**Isolation of Zika Virus from Febrile Patient, Indonesia**


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To the Editor: Arthropodborne viruses (arboviruses) cause substantial human disease worldwide and have a pronounced effect on public health throughout Asia. Zika virus, discovered in Uganda in 1947 (1), is a flavivirus related to the following viruses: dengue (DENV), West Nile, Japanese encephalitis, and yellow fever. Like DENV, Zika virus is a public health problem in 2007, when it caused an epidemic in Asia, including in Indonesia (2), has been serologic, but recent virus strains isolated from persons in Thailand (6), the Philippines (7), and Cambodia (8) have begun to clarify its genomic diversity. Phylogenetically, Zika virus appears to have 2 major lineages, African and Asian (9).

During December 2014—April 2015, a confirmed outbreak of dengue (determined by reverse transcription PCR [RT-PCR] for DENV and nonstructural protein 1 [NS1] antigen detection; data not shown) occurred in Jambi Province, central Sumatra, Indonesia. We received samples from 103 case-patients with clinically diagnosed dengue; these samples had been negative for DENV by RT-PCR, NS1 antigen detection, or evidence of seroconversion by ELISA (data not shown). We tested the samples for other viruses using alphavirus and flavivirus RT-PCR (targeting genome positions 6533–6999 and 8993–9258, respectively). In parallel, we attempted virus isolation using Vero cells.

One sample, JMB-185, came from a 27-year-old man who sought treatment at the Jambi city hospital 2 days after illness onset with a sudden high fever, headache, elbow and knee arthralgia, myalgia, and malaise. He did not exhibit some common clinical characteristics of Zika virus infection (10), including maculopapular rash, conjunctivitis, or peripheral edema. Hematologic testing revealed lymphopenia and monocytosis; platelet count was within reference range. Results of all assays were negative for DENV, including NS1 antigen detection with NS1 Ag Rapid Test (SD Bioline, Kyong, South Korea); PanBio Dengue Early NS1 ELISA (Alere, Brisbane, Australia); PanBio Dengue Duo IgM and IgG ELISA (Alere); and Simplexa real-time RT-PCR (Focus Diagnostics, Cypress, CA, USA). The illness was self-limiting, and the patient recovered 2 days after he sought treatment without any complications.

Of the 103 DENV-negative specimens we tested, only JMB-185 was positive for flavivirus and displayed cytopathic effects when cultured in Vero cells for 10 days. A subsequent passage was performed, and supernatants from both passages were tested for flaviviruses by RT-PCR. A 265-bp amplicon was generated from JMB-185 by using flavivirus consensus primers. This consensus amplicon product had ≈85% nucleotide identity with the prototype Zika virus (strain MR 766, 1947, Uganda). An additional larger amplicon was generated (nt 9278–9808 of NS5 gene), and a phylogenetic tree was constructed based on the partial sequence of the NS5 region (530 bp) for JMB-185 (GenBank accession no. KU179098) and other Zika virus sequences, including those from Cambodia, Yap Island, Thailand, and the Philippines (Figure). Phylogenetic analysis indicated that JMB-185 belonged to the Zika virus Asian lineage and had 99.24% nucleotide identity to an isolate from a Japanese visitor to Thailand (10). It was also close to a Zika virus strain isolated from an Australian traveler who had visited Java (on the basis of a different NS5 region; data not shown). The original serum and passage samples were
further tested with Zika virus–specific real-time quantitative RT-PCR (2) by using the QuantiTect Probe RT-PCR Kit (QIAGEN, Valencia, CA, USA) with amplification in the iCycler iQ5 (Bio-Rad, Hercules, CA, USA), following the manufacturer’s instructions. Viral titers of JMB-185, as determined by real-time quantitative RT-PCR, were $4.25 \times 10^3$ PFU, $5.07 \times 10^7$ PFU, and $7.33 \times 10^6$ PFU for the clinical sample, first passage, and second passage, respectively.

The isolation and characterization of Zika virus from a resident with no travel history confirm that the virus is circulating in Indonesia and that, by mimicking mild dengue infection, this infection is likely contributing to the large number of undiagnosed cases of acute febrile illness. Although reported human cases of Zika virus infection have been rare in Southeast Asia (1), confusion with dengue and difficulty in obtaining a laboratory diagnosis are likely causing its incidence to be underestimated. Surveillance must be implemented to evaluate and monitor the distribution of Zika virus and the potential public health problems it may cause in Indonesia.

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Fatal Sickle Cell Disease and Zika Virus Infection in Girl from Colombia

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