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DOI: http://dx.doi.org/10.3201/eid2008.131308

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Chikungunya Outbreak in Bueng Kan Province, Thailand, 2013

To the Editor: Chikungunya fever is a dengue-like syndrome characterized by acute fever, arthralgia, and maculopapular rash. The causative agent is chikungunya virus (CHIKV), which is transmitted by Aedes aegypti and Aedes albopictus mosquitoes (1). Based on the genome and the viral envelope E1 sequences, CHIKV is classified into 3 genetic lineages: Asian, West African, and East/Central/South African (ECSA) genotypes (2).

In Thailand, the first report of CHIKV infection occurred in Bangkok in 1958 (3); later, sporadic cases of chikungunya fever occurred in many provinces during 1976–1995 (4). All of the CHIKV strains found in Thailand at that time were of the Asian genotype. The virus has since reemerged during 2008–2009 and caused large outbreaks in southern Thailand, affecting >50,000 persons (5). These outbreaks were attributed to the ECSA genotype. We report an outbreak of CHIKV infection in the northeastern province of Bueng Kan in 2013.

Bueng Kan Province is located on the Mekong River on the foothills of the mountainous region of Laos to the north. An outbreak of suspected dengue cases was reported during the rainy season during April–September 2013 (6). Beginning in September, however, hospital physicians noticed that patients were reporting fever with moderate to severe joint pain resulting in limitation of movement that lasted for weeks. Serum samples were collected from 109 persons (hospitalized and outpatient) in October. Clinical data showed that 38 (34.9%) had moderate to severe joint pain; median duration of illness was 4 days (range 1–7). Median timing of sample collection from the onset of illness was 8 days (range 1–21).

Samples were sent to Chulalongkorn University Hospital in Bangkok to screen for mosquitoborne viruses. The study protocol was approved by the Institutional Review Board of Chulalongkorn University and consents were waived because all samples were stored as anonymous. Viral genomic RNA was assayed by using seminested reverse transcription PCR (RT-PCR) for CHIKV nucleic acid (7). Serum samples were tested for IgM against CHIKV by using SD BIO-LINE Chikungunya IgM Test (Standard Diagnostics Inc., Kyonggi-do, South Korea) (8). Cases positive for CHIKV were confirmed by using a line enzyme immunoassay test (LIEIA) for anti–CHIKV IgM.

Of the 109 patients, 62 (57.2%) had CHIKV infection. Median age of patients with CHIKV infection was 40 years (range 15–75 years). Median illness duration was 4 days (range 1–17 days). Of the 62 patients, 48 (77.4%) were female. Median timing of sample collection from the onset of illness was 8 days (range 1–21).

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South Korea) (8). In our study, the criteria for diagnosis of CHIKV infection included the detection of CHIKV nucleic acid by RT-PCR or IgM antibodies against CHIKV.

Of the 109 samples tested, 51 (46.8%) had evidence of CHIKV infection, as 25 (22.9%) were positive for CHIKV RNA by RT-PCR, and 32 (29.3%) were positive for IgM antibodies. Both CHIKV nucleic acid and IgM were found in 6 samples. To further characterize the phylogenetic relationships between the CHIKV strains in this outbreak with strains previously found in Thailand and neighboring regions, we performed full-length viral genomic sequencing from strains from 4 samples by primer walking (4). We subjected sequences to BLAST analysis (BLAST, http://blast.ncbi.nlm.nih.gov), aligned using the BioEdit program v7.1.9 (http://www.mbio.ncsu.edu/bioedit/bioedit.html), and performed sequence assembly using the DNASTAR v6.0 (DNA Star, Madison, WI, USA). We performed maximum likelihood phylogenetic analysis of a set of 11,710 nt, including the sequences identified in this study (THA/Bueng Kan/BK46/2013, THA/Bueng Kan/BK57/2013, THA/Bueng Kan/BK63/2013, THA/Bueng Kan/BK68/2013; accession nos. KJ579184–7) by using the MEGA program v6.0 (http://www.megasoftware.net). Taking into consideration possible co-circulation of the Asian and ECSA genotypes in Thailand, we also examined intergenotypic recombination using the Recombination Detection Program, v4.22 (http://en.bio-soft.net/tree/RDP.html).

