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Full Length Research Paper

The first isolation of chikungunya virus from non-human primates in Malaysia

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Chikungunya is a mosquito borne disease caused by chikungunya virus (CHIKV). The virus is transmitted to human by Aedes genus mosquitoes. Transmission cycles of CHIKV can be man - mosquito - man (urban cycle) or animal - mosquito - man (sylvatic cycle). Sylvatic transmission cycle of CHIKV has been described in Africa and may play a role in re-emergence of CHIKV infection. In Malaysia, CHIKV- neutralizing antibodies have been detected among wild monkeys in mid 1960s but so far CHIKV has never been isolated in monkeys.

Key words: Chikungunya virus, sylvatic cycle, non human primates, CHIKV genotypes

INTRODUCTION

Sera samples were collected from wild monkeys from 3 states of Peninsular Malaysia. All were inoculated into Vero and BHK cells; isolates were then identified and confirmed by polymerase chain reaction (PCR) and DNA sequencing techniques. These four isolates from monkeys were blast searched in GenBank found to be CHIKV with higher similarity to CHIKV isolated from klang outbreak in 1998 and Bagan Panchor outbreak in 2006 but were totally different from outbreak in Peninsular Malaysia in 2008.

This is the first to report CHIKV isolated from non human primates, thus confirming the existence of sylvatic transmission cycle in Malaysia. However, their roles as reservoir of infection need to be further investigated.

Text

Chikungunya virus (CHIKV) is an arbovirus from the genus Alpha virus in the family of *Togaviridae* was first isolated in Tanzania in 1953 (Ross, 1956). Between the 1960s and 1980s, these viruses had been reported circulating in many countries in the central, southern and Western Africa and recently a large epidemic occurred in Indian Ocean Islands and India (Vidya et al., 2007). The spread of CHIKV infections are normally sustained by human-mosquito-human transmission (urban cycle) but reports suggest primates-mosquito transmission (sylvatic cycle) maintains the virus in the wild in Africa (sylvatic cycle). Here we present the first evidence for a sylvatic cycle for CHIKV primates in Asia.

Currently CHIKV can be grouped into three distinct genotypes as 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). Recent explosive epidemic of African genotype in Indian Ocean Islands and India since 2005 and other parts of Asia, Africa and Europe showed that international travelers have disseminated new strain of the virus, some into region from which CHIKV has been absent (Townson and Nathan, 2008). This scenario had changed the geographical origin of CHIKV worldwide.

Re-emergence of CHIKV infection has been reported in many parts of the world (Rao, 1971; Thuang et al., 1975; Thaikruea et al., 1997) and recently occurred in the India Ocean Islands and India (Schuffenecker et al., 2006; Vidya et al., 2007) with European countries such as France, Germany, Italy, Norway and Switzerland also experiencing imported cases of CHIKV infection from people returning from endemic areas (D'Ortenzio et al.,

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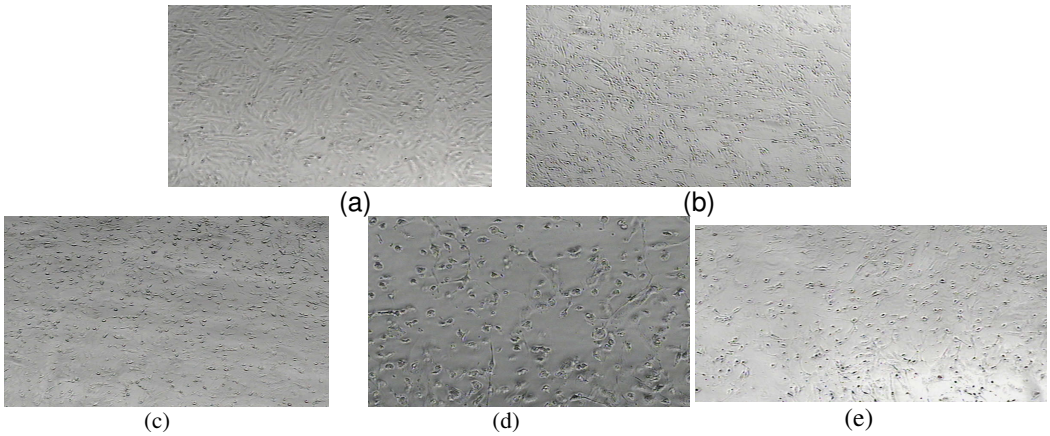

