

*Full Length Research Paper*

# Epidemiology and molecular characterization of chikungunya virus involved in the 2008 to 2009 outbreak in Malaysia

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The 2008 to 2009 outbreak of chikungunya was considered as the huge and worst outbreak of CHIKV (Chikungunya virus) infections in history of the country affecting all states in both Peninsular and East Malaysia. This was unlike the first outbreak in late 1998, which was only restricted to Klang district in Selangor and six years later the second outbreak which only involved the state of Perak. The objective of the study was to detect the presence of chikungunya antibody and antigen by immunofluorescence technique and RT-PCR from the sera samples. A total of 2,692 sera samples were received in 2008, in which 19.2% were positive by antibody detection and 42.6% were positive by RT-PCR. The following year in 2009, the samples size increased to 3,592, only 16.3% sample were positive by antibody detection and 31.7% were positive by RT-PCR. Majority of the hospitalized cases were adults between 30 to 60 years of age and the highest incidence rate was amongst patients' age between 40 to 49 year old. In 2008, most of the confirmed CHIKV infection cases were female but the opposite was seen in 2009, where more male cases were reported. In this outbreak, the prominent ethnic group affected was the Malays. CHIKV involved in the 2008 to 2009 outbreaks was the new Central/East African genotype which was found to be similar with strains causing the outbreaks in the India Ocean and main CHIKV genotype circulating in the European countries from 2006 to 2009.

**Key words:** Chikungunya virus, genotype, epidemiology, outbreaks in Malaysia.

## INTRODUCTION

Chikungunya virus (CHIKV) is responsible for an acute infection of an abrupt onset of high fever, arthralgia, myalgia, headache and rash (Johnson and Peters, 1996). This small envelope and single stranded positive sense RNA virus is in the genus of "alphavirus" under the Togaviridae family. It was first isolated nearly six decades ago in Tanzania in 1953 (Ross, 1956). Until now, only *Aedes* mosquitoes either *Aedes aegypti* or *Aedes albopictus* were documented as the responsible vectors in transmitting the disease. However, several cases of CHIKV infections as the result of maternal-foetal

transmission have been reported (Geradin et al., 2008). During the past 50 years, emergence and re-emergence of CHIKV infection has occurred globally (Rao, 1971; Thuang et al., 1975; Thaikruea et al., 1997). Numerous CHIKV re-emergences have been reported in both Africa and Asia with the intervals of 2 to 20 years between the outbreaks (Powers and Logue, 2007). In the late 50s to 1970s, many African countries experienced the outbreaks of CHIKV and the new Central/East African strains were isolated in 2000. These viruses were believed to have originated from the outbreak in the Democratic Republic of Congo (Pastorini et al., 2004). Now, CHIKV disease has become extremely important in public health control programme as the disease could spread to several countries around the world in a short period of time. In the last decade,

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CHIKV infection had caused many outbreaks across the globe. Starting from the emergence of CHIKV in Kenya in 2004 (Chretien et al., 2007; Pialoux et al., 2007) then the outbreaks spread to Comoros and Seychelles in early 2005, followed by Mauritius (Beeson et al., 2008). Later the outbreaks were reported to have spread to other islands in the Indian Ocean, including La Reunion Islands which is part of France in 2005 to 2006 (Schuffenecker et al., 2006; Bonn, 2006) and causing major outbreaks in these regions (Renault et al., 2007).

Based on the viral genetic analysis, there was a strong link between the infection in La Reunion Islands in 2005 to 2006 with the outbreak in Kenya in 2004 (Kariuki et al., 2008) that were caused by Central/East African genotype. Then epidemic of CHIKV rapidly spread to Europe and the United States of America. Their activities were seen in countries such as 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 *Aed. Albopictus* (European Centre for Disease Prevention and Control, 2007; Watson, 2007; Vazeille et al., 2008). In 2008, CHIKV fever was listed by US National Institute of Allergy and Infectious Disease (NIAID) under category C priority pathogen (Powers and Logue, 2007; Staples et al., 2009). In Malaysia, the first ever outbreak of CHIKV was recorded in 1998 to 1999 in Port Klang, Selangor affecting more than 51 people (Lam et al., 2001). Six years later, CHIKV re-emerged 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). The third and largest outbreak so far, was first detected in Tangkak, Johor in April 2008 and over short period of time the number of cases drastically increased and spread beyond the states of Johor. By the end of 2008 a total of 2,692 cases were investigated and CHIKV activities were recorded in all states in Malaysia including Sabah and Sarawak. The trend of infections continued till end of 2009 with 3,592 cases being investigated.

