

IDENTIFICATION OF CHIKUNGUNYA VIRUS STRAINS CIRCULATING IN KELANTAN, MALAYSIA IN 2009

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Abstract. Malaysia experienced its first outbreak of chikungunya virus (CHIKV) infection in late 1998 in Klang District in Selangor; six years later the virus re-emerged in the state of Perak. All the CHIKV isolates in 1988 and 2006 shared high sequence similarities and belonged to the Asian genotype. In 2007 and 2008 CHIKV infection again reemerged but the genotype was the Central/East African genotype. This strain was found to be similar to the strains causing outbreaks in the India Ocean. In 2009, the strains circulating in Malaysia, including the state of Kelantan, based on the partial E1 gene, also belong to the Central/East African genotype.

Key words: chikungunya virus, circulating strain, genotype, Malaysia

INTRODUCTION

Chikungunya infection is caused by chikungunya virus (CHIKV), classified in the *Alphavirus* genus under the *Togaviridae* family. CHIKV was first isolated six decades ago in Tanzania in 1953 (Ross, 1956). Only *Aedes* mosquitoes have been documented as the responsible vectors in transmitting the disease.

Since its first isolation CHIKV infection has emerged and reemerged globally (Rao, 1971; Thuang *et al*, 1975; Thaikruea *et al*, 1997). Epidemics occurred in many African countries in the late 1950s to 1970s. The central African strains of CHIKV isolated in 2000 are from an outbreak of the virus in the Democratic Re-

public of Congo (Pastorino *et al*, 2004).

In Asia, CHIKV was first isolated in Bangkok in 1958 (Fields *et al*, 1996). It caused many outbreaks, such as in Kolkata and Southern India in 1963 and 1964, respectively, as well as other Indian states (Shah *et al*, 1964). Transmission was also reported in the Philippines in 1968 (Kalantari *et al*, 2006), Sri Lanka in 1969, Myanmar in 1975, Indonesia in 1982 and then in 2001-2003 after a lapse of 20 years (Laras *et al*, 2005; Yergolkar *et al*, 2006) and again in India during 2005-2006 (Yergolkar *et al*, 2006; Chhabra *et al*, 2008). A recent epidemic in the Indian Ocean originated from Kenya in 2004 (Chretien *et al*, 2007; Pialoux *et al*, 2007) before spreading to Comoros and Seychelles in early 2005, followed by Mauritius (Beeson *et al*, 2008) and the Reunion Islands in 2005-2006 causing major outbreaks in these regions (Renault *et al*, 2007).

In Europe, CHIKV infection has been

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recorded in France (Parola *et al*, 2006), Germany, Italy, Norway and Switzerland, due to imported cases from people returning from endemic areas (WHO, 2006). The best example was the transmission of CHIKV in Italy in 2007 where the index case was a traveller from Kerala, India and the virus was isolated from local *Ae. albopictus* (European Centre for Disease Prevention and Control, 2007).

In Malaysia, CHIKV was first recorded in 1998-1999 in Port Klang, affecting 51 people (Lam *et al*, 2001). This was followed six years later by an outbreak in Bagan Panchor, Perak in March 2006 (Kumarasamy *et al*, 2006; Abubakar *et al*, 2007) and Kinta District, Perak in December 2006 (Noridah *et al*, 2007). After a lapse of almost 2 years, the CHIKV infection re-emerged in July 2008 in Tangkak, Johor and from there spread to other states in Peninsular Malaysia, where more than 2,692 cases were reported (IMR, 2009). The greatest number of infections were seen in the Federal Territory Kuala Lumpur, Malacca, Johor and Selangor, while fewer were seen in the Kelantan and East Malaysian states of Sabah and Sarawak. However, a change in this was seen in 2009, when the most affected areas were Sarawak, Kedah and Kelantan. A total of 4,430 cases of CHIKV infection were reported in 2009 (CDC, 2010). The state of Kelantan had 566 cases, as compared to only 28 cases in 2008 (Institute for Medical Research. Number of chikungunya case investigated, 2010. Unpublished data).