Figure 1. (a) Uninfected African Green Monkey Kidney (Vero) cell at 10X, (b) Sample M125 after 4 days of inoculation in Vero cell at 10X; (c) Sample M127 after 4 days of inoculation in Vero cell at 10X; (d) Sample M128 after 4 days of inoculation in Vero cell at 10X and (e) Sample M129 after 4 days of inoculation in Vero cell at 10X.

2009; Townson and Nathan, 2008; Pialoux et al., 2007; WHO, 2006). In Asia particularly, CHIKV activity was documented from its isolation in Bangkok in 1958 (Fields et al, 1996) and since then the transmission of this virus from Asian genotype continued and caused many outbreaks such as in Kolkata and Southern India in 1963 and 1964 respectively, in Indonesia during 2001 - 2003 (Laras et al., 2005) even though the African genotype was responsible for outbreaks India and Indian Ocean Islands during 2005 - 2006 (Yergolkar et al., 2006).

In Malaysia, CHIKV infection was first recorded in 1998 - 1999 in Port Klang affecting more than 51 people (Lam et al., 2001), which was followed by the outbreak in Bagan Panchor, Perak in 2006 (Kumarasamy et al., 2006). Both outbreaks were attributed to the Asian genotype of CHIKV. After a period of almost 2 years, the re emergence of CHIKV infection occurred in July 2008 which started in Tangkak, Johor then spread to other states in Peninsular Malaysia such as Negeri Sembilan and Malacca, with more than 2000 cases recorded (MOH, 2008). In March 2009, more than 1600 cases had been reported, with the states of Kuala Lumpur, Selangor and Kelantan, recording the highest number of cases (MOH, 2009). This outbreak is very important since it was the first chikungunya outbreak caused by CHIKV belongs to the Central/East African genotype in Malaysia (Maizatul, 2009). Clinically, the patients presented with fever, arthralgia, myalgia and most often the symptoms were indistinguishable from dengue infection. However, unlike dengue which caused morbidity and mortality, there has never been any mortality from CHIKV infection in Malaysia, although mortality has been reported in Reunion Island (Michault and Staikowsky, 2009) and suspected in India (Mavalankar et al., 2008).

In this study, the main objective was to detect the presence of viruses especially arboviruses in non human primates. A total of 105 sera were collected from wild long tailed macaques (*Macaca fascularis*) from 3 states of Peninsular Malaysia namely Pahang, Selangor and Wilayah Persekutuan Kuala Lumpur from August 2007 till January 2008. All sera were inoculated onto 3 different cell lines: C6/36, Vero and BHK cells. Cultures were observed daily for cytopathic effect (CPE) and samples which did not exhibit any CPE after 2 passages or after a total of 14 days incubation were regarded as negative. Four from 5 sera collected from Kuala Lipis in Pahang showed CPE only in Vero cells 3 - 4 days after inoculation. The virus showed characteristic of enterovirus CPE in which the cells became refractile and rounded cells in loose clusters as shown in Figures 1a - e.

Based on the CPE characteristics, simian enterovirus was thought to be the most likely virus. Therefore published primers 188 - 222, 012-011, OL68-EVP2 and 189 - 222, which were reported by Oberste et al. (2002) to detect simian enteroviruses in non human primates were used for PCR-based identification. Unforyunately all these primers failed to amplify any product as shown in Figures 2a, b, c and d.

