ORIGINAL ARTICLE

Chikungunya Virus of Central/East African Genotype Detected in Malaysia

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SUMMARY

Since its isolation in Tanzania in 1953, chikungunya virus has caused periodic outbreaks in both tropical Africa and Asia. In the last decade, the virus has shown not only increased activity but has expanded its geographical locations, thus classical delineation of various genotypes of chikungunya virus to specific geographic locales no longer holds true. Rapid mass movement of people and the constant presence of the right vectors in this region could have contributed to the change in virus ecology. This paper documents the first detection of chikungunya virus of Central/East genotype in Malaysia from a patient who was most likely infected with the virus during her visit to India. Without good *Aedes* vector measures, only time will tell whether this genotype rather than the existing endemic genotype will subsequently cause the next chikungunya outbreak in Malaysia.

KEY WORDS:
Chikungunya virus, Central/East African genotype, Malaysia

INTRODUCTION

Chikungunya (CHIK) virus is a mosquito-borne togavirus belonging to the genus *Alphavirus* and is classified as a member of the Semliki Forest antigenic complex. It is a small envelope positive sense RNA virus. 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]. The virus is transmitted to human beings by the bite of infective mosquitoes of the *Aedes* genus (especially *Aedes* aegypti)[2-4].

The clinical features of CHIK virus infection are characterized by fever, headache, severe back and joint pain, cutaneous 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. Infection can also be asymptomatic[1,2].

CHIK virus was first isolated from human serum and *Aedes aegypti* mosquitoes during an epidemic in Tanzania in 1953. Hammon et al (1960) documented the first appearance of the virus in Southeast Asia by isolation during an intense epidemic of dengue fever in Bangkok, Thailand in 1958[5]. Since then, CHIK virus has caused occasional outbreaks and some larger epidemics throughout most of sub-Sahara Africa and tropical Asia. Historic evidence points to the spread of CHIK virus from Africa 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*[3-8].

In Peninsular Malaysia, a serological survey for alphaviruses conducted by Marchette *et al* (1978) showed that CHIK antibody was detected in persons older than 20 years with a proportionately larger number of seropositive individuals in the northern states bordering Thailand such as Perlis, Kedah and Kelantan. A follow up serological study by Marchette *et al* (1980) showed specific haemagglutination inhibition and neutralizing antibodies in a chicken in Kelantan and a pig in Kedah, further supporting CHIK activity occurred mainly along the Malaysia-Thailand border[9]. Despite the fairly high antibody prevalence in man in the surveyed areas, there was no report of clinical disease associated with the virus until 1998 where the first outbreak was reported involving residences staying in the suburb of Klang, a coastal city in the central western part of Peninsular Malaysia[10]. Following which, a second outbreak occurred in early 2006 involving residences staying in Bagan Panchor, a fishing village situated at approximately 15 kilometres from Taiping in the state of Perak, north western part of Peninsular Malaysia[11]. In both outbreaks, CHIK viruses of Asian genotype were isolated and nucleotide sequence analysis of the virus partial E1 gene strongly suggested that the 2006 virus strain most probably

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evolved from the 1998 outbreak strain which could have remained at low circulation between the outbreaks[11]. This paper documents the detection of CHIK virus of Central/East genotype for the first time in Malaysia from a patient who was most likely infected with the virus during her visit to India.

Patient RS, a 49-year-old Indian housewife was treated in a government district hospital (Hospital Batu Gajah), Perak on 27th August 2006 with a four days history of fever, joint pain and occasional vomiting. The fever was described as of high grade in nature but not associated with chill and rigor. The joint pain was severe involving bilateral knee, shoulder, wrist and phalange joints. The joint pain was associated with swelling of knee joints and severe backache, especially the lower back. Physical examination confirmed she was febrile with an oral temperature of 39.5°C. Her knee joints were swollen, tender with reduced range of movement but there was no obvious joint effusion. Mild swelling and tenderness was also noted over bilateral wrist and finger joints. There was no skin rashes nor petechiae noted. Systemic examination revealed no other significant abnormality.

Epidemiological investigation revealed that she visited southern India (Chennai) with her husband, son and brother-in-law (husband's brother) about three weeks prior to her onset of illness. They traveled to various parts of southern India and finally stayed in her relative's house in Puttu, Chennai for ten days. During the period of her stay, there was an outbreak of similar illness affecting residences of the local community. Towards the end of their visit, her husband and brother-in-law developed two days of fever which resolved without any joint involvement. She developed abrupt onset of high fever (23/8/2006), a day prior to her return to Malaysia. She developed crippling joint pain and severe backache the following days. So far, there was no similar illness affecting her neighbours within a month since her return. A provisional diagnosis of chikungunya with the differential of dengue fever was made and a blood sample was taken for laboratory confirmation of her illness.

