Chikungunya: an overview

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Chikungunya (CHIK), a mosquito borne debilitating disease, is caused by CHIK virus, an alphavirus belonging to the family Togaviridae. The sudden onset of very high fever along with rash, and severe arthralgia especially in the small joints of hands and toes are the characteristics of the disease. It was first reported from Tanzania in 1952-53 and spread subsequently to sub-Saharan Africa, South East Asia and Pacific causing large epidemics. The virus exists in three genotypes, the Asian, West African and East Central South African that are responsible for outbreaks in the respective areas. The first outbreak in Asia was in Bangkok in 1958 followed by other Asian countries. India experienced massive outbreaks of CHIK in the 1960s and early 70s mainly in cities. After a gap of 32 years an explosive outbreak of CHIK devastated the country affecting more than 1.4 million people in 13 states. The epidemic also witnessed many unusual clinico-pathological complications including CHIK associated deaths and mother to child transmission. High morbidity with severe arthralgia persisted for several months made the people mentally and physically weak. This review describes CHIK in general and highlights the various clinico-pathological aspects observed during the recent outbreak.

[Sudeep A B and Parashar D 2008 Chikungunya: an overview; *J. Biosci.* **33** 443–449]

1. Introduction

The resurgence of chikungunya (CHIK) in the Indian Ocean Islands and India has drawn worldwide attention due to its explosive nature, high morbidity and complex clinico-pathological manifestations. The disease, caused by the chikungunya virus (CHIKV) has already involved in many outbreaks in Africa and Asia ever since its discovery in 1952 (Edelman *et al* 2000; Powers and Logue 2007). CHIK produces a dengue-like illness in humans, characterized by fever, rash, and severe arthralgia persisting for a few weeks to several months. The most significant characteristic of CHIK is the prolonged arthralgic syndrome that primarily affects the peripheral small joints associated with excruciating pain (Powers and Logue 2007). The disease is generally non-fatal and the acute phase resolves within 3–4 days leaving the arthralgic syndrome persisting for some more time. The causative agent, CHIKV is an alphavirus of the family Togaviridae has a genome consisting of a linear, positive-sense, single stranded RNA molecule of approximately 11.8 kb. Man gets infection through the bite of infected *Aedes* mosquitoes mainly *Aedes aegypti*, the incriminated vector of CHIKV (Rao 1964). Comparing the earlier outbreaks, the recent episode was massive, spread at a fast pace to wider areas causing serious economic and social impact. Symptoms and complications uncharacteristic of CHIK including deaths were reported (Mavlankar *et al* 2008). The present review briefly describes the disease in general and highlights the various clinico-pathological aspects, observed during the recent outbreak.

2. Historical perspective

The first outbreak of CHIK was reported from the Makonde Plateau, along the border between Tanzania (formerly Tanganyika) and Mozambique, during 1952–1953 (Ross

Keywords. *Aedes* mosquitoes; arthralgia; chikungunya; clinico-pathological complications; real-time PCR; vaccines

Abbreviations used: CHIK, chikungunya; CHIKV, Chikungunya virus; ECSA, East Central South Africa; RT-PCR, reverse trascriptase-polymerase chain reaction

1956). The term Chikungunya is derived from the Makonde root verb kungunyala, meaning "that which bends up" in reference to the stooped posture developed due to the excruciating joint and muscle pain and other rheumatologic manifestations. Serogical and antigenic characterization of the etiological agent collected during the epidemic ruled out the involvement of dengue but attributed to an alphavirus closely related to Mayaro and Semliki Forest virus (Powers and Logue 2007). Subsequent studies have demonstrated it as a new virus which might have existed in East Africa and maintained probably in a nonhuman primate-sylvatic *Aedes* mosquito transmission Cycle (Powers *et al* 2001). Retrospective studies have suggested that CHIKV epidemics occurred as early as 1779 but were documented inaccurately as dengue outbreaks (Carey 1971; WHO Report 2006).

