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Chikungunya fever outbreak identified in North Bali, Indonesia

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Background: Chikungunya virus (CHIKV) infections have been reported sporadically within the last 5 years in several areas of Indonesia including Bali. Most of the reports, however, have lacked laboratory confirmation.

Method: A recent fever outbreak in a village in the North Bali area was investigated using extensive viral diagnostic testing including both molecular and serological approaches.

Results and conclusions: Ten out of 15 acute febrile illness samples were confirmed to have CHIKV infection by real-time PCR or CHIKV-specific IgM enzyme-linked immunosorbent assay (ELISA). The outbreak strain belonged to the Asian genotype with highest homology to other CHIKV strains currently circulating in Indonesia. The results are of public health concern particularly because Bali is a popular tourist destination in Indonesia and thereby the potential to spread the virus to non-endemic areas is high.

GenBank accession numbers: KY885022, KY885023, KY885024, KY885025, KY885026, KY885027.

Keywords: Bali, Chikungunya virus, House Index, Indonesia, Outbreak

Background

Chikungunya virus (CHIKV), an alphavirus, typically causes a clinical syndrome characterized by fever, rash and arthralgia. Recent CHIKV outbreaks have been reported in several areas of Indonesia, including Bali, from 2009 to 2011.[1] However, the determination of CHIKV outbreaks in Indonesia has been based mainly on clinical data and laboratory exclusion of dengue virus (DENV) infection. In addition, information on CHIKV characterization from Bali is limited except for reports from travel-acquired cases.[2] From December 2015 to January 2016, the Bali Provincial Health Office received reports of an outbreak of febrile illnesses with rash and arthralgia suspected to be caused by CHIKV infection in Nagasepaha Village, Buleleng, in North Bali. The objective of this study was to provide laboratory confirmation and characterization of this CHIKV outbreak in Bali using a range of standard assays.

Methods

A community-based study was carried out in patients suspected of recent CHIKV infection by the Bali Provincial Health Office through direct observation, interviews and collection of biological samples, entomological and environmental data. Out of the 96 suspected cases of CHIKV infection, blood samples were collected from 15 cases in the acute stage of illness (less than 7 days after onset of fever). Samples were sent to the Biomolecular Laboratory, Faculty of Medicine and Health Sciences, Warmadewa University, Bali for DENV reverse transcriptase real-time PCR (RT-PCR), and to the Eijkman Institute for Molecular Biology, Jakarta, for laboratory confirmation of CHIKV infection.

Chikungunya and dengue virus RT-PCR

Viral RNA was extracted from 140 µl of acute sera using the QIAamp® viral RNA mini kit (QIAGEN, Hilden, Germany). RNA was used as a template for CHIKV quantitative RT-PCR (qRT-PCR) and DENV nested RT-PCR according to published methods.[3,4]

Virus isolation and CHIKV genome sequencing

Virus isolation was performed in Vero-81 (African green monkey kidney) cells by inoculating the serum samples onto the monolayers followed by incubation at 37°C for up to 14 d. Cells were observed daily for any indication of cytopathic effects (CPEs)

and supernatant was assayed using CHIKV qRT-PCR as described above. Positive specimens were further assayed to amplify 1320 bases of the CHIKV genome envelope region (E1) as previously described[5] followed by DNA sequencing.

Anti-CHIKV IgM and IgG ELISA

IgM and IgG antibodies against CHIKV were detected using an in-house IgM/IgG capture enzyme-linked immunosorbent assay (ELISA) according to the published methods.[6] Samples containing an antibody titer of ≥40 units were considered positive.

Entomological survey

A house-to-house survey for larval mosquitoes in water containers in all houses in the village was conducted during the outbreak investigation. Data were used to calculate the House index (HI; number of houses with positive containers per 100 houses evaluated) to determine the potential risk of transmission.[7]

Results

From 14 December 2015 to 11 January 2016, a suspected CHIKV outbreak was reported in Nagasepaha Village, Buleleng, in the north of Bali. The outbreak reached its peak with 53 cases in the first week of January 2016. In total, 96 suspect cases were reported, with one patient requiring hospitalization, while the others recovered with symptomatic treatment.

Serum samples were collected from 15 individuals reported to be in the early phase of illness—53.3% (8/15) were female and 46.7% (7/15) were male between the ages of 19 and 73 years. Limited clinical data were available during the outbreak, but patients were reported to have fever (100%), arthralgia (100%) and skin rash (73.3%).

