Molecular Epidemiology of Chikungunya Virus in Malaysia Since its First Emergence in 1998

Y K Chem, BSc*, S Zainah, MSc, S J Berendam, BSc**, T A R Tengku Rogayah, BSc**, A H Khairul, MSc*, K B Chua, FRCPE***

*National Public Health Laboratory, Ministry of Health, Lot 1853, 47000 Sungai Buloh, Selangor, Malaysia, **Virology Unit, Infectious Disease Research Centre, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia

SUMMARY

Malaysia experienced the first outbreak of chikungunya (CHIK) in Klang in late 1998 due to CHIK virus of Asian genotype. The CHIK virus of Asian genotype reemerged causing outbreak in Bangan Panchor, Perak in March 2006. CHIK virus of Central/East African genotype was first detected from a patient who returned from India in August 2006. In December 2006, CHIK virus of Central/East African genotype was re-introduced into Malaysia from India and caused an outbreak in Kinta district, Perak but was successfully controlled following an early detection and institution of intensive vector control measures.

In late April 2008, CHIK virus of Central/East African genotype was laboratory confirmed as the cause of CHIK outbreak in Johore which spread to other parts of Malaysia by August 2008. Phylogenetic analysis based on the 254-bp fragment of the virus envelope protein gene as the genetic marker showed that three different strains of CHIK virus of Central/East African genotype were introduced into Malaysia on three separate occasions from 2006 to 2008. The strain that was introduced into Johor state was responsible for its subsequent spread to other parts of Malaysia, inclusive of Sarawak.

KEY WORDS:
Chikungunya, Molecular epidemiology, Malaysia

INTRODUCTION

Chikungunya (CHIK) virus is a small envelope positive sense RNA virus that belongs to the genus *Alphavirus* under the family *Togaviridae*. 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 vectors as of dengue viruses. Chikungunya (CHIK) virus was first isolated from human serum and *Aedes aegypti* mosquitoes during an epidemic in Tanzania in 1953. The name is derived from a local term for the illness and it means "that which bends up or curl up", referring to the crippling arthralgia and backache associated with infection by the virus[1]. 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 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 vertebrate 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*[2,3].

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[4].

Malaysia experienced the first outbreak of CHIK in late 1998 involving residents staying in suburb of Klang, Selangor. As it was the first outbreak, the diagnosis was only laboratory confirmed in January 1999 and CHIK virus of Asian genotype was isolated[5,6].

On 28 March 2006, the National Public Health Laboratory (NPHL) of Malaysia received a request to assist in laboratory investigation of possible outbreak of influenza, dengue, measles, or rubella affecting 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.* [6], and serological assay for dengue specific IgM by IgM-capture ELISA. On 30 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

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Corresponding Author: Chua Kaw Bing, National Public Health Laboratory, Ministry of Health, Lot 1853, 47000 Sungai Buloh, Selangor, Malaysia, Email: chuakawbing@yahoo.com.sg

Table I: Representative strains of chikungunya virus isolated from serum samples of patients with acute chikungunya staying in states (name of place within parenthesis) of Malaysia were used for phylogenetic study. The chronology of events is indicated by the respective dates in which the serum samples were collected.