3. Geographic distribution of CHIK

After the 1952–1953 outbreak, the virus has widely disseminated throughout sub-Saharan Africa, India, and countries of Southeast Asia, leading to numerous epidemics in the subsequent years (Edelman *et al* 2000). The virus has become endemic in Africa as evidenced by the frequent outbreaks in Uganda, Democratic Republic of Congo, Zimbabwe, Senegal, Nigeria, South Africa and Kenya (Powers and Logue 2007). The first outbreak in Asia was reported from Bangkok in 1958 (Aikat *et al* 1964) followed by a number of outbreaks in Cambodia, Vietnam, Malaysia, Taiwan etc. in the subsequent years. In the Bangkok epidemic, though three etiological agents were suspected, six isolates were identified as CHIKV indicating its active role in the Thai haemorrhagic fever. The first epidemic of CHIKV in

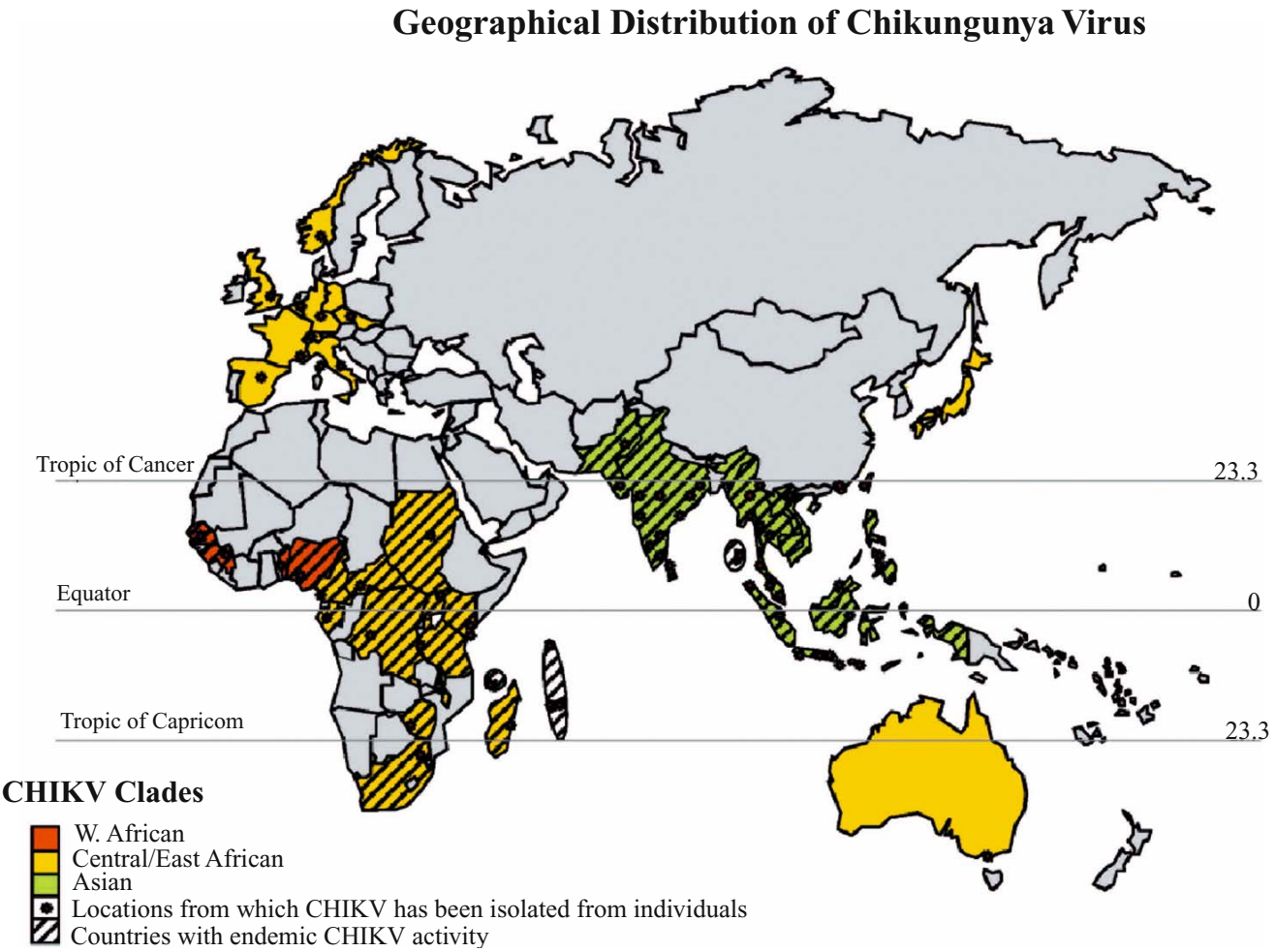

Figure 1. Worldwide distribution of CHIKV. Shading of countries indicates the predominant (or only) genotype reported to have been identified in a given country. India is shaded in green (Asian genotype) as outbreaks from 1963 to 1965 and 1973 were confirmed to have been caused by members of the Asian clade; however, reports from India during 2005–2007 indicate this outbreak was caused by the same CHIKV strains detected during the Indian Ocean outbreaks (Central/East African genotype). Asterisk indicates a location from which CHIKV was isolated (courtesy: Powers and Logue 2007).