Currently, only two laboratories in the Ministry of Health Malaysia are able to perform laboratory investigations for CHIKV, namely: the Institute for Medical Research (IMR) in Kuala Lumpur and the National Public Health Laboratory (NPHL) in Sungai Buloh, Selangor. IMR carries out laboratory tests for hospitalized cases, whereas NPHL carries out tests for outpatients, surveillance and outbreaks cases. Since this is third and the largest outbreak of CHIKV infection in Malaysia, in this study we attempt to identify, analyze and characterise all CHIKV strains circulating in each different states of Malaysia and also study the epidemiology of the CHIKV infection during the outbreak.

## MATERIALS AND METHODS

In year 2008 and 2009, a total of 2,692 and 3,592 samples respectively were received and tested in IMR. The RT-PCR, using primers E1-C and E1-S (Hasebe et al., 2002) was used to detect the nucleic acid of the E1 gene and the indirect immunofluorescence technique Nagasaki University (1999) was used to detect the presence of IgM antibody against CHIKV. Date of disease onset (such as fever, arthralgia, arthritis and rash) was the criteria used to determine the types of test to be conducted. Samples from patients with onset of disease of  $\leq 5$  days duration were subjected to RT-PCR whereas those with  $>5$  days of disease duration were screened for CHIKV IgM. CHIKV IgM detection was also performed on samples tested negative by RT-PCR test. Table 1 shows the number of samples received and tested at IMR in 2008 and 2009. There are 13 states and 3 federal territories (FT) in Malaysia. Representative samples from each state that were found positive by RT-PCR were inoculated into BHK (baby hamster kidney) cells and observed daily for cytopathic effect (CPE). RT-PCR, using E1-S and E1-C primers which amplified 294 bp glycoprotein E1 gene of CHIKV, was performed on the supernatant from cell culture samples that showed CPE (Hasebe et al., 2002). RNA was first extracted using QIAamp® Viral RNA Mini Kit from Qiagen (Hilden, Germany) prior RT-PCR amplification.

The resulting PCR product was subjected to agarose gel electrophoresis and the QIAquick® Gel Extraction kits from Qiagen (Hilden, Germany) was then used to extract the DNA from the gel. DNA sequencing was performed using both forward (E1-S) and reverse (E1-C) primers.

## RESULTS

In 2008, from a total of 2,692 sera samples received at IMR from patients showing signs and symptoms of CHIKV infection, 42.6% (1146/2692) were found positive for CHIKV infection by RT-PCR and 19.2% (517/2692) positive by antibody detection. The following year in 2009, there was a 33% (3592/2692) increased in number of samples received of which, 31.7% (1140/3592) were PCR positive for CHIKV and 16.3% (585/3592) were antibody positive. Overall, positivity detected by RT-PCR was higher compared to IgM detection (Table 1). Based on the number of samples received in IMR, FT of Kuala Lumpur recorded the highest cases of CHIKV infection with 1,134 suspected cases in 2008 followed by Melaka (475 cases), Johor (411 cases) and Selangor (331 cases). The trend of infection was slightly different in 2009 with Sarawak (566 cases) and Kelantan (566 cases) recorded a significant increased in number of cases whereas some states such as Johor and Melaka which recorded high number of cases in 2008, showed a significant decreased in number of suspected cases in 2009. However, FT of Kuala Lumpur still maintained as the state with the highest number of suspected chikungunya cases for both 2008 and 2009. A total of 54 CHIKV isolates, 14 isolates from 9 states received form suspected chikungunya cases in 2008 and 35 isolates from all states in Malaysia in 2009 were sequenced. Phylogenetic tree of these isolates was constructed together with isolates retrieved from GenBank for partial E1 gene.

