ORIGINAL ARTICLE

Re-Emergence of Chikungunya Virus in Malaysia

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Summary

An outbreak of Chikugunya (CHIK) fever occurred among the fishing community in Bagan Pancor, Perak. The outbreak was laboratory confirmed within 48 hours after the receipt of the specimens. Fifty-three patients' serum samples were submitted for laboratory investigation and 47 (88.7%) were confirmed to be positive for CHIK infection by RT-PCR, and/or virus isolation, and/or in-house immunoflourescent test. RT-PCR and virus isolation were the tests of choice for patients with illness of four days or less and detection of CHIK specific IgM for those with more than four days of fever. The nucleic acid sequence based on the 354- and 294-bp of the nsP1 and E1 genes of the CHIK virus detected from pools of adults Aedes aegypti mosquitoes were identical to those CHIKV virus isolated from humans in the same locality. Phylogenetic analysis of the CHIK virus based on the 257 nts partial E1 gene indicates that Bagan Panchor's strain was closely related to the first CHIK virus isolated during the outbreak in Klang in 1998.

Key Words: Chikungunya, Outbreak, Malaysia

Introduction

Chikungunya virus (CHIK) is a small envelope positive sense RNA virus which belongs to the genus *Alphavirus* under the family *Togaviridae*. The virion is essentially spherical, 60-65 nm in diameter and consists of three components: an outer glycoprotein shell, a lipid bilayer and an RNA-containing core or nucleocapsid[1]. Serologically, the virus is classified as a member of the Semliki Forest antigenic complex. It is transmitted to human beings by mosquitoes of the *Aedes genus* (especially *Aedes aegypti*), similar to the vectors of dengue viruses[2-4].

The symptoms of CHIK virus infection are characterized by fever, headache, severe back and joint pain, rash, and lymphadenitis. The incubation period varies but is usually between two to three days. In adults there is abrupt onset of fever, headache and severe joint pain without prodromal symptoms. The joint pains are the dominant complaint and affect mainly the small joints of the hands, wrists and feet. A maculopapular rash together with a generalized lymphadenopathy appears three to four days later. Although the arthritis may resolve within a few weeks, pain, swelling and morning stiffness may continue for months and even a year after infection. Petechiae and bleeding from gums may occur, but there are no significant haemorrhagic manifestations. Clinical illness in children tends to be less specific and may manifest as non-specific febrile viral illness with vomiting and abdominal pain[1,2].

CHIK virus was first isolated from human serum and *Aedes aegypti* mosquitoes during an epidemic in Tanzania in 1953. Since then, CHIK virus has caused occasional outbreaks and some larger epidemics throughout most of sub-Sahara Africa and tropical Asia including India and the Western Pacific. Historic evidence points to the spread of CHIK virus from Africa

This article was accepted: 11 May 2006

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to Asia, where it has caused outbreaks in the Philippines, Thailand, Indonesia, India, Sri Lanka, Vietnam, Kampuchea and Myanmar since 1954. The epidemiology of CHIK virus infection differs in Africa and Asia. In Africa, the most important vertebrae in maintaining the cycle of CHIK virus infection are the non-human primates such as baboons and *Cercopithecus* monkeys. Humans may be infected in African villages and rural areas, particularly where *Aedes aegypti* is present in large numbers. In contrast to the situation in Africa, transmission in Asia is primarily from human to human by *Aedes aegypti*[5-7]. Malaysia experienced the first outbreak of CHIK in late 1998 involving residences staying in the suburb of Klang, a coastal city in the central western part of Peninsular Malaysia. Because of its first occurrence, the diagnosis was only laboratory confirmed at the end of January 1999[8-10].

On 28th of March 2006, the National Public Health Laboratory Malaysia received a request from a health officer based in northern part of Peninsular Malaysia (Taiping, Perak) for laboratory confirmation of influenza, dengue, measles, or rubella for a cluster of patients who came down with febrile rash illness and sore-throat staying in Bagan Panchor, a fishing village situated at approximately 15 kilometres from Taiping. On reviewing the presenting clinical features, a provisional diagnosis of chikungunya virus as the cause of the outbreak was made. On the following day, the laboratory received twenty-five serum samples and twenty-five throat swab samples in viral transport medium from 25 patients with the illness. The serum samples were processed for virus isolation in C6/36 cells (ATCC CRL-1660) and Vero cells (ATCC CCL-81), molecular detection of CHIK virus genome by RT-PCR based on the published method by Hasebe *et al.*,[10] and serological assay for dengue specific IgM by IgM-capture ELISA. On the 30th of March 2006, RT-PCR amplification products corresponding to the expected 354- and 294-bp of the CHIK nsP1 and E1 genes were detected by using the CHIK virus specific primers pairs in acute serum samples of six patients with the history of fever of four days or less. Subsequent nucleic acid sequencing by ABI Prism Big-Dye (Pharmacia, USA) dideoxyl termination cycle on the same day confirmed the amplicons were of segments of CHIK virus non-structural (nsP1) and envelope protein (E1) genes. Based on the laboratory finding, drastic control measures were immediately taken by the public health authority to eradicate *Ae. Aegypti* mosquitoes present in the affected village and its surrounding areas. The outbreak was rapidly brought under control and the last laboratory confirmed acute case was on 10th of April 2006.

