

Original article

### **Chikungunya: A Remerging Arboviral Disease**

Shalley Dahiya<sup>1\*</sup>, Mahesh Kumar<sup>2</sup>, Bijender Singh<sup>3</sup>, Ruchi Dabas<sup>4</sup>, Surender Kumar<sup>1</sup>

<sup>1</sup>Department of Microbiology, BPSGMC, Khanpurkalan, Sonapat, Haryana, India

<sup>2</sup>Department of Paediatrics CMC, Ludhiana, Punjab, India

<sup>3</sup>GH Bahadurgarh, Haryana, India

<sup>4</sup>Department of Ophthalmology, BPSGMC, Khanpurkalan, Sonapat, Haryana, India

\*Corresponding Author: ShalleyDahiya

---

#### **Abstract**

Chikungunya fever is an arboviral disease characterised by triad of fever, rash and polyarthralgia. Chikungunya virus (CHIKV) is transmitted to humans primarily via the bite of an infected mosquito. The virus was first identified some 50 years back. The interest in the research of this disease increased after an important epidemiological outbreak in 2005 on the French metropolitan island of La Reunion. Currently, Chikungunya is spreading in Southeast Asian countries. In this review, we will discuss the virus, vector, clinical features, laboratory diagnosis and management of chikungunya fever.

**Keywords:** Chikungunya; Arbovirus; Aedes; Fever; Rash; Arthralgia.

#### **Introduction**

Chikungunya virus (CHIKV) was first reported in East Africa around five decades ago in 1952-53. It was reported during an epidemic of fever that developed along the border between Tanzania and Mozambique. This fever was again reported as "Dengue-like fever" by M. Robinson and W.H.R. Lumsden in 1955.[1] The name Chikungunya is derived from Makonde, a language spoken in Tanzania. It means "that which bends up" or "stooped over" or "walking bent over" or "bent walker", indicating the posture patients assume with the resulting arthralgia.[2] Due to rapid increase in global travel and environmental changes resulting in increased vector distribution, there is a huge list of new and re-emerging diseases. Thus, posing new hurdles for policy makers, researchers and modern medicine to overcome.

#### **The virus**

Chikungunya virus (CHIKV) is an arthropod borne virus (arbovirus) and belongs to the Alphavirus genus of the Togaviridae family.[3] The study of CHIKV by an electron microscope reveals a characteristic alphavirus morphology. It has a size of 50-70 nm and consists of an icosahedral-like nucleocapsid

surrounded by an envelope which is embedded with viral glycoproteins.[4,5]The genome is about 11.8Kb and has 2 open reading frames(ORF), that encodes a non-structural polyprotein and a structural polyprotein. The non-structural polypeptide is processed to form 4 non-structural proteins. The structural polyprotein cleaves to form protein C, E3, 6K, E2 and E1.The nucleocapsid complexes with multiple copies of a single species of capsid protein C of about 30 kDa. The virion envelope consists of a lipid bilayer which is derived from the plasma membrane of the host cell.Multiple copies of two major virus encoded glycoproteins E1 and E2 are embedded in the virion envelope.[3] Another small peptide, 6K, is associated with virus particles only at a very low level.[6,7] Conventional membrane-spanning anchors located in their C-terminal regions anchors E1 and E2 proteins in the membrane. These two polypeptides form a stable heterodimer. Three E2-E1 heterodimers interact to form the spike that is found on the virus surface. The genome consists of a non-segmented, single-stranded, positive-sense RNA. CHIKV genome transcription and replication occurs in cytoplasm. The virus enters the target cells by endocytosis of clathrin-coated vesicles. The assembled virus particles finally bud through the cell membrane as enveloped virions.[8]