Virus strain	Date Serum Collected	Source
MALh0198	1998	GenBank (AF394210)
BP146	28/3/2006	Perak (Bagan Panchor)
BP151	28/3/2006	Perak (Bagan Panchor)
BP158	28/3/2006	Perak (Bagan Panchor)
BatuG1/8/06	27/8/2006	Perak (Batu Gajah)
Kin13/12/06	12/12/2006	Perak (Kinta)
Kin14/12/06	12/12/2006	Perak (Kinta)
Joh4/4/08	18/4/2008	Johore (Sg. Choh)
Joh5/4/08	18/4/2008	Johore (Sg. Choh)
Joh15/4/08	21/4/2008	Johore (Kota Tinggi)
Joh53/6/08	22/6/2008	Johore (Muar)
Joh80/6/08	23/6/2008	Johore (Segamat)
NS142/6/08	26/6/2008	Negeri Sembilan (Jempol)
NS148/6/08	26/6/2008	Negeri Sembilan (Jempol)
Mel20/7/08	8/7/2008	Melaka (Jasin)
Mel21/7/08	8/7/2008	Melaka (Jasin)
Pah2/7/08	8/7/2008	Pahang (Kuantan)
FT1/7/08	18/7/2008	Federal Territory (Putrajaya)
Kel362/8/08	6/8/2008	Kelantan (Kota Bharu)
Sar1/8/2008	8/8/2008	Sarawak (Limbang)
Sel17/8/08	13/8/2008	Selangor (Petaling)
Ked616/8/08	25/8/2008	Kedah (Sg. Petani)
Per14/7/08	23/7/2008	Perak (Trolak Selatan)
Per15/7/08	25/7/2008	Perak (Slim River)
Per501/9/08	12/9/2008	Perak (Gopeng)
Per243/11/08	16/10/2008	Perak (Manjung)

virus specific primer pairs in acute serum samples of 6 patients with the history of fever of 4 days or less. Phylogenetic analysis showed that that CHIK virus causing the outbreak was again of the Asian genotype (Figure 1). 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 10 April 2006[7].

On 27 August 2006, a 49-year-old Indian housewife was treated in a government district hospital (Hospital Batu Gajah), Perak with a 4 days history of fever, joint pain and occasional vomiting. Epidemiological investigation revealed that she visited southern India (Chennai) with her husband, son and brother-in-law 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 10 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 2 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. Laboratory tests in NPHL confirmed she was having CHIK and CHIK virus of Central/East African genotype was detected for first time in Malaysia (Figure 1). Follow-up epidemiological investigation revealed there was no local transmission and spread[8].

On 12 December 2006, a health officer of Kinta district received an informal report of an outbreak of fever and joint pains affecting some residents staying along Jalan Bendahara in the suburb of Ipoh city. The first suspected case which was subsequently laboratory-confirmed, RMCC, was a 64-year old Indian man who visited his relatives in Paramakudi, Tamil Naidu, India on 28 June 2006. He returned to Malaysia on 9 November 2006 and came down with fever on 17 November 2006. The second such case, RSBC, was a 44-year Indian man who also visited Paramakudi, Tamil Naidu, India on 21 November 2006 and returned home on 30 November 2006. On returning home, he developed high fever and joint pain on the 3 December 2006. The rest of the affected residents did not leave Kinta district for the last two month prior to the occurrence of outbreak. CHIK virus of Central/East African genotype was isolated but it differed from the Central/East African CHIK virus detected in Batu Gajah by two nucleotides (in bold) based on the 254-bp fragment of the envelope protein gene (Figure 2). As soon as the CHIK virus infection was laboratory-confirmed, an intensive "search and destroy" surveillance for *Aedes* mosquito breeding sites and institution of chemical thermal fogging in the affected areas were simultaneously carried out. The outbreak was successfully controlled[9].

THE LATEST OUTBREAK

In late April 2008, NPHL received a request from an epi-officer in Johore for laboratory investigation of an outbreak of possible "slap-cheek" disease (due to parvovirus B19) in Sg. Choh. However, on reviewing the clinical features of the presenting illness, the officer was informed that he was probably facing an outbreak of CHIK. Twenty serum samples of patients were sent to NPHL and were rapidly confirmed that the outbreak was due to CHIK virus. CHIK virus of Central/East African genotype was isolated but differed from the Kinta Central/East African genotype of CHIK virus by two