During the outbreak, 53 patients' serum samples were submitted to the NPHL for laboratory investigation of the aetiology. The types of tests performed to support clinical diagnosis of CHIK and their respective results are shown in Table I. The assay of CHIK specific IgM was based on an in-house indirect immunofluorescent test using Vero cells infected with CHIK virus isolated from the patient as the source of antigens. Forty-seven patients were laboratory confirmed to have acute CHIK virus infection by either one or more of the tests performed (Table I). The findings indicated that molecular detection of CHIK genome by RT-PCR and virus isolation were the tests of choice for patients with acute illness of four days or less and detection of CHIK specific IgM was the test of choice for those patients with the duration of illness of five days or more.

Two batches of adult *Aedes aegypti* mosquitoes, consisting of eight pools, were also received by NPHL for virus isolation during the period of outbreak investigation. CHIK virus RNA was detected in three of the eight *Aedes aegypti* mosquito pools. Nucleic acid sequence information showed that CHIK nucleic sequence obtained from mosquitoes was identical to that of the nucleic acid sequence of CHIK virus isolated from humans from the same location. The phylogenetic relationship of the CHIK virus isolated in this outbreak with respect to other CHIK viruses based on the 257-nts partial E1 gene is shown in Figure 1. Analysis of the 257-nts partial E1 gene shows that the CHIK virus isolated in this outbreak was closely related to the CHIK virus (Figure 1, MALh0198) which caused the outbreak in Peninsular Malaysia in 1998 (>99%) and differed from it by only one nucleotide at position 10471(GenBank Accession No. DQ443544) (thymidine_cytosine). Both have the closest match to the CHIK virus isolated in Philippines in 1985 (H15483) and all belong to the Asian genotype (Figure 1). This finding strongly suggests the CHIK virus caused the Bagan Panchor's outbreak is most probably derived from the strain that caused the Klang's outbreak in 1998. The finding also suggests that after causing the outbreak in Klang, the CHIK virus most probably underwent low endemic transmission which caused sporadic cases until the right circumstances where it re-emerged again as an outbreak. The hypothesis is supported by a recent finding where positive anti-CHIK IgM serology were found in patients from other

locations in Perak with febrile rash illness associated arthralgia (unpublished data). Thus, practising clinicians should be watchful of CHIK virus infection as the cause of the patients' illness with the presenting features as mentioned earlier.