The serum sample was 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.*,[12] and serological assay for CHIK specific antibodies by indirect immunofluoresecent assay using in-house prepared antigen. Convalescent blood samples were collected from her and her husband on 14th October 2006 for serological assay of anti-CHIK specific antibodies. Low level of anti-CHIK IgM (1:10 dilution) was detected in both her acute and convalescent sera and her husband's serum sample (Table I). Her convalescent serum sample showed four-fold increased in anti-CHIK IgG (Table I). Though no CHIK virus was isolated in both Vero and C6/36 cells, RT-PCR amplification products corresponding to the expected 354- and 294-bp of the CHIK nsP1 and E1 genes were detected in her acute serum sample by using the CHIK virus specific primers pairs (Figure 1). Subsequent nucleic acid sequencing by ABI Prism Big-Dye (Pharmacia, USA) dideoxyl termination cycle confirmed the amplicons were segments of CHIK virus non-structural (nsP1) and envelope protein (E1) genes. The phylogenetic analysis based on the 257-nts (exclusion of primers' sequences) of the CHIK partial E1 gene detected in the patient RS's acute serum sample with respect to similar partial E1 gene sequences of other CHIK viruses isolated in different parts of the world at various period is shown in Figure 2. The finding supported that patient RS was infected with CHIK virus of Central/East African genotype, but the virus differed from its closest Central/East African genotype (an Uganda strain, GenBank accession no. AF192907) isolated in Africa in 1982 by 5 nucleotides based on the comparison of 257-nts of the E1 gene segment (data not shown).

Since the first description of epidemic and isolation in Tanzania in 1953, CHIK virus has caused periodic outbreaks in both tropical Africa and Asia. However, the last decade saw not only the resurgence of CHIK activity in the region, the virus has expanded into new areas such as Malaysia and

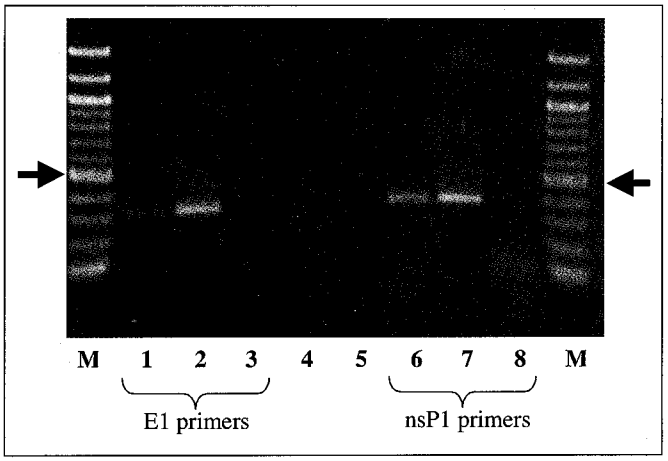

Fig. 1: Agarose gel analysis of RT-PCR amplified products for molecular detection of chikungunya virus non-structural protein 1 (nsP1) and glycoprotein E1 (E1) gene segments based on the published method by Hasebe *et al.* Lane 1 and 6: patient RS's serum samples. Lane 2 and 7: culture supernatant containing chikungunya virus from Taiping outbreak. Lane 3 and 8: negative serum control. Lane M: 100-bp ladder DNA molecular size marker (arrows indicate position of 500 base-pairs).

Table I: Serological response (IgM and IgG) of patient RS and her husband against chikungunya (CHIK) virus by indirect immunofluorescent assay.

Person	Titre of IgM against CHIK virus (dilution)	
	acute serum specimen	convalescent serum specimen
RS (patient)	1 : 10	1 : 10
SM (patient's husband)	-	1 : 10
	Titre of IgG against CHIK virus (dilution)	
	acute serum specimen	**convalescent serum specimen**
RS (patient)	1 : 40	1 : 160
SM (patient's husband)	-	1 : 320

