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Study of viremic profile in febrile specimens of chikungunya in Bandung, Indonesia

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ABSTRACT

Background: Data regarding the viremia profile of chikungunya virus (CHIKV) infected patients especially during the pre-febrile period is limited.
Objective: To obtain virological kinetic data on CHIKV infections.
Study design: A two-week community observation for dengue transmission was conducted in Bandung, Indonesia, from 2005 to 2009. Acute specimens from non-dengue febrile patients were screened by pan-alphavirus conventional RT-PCR. The positives were confirmed for CHIKV RNA by a specific RT-PCR followed by sequencing. Simultaneously these specimens were also cultured in Vero cells and tested for anti-CHIK IgM MAC-ELISA. All the available serial specimens,including the pre-febrile specimens, from confirmed CHIK cases, were tested by virus isolation, RT-PCR, qRT-PCR, and CHIK IgM ELISA.
Results: There were five laboratory confirmed CHIK cases identified and studied. Among these, viremia was determined to extend from as early as 6 days prior to until 13 days post fever onset. Quantitative RT-PCR showed viremia peaked at or near onset of illness.
Conclusion: In this study, individuals were identified with viremia prior to fever onset and extending beyond the febrile phase. This extended viremic phase has the potential to impact transmission dynamics and thus the public health response to CHIK outbreaks.

1. Background

Chikungunya virus (CHIKV), a positive-sense single stranded RNA alphavirus belonging to the family *Togaviridae*, is responsible for explosive outbreaks of debilitating arthralgia in many tropical countries. As illness caused by CHIKV and dengue viruses (DENV) are clinically undifferentiated, previously reported dengue (DEN) outbreaks might actually have been caused by CHIKV [1]. Human-human transmission of both CHIKV and DENV involves *Aedes aegypti* and *Aedes albopictus* mosquitoes, which can result in co-circulation as high endemicity of both viruses has been concurrently reported in the same area in Indonesia [2].

2. Objectives

To implement appropriate public health measures during outbreaks, it is important to understand the possible duration of transmission and the kinetics of CHIKV viremia. While there is some information regarding how long the virus may persist post

Abbreviations: CHIK, chikungunya; CHIKV, chikungunya virus; DEN, dengue; DENV, dengue virus; RT-PCR, reverse transcriptase-polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

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illness onset, there is limited knowledge on viremia prior to acute specimen acquisition [3–5]. For this reason, we conducted a retrospective study using serial specimens from several observed clusters collected during a DEN cluster study in Bandung, Indonesia to obtain the complete viremia profiles of infected patients.

3. Study design

The CHIKV retrospective cohort study was based on a DEN community cluster study conducted in Bandung, West Java, from 2005 to 2009 as described previously [6],in which blood specimens were collected to evaluate early dengue infection. Within 48 h of confirmation of a hospitalized dengue case (index case), community clusters were selected. For each cluster, a maximum of twenty individuals aged 4 years or older living with the index case or living within a 100 m radius from the index case's home, were invited to participate in the study. A total of 1928 individuals in 97 clusters were enrolled in the study. After signing informed consent documents, demographic information, clinical data, and blood samples were taken on day of enrollment and participants were monitored for 14 days for febrile episodes. Body temperature was measured daily by a research nurse or physician and blood specimens were taken every 3–4 days. Whenever fever occurred, another blood specimen was taken and the first day of onset of fever was defined as day 0. DENV infection was confirmed using IgM ELISA, reverse transcriptase-polymerase chain reaction (RT-PCR), and viral isolation assays. The DEN community cluster study was approved by the institutional review boards of the National Institute of Health Research and Development, Indonesian Ministry of Health, and the U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia in compliance with all applicable Federal regulations governing the protection of human subjects.

CHIKV infection in non-DENV acute febrile specimens from all clusters was identified by a combination of laboratory assays. Acute specimens from 102 cases were initially screened by pan-alphavirus conventional RT-PCR and those that tested positive were confirmed for CHIKV RNA by a specific RT-PCR targeting 1400 bp of the E1 gene followed by sequencing [7–9]. In parallel with RT-PCR, all acute sera were cultured in Vero cells in 24-well culture plates. Cells that were positive for cytopathic effects (CPE) were examined by immunofluorescent assay (IFA) using in-house generated anti-CHIKV hyper-immune mouse ascitic fluid (HMAF) antibodies to confirm that the CPE was caused by CHIKV [10,11]. All available specimens from the 102 non-dengue febrile cases were tested for anti-CHIK IgM using immunoglobulin M antibody capture ELISA (MAC-ELISA) [12]. To determine the antibody and virus kinetic profiles of CHIKV infection, all available serial specimens, including pre-febrile specimens, of CHIKV positive cases (identified by RT-PCR or culture) were tested by CHIK IgM ELISA, virus isolation, and RT-PCR. In all CHIKV positive cases, CHIKV viral load was also measured using real-time quantitative PCR (qRT-PCR) [13].