Rudnick and Lim in 1986 reported the presence of arboviruses, especially dengue virus, in non human primates in Malaysia. Flavivirus universal primers and dengue universal primers (Lanciotti et al., 1992) were thus used in PCR analysis of these isolates; however, none were able to amplify any PCR product. Due to re emergence of CHIKV infection, that in Malaysia started in mid 2008, the isolates were then amplified using the primers NSP1-C and NSP1-S and E1-C and E1-S (Hasebe et al., 2002), which amplify 354 bp of the non-structural protein P1 and 294bp of the glycoprotein E1 gene of CHIKV, respectively. The amplifications of PCR products are shown in Figures 3a and b.

These products were sequenced in both directions using NSP-1 and E-1 primers. Blast searches in Gen-Bank revealed that these isolates were CHIKV and were

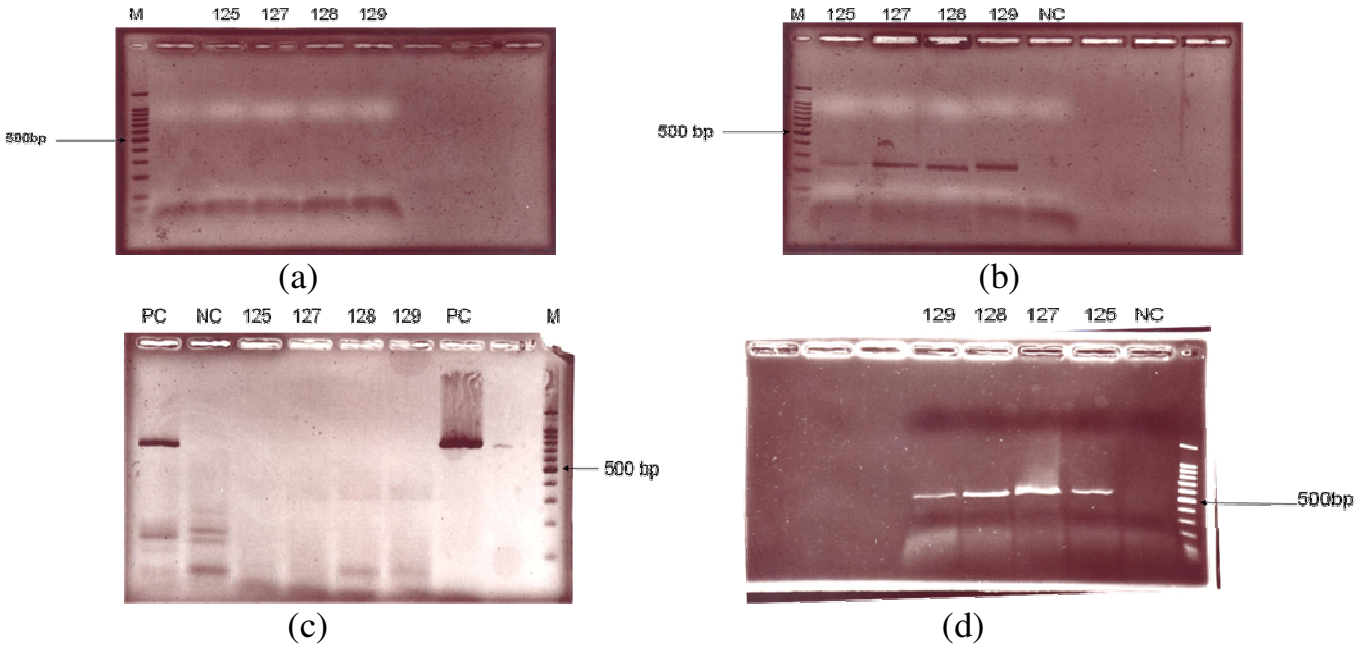

Figure 2. Amplification of four isolates by using (A): primer 188-222; (B) primer 012-011; (C) OL68-EVP2 and (D) primer 189-222. M: 1kb DNA ladder, BioLabs, New England.