India was reported from kolkatta (earlier Calcutta), West Bengal in 1963, which claimed nearly 200 lives mainly children (Sarkar *et al* 1964). The CHIKV isolates from the Calcutta epidemic were more closely related to the Thailand strain than to the African strain indicating its origin from South East Asia (Rao and Anderson 1964). In the subsequent years several cities in India had massive outbreaks of CHIK affecting lakhs of people (Mohan 2006). However, the 2006–2007 outbreak has shaken the country due to its high magnitude.

Studies with the CHIK virus isolates collected from different geographical areas have shown the prevalence of three lineages with distinct genotypic and antigenic characteristics (Arankalle *et al* 2007). In Africa, two genotypes viz. West African and East Central South African (ECSA) genotypes contributed to all the epidemics while the Asian genotype represented for whole of Asia (Powers and Logue 2007). However, the 2005-06 epidemic has seen the introduction of the ECSA genotype to the Asian continent for the first time (Yergolkar *et al* 2006). CHIK cases were also reported recently from Europe, USA and Australia through travelers returning from affected areas (Centers for Disease Control and Prevention 2006; Bonilauri *et al* 2008). The geographic distribution of CHIKV is shown in figure 1.

4. CHIKV vectors and virus transmission

The urban mosquito *Ae. aegypti* that are anthropophilic and maintain close associations with humans is the major vector of CHIK as CHIKV has been isolated repeatedly from this mosquito from the epidemic areas in Tanganyika, Thailand and Calcutta (Shah *et al* 1964; Pavri *et al* 1964). It has involved virtually in all the epidemics in India and other South East Asian countries (Pavri *et al* 1964; Rao 1964; Yergolkar *et al* 2006; Bonilauri *et al* 2008; Sang *et al* 2008). The vector potential of *Ae. aegypti* mosquitoes in the transmission of CHIKV has proved conclusively by experimental transmission studies also (Rao *et al* 1964; Shah *et al* 1964; Soekiman 1987). However, during the 2005-06 epidemic in certain Indian Ocean Islands and Kerala State of India *Ae. albopictus*, played an alternate role (Schuffenecker *et al* 2006; Santosh *et al* 2008). Experimental studies also substantiated its potential as a vector (Reiskind *et al* 2008; Tsetsarkin *et al* 2007).

CHIKV maintenance and transmission differed in Asia and Africa. In Asia CHIKV is maintained in a mosquito-human-mosquito cycle while in Africa the virus is maintained in a sylvatic cycle involving wild non-human primates and forest-dwelling *Aedes* Mosquitoes (McIntosh *et al* 1977; Diallo *et al* 1999). Though *Ae. aegypti* has been the major vector in Africa, CHIKV has been isolated from several sylvatic *Aedes* mosquitoes (*Ae. africanus, Ae. furcifer-taylori* etc.) and non *Aedene* mosquitoes (Mansonia, Culex, etc.) (Diallo *et al* 1999; Rao 1964). However, the vector potential of *Culex* and *Anopheles* mosquitoes needs further investigation as only a few isolations were reported from them and they failed to transmit the virus experimentally (Shah *et al* 1964; Rao *et al* 1964). It would also be remembered that detection of a virus from a wild caught arthropod does not mean that the species is a vector.

5. Mutated CHIKV genome

Schuffenecker *et al* (2006) demonstrated a specific change at position 226 of the E1 protein with the substitution of alanine with valine (E1: A 226 V) in the Reunion CHIK isolates. This change has been observed in 90% of the strains isolated after September 2005, i.e. towards the latter half of the epidemic. Similar results were also reported from India as E1: A 226V changes were observed in CHIK isolates collected towards the latter half of the epidemic (2007 isolates) but not in 2006 isolates (Arankalle *et al* 2007; Santosh *et al* 2008). This clearly demonstrated that in both the places, the specific mutation was observed with the progression of the epidemic i.e. towards the latter half of the epidemic.