To determine the possibility of CHIKV infection among non-febrile individuals in the cluster, paired sera (first and last collection) from individuals who were never febrile during the study period ($n = 26$) from community clusters were tested for CHIKV IgM antibodies. First and last collection sera were compared to identify seroconversion (two cases had no specimens available for testing).

4. Results

Dengue was confirmed in 29 of 143 febrile cases (20.2%) from a total of 97 community clusters with 1928 community volunteers in the original study. The etiology of febrile illness for the remaining 114 cases could not be attributed to dengue infection. Upon performing CHIKV testing on sera from these cases (102 cases with specimens available), a total of 5 febrile CHIK cases from 2 clusters (Cluster 40 and 61) were identified. In the first cluster, 4 CHIKV cases were identified: 2 were found positive by both RT-PCR and serology, 1 by RT-PCR only, and 1 by serology only. In the second cluster, one case was found positive (by RT-PCR) (Table 1). All 4 cases confirmed by alphavirus RT-PCR were also positive for CPE and CHIKV IFA. Two cases had their blood taken before day 0, and three starting from day 0 as they presented with fever on the day of enrollment. Among the 5 symptomatic CHIKV cases, clinical symptoms were moderate and non-specific with headache (4/5), myalgia (4/5), nausea (4/5) cough (2/5), coryza (2/5), vomiting (2/5), epigastric pain (1/5), gum bleeding (1/5), sore throat (1/5) and arthralgia involving multiple joints (1/5). All symptomatic laboratory confirmed cases had fever with a duration of 1 to 3 days. All cases recovered completely without sequelae and none were hospitalized.

In two cases (ID 4003 (Genbank accession KT175540) and 4010 (Genbank accession KT175541)), pre and post-febrile specimens were available for testing. CHIKV qRT-PCR testing on serial specimens from these cases indicated that viremia lasted for 11–13 days (Table.1). Viremia was detected by a CHIKV-specific qRT-PCR at low levels as early as 5 and 6 days before fever onset and peaked at 7 and 8 $\log_{10}$ PFU equivalents/mL on day 0 (fever onset). The viremia profiles of the 4 nucleic acid positive individuals are shown in Table 1 and Fig. 2.

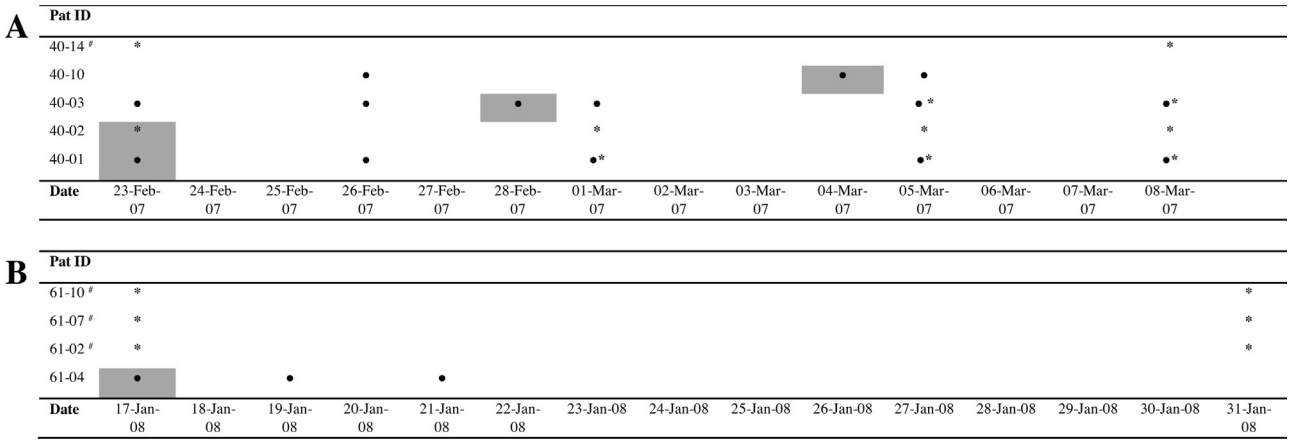

Fig. 1. Timeframe of chikungunya cases identified in two clusters. (A) cluster #40, (B) cluster #61. Symbol ● representing positive qPCR, * representing positive IgM ELISA and patient ID with # representing patients with recent infection prior to participation in study. Gray color ( ) is day of fever.

Table 1
Viremia and antibody profiles of symptomatic chikungunya cases.

