in Yucatán State. It also shows that a shell vial alternative method for *R. typhi* isolation is simple and effective.

Acknowledgments

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We dedicate this article to the memory of Jorge Zavala Velázquez, who pioneered *Rickettsia* research in Mexico and Latin America.

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Zika Virus Infection after Travel to Tahiti, December 2013

To the Editor: Zika virus (ZIKV), a member of the family *Flaviviridae*, is a mosquito-borne virus that is endemic to Africa and Southeast Asia. ZIKV causes illness that is similar to dengue fever, characterized by joint pain, myalgia, headache, and rash (1). ZIKV has caused several recent outbreaks, including one in Micronesia in 2007 (2) and one in French Polynesia (~30,000 cases) ongoing since October 2013 (3) and spreading to New Caledonia and Easter Island (4). We report the clinical and laboratory findings for a patient with ZIKV infection imported from Tahiti, French Polynesia.

The previously healthy 31-year-old woman from Norway was admitted to the Oslo University Hospital, Norway, on December 13, 2013. Six days earlier, she had returned from a 14-day vacation to Tahiti, where she mainly stayed in the capital, Pape’ete, and took a short trip to the island of Mo’orea. One day after her return to Norway, she experienced fever, intense joint pain, and myalgia. Subsequently, a maculopapular rash developed. At the time of admission, her temperature was 37.7°C, and she had enlarged nuchal lymph nodes; injected conjunctivae; and a maculopapular rash on her trunk, extremities, and face (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/20/8/14-0302-Techapp1.pdf). Clinical examination findings were otherwise unremarkable. Laboratory tests showed leukopenia of 2.7 × 10⁹ cells/L (reference range 3.5–10 × 10⁹/L), with mild lymphopenia of 1.0 × 10⁹ cells/L (reference range 1.5–4.0 × 10⁹/L) and neutropenia of 1.4 × 10⁹ cells/L (reference range 1.5–7.3 × 10⁹/L). No thrombocytopenia or elevated liver enzyme levels were detected. C-reactive protein levels (1.4 mg/L) were within reference range.

Because of the patient’s clinical picture and travel history, an acute ZIKV infection was suspected and several diagnostic tests were ordered. In a serum sample taken 5 days after symptom onset, no IgM or IgG against ZIKV, dengue virus (DENV), Japanese encephalitis virus, yellow fever virus, or chikungunya virus was detected by in-house indirect immunofluorescence (5, 6). Only a weak IgG titer of 1:20 (and no IgM) against tick-borne encephalitis virus was found (cutoff <1:20). Test results for DENV nonstructural protein 1 antigen (Plate; Bio-Rad, Hercules, CA, USA) and generic flavivirus reverse transcription PCR (RT-PCR) (6) were negative. Thus, for increased sensitivity, quantitative ZIKV-specific real-time RT-PCR (6) with the AgPath-ID One-Step RT-PCR Kit (Life Technologies, Carlsbad, CA, USA) was performed according to the manufacturer’s instructions, and results were positive. ZIKV RNA load was 1.6 × 10⁶ copies/mL; in vitro–transcribed RNA from a
Attempts to isolate ZIKV in cell culture failed. Therefore, the serum sample was used to obtain the partial ZIKV genome sequence with primers designed from multiple alignments of partial ZIKV genomes retrieved from databases. Primer sequences used for partial genome amplification of ZIKV are available on request (to J. S.-C.). The partial ZIKV genome (strain Tahiti, GenBank accession no. KJ461621) was successfully amplified from the serum sample, and phylogenetic analysis of an ≈200-bp long genomic fragment of the nonstructural protein 3 gene demonstrated that strain Tahiti clusters within the Asian ZIKV lineages and is closely related to a strain from Malaysia (Figure).

In a follow-up serum sample collected 36 days after symptom onset, IgG and IgM seroconversion against ZIKV was demonstrated; IgM titer was 1:1,280 and IgG titer was 1:2,560 (cutoff <1:20). In the same sample, low IgG titers against tick-borne encephalitis virus and DENV (1:40 and 1:80, respectively) were noted (cutoffs <1:20). Real-time RT-PCR for ZIKV in this serum sample was negative.

Travel-related imported ZIKV infections have been reported after travel from Thailand to Germany (6) and Canada (7), from Indonesia to Australia (8), and from Senegal to the United States (9). Linked to the current outbreak in French Polynesia, infections in 2 travelers who had returned from Bora Bora to Japan have recently been described (10). The clinical findings for the patient reported here (fever, rash, arthralgia, myalgia) were similar to those previously reported for patients with imported cases (6,10). Available laboratory data are meager, but mild thrombocytopenia has been reported for some patients with Zika fever (10), but not for others (6,8).

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 cases of human plague (1). Madagascar is 1 of 2 countries in Africa that have reported cases of human plague every year since 1991 (3).

In January 2013, a total of 9 suspected bubonic plague cases, 3 confirmed, were reported in Soavina, a rural area in the district of Ambatofinandrahana, 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%), E. 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 TTA CTT TTC TGG AGA AG-3′) and YP2 (5′-CTT GTA TGT TGA GCT 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 × 10^6 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

| Species                  | Total no. (%) | No. (%) inside | No. (%) outside |
|-------------------------|---------------|----------------|-----------------|
| Pulex irritans          | 233 (73.0%)   | 233 (95.5%)    | 0               |
| Ctenocephalides canis   | 1 (0.3%)      | 1 (0.4%)       | 0               |
| Echidnophaga gallinacea | 10 (3.1%)     | 10 (4.1%)      | 0               |
| Synopsyllus fonquerniei | 62 (19.4%)    | 0              | 62 (82.7%)      |
| Xenopsylla cheopis      | 13 (4.1%)     | 0              | 13 (17.3%)      |
| Total                   | 319 (100%)    | 244 (100%)     | 75 (100%)       |

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Zika Virus Infection after Travel to Tahiti, December 2013

Technical Appendix

Technical Appendix legend. Maculopapular rash on the trunk and extremities of a patient with Zika virus infection imported from Tahiti, French Polynesia, December 2013.