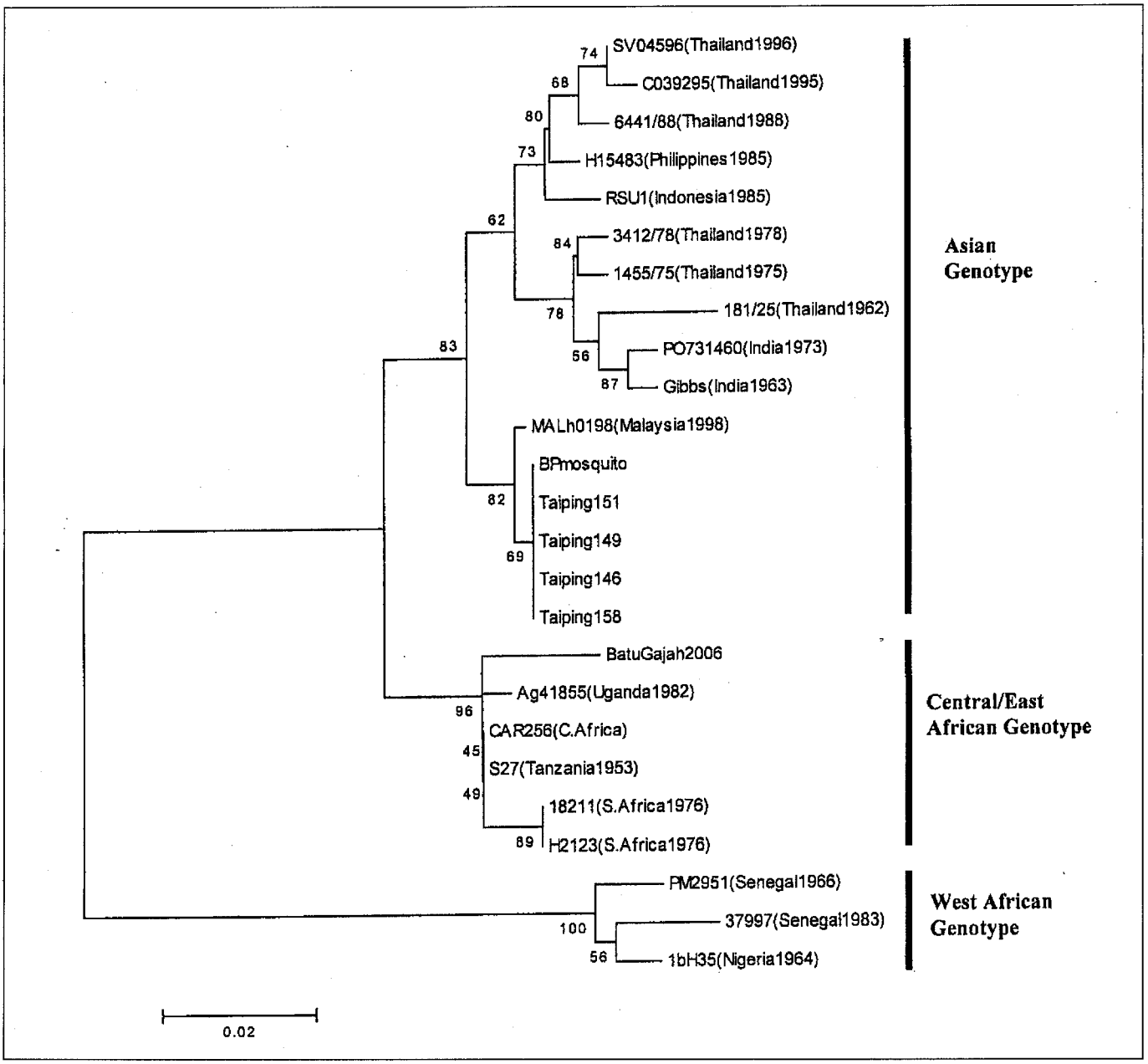

Fig. 2: Phylogenetic analysis of chikungunya (CHIK) virus based on 257-nt-long virus partial E1 gene sequence derived from acute serum sample of patient RS (BatuGajah2006) with respect to similar gene segments of 5 strains of CHIK virus [4 from human (Taiping146, Taiping149, Taiping151, Taiping158) and 1 from mosquito (BPmosquito)], isolated in the 2006 outbreak in northern part of Peninsular Malaysia and sequences of 19 CHIK virus strains deposited in the GenBank. 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).

Indian Ocean Islands. With rapid mass movement of people and the constant presence of CHIK vectors in this region, it would not be a surprise that the traditional delineation of various genotypes of CHIK virus to specific geographic locales will no longer hold true. Recently, the CHIK virus of Central/East genotype has spread to India and caused extensive outbreak[13]. The CHIK virus of Central/East genotype has not only spread to the Indian Ocean Islands, it has led to the emergence of new variant which may be of higher virulent[14]. This paper documents the first detection of CHIK virus of Central/East genotype in Malaysia. Without good *Aedes* vector measures, only time will tell whether this genotype will subsequently cause the next CHIK outbreak instead of the existing endemic genotype.

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