Pat ID	Sex/age	CHIKV assay	Days to the onset of fever												
			−9	−6	−5	−2	Day#0 (fever onset)	1	2	3	4	6	7	10	13
4001	Female/52	qRT (pfu equivalent/mL)	.	.	.	.	2,37E + 07	.	.	7,62E + 01	.	3,69E + 01		3,25E + 01	1,02E + 01
		CHIKV isolate	.	.	.	.	Pos	.	.	Neg	.	Neg		Neg	Neg
		Alphavirus RT PCR	.	.	.	.	Pos	.	.	Neg	.	Neg		Neg	Neg
		CHIKV IgM ELISA	.	.	.	.	Neg	.	.	Neg	.	Pos		Pos	Pos
4003	Male/35	qRT (pfu equivalent/mL)	.	.	5,47E + 01	1,79E + 03	1,06E + 07	5,08E + 06	.	.	7,59E + 01	.	4,33E + 01	.	.
		CHIKV isolate	.	.	Neg	Neg	Pos	Pos	.	.	Neg	.	Neg	.	.
		Alphavirus RT PCR	.	.	Neg	Neg	Pos	Pos	.	.	Neg	.	Neg	.	.
		CHIKV IgM ELISA	.	.	Neg	Neg	Neg	Neg	.	.	Pos	.	Pos	.	.
4010	Male/12	qRT (pfu equivalent/mL)	Neg	1,57E + 01	.	.	6,14E + 08	8,77E + 07	.	.	.	.	.	.	.
		CHIKV isolate	Neg	Neg	.	.	Pos	Pos	.	.	.	.	.	.	.
		Alphavirus RT PCR	Neg	Neg	.	.	Pos	Pos	.	.	.	.	.	.	.
		CHIKV IgM ELISA	Neg	Neg	.	.	Neg	Neg	.	.	.	.	.	.	.
6104	Male/16	qRT (pfu equivalent/mL)	.	.	.	.	3,98E + 06	.	4,68E + 05	.	4,05E + 02	.	.	.	.
		CHIKV isolate	.	.	.	.	Pos	.	Pos	.	Neg	.	.	.	.
		Alphavirus RT PCR	.	.	.	.	Pos	.	Pos	.	Neg	.	.	.	.
		CHIKV IgM ELISA	.	.	.	.	Neg	.	Neg	.	Neg	.	.	.	.
4002	Male/12	qRT (pfu equivalent/mL)	.	.	.	.	Neg	.	.	.	.	.	.	.	Neg
		CHIKV isolate	.	.	.	.	Neg	.	.	.	.	.	.	.	Neg
		Alphavirus RT PCR	.	.	.	.	Neg	.	.	.	.	.	.	.	Neg
		CHIKV IgM ELISA	.	.	.	.	Pos	.	.	.	.	Pos	.	Pos	Pos

Note: (.) = sample not available.

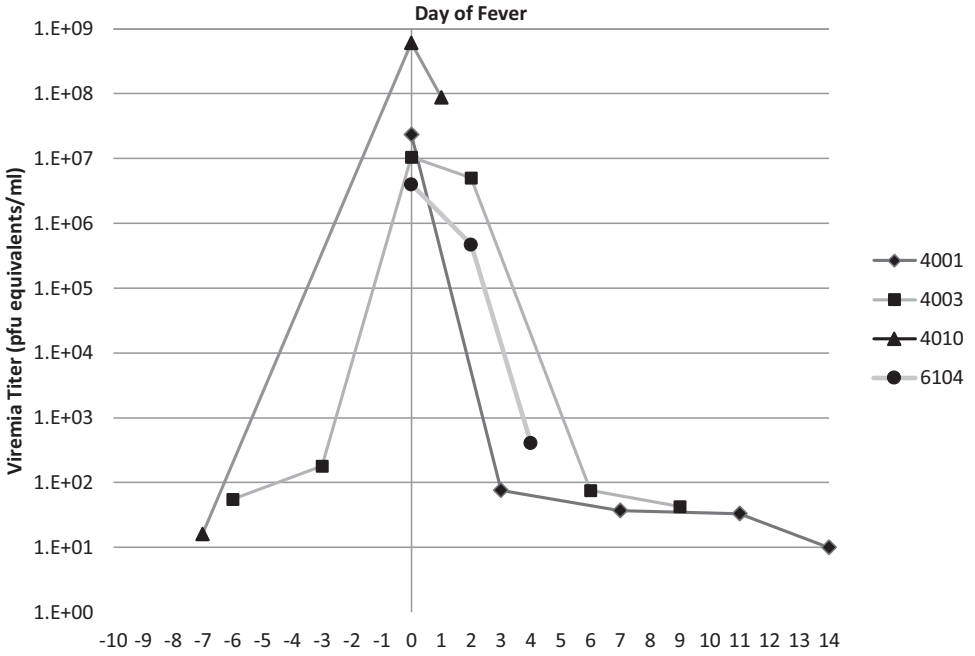

Fig. 2. Viremia kinetics in four confirmed chikungunya cases. The viremia titer (pfu equivalents/mL) during observation, day 0 = day of fever onset.