These changes had immense significance in the epidemiology of the virus providing selective advantage for transmission by mosquitoes. Schuffenecker *et al* (2006) speculated that the genetic change in position E1:A 226 V reduced their cholesterol dependence to infect mosquito hosts. Because mosquitoes often do not have enough cholesterol for viruses to efficiently infect the host cells, the E1:A226V mutation might have helped in replication and transmission of the virus. Tsetsarkin *et al* (2007) have experimentally demonstrated that the mutation at the E1: A 226 V was responsible for the enhanced infectivity and efficient transmission to mice by *Ae. albopictus* and *Ae. aegypti*. They also observed an association between cholesterol dependence and increased fitness of CHIKV during their studies with C6/36 cells.

5.1 *Clinical aspects of CHIKV infection*

CHIK causes debilitating and prolonged arthralgic syndrome incapacitating the affected population for longer periods. The disease etiology consisted of sudden onset of fever with arthralgia, which generally resolved within a few days. However, complicated clinico-pathologic manifestations were reported from Calcutta during the 1963 epidemic. Neurological (meningoencephalitis) and haemorrhagic manifestations (haematemesis and melaena) with a shock-like syndrome leading to death were reported for the first time (Aikat *et al* 1964; Sarkar *et al* 1964). In very severe clinical forms, purpuric spots and petechae were also observed. Sarkar *et al* (1964)

reported circulatory collapse with or without bleeding manifestations, leucopenia, thrombocytopenia etc during the same epidemic. Similar complexities were also observed in hospitalized cases of CHIK confirmed patients from Vellore, Tamil Nadu (Thiruvengadam *et al* 1965; Jadhav *et al* 1965). Since dengue virus activity was also reported during the time of CHIK outbreaks at both the places, the clinical manifestations observed during these epidemics therefore cannot be attributed to CHIKV alone.

5.2 *The recent epidemic*

The 2005–2006 epidemic in Indian Ocean Islands was the most devastating and had very complicated clinico-pathological manifestations associated with encephalopathy and hemorrhagic fever (Pialoux *et al* 2007). Arthraligia persisted for months and years with excruciating pain in joints and ankles making the people prostrate. The most affected were the aged adults and adults suffering from diabetes, alcoholic hepatopathy and impaired renal functions (Couderc *et al* 2008). The epidemic also witnessed the first ever CHIKV associated deaths and mother to child transmission (Pialoux *et al* 2007; Mavalankar *et al* 2008).

In India, Ahmedabad city of Gujarat and Kerala states experienced large scale out breaks with high morbidity and extensive incapacitation. Complications with involvement of the neurological and renal system leading to deaths were reported from hospitalized cases (Mavalankar *et al* 2007, 2008; Solanki *et al* 2007). Kerala state had the worst epidemic as the infection run through 2006 to 2008 affecting the whole state. Unique complications such as swollen limbs with painful arthralgia which persisted for long periods were witnessed among the patients. The most common and distinct disabilities during convalescence include acute difficulty in sitting, lying down, standing straight, and walking as well as general weakness of mind and body. Though several CHIK associated deaths were reported in kerala by the print media, the official website of Kerala government has not confirmed any deaths. Mavalankar *et al* (2008) carried out systematic studies and observed high incidence of deaths during the CHIK epidemic period in Ahmedabad city. Similar statistics would have given at least a fair picture of the CHIK associated deaths in Kerala. Ophthalmic involvements, hypokalemic paralysis, sensorineural hearing loss, Guillain Barre Syndrome, and acute flaccid paralysis were some of the other complications observed in the affected people across the country (Lalitha *et al* 2007, Mittal *et al* 2007; Rampal *et al* 2007; Singh *et al* 2007; Bhavana *et al* 2008). Significant abnormalities in CSF and CT scan with raised levels of hepatic enzymes, altered renal function tests and decreased electrolyte levels were also noted in large number of patients.

6. Animal pathogenicity and cell/tissue tropisms

Pathogenicity studies of CHIKV infection could not be studied due to the lack of a proper animal model. Couderc *et al* (2008) recently reported the development of a mouse model and observed that CHIK virus replicates first in the liver, and targets muscle, joints and skin, closely resembling the cell/tissue tropism observed in biopsy samples of CHIK infected humans. They also observed the dissemination of virus to choroids plexuses and the leptomeninges in the CNS in severe infections. Ziegler *et al* (2008) observed the presence of virus in the leg muscles even after the disappearance of viraemia, which lasted for 6-7 days. Histopathologic studies revealed focal necrosis and inflammation in the skeletal muscle followed by fibrosis and distrophic calcification. Distrophic calcification was also observed in the joint cartilages.