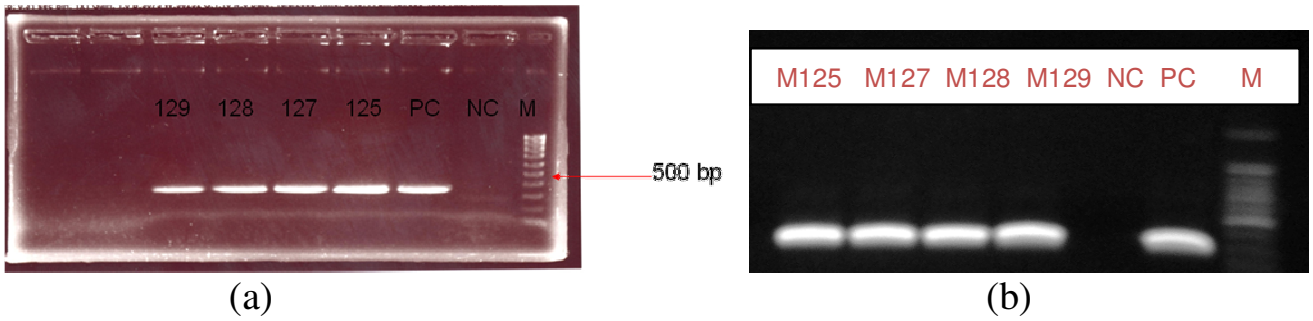

Figure 3. Amplification of four isolates by using (a): primer NSP1-C and NSP1-S; (b) primer E1-S and E1-C; M: 100bp DNA ladder, BioLabs, New England.

closely related to the human CHIKV isolated in Bagan Panchor in 2006 and Klang in 1998 outbreaks. They belonged to the Asian genotype clusters but distinct from the current CHIKV circulating in Malaysia in 2008, which has been identified as the Central/East African genotype (Maizatul, 2009). Phylogenetic analysis of these isolates together with CHIKV representatives from other regions is shown in Figure 4.

The human CHIKV isolates from the 2006 outbreak in Bagan Panchor, Malaysia were closely related to the isolates from the 1998 outbreak in Klang. Interestingly the four isolates from monkeys were closely related to these viruses, even though there were isolated from different hosts. The high sequence similarities suggests that cross transmission between human and non-human primates occurred in Malaysia and that the 2006 and 1998 outbreaks may have arisen from virus circulating in monkeys, supporting the view that CHIKV is endemic in Malaysia (Abubakar et al., 2007).

Before 1998, CHIKV had not been isolated from humans or animals and no clinical disease caused by CHIKV had been reported in Malaysia. However, serologic survey of human serum samples collected during 1965 - 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). Earlier studies also found evidence of CHIKV-neutralizing antibodies in wild monkeys, pigs, and chickens (Marchette et al., 1980) suggested that a CHIKV sylvatic transmission cycle may exists in Malaysia even though CHIKV never been isolated from Asian monkeys (Chhabra et al., 2008). In