CHIKV was detected by qRT-PCR in 46.7% (7/15) serum samples with viremia titers ranging from 10^5 to 10^7 plaque-forming unit (pfu) equivalents/mL (Table 1). Vero-81 cells inoculated with the RT-PCR-positive samples also showed CPEs on day 3 post-inoculation. CHIKV infection was confirmed from the virus isolation cultures by qRT-PCR assay of the supernatants. Genetic characterization of the CHIKV E1 gene showed the Asian genotype [GenBank accession nos KY885022–KY885027]. Phylogenetic tree analysis indicated that the CHIKV strains from the outbreak were closely related to previous isolates collected from various provinces in Indonesia, including Bali, in 2011 and 2013. Additionally, three cases with samples collected between days 5 and 7 after clinical onset were classified as recent CHIKV infection since samples were positive for anti-CHIKV IgM. Eight cases with sufficient remaining sample volume were tested for anti-CHIKV IgG; these showed no serological evidence of past CHIKV infection. All 15 specimens were negative for DENV by nested RT-PCR.

The mosquito larval survey conducted by the Bali Provincial Health Office showed multiple egg laying sites in the houses with a HI of 55%.

Discussion

In 2010, CHIKV infections were reported to have re-emerged sporadically in seven out of eight districts in Bali, including Denpasar municipality.[1] However, this is the first report where extensive diagnostic tools were used to confirm the circulation of CHIKV in Bali. Our study documents the re-emergence of CHIKV

Table 1. Chikungunya test results of 15 acute cases during a febrile outbreak in Bali, 2015

Case ID	Age/sex	Days after fever onset	CHIKV-qRT-PCR (pfu equivalents/mL)	Virus isolation (cytopathic effects)	Anti-CHIKV IgM	Anti-CHIKV IgG
2016-10122	37/F	5	Negative	Negative	Positive	Negative
2016-10123	73/F	7	Negative	Negative	Positive	Negative
2016-10124	33/M	3	Negative	Negative	Negative	Negative
2016-10125	19/M	2	Positive (1.47×10^7)	Positive	Negative	QNS
2016-10126	52/F	1	Positive (2.18×10^5)	Positive	Negative	QNS
2016-10127	45/M	2	Positive (4.11×10^6)	Positive	Negative	QNS
2016-10128	34/M	3	Negative	Negative	Negative	Negative
2016-10129	21/F	1	Positive (7.59×10^5)	Positive	Negative	QNS
2016-10130	28/F	2	Positive (6.88×10^6)	Positive	Negative	QNS
2016-10131	23/M	3	Negative	Negative	Negative	Negative
2016-10132	45/F	4	Negative	Negative	Negative	Negative
2016-10133	59/M	2	Positive (3.07×10^6)	Positive	Negative	QNS
2016-10134	43/F	3	Negative	Negative	Negative	Negative
2016-10135	66/M	6	Negative	Negative	Equivocal	Negative
2016-10136	43/F	2	Positive (2.12×10^5)	Positive	Negative	QNS

QNS: quantity not sufficient; pfu: plaque-forming unit.

in Nagasepaha Village, North Bali where 96 patients were clinically diagnosed, based on fever, rash and arthralgia. Laboratory data confirmed nearly half of the acute phase specimens as CHIKV infection with high viral titers of 10^5–10^7 pfu equivalents/mL. Similarly high titers have been noted elsewhere and reported to be associated with high dissemination rates in mosquitoes under appropriate environmental conditions.[8] DENV was not detected by RT-PCR in any of the acute specimens.

One limitation of our study was the small sample size. With the outbreak reported towards the end and with limited resources, only acute-phase specimens were collected to maximize the virus recovery.

The entomological survey revealed an abundance of egg-laying sites in the village with an HI of 55%. This level is considered by the Ministry of Health Mosquito Control Program to indicate a high risk of transmission. While this is only one value reflecting the complex vector dynamics, this program uses a cut-off value of 5% to indicate effective mosquito control and low risk of mosquito-transmitted disease.[7] The high number of houses with larvae could potentially increase the viral transmission risk given other appropriate vectorial capacity values.

The absence of anti-CHIKV IgG in the samples submitted from 15 cases suggests limited population exposure to CHIKV. However, sequencing of the CHIKV strain isolated here revealed that it belongs to the Asian genotype closely related to strains known to be circulating in Bali and other areas of Indonesia, and associated with relatively mild symptoms.[9] This suggests that there is ongoing, endemic activity occurring throughout Indonesia.

Conclusions

Our study confirmed CHIKV to be the cause of a febrile outbreak in Nagasepaha, North Bali, and highlights the re-emergence of CHIKV in Bali. Laboratory confirmation of CHIKV is important for public health purposes, especially in dengue-endemic areas, as both DENV and CHIKV share the same mosquito vectors and co-circulate. Considering the high viremia as found in our study, molecular techniques are clearly a useful tool for an outbreak investigation, particularly for samples collected during the acute phase of illness in which specific IgM may have not yet developed. Rapid, robust and affordable assays are necessary for this under-diagnosed arbovirus, especially in tourist destinations such as Bali.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

Author contributions: Outbreak investigation and specimen collection was undertaken by ARA and PDA. Laboratory experiments and analysis of data was undertaken by RD, AP, CNM and DM. The draft manuscript was prepared by UAJ, KSAM, AMP, NPDW and KS. All authors read and approved the final manuscript. The guarantor of the paper is KSAM.

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