

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

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References

- Gillespie JJ, Ammerman NC, Beier-Sexton M, Sobral BS, Azad AF. Louse- and flea-borne rickettsioses: biological and genomic analyses. *Vet Res.* 2009;40:12. <http://dx.doi.org/10.1051/vetres:2008050>
- Acuna-Soto R, Calderón-Romero L, Romero-López D, Bravo-Lindoro A. Murine typhus in Mexico City. *Trans R Soc Trop Med Hyg.* 2000;94:45. [http://dx.doi.org/10.1016/S0035-9203\(00\)90432-2](http://dx.doi.org/10.1016/S0035-9203(00)90432-2)
- Zavala-Castro JE, Zavala-Velázquez J, Sulú-Uicab J. Murine typhus in child, Yucatan, Mexico. *Emerg Infect Dis.* 2009;15:972-4. <http://dx.doi.org/10.3201/eid1506.081367>
- Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol.* 1991;173:1576-89.
- Williams SG, Sacci JB Jr, Schriefer ME, Anderson EM, Fujioka KK, Sorvillo FJ, et al. Typhus and typhus-like rickettsiae associated with opossums and their fleas in Los Angeles County, California. *J Clin Microbiol.* 1992;30:1758-62.
- Webb L, Carl M, Mallow DC, Dasch GA, Azad AF. Detection of murine typhus in fleas by using the polymerase chain reaction. *J Clin Microbiol.* 1990;28:530-4.
- Dzul-Rosado K, González-Martínez P, Peniche-Lara G, Zavala-Velázquez J, Zavala-Castro J. Murine typhus in humans, Yucatan, Mexico. *Emerg Infect Dis.* 2013;19:1021-2. <http://dx.doi.org/10.3201/eid1906.121400>
- Kordová N. Plaque assay of rickettsiae. *Acta Virol.* 1966;10:278.

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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 2.7×10^9 cells/L (reference range $3.5\text{--}10 \times 10^9$ /L), with mild lymphopenia of 1.0×10^9 cells/L (reference range $1.5\text{--}4.0 \times 10^9$ /L) and neutropenia of 1.4×10^9 cells/L (reference range $1.5\text{--}7.3 \times 10^9$ /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 (Plateia; 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 1.6×10^5 copies/mL; in vitro-transcribed RNA from a

reference plasmid was used as a quantification standard.

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).

Outbreaks of dengue fever also occur in French Polynesia (10), making dengue fever clinically and epidemiologically the most important differential diagnosis. Elevated liver enzymes, which are found in patients with acute dengue fever, are found in some, but not all, patients with Zika fever (6,8).

The measured viral load for the patient reported here (5 days after symptom onset) would not be high enough for efficient transmission of ZIKV to susceptible vectors such as *Aedes aegypti* or *Ae. albopictus* mosquitoes (S. Becker, pers. comm.). This finding is consistent with previously reported findings of ZIKV RNA loads of 930–728,800 copies/mL (2). However, *Ae. aegypti* and *Ae. albopictus* mosquitoes are not present in Norway; thus, transmission in Norway seems unlikely. ZIKV infection should be considered as a differential diagnosis for febrile dengue fever-like syndromes in travelers who have returned from Southeast Asia and the Pacific region.

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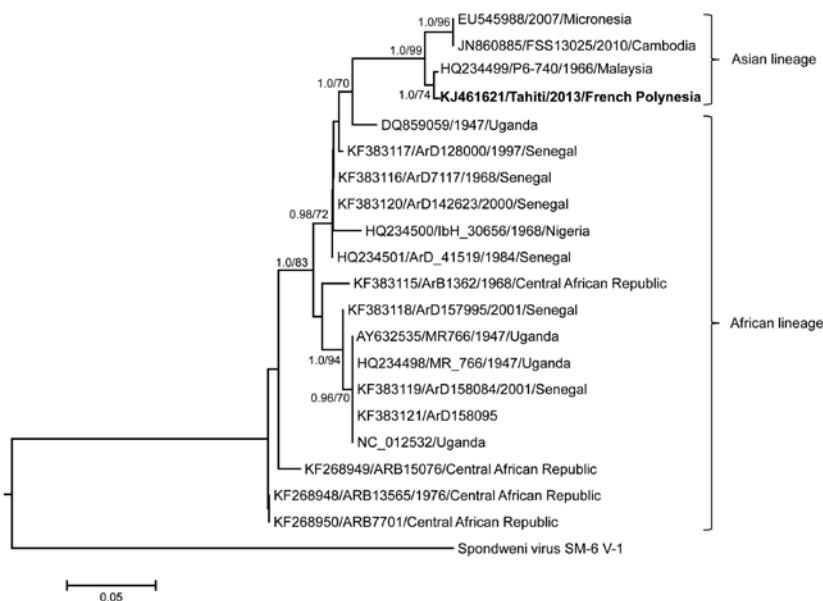