### **Vector and its transmission**

On the basis of differing partial CHIKV E1 protein sequences, three phylogenetically distinct groups with distinct antigenic properties are identified. The three groups are the Asian phylogroup, West African phylogroup and East, Central and Southern African(ECSA) phylogroup. CHIKV can be both endemic and epidemic. It is maintained by sylvatic and human–mosquito–human cycle.[9] In sylvatic cycles, the main reservoirs for CHIKV are mainly non-human primates, rodents, birds and other vertebrates.[10,11,12,13] During epidemics, human beings also become a reservoir. A range of vectors, reservoirs and a local population with high herd immunity is seen in endemic areas.[10] On the other hand, in epidemics, there is just one or two vectors, namely *Aedes.aegypti* and *Aedes.albopictus* with a local population of low herd immunity. For sylvatic cycles, the vectors implicated are *Aedes.furcifer*, *Aedes.taylori*, *Aedes.luteocephalus*, *Culex.ethiopicus*, *Anopheles.Coustani* and *Mansonia.fuscopennata*.[10,13] The main vectors in epidemics are the *Aedes. Aegypti* and *Aedes.albopictus*. Several properties of *A.aegypti* makes it an efficient vector for the chikungunya virus. It is highly susceptible to the virus, prefers to live close to people and seeks a blood meal during the day time. It bites painlessly many people in a short period for one blood meal.[14]The mosquito survives well in urban settings. It typically breeds in clean standing water and collections of water in artificial containers such as tin cans, pots, plastic containers, rain barrels, buckets and discarded tyres.[15]However, now there is data suggestive of *Aedes.albopictus* superseding *Aedes.aegypti* in different areas.[16]The recent 2005-2006 Reunion outbreak was primarily

caused by *Aedes.albopictus*. The virus strain implicated was also associated with a mutation in the E1 envelope gene, where there is a change of alanine to valine at position 226.[17,18] This mutation appeared to provide a selective advantage for replication and transmission via that species. *Aedes.albopictus* was also implicated as the primary vector in the 2007 Italy and 2007 Gabonese outbreaks.[18] *A.albopictus* was the principal vector in the outbreaks in the Indian Ocean islands and *A.aegypti* in the 2006 Indian epidemic.[19] Regional differences in the mosquito species exist: *Anopheles* is a predominant circulating vector species in the rural areas of Orissa and Madhya Pradesh and *A.albopictus* in Tamil Nadu and Southeast Asia.[20-23]

### **Clinical features**

The incubation period for CHIKV is usually 2-4 days but can vary from 1-12 days.[24,25] Viremia lasts for 2-10 days and is on the higher side in newborns and elderly.[25,26] CHIKV infection is mostly symptomatic and asymptomatic infection is reported only in 3-25% of people.[27,28,29] The classical triad of symptoms includes fever, arthralgia and rash distributed on the trunk, limbs and face. The most common clinical features are fever, arthralgia, rash and conjunctivitis. Less common symptoms observed are tenosynovitis, myalgia, headache, retro-orbital pain, pharyngitis, nausea, vomiting, lymphadenopathy, asthenia and dysgeusia.[30,31]

### **Fever**

Fever is usually acute in onset with chills and rigor. It occurs before the rash and joint pain and can be as high as 40°C. It remits after 4-5 days resulting in a "saddleback" fever.[24,32] Convulsions are also reported.[31]

### **Arthralgia**

There is usually symmetrical, migratory, poly-articular arthralgia involving peripheral joints, most commonly, the fingers, wrists, elbows, toes, ankles and knees.[33,34] It may be associated with atypical presentations such as Baker's cyst and hygromas.[34] There is severe pain that is worse in the morning. It improves with mild exercise but exacerbates with strenuous exercise.[35,36] There is great difficulty in sitting, lying down, standing straight as well as walking.[37] Usually the symptoms subside by tenth day of infection but arthralgia may persist for months to years in adults.[36-41] A prospective observational study was done in France in 2007 on 47 imported cases. They reported arthralgia in 80% of cases after 10 days.[34] In the 2005 outbreak of CHIKV in Indian Ocean islands, 69% of infected travellers reported arthralgia for more than 2 months and 13% reported arthralgia for more than 6 months.[40] In two other studies with 88 and 147 participants, 63.6% and 57% of participants reported arthralgia more than 1 year after infection.[38,41]

### **Rash**

The skin lesions are present mainly on the trunk and peripheries but may also be seen on the palms, soles and face. It may not be necessarily pruritic.[31,44,45] The rash is usually similar to the one seen in dengue fever. It shows the islands of sparing and is difficult to differentiate clinically.[46] The rashes usually manifests on day 3-4 but it can occur earlier.[24] It can start as a flush that later develops into a rash which lasts 3-4 days. The rash is usually morbilliform or maculopapular in nature affecting 35.7% of suspected CHIKV-infected cases.[45]

