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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.[1] 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.[1] Fleas are vectors of several important bacterial zoonoses, including plague (*Yersinia pestis*), bartonelloses (several *Bartonella* species), and rickettsioses such as murine typhus (*Rickettsia typhi*), flea-borne spotted fever (*Rickettsia felis*), and occasionally sylvatic epidemic typhus (*Rickettsia prowazekii*).[1,2]

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.[3,4] In addition, the seroprevalence rate of bartonellosis in dogs was estimated to be ~10%.[5] 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 ecologic 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 (*Mus musculus*), 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.[6] The detailed descriptions of the distribution and ecology of the collected fleas and their animal hosts are the subject of another study.[7] A total of 205 flea DNA samples extracted as previously described,[8] 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 gallinacea* (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′-GCAAACATCGGTGAAATTGA-3′, and Rfel_phosp_MBR, 5′-GCCACTGTGCTTCACAAACA-3′, and the probe Rfel_phosp_MBP, 6FAM-CCGCTTCGTT ATCCGTGGGACC, targeting the phosphatase gene. Positive results were confirmed by a second qPCR assay targeting the guanosine polyphosphate gene using the primers Rfel_ guano_ MBF, 5′GCATATACTTTATTGTGCGCAAGTT-3′, and Rfel_ guano_MBR, 5′-TTTATCGATTGACAGAAGAAGA AATCA-3′, and probe Rfel_ guano_MBP, 6FAM-TCGCT TTTTGGGATTGTTTGCCAGA. We screened the DNA samples by qPCR for typhus-group rickettsiae with a *Rickettsia*-specific glycosyltransferase gene-based Rpr331 system.[9] Positive samples were further confirmed with amplification of the Rpr 274P gene.[4] Samples were considered positive when two amplifications were obtained targeting two different specific genes. Subsequently, DNA samples were screened using *Bartonella* genus-specific qPCR with a Taqman probe targeting the 16S/23S rRNA gene intergenic spacer (ITS).[10]

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 (10.5%, 6 of 57) collected from 15 *R. rattus* of 59 infested small mammals (25%). *Rickettsia felis* was detected in 5 of 205 (2.44%) 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*

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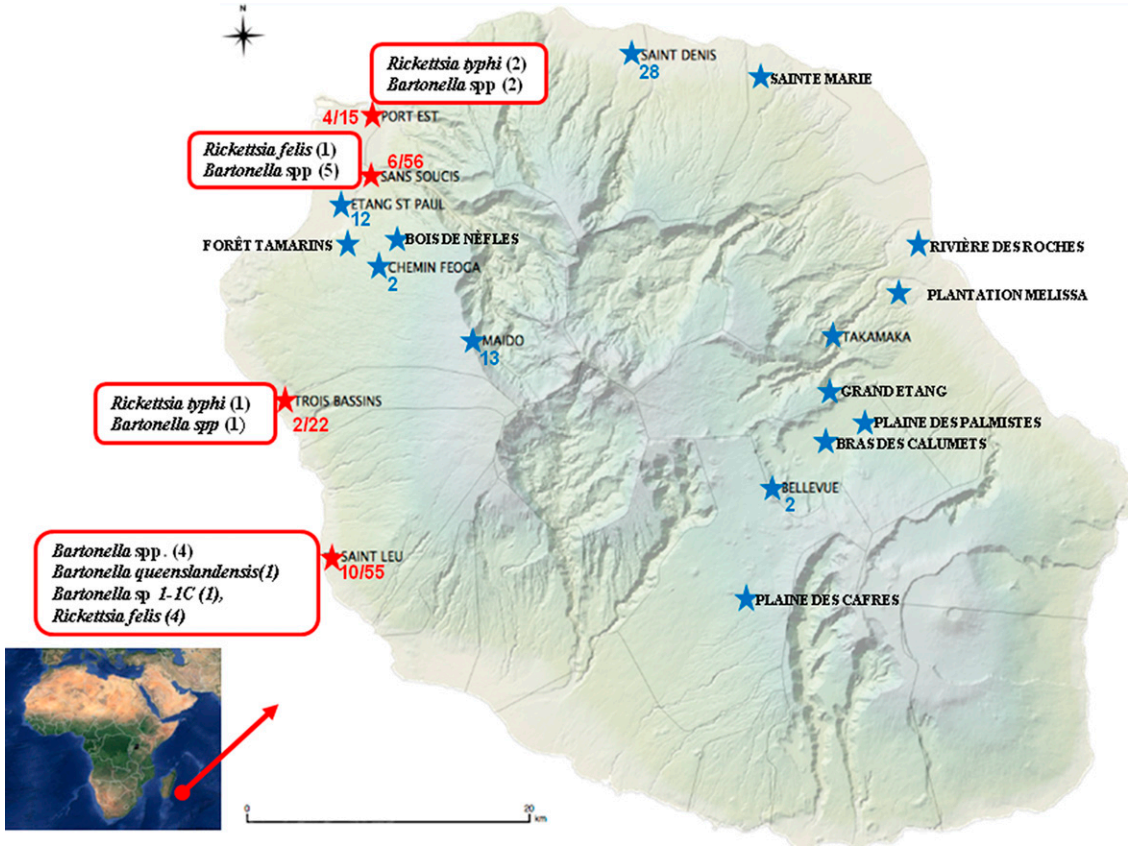

