We investigated recent outbreaks of CHIKV in Indonesia (1). Concern about transmissibility of the mutated ECSA strain through Ae. albopictus mosquitoes (9). The predominant strain during this epidemic was the ECSA genotype with the A226V mutation of the E1 protein (2), the transmission of which is reported to be facilitated by Ae. albopictus mosquitoes (3). The ECSA genotype has been reported to circulate in Southeast Asia, including Malaysia, but not in Indonesia (4). Concern about circulating ESCA strains triggered alerts in 2009, when the Indonesian Ministry of Health reported an increasing number of chikungunya cases (3,529 cases in 2008, 83,756 in 2009) (5). However, only Asian genotypes were detected (4). We investigated recent outbreaks of CHIKV in Indonesia and genotypes of associated CHIKV strains.

After chikungunya outbreaks were reported from 6 districts in Indonesia (Tangerang, Karang Anyar, Ngawi, Jembrana, Mataran, and Kubu Raya), a team from the National Institute of Health and Research Development, Indonesian Ministry of Health, conducted field investigations from April through October 2011. This study received institutional review board approval (KE.01.06/EC/373/2011).

Serum specimens from persons with fever ≥38°C who provided signed informed consent were tested at the Virology Laboratory, Center for Biomedical and Basic Technology of Health, National Institute of Health Research and Development, in Jakarta. Molecular examination by reverse transcription PCR (RT-PCR) of acute-phase serum specimens, selective for the E1 gene, was performed as previously described (6). Amplicons (330 bp) were sequenced for confirmation. The entire E1 gene of 2 identified ECSA genotypes was sequenced (7). A cladogram was created by using MEGA version 6.06 and the neighbor-joining method (8). The strength of the cladogram was estimated by bootstrap analyses that used 1,000 random samplings. To determine the circulating genotype of CHIKV in Indonesia, we compared these results with other reference sequences in GenBank.

RT-PCR confirmed CHIKV in 28 (26%) of 109 samples from 5 districts: 12 (50%) in Mataran, 8 (47%) in Jembrana, 2 (40%) in Tangerang, 4 (21%) in Ngawi, and 2 (9%) in Kubu Raya. No samples from Karang Anyer were positive for CHIKV. Sequencing analysis revealed the A226V mutant (alanine to valine) ECSA genotype in 2 (7%) specimens (GenBank accession nos. KJ729851, KJ729852) and the Asian genotypes (KJ729829–50, KJ729853–56) in 26 (93%) specimens. The Asian genotypes were closely related to those of CHIKV isolated from East Kalimantan, Bandung, Malaysia, and India (Figure).

The 2 cases associated with the A226V mutant ECSA genotype occurred in October 2011 in the Kubu Raya district, West Kalimantan, near the Malaysia border. Because both patients had no history of travel to Malaysia, where outbreaks involving the ECSA genotype had been reported, this finding demonstrates the emergence of the CHIKV A226V ECSA genotype in Indonesia. The 2008 nationwide outbreak of chikungunya in Malaysia proved that A226V mutation enhances transmissibility of CHIKV by Ae. albopictus mosquitoes (9). Population movement from this region might contribute to the spread of this virus to Indonesia, which is a concern because of the higher transmissibility of the mutated ECSA strain through the Ae. albopictus mosquito vector, which is prevalent throughout Indonesia.

That ECSA genotypes were not found in other districts during this investigation would suggest that this strain was not the source of the 2008–2009 outbreaks in Bandung, Malaysia, and India (Figure).
Indonesia, although this suggestion is by no means certain. The predominance of the Asian genotypes suggests endemicity of similar CHIKV strains.

A limitation of this study was the lack of serologic assays to confirm CHIKV infections, especially for those who sought care late after onset of illness. Because of the lack of reliable serologic assays with high sensitivity, some cases deemed by RT-PCR to be CHIKV negative might have been clinical cases of CHIKV infection (10).

Thus, the A226V ECSA genotype of CHIKV was circulating in Indonesia 5 years after the global pandemic and 2–3 years after the emergence of this strain from other Southeast Asian countries (4). Sensitive serology-based assays and rapid tests for different operational settings are needed, especially in those areas without molecular diagnostic capabilities. Also needed is surveillance of CHIKV throughout Indonesia so that health policy makers can have comprehensive data on the molecular epidemiology and prevalence of CHIKV infection. In addition, studies of CHIKV transmission by different vectors as well as virus and vector interactions are needed to provide an understanding of the emergence of the mutant strain across the region and to assist strategies for vector control and disease prevention and control.