Molecular analysis showed that all the isolates from

**Table 1.** Number of samples received and tested at IMR in 2008 and 2009.

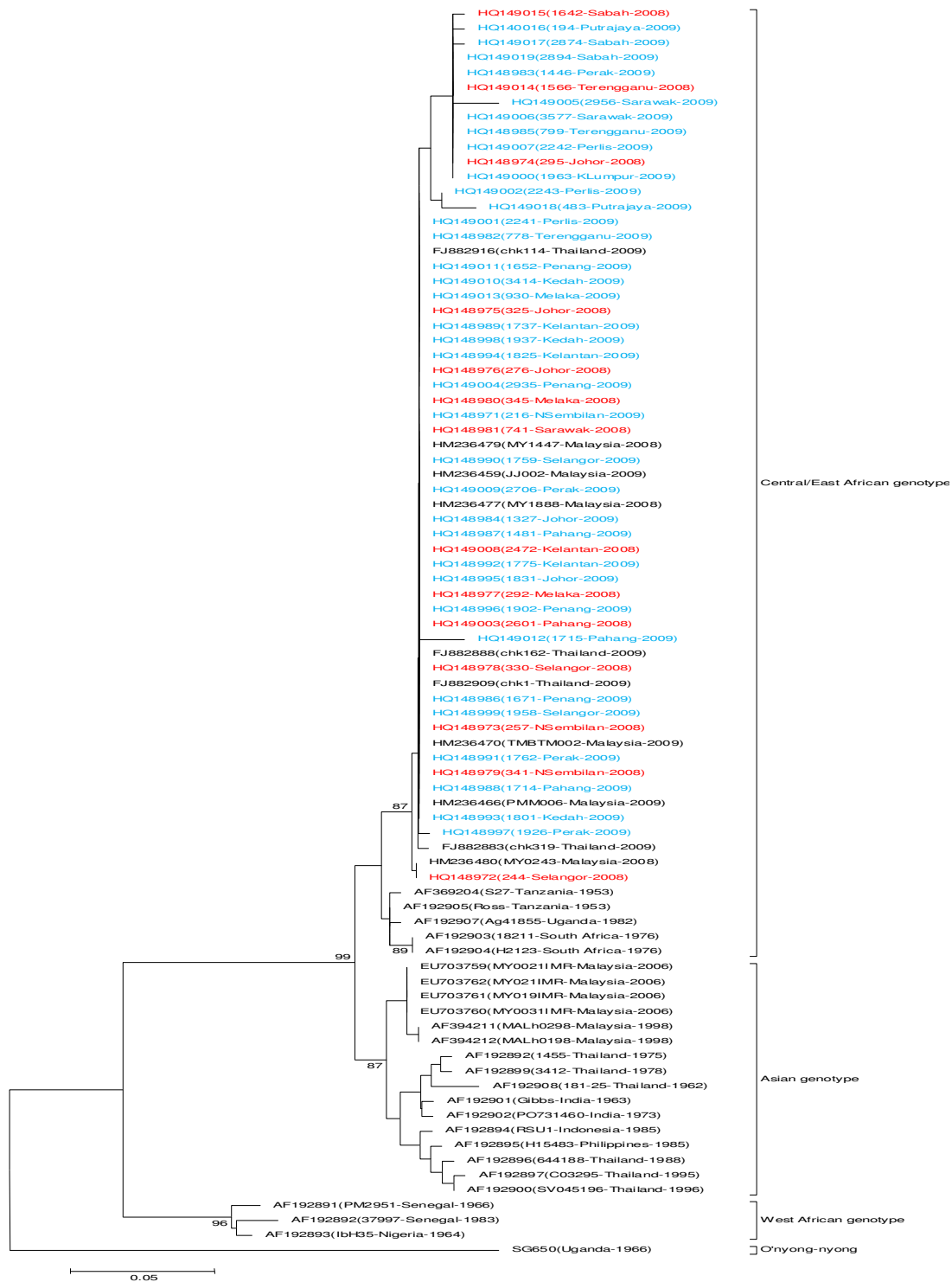
State	No. of sample tested in 2008			No. of sample tested in 2009		
	No. of sample	Positive by RT-PCR	Positive by IgM	No. of sample	Positive by RT-PCR	Positive by IgM
Johor	411	263	77	44	13	6
Kedah	58	31	10	308	174	36
Kelantan	28	18	5	566	230	136
FT Kuala Lumpur	1134	308	240	811	172	109
Melaka	475	301	58	43	6	3
Negeri Sembilan	44	24	8	2	0	0
Pahang	82	33	24	85	41	16
Perak	79	45	8	142	58	13
Perlis	4	1	0	23	9	2
Pulau Pinang	24	6	7	166	46	39
FT Putrajaya	0	0	0	21	5	2
Sabah and FT Labuan	8	2	1	47	27	5
Sarawak	3	1	0	566	262	89
Selangor	331	109	78	757	94	128
Terengganu	11	4	1	11	3	1
Total	2692	1146	517	3592	1140	585