Kelantan is a state in Peninsular Malaysia, located on the northeastern coast along the southern border of Thailand. During this time period, CHIKV infections were also reported in southern Thailand, especially in Narathiwat Province in 2009 (CDC, 2010). The Ministry of Health from both countries decided to collaborate on a

project to investigate CHIKV infection along the border of both countries: Narathiwat Province in Thailand and the state of Kelantan in Malaysia. The aims of the project were to identify CHIKV strains circulating along the border region between Thailand and Malaysia, to strengthen surveillance, to share investigation findings, improve control measures and apply correct preventive measures. This report describes the CHIKV strains circulating in Kelantan in 2009.

MATERIALS AND METHODS

Four districts bordering southern Thailand, Pasir Mas, Tanah Merah, Jeli and Tumpat, in the state of Kelantan, were chosen for this study. Serum samples were collected from patients showing signs and symptoms of CHIKV infection, such as acute onset of fever, arthralgia, arthritis and rash. All samples were screened by RT-PCR. RNA was extracted using QIAamp® Viral RNA Mini Kit from Qiagen (Hilden, Germany) followed by amplification by PCR using the NSP1-C and NSP1-S primers (Hasebe *et al*, 2002).

CHIKV IgM detection (Nagasaki University, 1999) was performed on samples negative for CHIKV by RT-PCR test. Samples positive by RT-PCR were inoculated into baby hamster kidney (BHK) cells and observed daily for cytopathic effects (CPE). For cell culture samples that showed CPE, RT-PCR using E1-C and E1-S primers, which amplify the 294 bp glycoprotein E1 gene of the CHIKV gene, was performed on the cell culture supernatant (Hasebe *et al*, 2002).

The resulting PCR product was subjected to agarose gel electrophoresis and the QIAquick® Gel Extraction kit from Qiagen (Hilden, Germany) was then used to extract the DNA from the gel. DNA se-

Table 1
Number of samples tested for CHIKV infection.

District	No. of samples tested	RT-PCR positive	Cell culture positive	CHIKV IgM positive	% Positivity by RT-PCR or CHIKV IgM
Pasir Mas	41	15	5	13	68.3
Tanah Merah	58	17	5	5	37.9
Jeli	29	17	7	2	65.6
Tumpat	2	0	0	1	50.0
Total	130	49	17	21	53.8

quencing was performed using both forward (E1-S) and reverse (E1-C) primers.

RESULTS

Between June and December 2009, a total of 130 serum samples were collected from subjects in Kelantan and screened for CHIKV infection by RT-PCR (Table 1). Thirty-seven point seven percent (49/130) of the samples collected were positive for CHIKV by RT-PCR. Of the 81 RT-PCR negative samples, 25.9% (21/81) were CHIKV IgM positive.

Pasir Mas District had the highest positivity for CHIKV (68.3%) followed by Jeli (65.6%) and Tanah Merah (37.9%). In Tumpat, only 2 samples were collected, one of which (50%) was positive for CHIKV infection.

A total of 49 samples positive by RT-PCR, were inoculated into BHK cells and CHIKV was isolated from 17 of these samples. The 17 isolates were sequenced and a phylogenetic tree of these isolates was constructed together with isolates retrieved from GenBank for partial E1 gene (Fig 1). Phylogenetic tree analysis showed all isolates from Kelantan state in 2009 were of the Central/East African genotype and belonged in the same cluster as isolates from Thailand in 2009 and other

Malaysian isolates in 2008. These isolates were completely different from the CHIKV isolated in the 1998 and 2006 Malaysian outbreaks which were of the Asian genotype.

DISCUSSION

Currently, CHIKV can be grouped into three distinct genogroups: Asian, Central/East African and West African genotypes based on the phylogenetic analysis of the E1 gene (Powers *et al*, 2000; Schuffenecker *et al*, 2006). Before 2006, these genotypes were restricted to the geographical areas denoted by their names. Recent explosive epidemics of the African genotype in the Indian Ocean Islands and India, other parts of Asia, Africa and Europe indicate international travellers have disseminated a new strain of the virus, into regions where the CHIKV has been absent (Townson and Nathan, 2008). This has changed the geographical distribution of CHIKV worldwide.