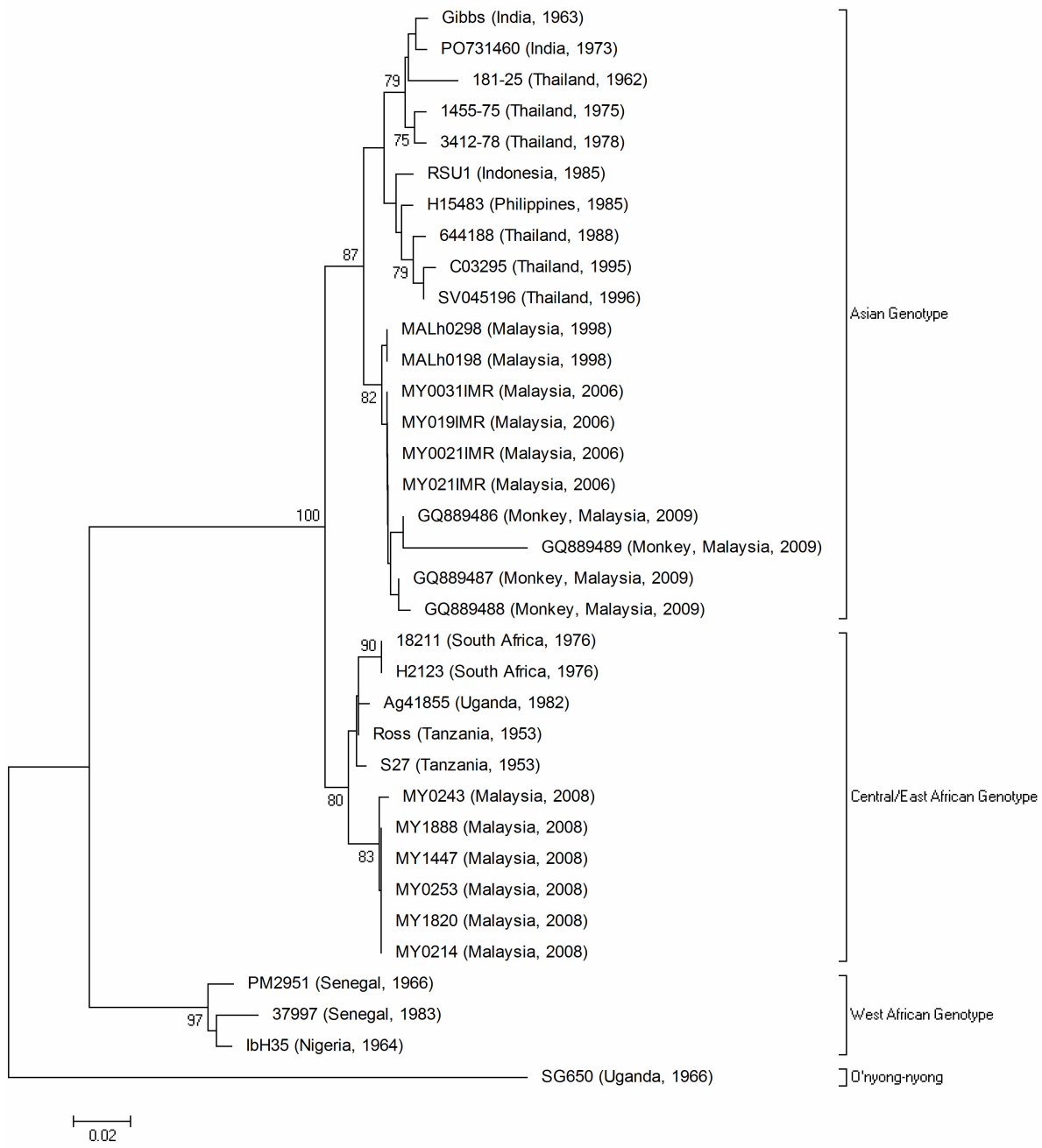

Figure 4. Phylogenetic tree of partial glycoprotein E1 sequences (257bp) of CHIKV inferred using the Neighbor-Joining method from the software MEGA 4. The evolutionary distances were computed using the Maximum Composite Likelihood method. Genotype Asian, Central/East African and West African are indicated by square brackets with O'nyong-nyong virus as an out-group. Four CHIKV isolated from monkeys labeled as GQ889486, GQ889487, GQ889488 and GQ889489. Representative strains of each genotype obtained from GenBank are labeled using the following format: 'isolate'-'Country of origin'- 'Year isolation'. Bootstrap values (> 75%) for 1,000 pseudo replicate dataset are indicated at branch nodes.

the absence of active surveillance since the 1965 study (Marchette et al., 1980), whether the apparent absence of CHIKV over the years and between the 2 recent out-breaks in Malaysia is due to an unidentified sylvatic transmission cycle or silent transmission among humans is difficult to establish.