Pan-alphavirus conventional RT-PCR was found to be less sensitive than the CHIKV-specific qRT-PCR (data unpublished). In this study, the pan-alphavirus RT-PCR detected only specimens with viremia at 5 $\log_{10}$ PFU equivalents/mL or higher. Using the CHIKV-specific qRT-PCR, viral RNA was detected for 8 days (mean) after onset compared with only 2 days (mean) for RT-PCR (Table 1). The sensitivity between virus isolation and the conventional RT-PCR assay were comparable (Table 1). Virus isolation was limited to those specimens drawn within the first 2 days of illness onset.

Two cases demonstrated unexpected laboratory results. In one case (ID 4001(Genbank accesion KT175539)), CHIKV RNA persisted for at least 13 days after illness and CHIK IgM antibodies were not detected until as late as 6 days after fever onset. Interestingly, another case (ID 4002) was already positive for CHIKV IgM antibody at day 0, but was negative by RT-PCR and isolation. However, because the patient was already febrile on day 0 and there was no information available for fever status prior to this point, it is conceivable that viremia had already subsided.

Genotype analysis of the CHIKV E1 gene fragment revealed that all isolates belonged to the Asian genotype and were closely related to the Bandung strains circulating in 2001–2008 [2] and Surabaya/Indonesia strains from 2010 to 2011 [14].

From clusters 40 and 61 (containing 33 total subjects, 26 with paired specimens available), a total of four additional subjects had a recent CHIKV infection as determined by the presence of IgM antibodies: one in cluster 40 and three in cluster 61 (Fig. 1). Apart from the index cases, no additional DEN infections were identified among subjects in these clusters during the two week observation period (Fig. 2).

5. Discussion

This study examined the viremia and antibody profiles of CHIKV infection based on archived specimens from an early dengue community study and revealed that CHIKV viremia preceded fever onset by up to 6 days. A previous study that included collection of pre-illness specimens demonstrated viremia only a single day before illness in two cases [8]. The kinetics of CHIKV viremia and antibodies after illness observed in this study were similar to previous reports [3]. Additionally, high peak titers and prolonged duration of viremia observed in this study are consistent with earlier studies and could be an important factor in facilitating the spread of CHIKV infection [3,13,15]. However, unlike in animal models [16] or DENV studies [17], high CHIKV viral loads did not seem to be correlated with increased disease severity since no prolonged illnesses were observed with these cases.

In this study, another 4 out of 26 subjects (15%) in clusters with confirmed CHIK infection had CHIKV-specific IgM antibodies from day 1 of participation (Fig. 1), but fever was not reported during 14 days observation period. This suggests that chikungunya infection had already occured in these communities prior to their participation in this study. Chikungunya has previously been reported as endemic in Bandung, Indonesia, with a yearly incidence rate of 10.1 per 1000 persons [2]. These data suggest that chikungunya should be considered in the differential diagnosis of acute febrile cases.

The current study did have some limitations in that there was a small number of CHIKV positive cases and only two confirmed cases with serial, pre-febrile specimens to study the full viremic profile. However, both cases revealed lengthy viremia before illness onset. In addition, due to limited personnel and resources, daily serial specimens were not collected as would have been ideal for a full kinetic profile. Compared with earlier reports on CHIKV infection kinetics, where specimens were primarily post-fever onset [4], the findings presented here reveal substantially extended viremia and information regarding pre-fever viremia. This is of public health significance as patients with a prolonged viremic period and extensive pre-febrile viremia may have the potential to spread the virus long before clinical presentation.

Earlier studies from Indonesia have reported the co-circulation of DENV and CHIKV since both viruses utilize the same vector species [14]. As no additional DENV cases were identified in these two clusters with CHIKV positive subjects, the co-existence of these two viruses was likely of a low level in this community. Furthermore, no CHIK cases were identified in the remaining 94 communities where DENV was detected, strengthening this speculation.

6. Conclusions

In this study, CHIK cases identified in a DEN cluster study manifested with a milder spectrum of clinical manifestations, with only one subject presenting with arthralgia, the hallmark of CHIKV infection. Physicians should consider CHIKV infection even in patients who present without polyarthralgias especially those who have recently traveled to CHIKV endemic areas. With the evidence for lengthy viremia found in this study, the CHIKV infectious period may be greater than realized and should be considered as a factor impacting transmission in outbreak areas.

Competing interest

None.

Authors' disclosures

The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, Centers for Disease Control and Prevention, nor the U.S. Government. MW is a military service member. This work was prepared as part of her official duties. Title 17 U.S.C. section 105 provides that 'Copyright protection under this title is not available for any work of the United States Government' Title 17 U.S.C. section 101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties.

Ethical approval

Testing of archived specimens was approved by the Eijkman Institute Research Ethics Commission.

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