7. Diagnosis of CHIK Virus infection

Since no effective vaccines or therapeutics are available, early detection and proper diagnosis plays the key role in the effective control of the infection. Infant mice inoculation and serological techniques [haemagglutination, Haemagglutination Inhibition assay, complement fixation and neutralization test (NT)] were used effectively in the identification and characterization of viruses (Clarke and Casals 1958; Pavri 1964). The development of immunoglobulin M antibody (IgM) capture enzyme-linked immunosorbent assay (MAC-ELISA) has been a major achievement in serology as it provided a rapid and reliable technique for the diagnosis of arboviruses (Gadkari and Sheikh 1984; Bodemann and Genton 2006). Indirect immunofluorescent antibody technique is another reliable technique for detection and identification of viral antigens from clinical samples (Kuberski and Rosen 1977; Yergolkar *et al* 2006).

In the event of a viral outbreak, the situation warrants rapid detection and identification of the etiological agent. The molecular devices therefore become handy for the detection and characterization. Reverse transcription polymerase chain reaction (RT-PCR) using primers designed for structural and non-structural domains has been found useful in the rapid diagnosis of CHIKV (Pastorino *et al* 2005). The combination of RT-PCR/nested PCR has proved efficient for specific detection and genotyping of CHIKV (Pfeffer *et al* 2002; Hasebe *et al* 2002). Recently, real time RT-PCR, has revolutionized the field with its unique advantages i.e. rapidity, sensitivity, reproducibility and reduced risk of contamination and is being routinely used for detection and quantitation of viruses (Parida *et al* 2008).

8. Therapeutics against CHIK virus

Since no specific drugs are available, supportive treatment for the symptoms i.e. analgesics, antipyretics, anti-inflammatory agents etc is generally administered. Brighton (1984) observed chloroquine phosphate effective for chronic CHIK arthritis as they observed significant improvement in Ritchie articular index and morning stiffness in the patients. The recent outbreak of CHIKV has stimulated renewed interest in developing new antiviral agents. RNAi, the self defense mechanism of eukaryotic cells which specially prevent infection evoked by viruses by selectively shutting off the post transcriptional expression of mRNA in several viruses is emerging as a new strategy to combat virus infections (Tan and Yin 2004; Cullen 2006; McManus *et al* 2007).

9. Vaccines

The widespread geographic distribution and recurrent epidemics causing severe morbidity have necessitated the need for an efficient vaccine. Levitt *et al* (1986) reviewed the developments in vaccine development taken place through sixties and early 1970s. Among the many preparations, the cell culture based formalin inactivated vaccine developed by Harrison *et al* (1971) was the most promising as it elicited high levels of neutralizing antibody in human volunteers in a phase 1 trial. The study also demonstrated excellent immunogenic response without showing any side effects or untoward reactions in the vaccinees making it an ideal candidate for future. Edelman *et al* (2000) pursued the work and developed an attenuated vaccine by serial passaging in MRC-5 cell line. The vaccine was highly immunogenic and well within the tolerable limits. In the phase II safety and immunogenicity study the investigators observed sero-conversion in 98% of the vaccinees by day 28 and the presence of N-antibodies in 85% volunteers for 12 months. Attempts are being made to revive the project by the US Army Medical Research Institute for Infectious Diseases on a request from the French government after the 2005-06 epidemic (Powers and Logue 2007).

10. Prospective study

Until the recent explosive epidemic, CHIKV did not receive much attention due to low mortality, infrequent occurrence and absence in the developed countries. The re-emergence of CHIK in an explosive form has raised many questions that need urgent attention. They are:

(i) Why CHIKV disappeared from India, despite the vector being prevalent?
(ii) In nature, how the virus survives and what factors trigger the outburst?
(iii) Why African genotype replaced the Asian and what would be next?
(iv) Is there any correlation between CHIK activity in the vectors and human?
(v) Was CHIK present as a sporadic disease? If yes, at what level?
(vi) How will the current strain (African genotype) behave in future? What needs to be done in the inter epidemic periods?

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*e*Publication: 15 October 2008