Phylogenetic analysis, comparing the 4 strains of CHIKV identified from Bueng Kan to 52 additional whole genome sequences from GenBank, revealed that the 4 strains are closely related and share >99.8% pairwise nucleotide identity (Figure). The Bueng Kan isolates grouped to the ECSA genotype within the recent Indian Ocean clade (9). This relationship was confirmed by analysis of the non-structural, structural, and E1 encoding regions (data not shown). The THA/Bueng Kan strains all possessed an alanine to valine change at residue 226 (A226V) in the E1 gene, which was noted as one of the crucial substitutions for increased transmissibility by Aedes albopictus mosquitoes reported for the Réunion Island isolates (9). This substitution was shared among strains isolated in Thailand in 2008, which were responsible for the previous CHIKV outbreaks in southern Thailand (7), and those in the recent outbreak in Cambodia (10). This suggests the presence and continued circulation of the E1-A226V virus in Thailand since 2008. However, another mutation specific to the Indian Ocean isolates, D284E in the E1 gene, was not found in THA/Bueng Kan strains. No evidence of recombination has been found in the THA/Bueng Kan strains and other strains identified in Thailand thus far.
Viral infections by mosquitoes continue to challenge public health in Thailand. In evidence of this, we demonstrated that CHIKV has become established in northeastern Thailand. Surveillance and clinical recognition will not prevent future outbreaks, but rather will assist in organizing an early response to outbreaks and thus minimize unnecessary illness and death.

Acknowledgments

We thank Padet Siriyasatien for providing us with laboratory test kits and protocols. We thank the staff of Bueng Kan Provincial Hospital for their contribution in sample collection and the staff at the Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University, for their hard work in performing laboratory tests.

This research is supported by National Research University Project; Office of Higher Education Commission (WCU-007-HR-57); Integrated Innovation Academic Center, Chulalongkorn University; Centenary Academic Development Project (CU56-HR01); the Ratchadaphiseksomphot Endowment Fund of Chulalongkorn University (RES560530093); the Outstanding Professor of the Thailand Research Fund (DPG5480002); and King Chulalongkorn Memorial Hospital Thai Red Cross Society; the Ratchadaphiseksomphot Endowment Fund (S. Vongpunsawad and P. Linsuwanon); MK Restaurant Company Limited; and The Siam Cement Pel.

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DOI: http://dx.doi.org/10.3201/eid2008.140481

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Decline in Japanese Encephalitis, Kushinagar District, Uttar Pradesh, India

To the Editor: Kakkar et al. recently concluded that the low-quality surveillance data on acute encephalitis syndrome (AES)/Japanese encephalitis (JE) in Kushinagar District, India, provide little evidence to support development of prevention and control measures and to estimate the effect of interventions (1). Analysis of the surveillance data, however, does provide evidence supporting the effect of an ongoing intervention (i.e., JE vaccination).

In accordance with the surveillance protocol, cerebrospinal fluid and/or serum samples from AES patients admitted to the Baba Raghav Das Medical College (Gorakhpur, India) are tested for IgM against JE at the field laboratory of National Institute of Virology (NIV) at Gorakhpur (2). The samples are tested by using the ELISA developed by NIV Pune (Pune, India), which has a specificity of 85% (range 77%–95%) and sensitivity of 71% (range 71%–75%) (3). Patients with samples negative for JE are considered to have JE-negative AES.

We obtained the line-list of AES patients from the NIV laboratory at Gorakhpur for 2008–2012. Analysis of the surveillance data indicated that 251 (8.2%, range 4%–14.7%) of the 3,047 AES patients from Kushinagar...