Africa sylvatic transmission involving wild primates may play a role in the emergence and re-emergence of CHIKV infection (Diallo et al., 1999). The virus likely circulates among wild primates and many species of Aedes mosquitoes, with serological evidence demonstrating the presence of antibodies in humans and wild primates

(Adesina and Odelola, 1991; Jupp and McIntosh, 1998; Rodhain et al., 1989). This zootic cycle probably supports the virus with occasional human leading to epidemic (Lumsden, 1955). To date, a vertebrate reservoir has not been identified outside Africa to support the historical evidence by Carey (1971) that CHIKV originated in Africa. The evidence presented herein illustrates that viruses from the 2006 and 1998 CHIKV outbreaks in Malaysia are closely related to CHIKV isolates from monkeys suggesting the existence of sylvatic cycle for CHIKV in Malaysia

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REFERENCES

Abubakar S, Sam IC, Wong PF, MatRahim NA, Hooi PS Roslan N (2007). Reemergence of endemic chikungunya, Malaysia. Emer. Infect. Dis. 13(1): 147-149.

Adesina OA, Odelola HA (1991). Ecological distribution of Chikungunya haemagglutination inhibition antibodies in human and domestic animals in Nigeria. Trop. Geogr. Med. 43: 271-275.

Carey DE (1971). Chikungunya and dengue: a case of mistaken identity? J. Hist. Med. Allied Sci. 26: 243-262.

Chhabra M, Mittal V, Bhattacharya D, Rana UVS, Lal S (2008). Chikungunya fever: A re-emerging viral infection. Indian J. Med. Microbiol. 26(1): 5-12.

Diallo M, Thonnon J, Traore-Lamizana M, Fontenille D (1999). Vectors of chikungunya virus in Senegal: current data and transmission cycles. Am. J. Trop. Med. Hyg. 60: 281–6.

D'Ortenzio E, Grandadam M, Balleydier E, Dehecq JS, Jaffar-Bandjee MC, Michault A, Andriamandimby SF, Reynes JM, Filleul L (2009). Sporadic cases of chikungunya, Réunion Island, Eurosurveillance 14 (35): 1-2.

Fields BN, Knipe DM, Howley PM (1996). Alphavuruses. In: Fields of Virology Third Edition; V 1. Lippincott-Raven Publishers pp.858-898.

Hasebe F, Parquet MC, Pandey BD, Mathenge EG, Morita K, Balasubramaniam V, (2002). Combined detection and genotyping of Chikungunya virus by a specific reverse transcription polymerase chain reaction. J. Med Virol: 67: 370-374.

Jupp PG, McIntosh BM (1988). Chikungunya virus disease. In The Arboviruses : Epidemiol. Ecol. Edited by T. P. Monath. Boca Raton, FL: CRC Press. 2: 137-157.

Kumarasamy V, Prathapa S, Zuridah H, Chem YK, Norizah I, Chua KB (2006). Re-Emergence of Chikungunya Virus in Malaysia. Med. J. Malaysia 61(2): 221-225.

Lam SK, Chua KB, Hooi PS, Rahimah MA, Kumari S, Tharmaratnam M (2001). Chikungunya infection – an emerging disease in Malaysia. Southeast Asian J. Trop. Med. Public Health. 32: 447–51.

Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam AV (1992). Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J. Clin. Microbiol. 30(3): 545-551.

Laras K, Sukri NC, Larasati RP, Bangs MJ, Kosim R, Djauzi Wandra T, Master J, Kosasih H (2005). Tracking the re-emergence of epidemic chikungunya virus in Indonesia. Trans. R. Soc. Trop. Med. Hyg. 99: 128–141.

Lumsden WH (1955). An epidemic of virus disease in Southern Province, Tanganyika territory in 1952-53 II: General description and epidemiology. Trans. R. Soc. Trop. Med. Hyg. 49: 33-57.

