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**DETECTION AND ISOLATION OF CHIKUNGUNYA  
VIRUS FROM FIELD COLLECTED *Aedes*  
*ALBOPICTUS* SKUSE IN SELECTED SITES,  
PENINSULAR MALAYSIA**

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**ABSTRACT.**

Chikungunya fever, an *Aedes* borne viral disease, is becoming a serious public health concern today since the first reported outbreak in Port Klang in 1998/99. Recently, more outbreaks were reported in Malaysia. Entomological investigations were conducted in Chikungunya virus cases localities in Peninsular Malaysia which cover Johor, Negeri Sembilan, Melaka, Perak, Pahang and Selangor state in order to identify the vector responsible for transmitting the Chikungunya virus. The adult mosquitoes were collected using modified aspirator and sweep net methods, whereas water holding containers were inspected for larvae. Reverse transcriptase polymerase chain reaction (RT-PCR) were used as the detection of the virus. Positive samples were inoculated on the cell to isolate the virus. The most

common species collected at the localities was *Aedes albopictus* followed by *Culex quinquefasciatus*, *Aedes aegypti* and *Armigeres sp.* Five pools (n=78) of female, adult *Aedes albopictus* collected from Tangkak, Johor were positive for the Chikungunya virus as detected by reverse transcription-polymerase chain reaction. Three isolates were obtained and grouped with Central/East African genotype. The presence of Chikungunya virus in wild *Aedes albopictus* indicated that this mosquito is the most likely vector responsible for the transmission of virus to humans in Johor during the outbreak.

**Keywords:** Chikungunya Virus, *Aedes albopictus* Skuse, Peninsular Malaysia

### ABSTRAK

Demam Chikungunya merupakan penyakit yang disebabkan oleh virus *Aedes* menjadi masalah kesihatan awam serius sejak wabak itu mula-mula dilaporkan di Pelabuhan Klang pada tahun 1998/99. Baru-baru ini, lebih banyak kes wabak Chikungunya dilaporkan di Malaysia. Siasatan telah dijalankan di negeri-negeri yang telah dilaporkan dengan wabak ini seperti di Johor, Negeri Sembilan, Melaka, Perak, Pahang dan Selangor untuk mengenalpasti vektor yang bertanggungjawab dalam menyebarkan virus Chikungunya ini. Nyamuk dewasa dikumpulkan dengan menggunakan aspirator terubah suai dan jaring sauk, manakala bekas air diperiksa untuk mengenal pasti kehadiran larva. Tindak balas rantaian polimerase (RT-PCR) dengan menggunakan transkripsi terbalik digunakan untuk mengesan virus. Sampel positif telah disuntik ke dalam sel untuk memencilkan virus tersebut. Spesies yang paling banyak dikutip adalah *Aedes albopictus* diikuti oleh *Culex quinquefasciatus*, *Aedes aegypti* dan *Armigeres sp.* Lima longgokan (n = 78) *Aedes albopictus* betina dewasa yang dikutip daripada Tangkak, Johor adalah positif dengan virus Chikungunya dan dikesan melalui transkripsi terbalik tindak balas rantaian polimerase (RT-PCR). Tiga pemencilan telah

diperoleh dan dibandingkan dengan genotip virus dari Afrika Tengah/Timur. Kesimpulannya, kajian membuktikan kehadiran virus Chikungunya pada spesies liar *Aedes albopictus* merupakan vektor utama penyebab wabak Chikungunya di Johor semasa wabak terjadi.

**Kata kunci:** Virus Chikungunya, *Aedes albopictus* Skuse, Semenanjung Malaysia

## INTRODUCTION

Chikungunya fever is an arthropod-borne viral disease, caused by Chikungunya virus (CHIKV) of the genus *Alphavirus* in the family *Togaviiridae* (Pialoux, Gaüzère, Jauréguiberry, & Strobel, 2007). CHIKV is transmitted through the bites of infective *Aedes sp.* mosquitoes (Martin, Moutailler, Madec, & Failloux, 2010). In Malaysia, the first CHIKV outbreak occurred in Klang, between December 1998 and February 1999 (Lam et al., 2001) followed in Bagan Panchor, Perak in March 2006 (AbuBakar et al., 2007; Kumarasamy et al., 2006) and Kinta district, Perak in December 2006 (Noridah et al., 2007). The third outbreak initially started in early April 2008, where an increasing number of CHIKV infections was first detected in Johor State (Apanidi et al., 2011). CHIKV has been known to be enzootic in many countries in Asia and Africa, transmitted mainly by various wild *Aedes* mosquitoes (Diallo, M, Thonnon, Traoré-Lamizana, & Fontenille, 1999; Pfeffer, Lissen, Parker, & Kinney, 2002). In Asia and the Indian Ocean region the main chikungunya virus vectors are *Aedes aegypti* and *Aedes albopictus* (Pialoux et al., 2007). Both *Ae. aegypti* and *Ae. albopictus* are abundant in Malaysia and with the abundance of both mosquito species, it is possible that outbreaks of CHIKV can occur anywhere, anytime in the country (Rozilawati et al., 2011, 2014). Information on the ability of the local vectors to transmit CHIKV will be useful in assisting the public health personnel and the general public in implementing a more effective campaign against the vectors in order to reduce the