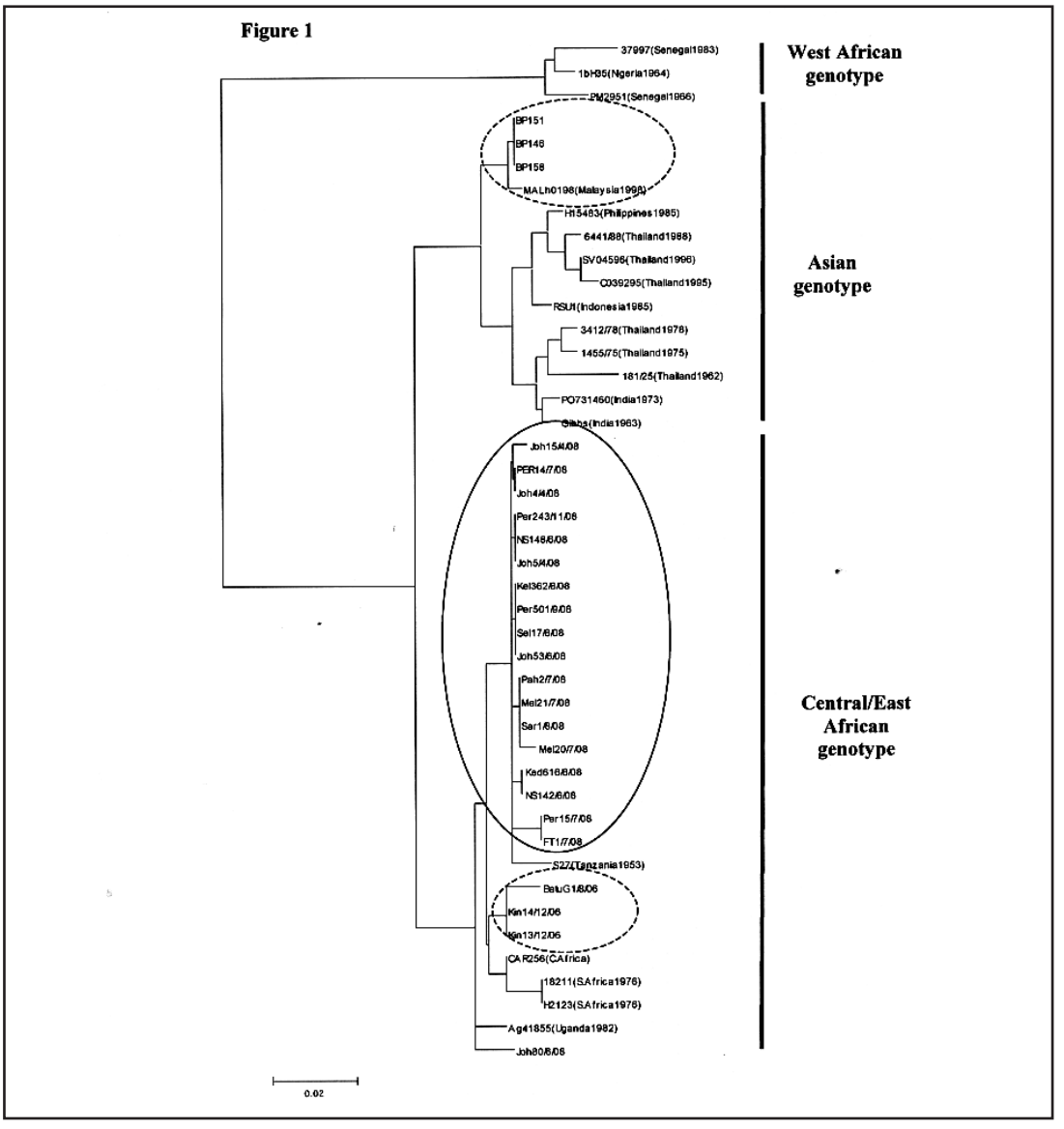

Fig. 1: Phylogenetic analysis based on the 254-bp fragment of the virus envelope protein gene as the genetic marker was carried on representative chikungunya (CHIK) viruses (Table I) isolated from acute serum samples of patients from various parts of Malaysia since its first outbreak in 1998. Similar segments of gene sequences of 19 CHIK virus strains deposited in the GenBank were used for the comparative analysis. 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). The bootstrapped consensus tree was constructed for a 257-nt-long E1 gene sequence using the MEGA programme. Identity of each isolate, location and year of isolation was indicated in the figure. Identity of CHIK viruses within the broken circles represents the strains isolated prior to 2008 outbreak. The CHIK viruses isolated in 2008 were all related to the strains which were first isolated in Johore, as indicated within the complete circle.