each states in Malaysia including Sabah and Sarawak for both years were of the Central/Eastern African genotype and belongs in the same cluster with isolates from Thailand in 2009 and other Malaysian isolates in 2008 (Figure 1). These isolates were totally different from the CHIKVs isolated in the Malaysian 1998 and 2006 outbreaks which were of the Asian genotype. Throughout the outbreak which started in April in 2008, based on the number of samples received, there were 3 peaks of CHIKV infections as shown in Figure 2. The first peak was in July 2008 to August 2008 which recorded more than 500 samples received from suspected chikungunya cases; the second peak which was the highest from December 2008 to January 2009 with nearly 1,000 suspected cases and the third peak with 300 suspected cases from October 2009 to November 2009. Majority of the CHIKV infections were in adults between the ages of 30 to 60 years with highest incident in the 40 to 49 years age group (Figure 3). There were more female cases in 2008 but the reverse was recorded in 2009, where more male was affected compared to female (Figure 4). Among the racial groups in Malaysia, the Malays were the highest racial group affected in this outbreak (Figure 5).

## DISCUSSION

The first outbreak of chikungunya in Malaysia was in Klang, Selangor in late 1998 with more than 51 people

infected (Lam et al., 2001). Six years later, the second outbreak occurred 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). The third outbreak initially started in early April 2008, where increasing number of CHIKV infections were first detected in state of Johor. Following this, neighbouring states Kuala Lumpur and Melaka were also affected with increased in number of chikungunya cases. By July 2008, the numbers of cases drastically increased and later spread to other states in Peninsular Malaysia where more than 2,692 cases were reported (IMR, 2009). Highest activities were recorded in the Federal Territory Kuala Lumpur, Malacca, Johor and Selangor; but lower in Kelantan and East Malaysia states of Sabah and Sarawak. However, the scenario was different the following year, where states like Kelantan, Sarawak and Kedah which had low number of cases in 2008, recorded high number of cases in 2009. Of the 5,430 chikungunya cases reported by Control of Disease Division, Malaysia in 2009 (MOH, 2010), 2,505 cases were from Sarawak. The two earliest outbreaks in 1998-1999 and 2006 were due to CHIKV belonging to Asian genotype strain (Lam et al., 2001; Kumarasamy et al., 2006; Abubakar et al., 2007). All the CHIKV isolates in 1988 and 2006 shared high sequence similarities. However, the recent CHIKV circulating in 2008 and 2009 were totally different from the 1998 to 1999 or 2006 isolates. The recent outbreak in Malaysia recorded the emergence of new Central/East



**Figure 1.** Phylogenetic tree of partial glycoprotein E1 sequences (257 bp) of CHIKV inferred using the Neighbor-Joining method from the software MEGA 4. The evolutionary distances were computed using the "maximum composite likelihood" method. Genotype Asian, Central/East African and West African are indicated by square brackets with O'nyong-nyong virus as an outgroup. 49 CHIKV isolates from Malaysia in 2008 and 2009 are indicated in red and blue underline words respectively. Representative strains of each genotype obtained from GenBank are labeled using the following format: 'Accession number'- 'isolate'-'Country of origin'- 'Year isolation'. Bootstrap values (>75%) for 1,000 pseudoreplicate dataset are indicated at branch nodes.

