References

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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, pneumonic, 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, were reported in Soavina, a rural area in the district of Ambatofinandranana, Madagascar. Domestic fleas were collected with candle traps inside 5 houses during 3 nights (Table). Fleas were also caught on small mammals trapped inside houses and outside in the sisal fences and rice fields (Table). A total of 319 fleas belonging to 5 species in 5 genera were collected inside and outside the houses, an average of 44 per house (maximum 71): Pulex irritans, Echidnophaga gallinacea, and Ctenocephalides canis were collected inside the houses (244, 76.5%), and S. fonquerniei and X. cheopis fleas were collected outside (75, 23.5%). The human flea, P. irritans, was the most collected flea species (233, 73.3%), followed by S. fonquerniei (62, 19.4%), X. cheopis (13, 4.1%), C. gallinacea (10, 3.1%), and C. canis (1, 0.3%).

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 GTA CTT TCC GTG AGA AG-3′) and YP2 (5′-TTG AGA GGT GTC GCC 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 × 10⁶ 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.

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Serologic Surveillance for West Nile Virus in Dogs, Africa

To the Editor: West Nile fever is caused by the West Nile virus (WNV), a mosquito-borne member of the genus *Flavivirus*. Birds are the natural reservoir of the virus, which is maintained in nature in a mosquito–bird–mosquito transmission cycle. WNV has been detected in many regions worldwide, including North America, Europe, Africa, the Near East, and Asia (1). WNV has been shown to cause meningoencephalitis in humans and horses. In the United States, seroconversion in dogs was detected 6 weeks before a human case was reported (2). Thus, dogs could be considered as sentinels for WNV infection, but their role as reservoir is unlikely because of short-term and low levels of viremia (3).

In this study, we determined the seroprevalence of WNV in dogs living close to humans in different environments to assess their role as sentinels of this potentially severe zoonosis.

During 2003–2012, blood samples were collected from 753 adult dogs from France and 6 countries in Africa (Table). Samples were centrifuged within 24 h after collection, separated, frozen at −20°C, and sent to the virology laboratory of the Institut de Recherche Biomédicale des Armées (Marseille, France). Each sample was systematically tested for IgG against WNV by using an in-house ELISA with inactivated WNV as antigen. Serum samples were considered positive if the optical density at 450 nm was >3-fold the mean of that for negative antigen. Because of the antigenic cross-reactivity among flaviviruses, all positive samples were further tested by Western blot for WNV-specific antibodies (4); seroprevalence was calculated on the basis of Western blot–confirmed cases only.
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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.