nucleotides (in bold and italic) and four nucleotides (in bold, bold and italic) from the Central/East African genotype of CHIK virus detected in Batu Gajah based on the 254-bp fragment of the envelope protein gene (Figure 2). By inference, the CHIK virus of Central/East African genotype isolated in Johor was a separate introduction into Malaysia. Apparently, this new strain of CHIK virus could have been introduced into Johor in February 2008 which concurred with a report of a similar outbreak in Singapore during that period (ProMed news). Subsequently, the outbreak of CHIK was noted in Negeri Sembilan and Melaka, two adjoining states to the north of Johor in late June and early July (Table I). By August 2008, the outbreak had spread to various parts of Malaysia including Sarawak in East Malaysia. Genetic analysis based on the 254-bp fragment of the envelope protein gene supported that the CHIK viruses isolated from other parts of Malaysia were related to the strains isolated in Johor.

Figure 2:

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BatuG1/8/06       1 ggcgcctactgcttctgcgacgctgaaaacacgcagttgagcgaagcacatgtggagaagtccgaatcaagcaaaacagaatttgcatca
Kin13/12/06       1 ..................................................................t.....................
Kin14/12/06       1 ..................................................................t.....................
Joh4/4/08         1 ..........................t.......................................t.....................
Joh5/4/08         1 ..........................t.......................................t.....................
Joh15/4/08        1 ..........................t.......................................t.....................
Joh53/6/08        1 ..........................t.......................................t.....................
Joh80/6/08        1 ..........t.............t.......................................t.....................
Mel20/7/08        1 ..........................t.....................c.................t.....................
Mel21/7/08        1 ..........................t.......................................t.....................
NS142/6/08        1 ..........................t.......................................t.....................
NS148/6/08        1 ..........................t.......................................t.....................
Pah2/7/08         1 ..........................t.......................................t.....................
FT1/7/08          1 ..........................t.......................................t.....................
Sel17/8/08        1 ..........................t.......................................t.....................
Sar1/8/08         1 ..........................t.......................................t.....................
PER14/7/08        1 ..........................t.......................................t.....................
Per15/7/08        1 ..........................t.......................................t.....................
Per501/9/08       1 ..........................t.......................................t.....................
Per243/11/08      1 ..........................t.......................................t.....................
Kel362/8/08       1 ..........................t.......................................t.....................
Ked616/8/08       1 ..........................t.......................................t.....................

BatuG1/8/06      91 gcatacagggctcataccgtatctgcatcagctaagctccgcgtcctttaccaaggaaataacatcactgtaactgcctatgcaaacggc
Kin13/12/06      91 ................c.....................................................................
Kin14/12/06      91 ................c.....................................................................
Joh4/4/08        91 ..g.............c.....................................................................
Joh5/4/08        91 ..g.............c.....................................................................
Joh15/4/08       91 ..g..t..........c.....................................................................
Joh53/6/08       91 ..g.............c.....................................................................
Joh80/6/08       91 ..g.............c.....................................................................
Mel20/7/08       91 ..g.............c.....................................................................
Mel21/7/08       91 ..g.............c.....................................................................
NS142/6/08       91 ..g.............c.....................................................................
NS148/6/08       91 ..g.............c.....................................................................
Pah2/7/08        91 ..g.............c.....................................................................
FT1/7/08         91 ..g.............c.....................................................................
Sel17/8/08       91 ..g.............c.....................................................................
Sar1/8/08        91 ..g.............c.....................................................................
PER14/7/08       91 ..g.............c.....................................................................
Per15/7/08       91 ..g.............c.....................................................................
Per501/9/08      91 ..g.............c.....................................................................
Per243/11/08     91 ..g.............c.....................................................................
Kel362/8/08      91 ..g.............c.....................................................................
Ked616/8/08      91 ..g.............c.....................................................................
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Fig. 2: Nucleic acid sequence of the virus partial envelope (E1) gene (180-nt) of the Central/East African genotype chikungunya viruses isolated or first detected in Malaysia that were used in phylogenetic analysis were aligned using the same MEGA programme. The CHIK virus isolated in the Kinta outbreak differed from the CHIK virus detected in Batu Gajah by two nucleotides (in bold). The CHIK virus isolated in the Johore outbreak and later in other parts of Malaysia in 2008 differed from the CHIK virus isolated in Kinta outbreak by two nucleotides (in bold and italic) and four nucleotides (in bold, bold and italic) from the CHIK virus detected in Batu Gajah based on this 180 nucleotides.