Maizatul A (2009). Analisa jujukan gen glikoprotein envelop E1 virus chikungunya Malaysia bagi tahun 2008. Thesis B.Sc (Hons). Universiti Putra Malaysia.

Mavalankar D, Shastri P, Bandyopadhyay T (2008). Increased mortality rate associated with chikungunya epidemic, Ahmedabad, India. Emerg. Infect. Dis. Mar; 14(3): 412-5.

Marchette NJ, Rudnick A, Garcia R (1980). Alphaviruses in Peninsular Malaysia: II. Serological evidence of human infection. Southeast Asian J. Trop. Med. Public Health. 11: 14–23.

Michault A, Staikowsky F (2009). Chikungunya: First step toward specific treatment and prophylaxis. J. Infec. Dis. 200: 489-491.

MOH (2008). Daily Report on Chikungunya outbreak, Ministry of Health, Malaysia.

MOH (2009). Daily Report on Chikungunya outbreak, Ministry of Health, Malaysia.

Oberste MS, Maher J, Pallansch MA (2002). Molecular phylogeny and proposed classification of the simian picornaviuses. J. Virol. 76(3): 1244-1251.

Pialoux G, Gauzere BB, Jaureguiberry S, Strobel M (2007). Chikungunya, an epidemic arbovirosis. Lancet Infect. Dis. 7(5): 319-327.

Powers AM, Brault AC, Tesh RB, Weaver SC (2000). Re-emergence of chikungunya and o'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. J. Gen Virol. 81: 471–479.

Rao TR (1971). Immunological surveys of arbovirus infections in South East Asia, with special reference to dengue, chikungunya and Kyasanur Forest disease. Bull. WHO 44: 585-591.

Rodhain F, Gonzalez JP, Mercier E, Helynck B, Larouze B, Hannoun C (1989). Arbovirus infections and viral haemorrhagic fevers in Uganda: a serological survey in Karamoja district, 1984. Trans. R. Soc. Trop. Med. Hyg. 83: 851-854.

Ross RW (1956). A laboratory technique for studying the insect transmission of animal viruses, employing a bat-wing membrane, demonstrated with two African viruses. J. Hyg. (Lond) 54: 192–200.

Rudnick A, Lim TW (1986). Dengue fever studies in Malaysia. Ins. Med. Res Malaysia Bulletin 23: 127-147.

Schuffenecker I, Iteman I, Milchault A, Murri S, Frangeul L, Vaney MC, Lavenir R, Pardigon N, Reynes JM (2006). Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. PLoS Med 3, e263.

Thaikruea L, Cheareansook O, Reanphumkarkit S (1977). Chikungunya in Thailand: a re-emerging disease?. SEA J. Trop. Med Pub. Health. 28: 358-364.

Thuang U, Ming CK, Swe T, Thein S (1975). Epidemiological features of dengue and chikungunya infections in Burma. SEA J. Trop. Med Pub Health 6: 276-283.

Townson H, Nathan MB (2008). Resurgence of chikungunya. Trans. R. Soc. Trop. Med. Hyg. 102(4): 308-309.

Vidya AA, Shubham S, Sarah C, Rashmi SG, Atul MW, Santosh MJ, Sudeep AB, Akhilesh CM (2007). Genetic divergence of chikungunya viruses in India (1963-2006) with special reference to the 2005-2006 explosive epidemic. J. Gen. Virol. 88: 1967-1976.

WHO (2006). Outbreak news. Wkly Epidemiol Rec. 81, 105–116.

Yergolkar PN, Tandale BV, Arankalle VA, Sathe PS, Sudeep A, Gandhe SS, Gokhle MD, Jacob GP, Hundekar SL, Mishra AC (2006). Chikungunya outbreaks caused by African genotype, India. Emerg. Infect. Dis. 12: 1580–1583.