### **Chronic tenosynovitis**

Chronic hypertrophic tenosynovitis is a very common problem in the chronic stage. It may end up as nerve tunnel syndromes in the wrists and ankles. It is associated with peripheral vascular disorders such as Raynaud's phenomenon in some cases.[34]

### **Chronic myalgia**

Patients may also suffer from persistent myalgia.[25,42] By immuno-fluorescence and immuno-peroxidase staining, CHIKV antigens have been isolated in human muscle satellite cells.[43]

### **Atypical presentation**

CHIKV is a benign and non-life-threatening disease. But recent outbreaks reported atypical presentations of the disease. Severe cases of CHIKV were reported for the first time in the Reunion outbreak. It was associated with complications like respiratory failure, heart failure, meningoencephalitis, acute hepatitis, severe skin involvement and kidney failure. Most of the complications were encountered in elderly patients with underlying medical conditions.[47] Reports in the 1960s-1970s from Asia have documented neurological complications of CHIKV infection including seizures, meningitis and encephalitis.[46,48] Other reported complications include petechiae, purpura, epistaxis, mucosal bleeding, hematemesis and melena, myocarditis, arrhythmia, pericarditis and myocardial infarction.[38,49,50]

### **Pregnancy and newborns**

However, most maternal CHIKV infections do not result in vertical transmission, with an overall transmission rate of only about 2.5%. Unfortunately, if delivery occurs during viremia, transmission rate may increase up to around 50%.[51,52]

### **LABORATORY DIAGNOSIS**

Chikungunya virus can be confirmed by laboratory tests, but should be suspected in cases with triad of fever, rash and arthralgia.

Case definition of chikungunya fever as per Who guidelines.[53]

- Clinical criteria: Acute onset of fever > 38°C and severe arthralgia/arthritis not explained by other medical conditions.
- Epidemiological criteria: Residing or having visited epidemic areas, having reported transmission within 15 days prior to the onset of symptoms.
- Laboratory criteria: At least one of the following tests in the acute phase
  - Virus culture, isolation.
  - Presence of viral RNA by RT-PCR.
  - Presence of virus-specific IgM antibodies in single serum sample collected in acute or convalescent stage.
  - Four-fold increase in IgG antibody values in samples collected at least three weeks apart.
- On this basis, cases are to be categorized as:
  - Possible case: A patient meeting clinical criteria.
  - Probable case: A patient meeting both clinical and epidemiological criteria.
  - Confirmed case: A patient meeting the laboratory criteria, irrespective of the clinical presentation.

Infections with CHIKV can be confirmed by the detection of the virus, viral RNA or CHIKV-specific antibodies in patient samples. However, the type of test is decided according to the timing and volume of samples available.

**Detection of the virus:**

CHIKV infections are associated with high levels of viremia (up to  $1 \times 10^6$ -8 plaque-forming units per mL). It typically last for 4–6 days but can persist for up to 12 days after the onset of illness.[26,54] Virus isolation can be done from serum specimens collected during the first 7 days of the illness. The isolation can be done by intra cerebral inoculation of mice and mosquito inoculation. In vitro cell culture methods have comparable sensitivity to *in vivo* methods. These include mosquito cell lines (C6/36) and other mammalian cell lines like BHK-21, HeLa and Vero cells that produce cytopathic effects. The cytopathic effects are confirmed by CHIK-specific antiserum. It takes one to two weeks to give positive result. Virus isolation is carried in BSL-3 laboratories to reduce the risk of viral transmission.[55]

**Molecular methods:**

Molecular tests can detect the viral RNA only during the viremic phase in patients, lasting from day 0 to day 6 after the clinical onset.[56,57,58] The presence of viral RNA is detected using several real-time RT-PCR protocols, targeting the nsP1, E1 and E2 gene.[58] Recently, sensitive and specific one-step Taq-Man

RT-PCR assay is developed as a tool for diagnosis of CHIKV and rapid indicator by quantifying viral load in clinical samples and cell culture supernatant.[57] Real-time loop-mediated isothermal amplification (RT-LAMP) assay has also been found to be a useful molecular tool for rapid diagnosis.[59] Molecular assays give results within 1 day, whereas viral isolation often takes 48 hours.