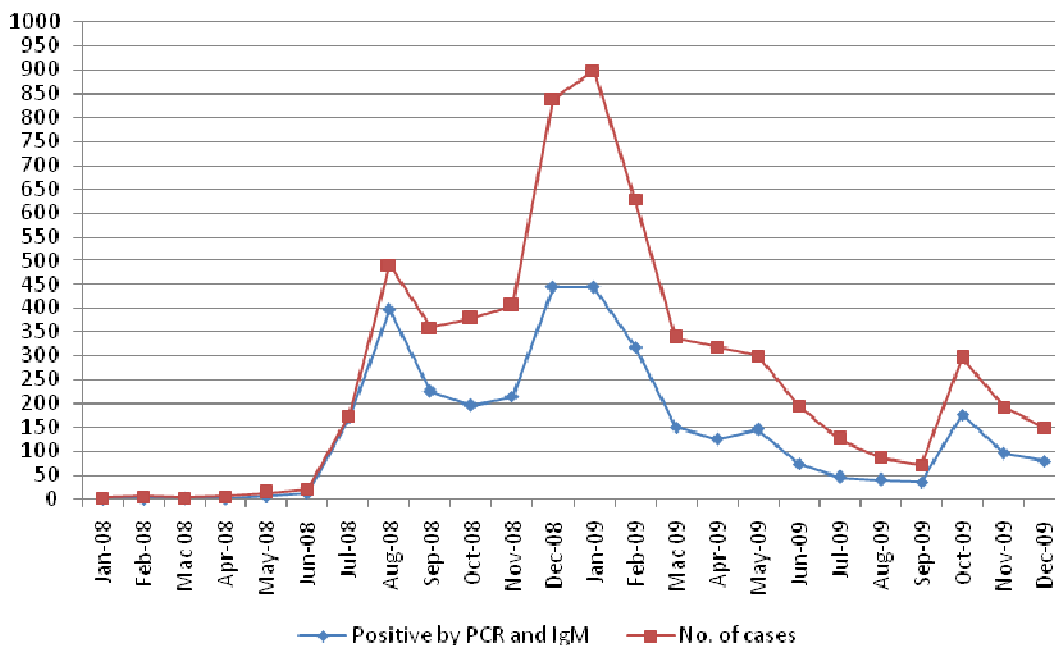


Figure 2. Distribution of CHIKV infection and positive cases by month from 2008 to 2009.

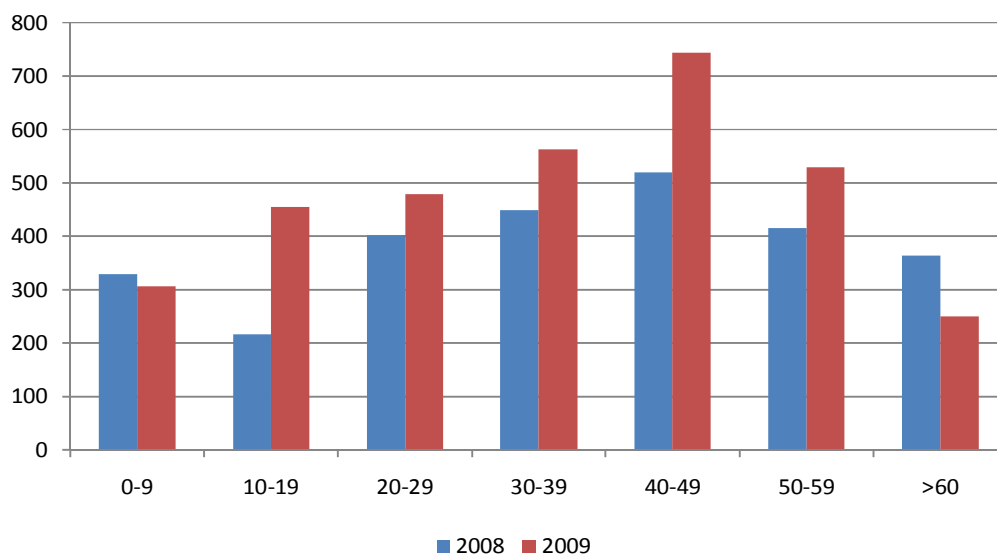


Figure 3. CHIKV infections during 2008 to 2009 outbreaks by age groups.