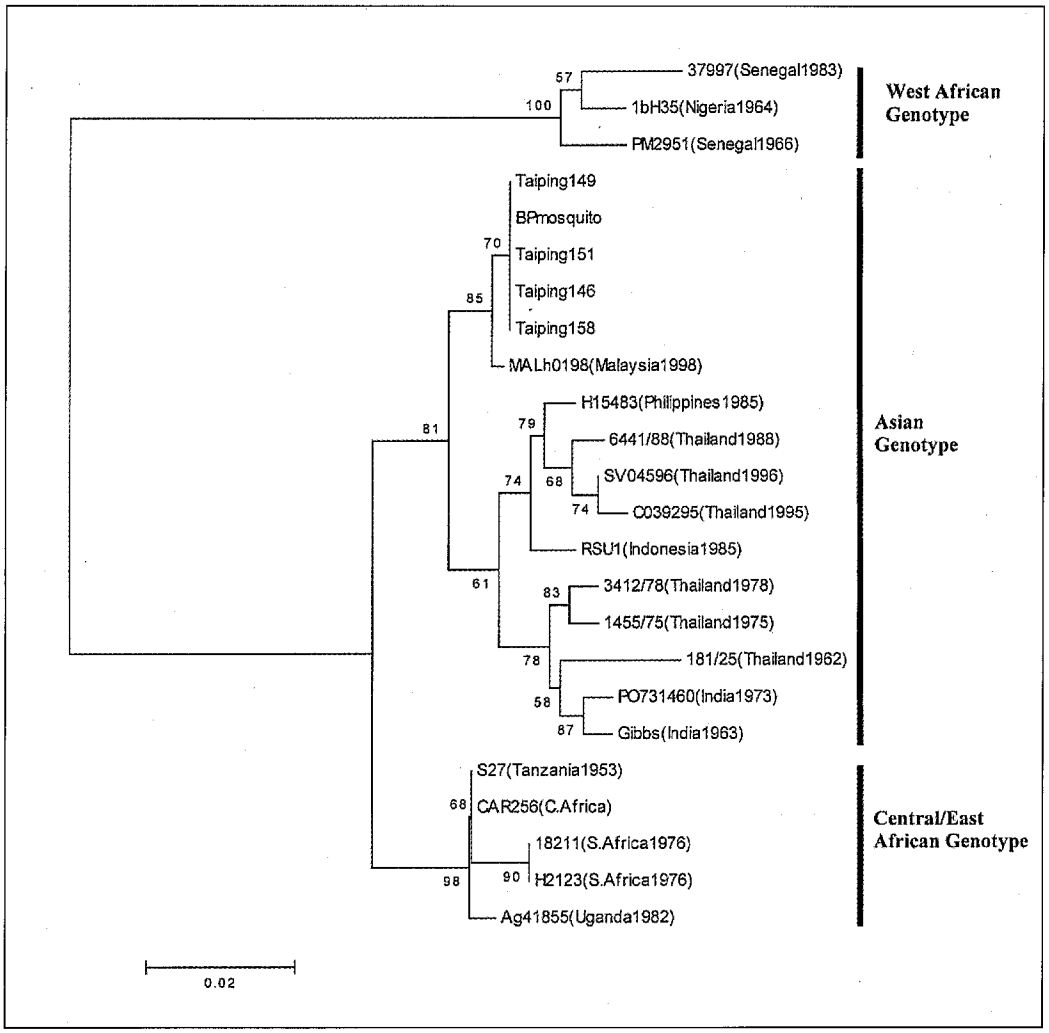

Fig. 1: Phylogenetic analysis of 19 strains of Chikungunya (CHIK) virus with respect to 5 strains of CHIK virus, 4 from human (Taiping 146, Taiping 149, Taiping 151, Taiping 158) and 1 from mosquito (BPmosquito), isolated in the 2006 outbreak in northern part of Peninsular Malaysia. The bootstrapped consensus tree was constructed for a 257-nt-long E1 gene sequence using MEGA programme. Identity of each isolate, location and year of isolation was indicated in the figure. Their respective Genbank accession numbers are as follow: 37997 (AF192892), PM2951 (AF192891), IbH35 (AF192893), MALh0198 (AF394210), H15483 (AF192895), 6441/88 (AF192896), SV045196 (AF192900), C039295 (AF192897), RSU1 (AF192894), 3412/78 (AF192899), 1455/75 (AF192898), 181/25 (AF192908), PO731460 (AF192902), Gibbs (AF192901), Ag41855 (AF192907), S27 (L37661), CAR256 (AF192906), 18211 (AF192903), H2123 (AF192904).

Table I: Result of laboratory investigation of patients' acute serum samples from Bagan Panchor (Perak, Peninsular Malaysia) during an outbreak of Chikungunya.

No.	Lab. ID	*Sample age (day)	RT-PCR	Virus isolation	CHIK IgM
1	GV140	8	-	-	Positive
2	GV141	7	-	-	Positive
3	GV142	5	-	-	Positive
4	GV143	5	-	-	Positive
5	GV144	14	-	-	Positive
6	GV145	5	-	-	Positive
7	GV146	7	Detected	Virus isolated	-
8	GV147	14	-	-	Positive
9	GV148	unknown	-	-	Positive
10	GV149	unknown	Detected	Virus isolated	-
11	GV150	2	-	Virus isolated	-
12	GV151	2	Detected	Virus isolated	-
13	GV152	21	-	-	Positive
14	GV153	unknown	Detected	Virus isolated	Borderline
15	GV154	7	-	-	Positive
16	GV155	14	-	-	Positive
17	GV156	4	-	-	Positive
18	GV157	8	Detected	-	Positive
19	GV158	unknown	Detected	Virus isolated	-
20	GV159	3	-	-	-
21	GV160	4	-	-	Positive
22	GV161	8	-	-	-
23	GV162	30	-	-	Positive
24	GV163	3	-	-	-
25	GV164	7	-	-	-
26	GV183	4	-	-	Positive
27	GV184	3	Detected	Virus isolated	-
28	GV185	1	-	-	-
29	GV186	7	-	-	-
30	GV187	2	Detected	-	-
31	GV188	5	-	-	Positive
32	GV206	4	-	-	Borderline
33	GV207	7	-	-	Positive
34	GV208	unknown	-	-	Borderline
35	GV209	unknown	Detected	-	-
36	GV212	5	-	-	Positive
37	GV213	5	-	-	Positive
38	GV214	unknown	-	Virus isolated	Borderline
39	GV215	3	-	Virus isolated	Positive
40	GV219	3	Detected	Virus isolated	Borderline

No.	Lab. ID	*Sample age (day)	RT-PCR	Virus isolation	CHIK IgM
41	GV226	3	Detected	Virus isolated	-
42	GV227	3	-	-	Positive
43	GV228	3	Detected	Virus isolated	Positive
44	GV237	4	-	Virus isolated	-
45	GV238	unknown	-	-	-
46	GV239	unknown	-	-	Positive
47	GV240	7	-	-	Positive
48	GV241	3	Detected	-	-
49	GV242	unknown	Detected	-	-
50	GV260	3	-	Virus isolated	-
51	GV261	4	-	Virus isolated	Positive
52	GV262	unknown	-	-	Positive
53	GV263	unknown	-	-	Positive

*Sample age was defined as the interval in days between the collection of blood sample and the date of onset of fever.

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