#### **Serological diagnosis:**

A commercial antigen based detection test is not available for CHIKV, and the ones described thus far in the literature have unclear established performance characteristics.[60,61] CHIKV specific IgM and IgG antibodies are detected in plasma and serum samples from acutely infected and convalescent patients by various classic serological methods. Inhibition of the haemagglutination, complement fixation, immuno-fluorescence (IIF) and immuno-enzymatic assays (ELISA) are few important tests.[62,63] The IgM specific response against CHIKV can be detected starting from two to six days after the onset of symptoms by ELISA and IIF, and could persist for several weeks up to three months.[64] The IgG antibodies can be detected in sera from convalescent stage patients and are present for several years. Demonstration of CHIKV IgM antibodies in acute-phase serum sample or demonstration of a 4-fold increase in CHIKV IgG antibody titre between the acute and convalescent phase serum indicates the infection. However, the drawback is the moderate specificity of these tests due to the antigenic cross-reactivity between CHIKV and other arboviruses such as Dengue virus, o'nyong-nyong virus, Sindbis virus, etc.[65] Other tests available for the detection of CHIKV antibodies include immunofluorescence assays and a plaque reduction neutralization test (PRNT). Immuno-fluorescence assays are sensitive and specific. However, they lack the ability to quantify antibodies, are subjective, and require special equipment and training. PRNTs are quite specific for alphaviruses and are the gold standard for confirmation of serologic test results. The major drawback is the use of live virus. Hence, this test is available only in a limited number of reference laboratories.[66,67]

#### **Future prospects:**

The upcoming techniques in arbovirology are microsphere-based immunoassay (MIA) and microarray technology. MIA is based on detection by flow cytometry of antigen or antibody attached to microspheres or beads. It is a rapid test and can perform in multiplex.[68] Microarray technology focuses on detection of nucleic acid fragments corresponding to different pathogens. It is a useful tool to screen a sample for many pathogens at a time.[69]

#### **TREATMENT**

There is no targeted antiviral treatment for CHIKV infection. The treatment is mainly supportive. However anti-viral treatment plays a crucial role in prophylaxis in vulnerable groups such as the

immunocompromised and for management of severe cases. Main drugs used currently include paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs) for alleviation of symptoms and corticosteroids. Short term and low dose systemic corticosteroids can be initiated during relapse. However, abuse of these drugs can result in severe side effects.[70] Ribavirin coupled with interferons may have a synergistic effect on CHIKV in vitro.[71] However, use of this comes with the difficulty in administration. It is ill suited to use in outbreak on large scale.[72] More research is required for conclusive evidence of the use of ribavirin. Immunotherapy, in the form of human polyclonal antibody can be another modality of treating human infections.[73] Human polyclonal antibodies (CHIKV Ig) are purified from plasma of convalescent donors and used in mouse models of CHIKV infection. CHIKV Ig is associated with both prophylactic and therapeutic potential.[74] This mode of treatment is feasible in areas with high prevalence of CHIKV infection. Large amounts of CHIKV Ig can be produced from plasma donors. It can be used for prophylaxis and treatment of neonates and in persons with underlying medical conditions.

### **VACCINES**

With the increasing numbers of widespread epidemics with significant sequelae, there is a need for an effective vaccine. CHIKV elicits lasting protective immunity. There have been no reports on recrudescence or reinfection and epidemic peaks decrease as the population gains immunity. Development of vaccines inducing similar lasting protective immunity are in the pipeline.[25] Around twenty years ago, a live CHIKV vaccine was developed via 18 serial passages through MRC-5 cell cultures by the U.S. Army but phase II safety trials resulted in transient arthralgia in 8.5% participants.[75,76] Recently three chimeric alphavirus/CHIKV vaccine viruses, VEE/CHIKV, EEE/CHIKV and SIN/CHIKV were created using recombinant DNA technology. They have been used in mice with efficient immune response and offered little reactivity and significant protection.[77]

### **Conclusion**

With the study of literature of previous epidemics, it is clear that CHIKV can spread and infect large proportions of the populations. There is a need to take measures to improve the disease recognition and control the disease transmission by effective vector control programmes. The disease should be notified to higher authorities. Timely sharing of the appropriate information can help control the spread of disease and prevent its outbreak.

### **References**

1. Cavrini F, Gaibani P, Pierro AM, Rossini G, Landini MP, Sambri V. Chikungunya: an emerging and spreading arthropod-borne viral disease. *J Infect Dev Ctries.* 2009; 3(10):744-52.