transmission of the virus. With this information, we have conducted an entomological investigation in CHIKV outbreaks and cases areas in several states in Peninsular Malaysia, in order to identify the most possible vector responsible for the transmission of the virus.

## MATERIAL AND METHODS

### **Study sites**

All study areas selected were based on chikungunya outbreaks/cases areas reported. Six states were involved in this study: Johor, Negeri Sembilan, Melaka, Perak, Pahang and Selangor.

### **Mosquito collection**

Larval survey was conducted to collect the immature mosquitoes whereas adult collection was conducted using sweep net and modified aspirator (Rozilawati et al., 2011).

### **Mosquitoes processing, Virus detection and isolation**

All mosquitoes sampled were pooled in sterile 2.0 ml tubes labeled accordingly to the study localities and species (both for adult and larvae) and sex (for adults) with a range of 1 to a maximum of 30 individuals per pool before transported to the laboratory in dry ice. The RNA was extracted using the QIAamp® Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The RT-PCR assay was conducted using the Titan One Tube RT-PCR kit (Roche, Germany). The cDNA was amplified by using primers E1-C and E1-S (Hasebe et al., 2002) which amplified a 354 bp region of the non-structural protein 1 (nsP1) and 294 bp region of the glycoprotein E1 gene of CHIKV respectively. Amplified products were analysed in 2% agarose gels. Samples positive by RT-PCR were inoculated into C6/36 cell line and observed daily for cytopathic effects (CPE) For cell culture samples that showed CPE, RT-PCR was once again conducted on the cell

culture supernatant using E1-C and E1-S primers. The positive DNA samples were then extracted from the gel using the QIAquick® Gel Extraction kit from Qiagen (Germany). The partial E1 genes were sequenced on both strands by using PCR primers (Apandi et al., 2010) . Phylogenetic trees were constructed by using the neighbor-joining method from the software MEGA4. The O'nyong-nyong virus was used as the outgroup for phylogenetic analysis together with chikungunya E1 genes obtained for the purpose of generating dendograms.

## RESULTS AND DISCUSSION

A total of 5, 668 mosquitoes comprising 1, 525 adult mosquitoes and 4, 143 larvae were collected during the study period. Table 1 described the mosquito's species collected by localities. However, from this collection, only five pools (n=78) which contained only adult female *Ae. albopictus* from Kg Sawah and Kg Teratai, Tangkak, Johor were positive for CHIKV as detected by RT-PCR, whereas all other mosquito species either from the adults (males and females) or from larvae were found to be negative for this virus. From these positive samples, only three isolates were obtained and a phylogenetic tree of these isolates for partial E1 gene and showed that the isolates were related to the Central/East/South African genotype (Figure: I).

A total of 343 containers containing water were inspected during the investigations and 112 containers were found to be positive with *Aedes* larvae. Among all the domestic containers inspected during the study, plastic containers (39.29%) were identified as the key breeding containers infested by *Ae. albopictus* and *Ae. aegypti*, followed by latex collection cups (13.39%), tires and aluminum cans/tins (both 10.71%), plastic covers and rubber boots (both 4.46%), porcelains (3.57%), cement/concrete pools and polystyrenes (both 2.67%), and others (8.07%).

Other researchers also reported the detection of CHIKV from field caught *Ae. albopictus* in Madagascar during the 2006 outbreak (Ratsitorahina et al., 2008), also during the 2007 CHIKV outbreak in Italy (Bonilauri et al., 2008) and both *Ae. aegypti* and *Ae. albopictus* in Thailand (Thavara et al., 2009). All the other species of adult and immature mosquitoes collected during this survey were not positive for CHIKV, even though previous studies have suggested the possibility of vertical transmission of CHIKV in the field (Bonilauri et al., 2008; Thavara et al., 2009). In the recent years, CHIKV has been spreading rapidly therefore with *Ae. albopictus* as the main vector, more outbreaks are likely to occur since this “Asian tiger” mosquito is widely distributed in Malaysia. There is currently no effective antiviral treatment or vaccine for chikungunya fever (Pialoux et al., 2007) . The only preventive measures depend on suppressing the vector.