African genotype (Noridah et al., 2007; Sam et al., 2009; Maizatul., 2009), and was found to be similar with strains causing outbreaks in the Indian Ocean (Vidya et al., 2007) and also the same strains found to be circulating in the state of Kelantan in 2009 (Apandi et al., 2010). The phylogenetic tree based on partial E1 gene of the isolates involved in 2008 to 2009 outbreaks in Figure 2 showed all were from Central/East African genotype.

The different strains of CHIKV involved in the three outbreaks in Malaysia raised concern regarding the epidemiology and the capability of CHIKV to change from one genotype to another. The Asian strains involved in 1998 to 1999 and re-emerged in 2006 was thought to be probably due to CHIKV which was already endemic in Malaysia as suggested by Abubakar et al. (2007). But prior to 1998, CHIKV has never been isolated from humans or animals

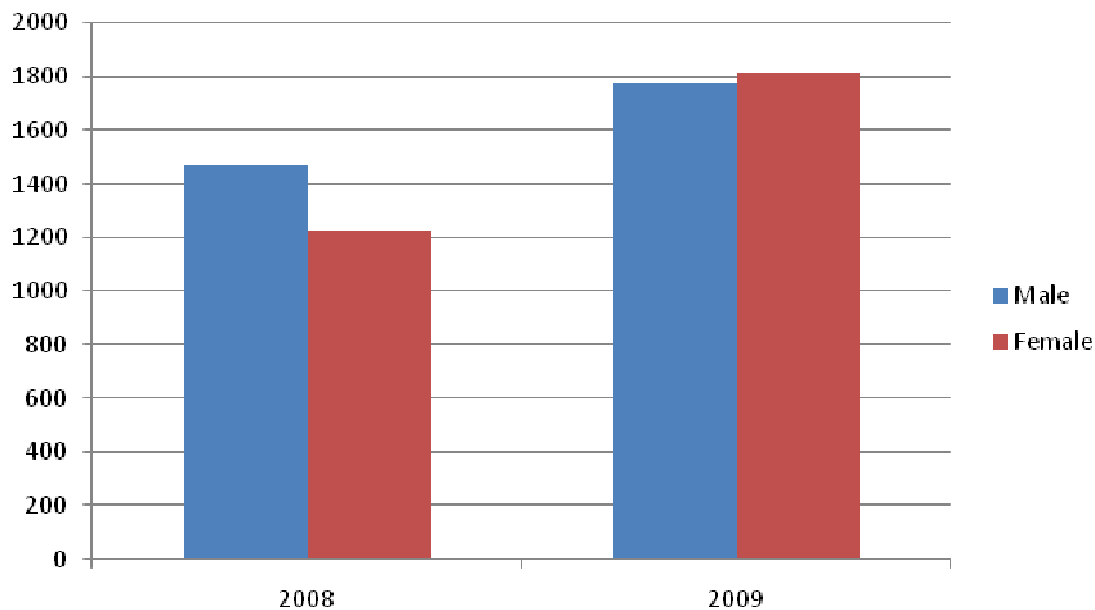


Figure 4. CHIKV infections during 2008 to 2009 outbreaks by gender.

