Short communication

Chikungunya virus of Asian and Central/East African genotypes in Malaysia

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ABSTRACT

Background: Chikungunya virus (CHIKV) of the Central/East African genotype has caused large outbreaks worldwide in recent years. In Malaysia, limited CHIKV outbreaks of the endemic Asian and imported Central/East African genotypes were reported in 1998 and 2006. Since April 2008, an unprecedented nationwide outbreak has affected Malaysia.

Objective: To study the molecular epidemiology of the current Malaysian CHIKV outbreak, and to evaluate cross-neutralisation activity of serum from infected patients against isolates of Asian and Central/East African genotypes.

Study design: Serum samples were collected from 83 patients presenting in 2008, and tested with PCR for the E1 gene, virus isolation, and for IgM. Phylogenetic analysis was performed on partial E1 gene sequences of 837 bp length. Convalescent serum from the current outbreak and Bagan Panchor outbreak (Asian genotype, 2006) were tested for cross-neutralising activity against representative strains from each outbreak.

Results: CHIKV was confirmed in 34 patients (41.0%). The current outbreak strain has the A226V mutation in the E1 structural protein, and grouped with Central/East African isolates from recent global outbreaks. Serum cross-neutralisation activity against both Central/East African and Asian genotypes was observed at titres from 40 to 1280.

Conclusions: The CHIKV strain causing the largest Malaysian outbreak is of the Central/East African genotype. The presence of the A226V mutation, which enhances transmissibility of CHIKV by Aedes albopictus, may explain the extensive spread especially in rural areas. Serum cross-neutralisation of different genotypes may aid potential vaccines and limit the effect of future outbreaks.

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1. Background

Chikungunya virus (CHIKV) causes epidemic fever and arthralgia, and is transmitted by the mosquitoes Aedes aegypti and Ae. albopictus. There are 3 genotypes, West African, Central/East African, and Asian, initially named according to the regions where the strains were reported.1 However, since 2005, CHIKV of the Central/East African genotype has caused large outbreaks in several countries worldwide and beyond the African continent, notably in the Indian Ocean and India.2

Malaysia, in Southeast Asia, has a population of approximately 27 million. Low-level CHIKV seroprevalence was noted in the 1960s,3 although the first 2 confirmed CHIKV outbreaks occurred only in the last decade, in Klang (Selangor state, 1998)4 and Bagan Panchor (Perak state, March 2006).5 Both were caused by the endemic Asian genotype; of note, the second outbreak occurred during the global outbreaks of the Central/East African genotype. A third outbreak, in Ipoh (Perak, December 2006), was caused by a Central/East African strain imported from India.6 These 3 outbreaks were each restricted to a single area, affecting about 300 people in total. However, since April 2008, Malaysia has been experiencing a nationwide outbreak. Initial outbreaks in Johor state have spread to 14 out of 15 states and federal territories, involving at least 7100 people to date.7

2. Objectives

To study the molecular epidemiology and neutralising immune responses of this latest outbreak, the most extensive yet in Malaysia.

3. Study design

The study was carried out from April to December 2008 at the University Malaya Medical Centre in Kuala Lumpur, Malaysia.
Serum samples were collected from 83 inpatients and outpatients with symptoms of suspected CHIKV infection, such as fever, rash, and arthralgia. Clinical diagnoses and the decision to test for CHIKV were made at the discretion of the attending physicians. The choice of diagnostic tests was based on the timing of the sample with respect to the onset of disease. Acute samples taken from day 3 onwards were tested by indirect immunofluorescence for IgM. If negative for CHIKV IgM, acute samples were cultured in Vero cells, and convalescent samples were requested for repeat IgM testing. Samples taken 6 days or less from onset of illness were tested by RT-PCR to detect a 257 bp fragment of the E1 gene, and virus isolation. Some PCR-positive samples and isolates were selected to span the 8-month period and different geographical sources. For these selected samples, published primers 1014F and 11158R were used to amplify and sequence a 1013 bp fragment of the E1 gene. Two representative CHIKV isolates of the Asian genotype from the Bagan Panchor outbreak, MY/06/37348 and MY/06/37350 were also sequenced.

The longer E1 sequences were used for phylogenetic analysis. Consensus sequences were generated using Geneious Pro version 4.0.4 (Biomatters Ltd., Auckland, New Zealand), and aligned against other CHIKV sequences retrieved from GenBank using ClustalX version 2.09. A phylogenetic tree was constructed using the neighbour-joining method and Kimura two-parameter distance model. The tree was rooted with O’nyong-nyong virus strain SG650 and displayed using the MEGA version 4.11. The strength of the phylogenetic tree was estimated by bootstrap analyses using 1000 random samplings.