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References


Co-infection with Zika and Dengue Viruses in 2 Patients, New Caledonia, 2014

Myrielle Dupont-Rouzyrol, Olivia O’Connor, Elodie Calvez, Maguy Daures, Michèle John, Jean-Paul Grangeon, Ann-Claire Gourinat

To the Editor: Dengue is the most prevalent arthropod-borne viral disease in tropical and subtropical countries. Every year, dengue virus (DENV) infections cause more than 50 million cases, 500,000 hospitalizations, and 12,500 deaths worldwide (1). DENV belongs to the genus *Flavivirus* and is transmitted by *Aedes* spp. mosquitoes. There are 4 distinct serotypes (DENV-1 to DENV-4), and infection with 1 serotype does not provide long-term, cross-protective immunity against the other 3 serotypes.

In New Caledonia, DENV outbreaks have occurred since World War II and have been caused mainly by 1 serotype/genotype introduced from a country to which dengue is hyperendemic. Since 2000, New Caledonia has had recurrent DENV-1 outbreaks (2). In 2014, circulation of DENV-1 and DENV-3 was still reported in this country (3).

Zika virus (ZIKV) is an emerging mosquito-borne virus that belongs to the genus *Flavivirus* and was first isolated in Uganda (4). ZIKV is believed to be transmitted to humans by *Aedes* spp. mosquitoes. Before 2007, few human cases of infection had been reported. In 2007, the first Zika epidemic occurred in Yap, Federated States of Micronesia (5). In October 2013, a ZIKV outbreak was reported in French Polynesia (6).

In New Caledonia, the first cases of ZIKV infection imported from French Polynesia were confirmed at the end of November 2013, and the first autochthonous cases were reported by mid-January 2014. Early in February 2014, the New Caledonia Health Authority declared an outbreak situation. Since February 2014, a total of 1,385 ZIKV laboratory-confirmed cases have been detected, including 35 imported cases (32 from French Polynesia, 2 from Vanuatu, and 1 from the Cook Islands). Concomitant with this ZIKV outbreak, circulation of DENV-1 and DENV-3 was reported; >150 cases were biologically confirmed during January–September 2014 (3). Thus, New Caledonia currently has 3 arboviruses co-circulating.

In recent years, co-circulation of multiple DENV serotypes or DENV and chikungunya virus has been reported. Although rare co-infections have been identified (7,8), given the similar clinical features and lack of concurrent testing, co-infections might not be identified. We report detection of ZIKV and DENV genomes in serum of a traveler (patient 1) who returned from French Polynesia where ZIKV and DENV were co-circulating (6,9) and in serum of a person (patient 2) in New Caledonia who had no travel history. The traveler was co-infected with ZIKV and DENV-3, and the local patient was co-infected with ZIKV and DENV-1.

Patient 1 was a 14-year-old boy who had fever (39.5°C), headache, arthralgia, asthenia, and myalgia. No hemorrhagic or neurologic findings were reported, and the patient recovered within 3 days. A complete blood count showed mild thrombocytopenia (platelet count 129 × 10^9 cells/L; reference range 150–400 × 10^9 platelets/L), leukopenia (leukocyte count 2.75 × 10^9 cells/L; reference range 4–10 × 10^9 cells/L) with associated stimulated lymphocytes, and discreet hepatic cytolysis (aspartate aminotransferase 55 IU/L; reference value <34 UI/L, and alanine aminotransferase 51 IU/L; reference value <55 IU/L). Serum was analyzed by using real-time reverse transcription PCR (RT-PCR) and was positive for ZIKV as recommend; by Lanciotti et al. (5) and DENV-3.

Patient 2 was a 38-year-old woman who had fever (40°C), headache, arthralgia, asthenia, myalgia, retroorbital pain, conjunctivitis, diarrhea, nausea, and a diffuse pruritic maculopapular rash. No hemorrhagic or neurologic findings were reported, but signs of illness lasted ≈1 week. The patient had mild thrombocytopenia (platelet count 123 × 10^9 platelets/L) and leukopenia (leukocyte count 2.65 × 10^9 cells/L). Serum was analyzed by using RT-PCR (5) and was positive for ZIKV and DENV-1.

Co-infections were assessed by sequencing partial ZIKV membrane–envelope gene regions for isolates (GenBank accession nos. KM212963 and KM212967) from both patients, partial DENV-1 envelope gene for an isolate (KM212960) from patient 2, and partial DENV-3 nonstructural protein 5 gene for an isolate (KM212962) from patient 1. Sequencing was conducted at La Plateforme du Vivant (Noumea, New Caledonia).

DENV-1 sequence obtained belonged to genotype I and clustered with DENV-1 sequences isolated in New Caledonia (2). DENV-3 sequence obtained belonged to genotype III, similar to DENV-3 recently isolated in French Polynesia (9). ZIKV sequences obtained belonged to the

Address for correspondence: Masri Sembiring Maha, Center for Biomedical and Basic Technology of Health, National Institute of Health Research and Development, Ministry of Health, Republic of Indonesia, Jl. Percetakan Negara 23, Jakarta Pusat 16417 Indonesia; email: masrimaha@yahoo.com