Prior to 1998, CHIKV had never been isolated from humans or animals in Malaysia, nor had any clinical disease due to CHIKV infection been reported in Malaysia. However, in serological surveys of human sera collected from 1965 to 1969 in West Malaysia, neutralizing antibodies

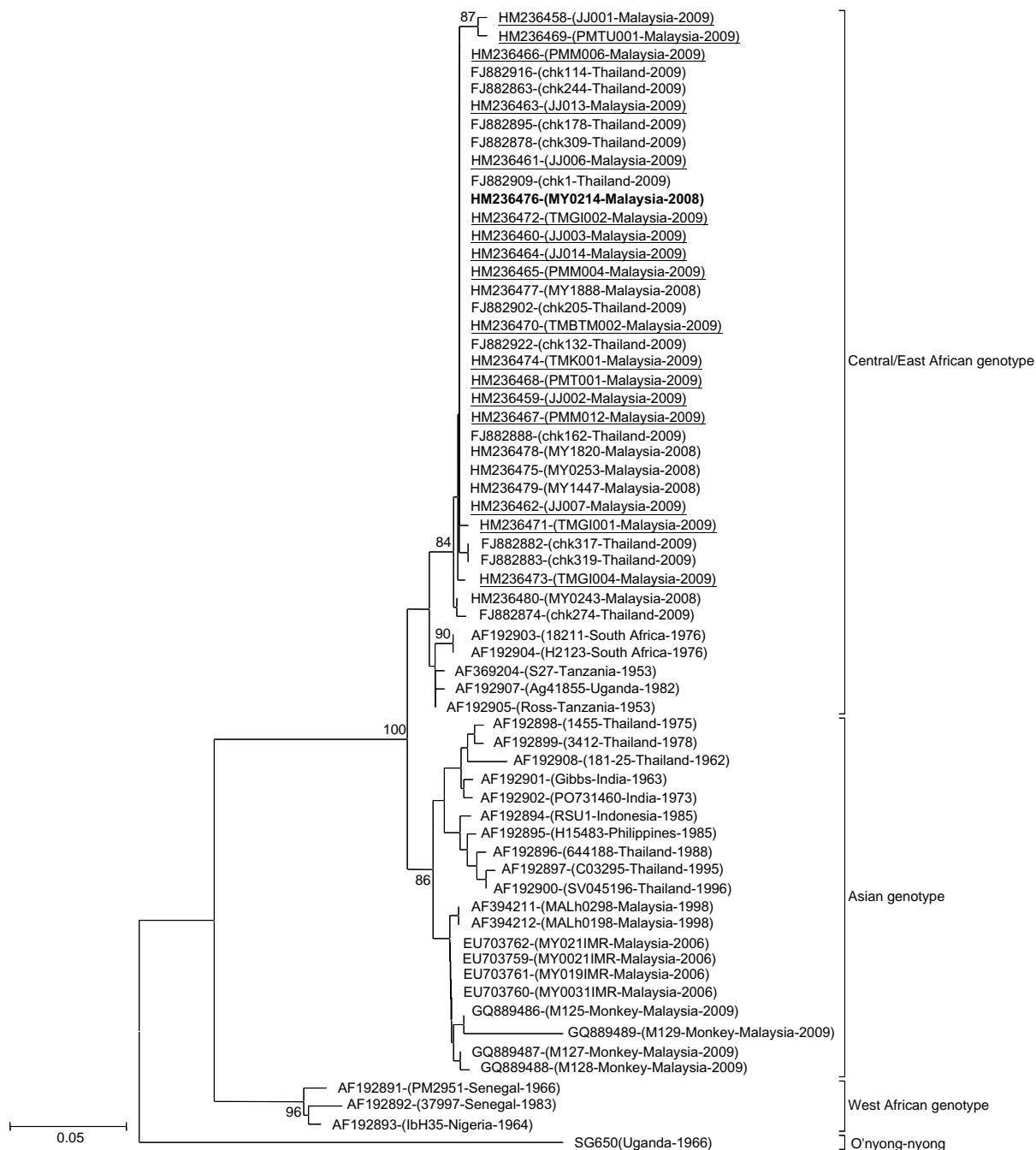


Fig 1–Phylogenetic tree of partial glycoprotein E1 sequences (257bp) of CHIKV inferred using the Neighbor-Joining method from MEGA 4 software. The evolutionary distances were computed using the Maximum Composite Likelihood method. Genotype Asian, Central/East African and West African types are indicated by square brackets with O'nyong-nyong virus as an outgroup. Seventeen CHIKV isolates from the state of Kelantan are indicated in underlined words. Representative strains of each genotype obtained from GenBank are labeled using the following format: “accession number”, “isolate”, “country of origin” and “year isolation”. Bootstrap values (>75%) for 1,000 pseudoreplicate dataset are indicated at branch nodes.

to CHIKV were found among adults, especially those inhabiting the rural northern and eastern states bordering Thailand (Marchette *et al*, 1980).

Malaysia experienced its first outbreak of CHIKV in late 1998 in the Klang District of Selangor; six years later the virus reemerged in the state of Perak. The outbreaks in Perak (March to December, 2006) and Klang (late 1998) were identified as the Asian genotype (Lam *et al*, 2001; Kumarasamy *et al*, 2006; Abubakar *et al*, 2007). All the CHIKV isolates in 1998 and 2006 shared high sequence similarities. However, the CHIKV isolates circulating in 2007 and 2008 were totally different from the 1998 or 2006 isolates. These new outbreaks in Malaysia were caused by the Central/East African genotype (Noridah *et al*, 2008; Maizatul, 2009; Sam *et al*, 2009), similar to the strains causing outbreaks in the India Ocean (Vidya *et al*, 2007). The strains circulating in the state of Kelantan are shown in Fig 1, which shows the phylogenetic tree based on the E1 gene, which belongs to the cluster of Central/East African genotype.

The disease is self limited and rarely fatal (Fields *et al*, 1996). In Malaysia, no deaths have been reported since its first appearance in 1998. However, a death due to CHIKV was reported from Reunion Island (Michault and Staikowsky, 2009) and another death suspected to be due to CHIKV was reported from India (Mavalankar *et al*, 2008).