## CONCLUSION

Findings of this study indicated that *Ae. albopictus* is the most potential primary vector of CHIKV in Malaysia during 2008 outbreak and vector control efforts should target this species.

Table 1 Mosquitoes species collected and total no. of pools accordingly to species, stages, and sexes in all study localities

State	Species	Total adult		Total pool	Total larvae	Total pool
		Female	male			
Johor	<i>Ae.albopictus</i>	200	180	96	1776	172
	<i>Cx.</i>					
	<i>quinquefasciatus</i>	0	0	0	14	1
	<i>Ae.aegypti</i>	0	0	0	16	1
	<i>Armigeres sp.</i>	5	0	1	0	0
Negeri Sembilan	<i>Ae.albopictus</i>	23	37	5	182	18
	<i>Cx.quinquefasciatus</i>	0	0	0	13	1
	<i>Ae.aegypti</i>	0	0	0	0	0
	<i>Armigeres sp.</i>	0	0	0	0	0
Melaka	<i>Ae.albopictus</i>	79	314	20	982	74
	<i>Cx.</i>					
	<i>quinquefasciatus</i>	0	16	1	62	3
	<i>Ae.aegypti</i>	0	0	0	85	14
Perak	<i>Armigeres sp.</i>	0	0	0	0	0
	<i>Ae.albopictus</i>	92	48	10	141	11
	<i>Cx.</i>					
	<i>quinquefasciatus</i>	0	0	0	0	0
Pahang	<i>Ae. aegypti</i>	0	0	0	30	1
	<i>Armigeres sp.</i>	6	1	3	0	0
	<i>Ae.albopictus</i>	156	312	34	585	44
	<i>Cx.</i>					
Selangor	<i>quinquefasciatus</i>	0	0	0	39	2
	<i>Ae. aegypti</i>	0	0	0	0	0
	<i>Armigeres sp.</i>	2	1	2	14	1
	<i>Ae.albopictus</i>	17	30	3	204	8
	<i>Cx.</i>					
TOTAL	<i>quinquefasciatus</i>	0	0	0	0	0
	<i>Ae. aegypti</i>	0	0	0	0	0
	<i>Armigeres sp.</i>	3	3	2	0	0
		583	942	177	4143	351

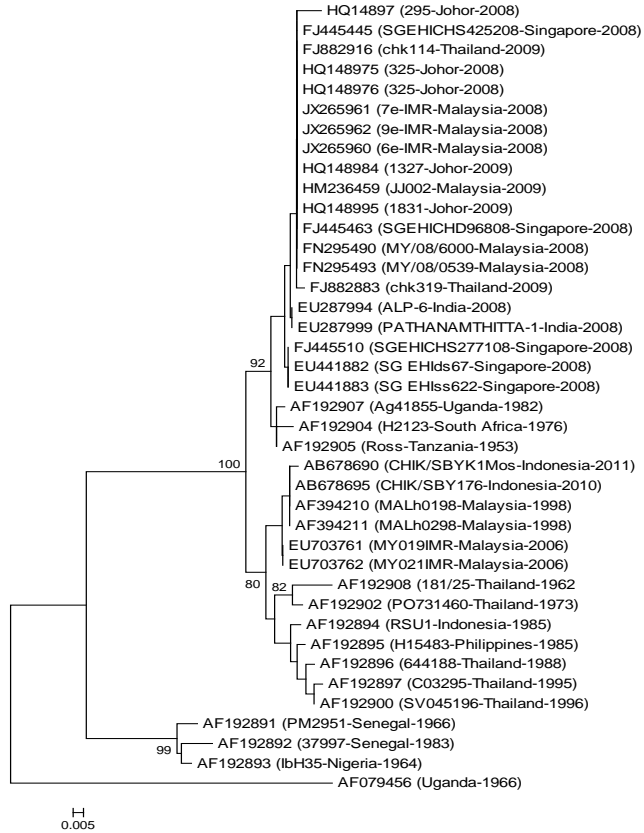


Figure 1 Phylogenetic tree of partial glycoprotein E1 sequences (257 bp) of CHIKV inferred using the Neighbour-Joining method from the software MEGA 4. The evolutionary distances were computed using the Maximum Composite Likelihood method. Genotypes Asian, Central/East African and West African are indicated by square brackets with O'nyong-nyong virus as the outgroup. Three representative isolates from this study are indicated as 6e-IMR-2008, 7e-IMR-2008 and 9e-IMR-2008 respectively. Representative strains of each genotype obtained from GenBank are labeled using the following format: 'Accession number-'isolate'-'Country of origin'-Year isolation'.



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