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Rickettsia and Bartonella Species in Fleas from Reunion Island

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Abstract. Rickettsia felis, Rickettsia typhi, and Bartonella DNA was detected by molecular tools in 12% of Rattus rattus fleas (Xenopsylla species) collected from Reunion Island. One-third of the infested commensal rodents captured during 1 year carried at least one infected flea. As clinical signs of these zoonoses are non-specific, they are often misdiagnosed.

INTRODUCTION

Adult male and female fleas are obligate hematophagous ectoparasites of mammals and birds throughout the world and can contaminate their hosts with bacteria, viruses, and blood-borne parasites. The two most common routes of pathogen transmission by fleas are 1) oral route, by the regurgitation of blood meals at the flea-bite site; and 2) fecal, though skin lesions contaminated with infected fecal pellets by scratching. Fleas are vectors of several important bacterial zoonoses, including plague (Yersinia pestis), bartonellosis (several Bartonella species), and rickettsioses such as murine typhus (Rickettsia typhi), flea-borne spotted fever (Rickettsia felis), and occasionally sylvatic epidemic typhus (Rickettsia prowazekii). Reunion is a tropical oceanic island of volcanic origin located in the Indian Ocean, East of Madagascar. To date, no information is available on the distribution of flea species and flea-borne zoonoses on this island. Several murine typhus cases were recently confirmed using serological and molecular tools in travelers and autochthonous people from Reunion Island. In addition, the seroprevalence rate of bartonellosis in dogs was estimated to be ~10%. The aim of this study was to analyze the presence of Rickettsia and Bartonella species in fleas sampled from small mammals on this island, which has favorable climatic and ecological conditions for the proliferation of fleas and their hosts.

THE STUDY

During a 1-year survey (2012–2013), fleas were collected from small terrestrial mammals, including the black rat (Rattus rattus), the brown rat (Rattus norvegicus), the Asian house shrew (Suncus murinus), the house mouse (Musmusculus), and the tailless tenrec (Tenrec ecaudatus), captured at 19 localities (Figure 1) on Reunion Island in various biotopes. Wire cage live traps (29 × 18 × 12 cm) were used for rat and tenrec trapping, and Sherman live traps were used for mice and shrews. All animal procedures carried out in this study were approved by the French Institutional Ethical Committee (CYROI) under no. 114. Fleas were manually collected with a brush or forceps and identified to the species level using the morphological criteria. The detailed descriptions of the distribution and ecology of the collected fleas and their animal hosts are the subject of another study. A total of 205 flea DNA samples extracted as previously described, were sent in dry ice to the World Health Organization (WHO) Center for Rickettsial Diseases, Marseille. These fleas were collected from 59 small mammals including 52 R. rattus, two R. norvegicus, four S. murinus, and one M. musculus. We analyzed four flea species, i.e., Xenopsylla cheopis (134/205), Xenopsylla brasiliensis (57/205), Leptopsylla segnis (13/205), and Echidnophaga gallinaceae (1/205), for the presence of Rickettsia and Bartonella DNA by quantitative polymerase chain reaction (qPCR) using a CFX96qPCR Detection System (Bio-Rad, Marnes-la Coquette, France). All positive (R. felis, R. typhi, and Bartonella elizabethae DNA) and negative (qPCR mix and DNA extracted from laboratory free bacteria fleas) controls used in the qPCR and standard PCR assays showed the expected results.