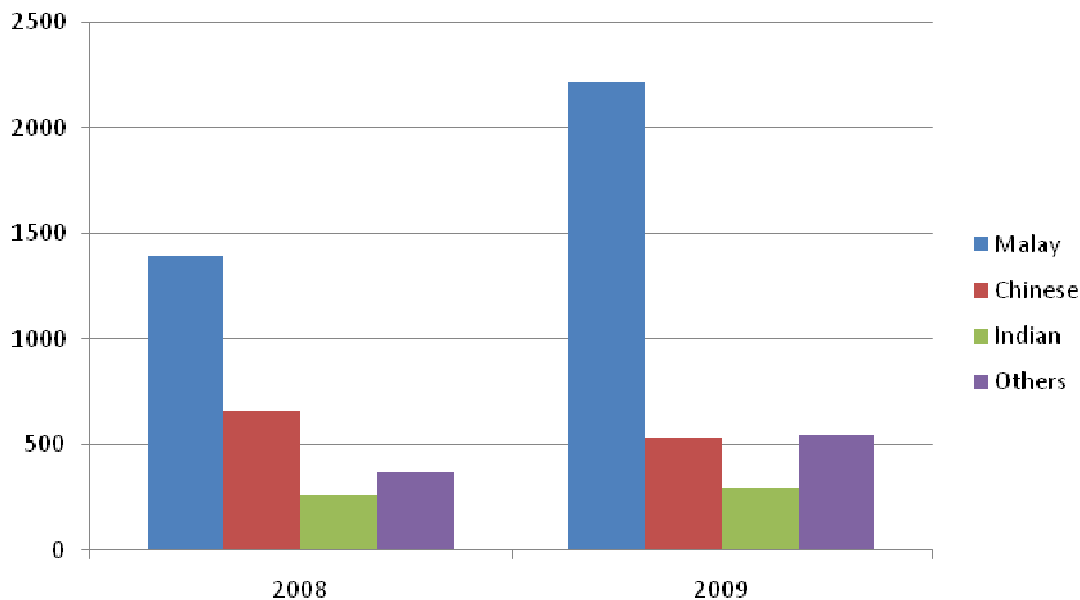


Figure 5. CHIKV infections during 2008 to 2009 outbreaks by ethnic groups.

in Malaysia. Neither was there any clinical disease due to CHIKV infection reported in Malaysia during that time period, even though earlier serological survey of human sera collected from 1965 to 1969 in West Malaysia showed neutralizing antibodies to CHIKV among adults, especially those inhabiting the rural northern and eastern

states bordering Thailand (Marchette et al., 1980). 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 (Apandi et al.,

2009) suggested that a CHIKV sylvatic transmission cycle may exist in Malaysia and could possibly contribute to the outbreaks. The emergence of Central/East African genotype in the third outbreak could probably originate from strain circulating in the Indian Ocean outbreaks in 2007 and responsible for other outbreaks in several regions (Vidya et al., 2007; Xavier et al., 2008; D'Ortenzia et al., 2009; CDC, 2010) including Thailand, (Theamboonlers et al., 2009). It was noted that before 2006, all the three CHIKV genogroups; namely: Asian, Central/East African and West African genotypes (Powers et al., 2000; Schuffenecker et al., 2006) were restricted to the geographical areas denoted by their names.

The recent explosive epidemics of African genotype in Indian Ocean Islands and India; and other parts of Asia, Africa and Europe have indicated that international travellers have disseminated new strain of the virus, some into region from which CHIKV has been absent (Townson and Nathan, 2008). This scenario has changed the geographical distribution of CHIKV worldwide and endemicity of CHIKV as previously stated 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; Rianthavorn et al., 2010) resulting in the virus becoming more susceptible to the new host of *Aedes* spp. especially *A. albopictus* which is found in high density in Malaysia. This mutation enables them to be more adaptive to new vector (Xavier et al., 2008) and could facilitate the spread of CHIKV. Another possibility was probably due to the presence of a large naive population who do not have prior exposure to and immunity against the virus. These could probably be the main factors causing re-emerging of CHIKV in Malaysia especially in rural areas. Also, the present of high viral load in patients travelling from epidemic areas could be an added factor (Parola et al., 2006) that been seen in Italy (Bordi et al., 2008).

The disease is almost self limiting and rarely fatal (Fields et al., 1996) and so far in Malaysia, no mortality was ever reported since its first appearance in 1998. However, mortality has been reported in Reunion Island (Michault and Staikowsky, 2009) and suspected in India (Mavalankar et al., 2008).

## Conclusion

The CHIKV strains circulating in all states of Malaysia during the outbreak in 2008 to 2009 were from the Central/East African genotype and were different from CHIKV strains previously isolated in 1998 to 1999 and 2006 outbreaks. Majority of the cases were adults between the age of 30 to 60 years with highest incident at

amongst the 40 to 49 age group and the Malays were the major race affected in the 2008 to 2009 outbreak.

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