Figure. Phylogenetic analysis of partial (≈ 200 bp) nonstructural protein 3 gene sequences of Zika virus strains performed by using maximum-likelihood and Bayesian methods. A substitution model was based on a general time-reversible model with gamma-distributed rate variation and a proportion of invariant sites. Numbers at the nodes represent posterior probability values (clade credibilities $\geq 90\%$) and percentage bootstrap support values ($\geq 70\%$) based on 1,000 replicates. GenBank accession numbers, strain name, year of isolation, and country of origin for sequences used to construct the tree are indicated on the branches. The tree was rooted with Spondweni virus (GenBank accession no. DQ859064). Strain Tahiti (from patient who had traveled to Tahiti, this study) is indicated in boldface. The scale bar represents genetic distance in nucleotide substitutions per site. The lineage of each virus is indicated to the right of the tree.

References

- Hayes EB. Zika virus outside Africa. *Emerg Infect Dis*. 2009;15:1347–50. <http://dx.doi.org/10.3201/eid1509.090442>
- Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis*. 2008;14:1232–9. <http://dx.doi.org/10.3201/eid1408.080287>
- European Centre for Disease Prevention and Control. Zika virus—Pacific (08). *ProMed*. 2014 Mar 16. <http://www.promedmail.org>, archive no. 20140316.2335754.
- Schwan K. Zika virus—Pacific (07). *ProMed*. 2014 Mar 9. <http://www.promedmail.org>, archive no. 20140309.2322907.
- Tappe D, Schmidt-Chanasit J, Ries A, Ziegler U, Müller A, Stich A. Ross River virus infection in a traveller returning from northern Australia. *Med Microbiol Immunol (Berl)*. 2009;198:271–3. <http://dx.doi.org/10.1007/s00430-009-0122-9>
- Tappe D, Rissland J, Gabriel M, Emmerich P, Gunther S, Held G, et al. First case of laboratory-confirmed Zika virus infection imported to Europe, November 2013. *Euro Surveill*. 2014;19:20685.
- Fonseca K. Zika virus—Canada ex Thailand. *ProMed*. 2013 May 29. <http://www.promedmail.org>, archive no. 20130529.1744108.
- Kwong JC, Druce JD, Leder K. Zika virus infection acquired during brief travel to Indonesia. *Am J Trop Med Hyg*. 2013;89:516–7. <http://dx.doi.org/10.4269/ajtmh.13-0029>
- Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddow AD, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. *Emerg Infect Dis*. 2011;17:880–2. <http://dx.doi.org/10.3201/eid1705.101939>
- Kutsuna S, Kato Y, Takasaki T, Moi ML, Kotaki A, Uemura H. Two cases of Zika virus imported from French Polynesia to Japan, December 2013 to January 2014. *Euro Surveill*. 2014;19:pi=20683.

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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 (National Plague Laboratory of the Ministry of Health, pers. comm.). Most (>83%) confirmed cases were bubonic plague, which most commonly results from flea bites, suggesting that these bites were the most common mode of *Y. pestis* transmission. In Madagascar, *Xenopsylla cheopis* fleas have been known as the primary plague vector in urban areas, whereas *Synopsyllus fonquerniei* fleas have been usually involved in plague transmission in rural areas (2).

In January 2013, a total of 9 suspected bubonic plague cases, 3 confirmed, were reported in Soavina, a

rural area in the district of Ambatofinandrana, 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* fleas 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 TCC GTG AGA AG-3') and YP2 (5'-CTT GGA 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, 3×10^8 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
<i>Pulex irritans</i>	233 (73.0)	233 (95.5)	0
<i>Ctenocephalides canis</i>	1 (0.3)	1 (0.4)	0
<i>Echidnophaga gallinacea</i>	10 (3.1)	10 (4.1)	0
<i>Synopsyllus fonquerniei</i>	62 (19.4)	0	62 (82.7)
<i>Xenopsylla cheopis</i>	13 (4.1)	0	13 (17.3)
Total	319 (100)	244 (100)	75 (100)

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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.