In Malaysia, the spread of CHIKV appears to be maintained by human-mosquito-human transmission (urban cycle). However, the presence of neutralizing antibodies in wild monkeys, pigs and chickens (Marchette *et al*, 1980) and recently isolated CHIKV from non-human primates (Apani *et al*, 2009) suggests a CHIKV sylvatic transmission cycle may exist in

Malaysia and could possibly contribute to outbreaks.

The earlier outbreaks in 1998 and 2006 caused by the same Asian strains raise the possibility of CHIKV being endemic in Malaysia (Abubakar *et al*, 2007). During the same time period an outbreak of CHIKV was also reported in the Indian Ocean, and unlike Malaysia, it was due to the Central/East African strain. However in Malaysia, the 2008-2009 outbreak of CHIKV was due to the Central/East African genotype, which has been responsible for outbreaks in several regions (Powers *et al*, 2000; Vidya *et al*, 2007; Xavier *et al*, 2008; D'Ortenzia *et al*, 2009) including Thailand (Theamboonlers *et al*, 2009), suggesting the endemicity of CHIKV was not the only factor involved in the epidemics. Other factors include point mutation of the virus, with the presence of A226V (Tsetsarkin *et al*, 2007; Bordi *et al*, 2008) resulting in the virus becoming more susceptible to the new *Aedes* spp host, especially *Aedes albopictus*, which is found in high density in Malaysia. The spread of the virus was also facilitated by international travellers who disseminated the new strains of virus into the region in which CHIKV had been absent (Townson and Nathan, 2008). Another factor, especially in Kelantan in 2009, was the presence of a large number of local residents who had no prior exposure to or immunity against CHIKV. The presence of high viral loads in patients travelling from epidemic areas could be an additional factor (Parola *et al*, 2006) as was seen in Italy (Bordi *et al*, 2008).

In summary, the CHIKV strains circulating in the state of Kelantan in 2009 were from the Central/East African genotype; the same strains were also found to be circulating in southern Thailand. A different genotype of CHIKV was seen in the 1998 and 2006 outbreaks in Malaysia.

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