FIGURE 1. Map of risk for *Rickettsia* and *Bartonella* species on Reunion Island. Red stars: the localities (Port: 20°55′S, 55°19E, Sans Souci: 21°01′S, 55°30′E, Trois Bassin: 21°06′S, 55°17′E, Saint Leu: 21°10′S, 55°17′E) where fleas were found infected for *Rickettsia* and *Bartonella* species. Blue stars: the localities where no fleas or non-infected fleas were collected on captured mammals.

individuals. Among these positive samples, two samples tested positive by standard PCR targeting the 972-bp ITS fragment.[10] 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.[11] 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.[12] The pathogenicity of *B. queenslandensis*, which has been isolated from small mammals from several Asian countries and detected in *X. cheopis* fleas, is unknown.[13] 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.[12] 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 if 960) of the captured mammals were infested with fleas. As

TABLE 1
Detection of *Rickettsia* and *Bartonella* species in fleas, Reunion Island

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

Xenopsylla fleas are competent vectors, these *Bartonella* species could be incidentally transmitted to other hosts such as humans on Reunion Island.

Rickettsia species are obligate gram-negative intracellular bacteria vectorized only by hematophagous arthropods.[1] *Rickettsia felis* is an emergent pathogen belonging to the Spotted Fever Group *Rickettsia*, with a worldwide distribution.[2] It has previously been detected in several non- and hematophagous ectoparasites, including *Xenopsylla* fleas[14]; however, the only known biological vector is the cat flea *Ctenocephalides felis*.[2]

The identification of *R. typhi* in 2% of *X. cheopis* (Oriental rat flea) collected from the commensal rat *R. rattus,* including in the neighborhood of murine typhus cases, illustrates the life cycle of this pathogen (rat-flea-rat) on this island. *Xenopsylla cheopis* remains infectious throughout its life, from 10 days to a year after an infected blood meal.[15] Experimental and field studies have shown that *X. cheopis* is the main vector of murine typhus.[15] The clinical signs of murine typhus and *R. felis* infection are quite similar: high fever, headache, weakness, generalized pain, and sometimes a generalized rash.[2,4] Murine typhus has been diagnosed in recent years on Reunion Island,[3,4] but *R. felis* infection has not, even though 15% of febrile patients in Senegal, with the same climate conditions, tested positive for the latter.[16] In addition, the previous group of symptoms is similar to those of a range of other bacterial and viral infectious diseases.[4] Recently, a Chikungunya infection study in Reunion Island reported that 30% of patients recruited on the basis of clinical presentation with acute febrile arthralgia during an epidemic period were excluded from the diagnosis of the viral infection, which may reflect that many cases of other infections such as rickettsioses may be responsible for these symptoms, including severe ones, which are likely misdiagnosed.[17] Within this context, preventive measures should rely on arthropod surveillance and minimizing the risk of exposure in areas of endemicity.

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