Convalescent serum from 4 patients from each of the Bagan Panchor and current outbreaks were also assayed for cross-neutralisation activity against representative isolates from each outbreak. The Bagan Panchor serum samples were obtained from patients 1 year after the outbreak in 2006. For the current outbreak, convalescent serum was collected between 3 and 7 weeks after onset of symptoms. The isolates used were MY/06/37348 from Bagan Panchor, and MY/08/065 from Johor. A previously described neutralisation assay, with minor modifications, was carried out in duplicate using an initial serum dilution of 1:10. The virus-serum mixtures were inoculated on 70%-confluent Vero cells and monitored for cytopathic effect for 7 days. The neutralizing titer was taken as the highest dilution of serum that inhibited virus growth.

### 4. Results

Of 83 patients tested, 34 (41.0%) had laboratory confirmation of CHIKV infection by detectable IgM (22 patients), PCR (18), virus isolation (16), or a combination of these. IgM was only detectable at least 5 days after symptom onset, while PCR and culture were positive at 5 days or less.

For phylogenetic analysis, longer E1 sequences were obtained for 11 patients infected at 8 different locations and time points throughout the outbreak, spanning 8 months in 2008. A phylogenetic tree was drawn using the partial E1 region of 837 bp, at nucleotide positions 10,264–11,100, numbered according to the CHIKV prototype S27 (Fig. 1). This confirmed that CHIKV isolates formed 3 distinct genotypes, West African, Central/East African, and Asian. The 11 isolates from the current Malaysian outbreak grouped together with recent Central/East African isolates from 2005 to 2008, sharing 98–99% nucleotide identity. All 11 Malaysian isolates had alanine replaced by valine at position 226 (A226V) of the E1 structural protein. There were also 2 nucleotide substitutions not seen in other Central/East African isolates, C300T and G693T, seen in 7/11 and 4/11 isolates, respectively (Table 1). Isolates MY/06/37348 and MY/06/37350 from the Bagan Panchor outbreak in 2006 were confirmed as the Asian genotype, and did not cross-react with other CHIKV isolates of different genotypes.

### Table 1

<table>
<thead>
<tr>
<th>CHIKV isolate (accession number)</th>
<th>Nucleotide position in E1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>300</td>
</tr>
<tr>
<td>MY/06/065 * (FN295485)</td>
<td>C</td>
</tr>
<tr>
<td>MY/06/066 * (FN295486)</td>
<td>C</td>
</tr>
<tr>
<td>MY/06/068 * (FN295487)</td>
<td>C</td>
</tr>
<tr>
<td>MY/08/5369 * (FN295491)</td>
<td>C</td>
</tr>
<tr>
<td>MY/08/6000 * (FN295490)</td>
<td>T</td>
</tr>
<tr>
<td>MY/08/7913 * (FN295492)</td>
<td>T</td>
</tr>
<tr>
<td>MY/08/6008 * (FN295495)</td>
<td>T</td>
</tr>
<tr>
<td>MY/08/2868 * (FN295488)</td>
<td>T</td>
</tr>
<tr>
<td>MY/08/2844 * (FN295489)</td>
<td>T</td>
</tr>
<tr>
<td>MY/08/2561 * (FN295494)</td>
<td>T</td>
</tr>
<tr>
<td>MY/08/0539 * (FN295493)</td>
<td>T</td>
</tr>
<tr>
<td>Other Central/East African genotype</td>
<td>C</td>
</tr>
<tr>
<td>Bagan Panchor, Malaysia (Asian genotype)</td>
<td>T</td>
</tr>
</tbody>
</table>

* Isolates from the current Malaysian outbreak, all from the Central/East African genotype.
The current Malaysian outbreak mainly involves rural areas, with numerous rubber and palm oil plantations, where \textit{Ae. albopictus} predominates over \textit{Ae. aegypti}.\textsuperscript{19,20} Therefore we suggest that in this setting, the current strain was able to cause an outbreak of unprecedented scale in Malaysia.

We also showed that serum from CHIKV-infected patients cross-neutralises CHIKV isolates from Central/East African and Asian genotypes. In an earlier study, sera from CHIKV-vaccinated mice and monkeys cross-neutralised different CHIKV strains from Africa, Asia, and India.\textsuperscript{21} Cross-neutralisation may limit the impact of a new CHIKV genotype being introduced into a population already immunised by natural infection or vaccination. Studies of potential vaccines\textsuperscript{22,23} should confirm this by testing efficacy against all CHIKV genotypes.

In conclusion, the largest ever reported outbreak of CHIKV in Malaysia is caused by an Central/East African strain with the A226V mutation in the E1 protein. Serum cross-neutralisation against Central/East African and Asian genotypes was shown, which has positive implications for vaccine development and future outbreaks.

### Conflict of interest

None declared.

### Funding

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### References