Phylogenetic analysis based on the 254-bp fragment of the envelope protein gene as the genetic marker of the representative CHIK viruses isolated in Malaysia since 1998 (Table I) showed that CHIK virus of Asian genotype caused the first two outbreaks and CHIK virus of Central/East African genotype caused the later two outbreaks. Three different strains of CHIK virus of Central/East African genotype were introduced into Malaysia on three separate occasions from 2006 to 2008. The strain that was introduced into Johor state was responsible for its subsequent spread to other parts of Malaysia, inclusive of Sarawak (Figure 2). The following factors may have contributed to the rapid spread of this CHIK virus from Johor to other parts of Malaysia: (i) The presence of a large naïve population in Malaysia who do not have prior exposure to and immunity against the virus; (ii) The failure of health-care workers in Johor to recognise and detect early the occurrence of CHIK that subsequently led to the failure of instituting appropriate intensive vector control measures to stamp out the outbreak as it was the case in Kinta, Perak. A single amino acid mutation in the envelope protein of the CHIK virus has been reported that led to the adaptation of the virus to use *Aedes albopictus* as vector besides *Aedes aegypti*[10]. Thus, together with the present of high vector density (*Aedes aegypti* and *Aedes albopictus*) and ferocious biting behaviour of *Aedes albopictus*, this may have accounted for the occurrence of a large number of cases of CHIK, especially in the rural areas and rubber plantations.

REFERENCES

1. Brink NS, Lloyd G. Alphaviruses. In: Zuckerman AJ, Banatvala JE, Pattison JR. Principles and Practice of Clinical Virology. 3rd edition. Chichester. John Wiley & Sons. 1994; 467-84.
2. Karabatsos N. Antigenic relationships of group A arboviruses by plaque reduction neutralization testing. Am J Trop Med Hyg 1975; 24: 527-32.
3. Turell MJ, Beaman JR, Tammariello RF. Susceptibility of selected strains of Aedes aegypti and Aedes albopictus (Diptera: Culicidae) to Chikungunya virus. 1992; 29: 49-53.
4. Marchette NJ, Rudnick A, Garcia R. Alphaviruses in Peninsular Malaysia. II: Serological evidence of human infection. Southeat Asian J Trop Med Public Health 1980; 2: 14-23.
5. Lam SK, Chua KB, Hooi PS *et al*. Chikungunya infection – an emerging disease in Malaysia. Southeast Asian J Trop Med Public Health 2001; 32: 447-51.
6. Hasebe F, Parquet MC, Pandey BD *et al*. Combined detection and genotyping of Chikungunya virus by a specific reverse transcription-polymerase chain reaction. J Med Virol 2002; 67: 370-74.
7. Kumarasamy V, Prathapa Senan, Zuridah bt Hassan, Chem YK, Norizah bt Ismail, Chua KB. Re-emergence of Chikungunya virus in Malaysia. Med J Malaysia 2006; 61: 221-5.
8. Soon YY, Junaidi I, Kumasamy V, Chem YK, Juliana R, Chua KB. Chikungunya virus of Central/East African genotype detected in Malaysia. Med J Malaysia 2007; 62: 214-7.
9. Noridah O, Paranthaman V, Nayar SK *et al*. Outbreak of Chikungunya due to virus of Central/East African genotype in Malaysia. Med J Malaysia 2007; 62: 323-8.
10. Tsetsarkin KA, Vaniandingham DL, McGee CE, Higgs S. A single mutation in Chikungunya virus affects vestor specificity and epidemic potential. PLoS Pathog 2007; 3(12): e201 (1-20).