Rickettsia felis DNA was assessed using primers: Rfel_phosp_MBF, 5′-GCAAAACATCGGTGAAATTGA-3′, and Rfel_phosp_MBR, 5′-GCCACTGTTCCTCACAAAACA-3′, and the probe Rfel_phosp_MBP, 6FAM-CCGCTTCTGTT ATCCGTGGGACC, targeting the phoshatase gene. Positive results were confirmed by a second qPCR assay targeting the guanosine polyphosphate gene using the primers Rfel_guano_ MBF, 5′-GCATATACCTTATTTGCGCAGTT-3′, and Rfel_guano_MBR, 5′-TATCCATATGTACCAAGAGA AATCA-3′, and the probe Rfel_guano_MBP, 6FAM-TCGCT TTTGGGGATTGTTTGGCAGA. We screened the DNA samples by qPCR for typhus-group rickettsiae with a Rickettsia-specific glycosyltransferase gene-based Rpr331 system. Positive samples were further confirmed with amplification of the Rpr 274P gene. Samples were considered positive when two amplifications were obtained targeting two different species. Subsequently, DNA samples were screened using Bartonella genus-specific qPCR with a Taqmam probe targeting the 16S/23S rRNA gene intergenic spacer (ITS). Bacterial DNA was detected in 10.73% (22 of 205) of the fleas by qPCR, including X. cheopis (12%, 16 of 134) and X. brasiliensis fleas (10.6%, 6 of 57) collected from 15 R. rattus of 59 infested small mammals (25%). Rickettsia felis was detected in 5 of 205 (2.4%) flea specimens, including four X. cheopis and one X. brasiliensis collected from three different R. rattus individuals. Rickettsia typhi was detected in three (1.46%) X. cheopis fleas collected from three different R. rattus individuals. Bartonella DNA was detected by qPCR in 14 (6.83%) flea specimens, including nine X. cheopis and five X. brasiliensis collected from 11 different R. rattus individuals.
individuals. Among these positive samples, two samples tested positive by standard PCR targeting the 972-bp ITS fragment. Sequence analyses using CHROMAS-PRO version 1.5 showed that one sequence harbored 99.56% (691 of 694) similarity with *Bartonella queenslandensis* (GenBank accession no.: EU111800); the second had 99.89% (971 of 972) homology with *Bartonella* sp. 1.1C (GenBank accession no.: FN645496) from a *R. norvegicus* isolated from Taichung, Taiwan. The geographical distribution of the infected fleas is shown in Figure 1 and Table 1.

**CONCLUSION**

In this study, *R. felis*, *R. typhi*, and *Bartonella* spp., including *Bartonella queenslandensis*, and *Bartonella* sp. 1.1C, were detected using molecular tools in *Xenopsylla* fleas collected from *R. rattus* on Reunion Island. Almost one-third of the infested rats (15 of 54) carried at least one infected *Xenopsylla* flea. *Bartonella* species are zoonotic facultative intracellular parasites of both wild and domestic animals, and more than 20 species have been described. The pathogenicity of *B. queenslandensis*, which has been isolated from small mammals from several Asian countries and detected in *X. cheopis* fleas, is unknown. The analysis of the genome of *Bartonella* sp. 1.1C, isolated from *R. norvegicus*, revealed that this species belongs to lineage 3, which contains some zoonotic pathogens. Unfortunately, the *Bartonella* DNA load that was detected using qPCR was low, and we failed to amplify and sequence the standard PCR product. Further study is needed to test the tissues of these small animals for the existence of other *Bartonella* species. Ten percent (95 of 960) of the captured mammals were infested with fleas. As

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**Table 1**

<table>
<thead>
<tr>
<th>Flea species</th>
<th>No. of tested fleas</th>
<th>No. (%) of positive fleas</th>
<th>Rickettsia spp. no. of infected fleas, localities</th>
<th>Bartonella spp. no. of infected fleas, localities</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Xenopsylla cheopis</em></td>
<td>134</td>
<td>16 (11.94)</td>
<td><em>R. felis</em> (4, St. Leu) <em>R. typhi</em> (3, Trois Bassin - 1; Port - 2)</td>
<td><em>Bartonella</em> spp. (7: Port - 2; St; Leu - 4; Trois Bassin - 1)</td>
</tr>
<tr>
<td><em>Xenopsylla brasiliensis</em></td>
<td>57</td>
<td>6 (10.52)</td>
<td><em>R. felis</em> (1, Sans Souci)</td>
<td><em>Bartonella</em> spp. 1-1C (1, St. Leu)</td>
</tr>
<tr>
<td><em>Leptopsylla segnis</em></td>
<td>13</td>
<td>-</td>
<td><em>R. felis</em></td>
<td><em>Bartonella</em> spp. (5, Sans Souci)</td>
</tr>
<tr>
<td><em>Echidnophaga gallinacea</em></td>
<td>1</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>205</td>
<td>22 (10.73)</td>
<td>8 (3.90)</td>
<td>14 (6.83)</td>
</tr>
</tbody>
</table>


**REFERENCES**


