References


Address for correspondence: Jonas Schmidt-Chanasit, Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Bernhard-Nocht-Strasse 74, 20359 Hamburg, Germany; email: jonassi@gmx.de

Yersinia pestis in Pulex irritans Fleas during Plague Outbreak, Madagascar

To the Editor: Yersinia pestis (family Enterobacteriaceae) is a bacterium that can cause high rates of death in susceptible mammals and can provoke septicemic, pneumatic, and bubonic plague in humans (1). This zoonotic pathogen can be transmitted directly by infectious droplets or by contact with contaminated fluid or tissue or indirectly through flea bites (1).

Plague was introduced into Madagascar in 1898 from rat-infested steamships that had sailed from affected areas (2). Now, Madagascar is 1 of 2 countries in Africa that have reported cases of human plague every year since 1991 (3). During January 2008–January 2013, the number of human plague cases reported in Madagascar ranged from 312 to 648 per year. Of these, 61.8%–75.5% were laboratory confirmed (National Plague Laboratory and genotyping by Beckman Coulter Genomics Inc. (Takeley, United Kingdom). The positive control was Y. pestis reference strain (strain 6/69, 3 × 108 bacteria/mL; Institut Pasteur de Madagascar).

Bacterial DNA was extracted from 277 fleas of 5 species: 233 P. irritans, 24 S. fonquerniei, 9 X. cheopis, 10 E. gallinacea, and 1 C. canis. PCR to detect Y. pestis was performed by using primers YP1 (5′-ATC TTA CTT TCC GTG AGA AG-3′) and YP2 (5′-CTT GTA GGT GTG TCC TA-3′) to amplify a 478-bp fragment (4). Y. pestis DNA was then amplified and genotyped by Beckman Coulter Genomics Inc. (Takeley, United Kingdom). The positive control was Y. pestis reference strain (strain 6/69, 3 × 108 bacteria/mL; Institut Pasteur de Madagascar).

Detection of Y. pestis was carried out on 274 fleas belonging to 5 flea species: 230 P. irritans (181 unfed and 49 engorged), 24 S. fonquerniei (15 unfed and 9 engorged), 9 X. cheopis (8 unfed and 1 engorged), 10 E. gallinacea (blood-feeding status not identified), and 1 unfed C. canis. Y. pestis

Table. Fleas collected inside and outside houses in Soavina, Madagascar, January 2013

<table>
<thead>
<tr>
<th>Species</th>
<th>Total no. (%)</th>
<th>No. (%) inside</th>
<th>No. (%) outside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulex irritans</td>
<td>233 (73.0%)</td>
<td>233 (95.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Ctenocephalides canis</td>
<td>1 (0.3%)</td>
<td>1 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>Echidnophaga gallinacea</td>
<td>10 (3.1%)</td>
<td>10 (4.1%)</td>
<td>0</td>
</tr>
<tr>
<td>Synopsyllus fonquerniei</td>
<td>62 (19.4%)</td>
<td>0</td>
<td>62 (82.7%)</td>
</tr>
<tr>
<td>Xenopsylla cheopis</td>
<td>13 (4.1%)</td>
<td>0</td>
<td>13 (17.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>319 (100%)</td>
<td>244 (100%)</td>
<td>75 (100%)</td>
</tr>
</tbody>
</table>
was detected in 9 *P. irritans* fleas (7 male [6 unfed and 1 engorged] and 2 [engorged] female) from 3 houses, including the house where a confirmed human case of plague had occurred (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/20/8/13-0629-Techapp1.pdf). Eight sequences (GenBank accession nos. KJ361938–KJ361945) were obtained and share 99% nucleotide homology with plasminogen activator genes of *Y. pestis* published in GenBank (accession nos. AF528537, AY305870). No *Y. pestis* was detected in the 24 *S. fonquerniei*, 9 *X. cheopis*, 10 *E. gallinacea*, or 1 *C. canis* fleas collected.

Although only *X. cheopis* and *S. fonquerniei* fleas had previously been described as plague vectors in Madagascar, *P. irritans* fleas were most commonly collected during this field study; engorged and unfed male and female *P. irritans* fleas carried *Y. pestis*. Other studies have found *P. irritans* fleas in the plague risk area in other countries in Africa (5,6); one study found that *P. irritans* fleas may play a role in plague epidemiology in Tanzania (5). Data on *P. irritans* fleas in rats make it unlikely that these fleas are involved in rat-to-human transmission of *Y. pestis* in Madagascar. During 1922–1995, a total of 118,608 rats were caught and examined in Madagascar, but only 148 *P. irritans* fleas were identified, and none have been found on rats since 1996 (http://www.pasteur.mg/spip.php?rubrique124).

The high density of *P. irritans* fleas we observed in villages where plague outbreaks occurred in late 2012 and early 2013 (http://www.pasteur.mg/spip.php?rubrique124) supports the possibility that *P. irritans* fleas played a role in domestic human-to-human transmission of *Y. pestis* during these outbreaks.

Jocelyn Ratovonjato, Minoarisoa Rajerison, Soanandrasana Rahelinirina, and Sébastien Boyer

Author affiliations: Institut Pasteur de Madagascar, Antananarivo, Madagascar (J. Ratovonjato, S. Boyer); and World Health Organization Collaborating Centre, Institut Pasteur de Madagascar, Antananarivo (M. Rajerison, S. Rahelinirina)

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**References**


**Address for correspondence:** Jocelyn Ratovonjato, Medical Entomology Unit, Institut Pasteur de Madagascar, BP 1274, Antananarivo 101, Madagascar; email: rato@pasteur.mg
Technical Appendix

Technical Appendix Figure. Agarose gel electrophoresis showing positive PCR results for *Yersinia pestis* in DNA of 9 *Pulex irritans* fleas collected from 3 houses in an area of Madagascar where plague outbreaks occurred in late 2012 and early 2013. Expected size = 478 bp; 100 bp = base ladder.