstrate an extremely high prevalence of Bartonella DNA in rat-associated fleas.

Of 200 individual Xenopsylla cheopis fleas removed from Rattus norvegicus rats trapped in downtown Los Angeles, CA, 190 (95%) were positive for the presence of Bartonella DNA. Ninety-one amplicons were sequenced: Bartonella rochalimae-like DNA was detected in 66 examined fleas, and Bartonella tribocorum-like DNA was identified in 25 fleas. The data obtained from this study demonstrate an extremely high prevalence of Bartonella DNA in rat-associated fleas.

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presence of Bartonella DNA are described elsewhere (Gundi et al., submitted).

Prevalence and genetic heterogeneity of Bartonella in X. cheopis fleas. A total of 200 individual X. cheopis fleas, collected from 55 R. norvegicus rats, were examined for the presence of Bartonella DNA. Of those, 95% were PCR positive using gltA-specific primers. Sequencing and further phylogenetic analysis of 91 fleas revealed that 72.5% (66/91) harbored B. rochalimae-like DNA versus 27.5% (25/91) that contained DNA most closely related to B. tribocorum (H92732/H1100514, P/H110210.01).

Of the 91 sequences examined, 8 genotypes with 86.8 to 99.7% similarity were found. The 8 genotypes were clustered around either B. rochalimae BMGH (DQ683195) (genotypes 1 to 3, GenBank accession numbers JF522364 to JF522366) or B. tribocorum IBS5067 (AJ005494) (genotypes 4 to 8, GenBank accession numbers JF522367 to JF522371) with sequence identities ranging from 98.5 to 98.8% and 97.9 to 99.7%, respectively. The B. rochalimae group (genotypes 1 to 3), detected in fleas recovered from 40 R. norvegicus rats, contained gltA sequences that were 99.4 to 99.7% homologous. Genotype 1 contained 64 identical sequences and genotypes 2 and 3 were both single sequences. The B. tribocorum group (genotypes 4 to 8), found in fleas removed from 20 rats, harbored sequences that were 98.1 to 99.7% homologous. Genotype 4 consisted of 2 sequences, genotype 5 contained 20 identical sequences, and genotypes 6 to 8 represented single sequences (Fig. 1).

Comparison between Bartonella spp. detected in fleas and those detected in their respective rat hosts. As depicted in Table 1, 27 B. rochalimae-positive fleas and 8 B. tribocorum-positive fleas were collected from 23 rats that were PCR positive for the presence of B. tribocorum only. Eight B. rochalimae-positive fleas and 1 B. tribocorum-positive flea were collected from 6 rats that harbored only detectable B. rochalimae DNA. Twenty B. rochalimae-positive fleas and 7 B. tribocorum-positive fleas were collected from 12 rats that had detectable DNA of both Bartonella spp. Eleven B. rochalimae-

TABLE 1. Comparison between Bartonella spp. detected in rats and in their respective fleas

<table>
<thead>
<tr>
<th>Bartonella DNA detected in rats</th>
<th>% (no.) of fleas PCR positive for B. rochalimae</th>
<th>% (no.) of fleas PCR positive for B. tribocorum</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. tribocorum positive only (23)</td>
<td>29.7 (27)</td>
<td>8.8 (8)</td>
</tr>
<tr>
<td>B. rochalimae positive only (6)</td>
<td>8.5 (8)</td>
<td>1.1 (1)</td>
</tr>
<tr>
<td>Positive for both agents (12)</td>
<td>22.0 (20)</td>
<td>7.7 (7)</td>
</tr>
<tr>
<td>Negative (14)</td>
<td>12.1 (11)</td>
<td>9.9 (9)</td>
</tr>
<tr>
<td>Total (55)</td>
<td>72.5 (66)</td>
<td>27.5 (25)</td>
</tr>
</tbody>
</table>

* Blood collected from 55 R. norvegicus rats and individual X. cheopis fleas were examined by PCR analysis for the presence of the Bartonella gltA gene. Data from 91 PCR positive fleas is represented in the table.
positive fleas and 9 B. tribocorum-positive fleas were found on 14 R. norvegicus rats that were PCR negative for the presence of Bartonella DNA.

From 91 fleas, B. tribocorum was detected in 27.5% and B. rochalimae was identified in 72.5% of these samples. A separate report (Gundi et al., submitted) also examined R. norvegicus rats, which served as hosts for fleas in this study, for the presence of Bartonella by culture and PCR analysis. Bartonella tribocorum was isolated from the blood of over half (56.4% of 55 rats) of R. norvegicus rats, while only 2 harbored viable B. rochalimae organisms. By PCR analysis, 41.8% (23 of 55) of rats harbored B. tribocorum only, 10.9% (6 of 55) had only detectable B. rochalimae DNA, and 21.8% (12 of 55) of rats had detectable DNA from both agents when screened using gltA-specific PCR primers (Gundi et al., submitted). In contrast, the majority of X. cheopis fleas examined in this study harbored B. rochalimae-like DNA. The reason for the disparity in Bartonella sp. composition between rats and their respective fleas remains unclear. As speculated by Tsai et al. (8), it is possible that selective pressure or competition allows certain species to adapt to the mammalian host, while others dominate in the potential arthropod vector. Because PCR products were not cloned and sequenced, it cannot be ruled out that fleas harbored both B. rochalimae and B. tribocorum DNA. Furthermore, detection of DNA in the flea does not demonstrate viability of organisms or infection, and the Bartonella spp. may have been acquired during a previous blood meal.

From a public health standpoint, it is important to ascertain whether R. norvegicus rats serve as reservoirs for B. rochalimae due to their close contact with humans and their pets. Furthermore, R. norvegicus rats are infested with ectoparasites, such as X. cheopis fleas, that will feed on humans and can transmit potentially deadly zoonotic pathogens. Further investigation is warranted to identify if X. cheopis fleas are competent vectors of Bartonella spp. and if R. norvegicus rats serve as reservoir hosts for these organisms.

**Nucleotide sequence accession numbers.** Sequences, representative of genotypes obtained from this study, were deposited in GenBank under accession numbers JF522364 to JF522371.

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**REFERENCES**