2. Kondekar S, Gogtay NJ. Why Chikungunya is called Chikungunya. *J Postgrad Med.* 2006;52:307.
3. Strauss JH, Strauss EG. The alphaviruses: gene expression, replication, and evolution. *Microbiol Rev.*1994;58:491-562.
4. Simizu B, Yamamoto K, Hashimoto K, Ogata T. Structural proteins of Chikungunya virus. *J Virol.*1984;51:254-8.
5. Higashi N, Matsumoto A, Tabata K, Nagatomo Y. Electron microscope study of development of Chikungunya virus in green monkey kidney stable (VERO) cells. *Virology.*1967;33:55-69.
6. Gaedigk-Nitschko K, Schlesinger MJ. The Sindbis virus 6K protein can be detected in virions and is acylated with fatty acids. *Virology.*1990;175:274-81.
7. Lusa S, Garoff H, Liljeström P. Fate of the 6K membrane protein of Semliki Forest virus during virus assembly. *Virology.*1991;185:843-6.
8. Chevillon C, Briant L, Renaud F, Devaux C. The Chikungunya threat: an ecological and evolutionary perspective. *Trends Microbiol.*2008;16:80-88.
9. Powers AM, Brault AC, Tesh RB, Weaver SC. Re-emergence of Chikungunya and O'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. *J Gen Virol.*2000;81:471-9.
10. Diallo M, Thonnon J, Traore-Lamizana M, Fontenille D. Vectors of Chikungunya virus in Senegal: current data and transmission cycles. *Am J Trop Med Hyg.*1999;60:281-6.
11. Inoue S, Morita K, Matias RR, Tuplano JV, Resuello RR, Candelario JR, et al. Distribution of three arbovirus antibodies among monkeys (*Macaca fascicularis*) in the Philippines. *J Med Primatol.*2003;32:89-94.
12. Wolfe ND, Kilbourn AM, Karesh WB, Rahman HA, Bosi EJ, Cropp BC, et al. Sylvatic transmission of arboviruses among Bornean orangutans. *Am J Trop Med Hyg.*2001;64(5-6):310-6.
13. McCrae AW, Henderson BE, Kirya BG, Sempala SD. Chikungunya virus in the Entebbe area of Uganda: isolations and epidemiology. *Trans R Soc Trop Med Hyg.*1971;65:152-68.
14. Gibbons RV, Vaughn DW. Dengue: An escalating problem. *BMJ*2002;324:1563-6.
15. Wilder-Smith A, Schwartz E. Dengue in travellers. *N Engl J Med*2005;353: 924-32.
16. Juliano SA, Lounibos LP. Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecol Lett.*2005;8:558-74.
17. Vazeille M, Moutailler S, Coudrier D, Rousseaux C, Khun H, Huerre M, et al. Two Chikungunya isolates from the outbreak of La Reunion (Indian Ocean) exhibit different patterns of infection in the mosquito, *Aedes albopictus*. *PLoS One.*2007;2:e1168.



18. Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. *Microbes Infect.* 2009;11(14-15):1177-85.
19. Yergolkar PN, Tandale BV, Arankalle VA, Sathe PS, Sudeep AB, Gandhe SS, et al. Chikungunya outbreaks caused by African genotype, India. *Emerg Infect Dis* 2006;12:1580-3.
20. Sharma SK, Tyagi PK, Padhan K, Upadhyay AK, Haque MA, Nanda N, et al. Epidemiology of malaria transmission in forest and plain ecotype villages in Sundargarh District, Orissa, India. *Trans R Soc Trop Med Hyg* 2006;100:917-25.
21. Singh N, Shukla MM, Mishra AK, Singh MP, Paliwal JC, Dash AP. Malaria control using indoor residual spraying and larvivorous fish: A case study in Betul, central India. *Trop Med Int Health* 2006;11:1512-20.
22. Paramasivan R, Thenmozhi V, Hiriyan J, Dhananjeyan K, Tyagi B, Dash AP. Serological and entomological investigations of an outbreak of dengue fever in certain rural areas of Kanyakumari district, Tamil Nadu. *Indian J Med Res* 2006;123: 697-701.
23. Gratz NG. Critical review of the vector status of *Aedes albopictus*. *Med Vet Entomol* 2004;18:215-27.
24. Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. I. Clinical features. *Trans R Soc Trop Med Hyg.* 1955;49:28-32.
25. Kam YW, Ong EK, Rénia L, Tong JC, Ng LF. Immuno-biology of Chikungunya and implications for disease intervention. *Microbes Infect* 2009;11(14-15):1186-96.
26. Laurent P, Le Roux K, Grivard P, Bertil G, Naze F, Picard M, et al. Development of a sensitive real-time reverse transcriptase PCR assay with an internal control to detect and quantify chikungunya virus. *Clin Chem.* 2007;53:1408-14.
27. Gérardin P, Guernier V, Perrau J, Fianu A, Le Roux K, Grivard P, et al. Estimating Chikungunya prevalence in La Réunion Island outbreak by serosurveys: two methods for two critical times of the epidemic. *BMC Infect Dis.* 2008;8:99.
28. Queyriaux B, Simon F, Grandadam M, Michel R, Tolou H, Boutin JP. Clinical burden of chikungunya virus infection. *Lancet Infect Dis.* 2008;8:2-3.
29. Sissoko D, Moendandze A, Malvy D, Giry C, Ezzedine K, Solet JL, et al. Seroprevalence and risk factors of chikungunya virus infection in Mayotte, Indian Ocean, 2005-2006: a population-based survey. *PLoS One.* 2008;3:e3066.
30. Couderc T, Lecuit M. Focus on Chikungunya pathophysiology in human and animal models. *Microbes Infect.* 2009;11(14-15):1197-1205.

31. Borgherini G, Poubeau P, Staikowsky F, Lory M, Le Moullec N, Becquart JP, et al. Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. *Clin Infect Dis.*2007;44:1401-7.
32. Powers AM, Logue CH. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. *J Gen Virol.*2007;88:2363-77.
33. Tesh RB. Arthritides caused by mosquito-borne viruses. *Annu Rev Med.*1982;33:31-40.
34. Simon F, Parola P, Grandadam M, Fourcade S, Oliver M, Brouqui P, et al. Chikungunya infection: an emerging rheumatism among travellers returned from Indian Ocean islands. Report of 47 cases. *Medicine (Baltimore).*2007;86:123-37.
35. Chhabra M, Mittal V, Bhattacharya D, Rana U, Lal S. Chikungunya fever: a re-emerging viral infection. *Indian J Med Microbiol.*2008;26:5-12.
36. Brighton SW, Prozesky OW, de la Harpe AL. Chikungunya virus infection. A retrospective study of 107 cases. *S Afr Med J.*1983;63:313-5.
37. Sudeep AB, Parashar D. Chikungunya: an overview. *J Biosci.*2008;33:443-9.
38. Borgherini G, Poubeau P, Jossaume A, Gouix A, Cotte L, Michault A, et al. Persistent arthralgia associated with chikungunya virus: a study of 88 adult patients on reunion island. *Clin Infect Dis.*2008;47(4):469-75.
39. Fourie ED, Morrison JG. Rheumatoid arthritic syndrome after chikungunya fever. *S Afr Med J.* 1979;56:130-2.
40. Taubitz W, Cramer JP, Kapaun A, Pfeiffer M, Drosten C, Dobler G, et al. Chikungunya fever in travellers: clinical presentation and course. *Clin Infect Dis.* 2007;45:e1-4.
41. Sissoko D, Malvy D, Ezzedine K, Renault P, Moschetti F, Ledrans M, et al. Post-epidemic Chikungunya disease on Reunion Island: course of rheumatic manifestations and associated factors over a 15-month period. *PLoS Negl Trop Dis.* 2009;3:e389.
42. Jaffar-Bandjee MC, Das T, Hoarau JJ, Krejbich-Trotot P, Denizot M, Ribera A, et al. Chikungunya virus takes centre stage in virally induced arthritis: possible cellular and molecular mechanisms to pathogenesis. *Microbes Infect.*2009;11(14-15):1206-18.
43. Ozden S, Huerre M, Riviere JP, Coffey LL, Afonso PV, Mouly V, et al. Human muscle satellite cells as targets of Chikungunya virus infection. *PLoS One.* 2007;2:e527.
44. Hochedez P, Jaureguiberry S, Debryne M, Bossi P, Hausfater P, Brucker G, et al. Chikungunya infection in travelers. *Emerg Infect Dis.*2006;12:1565-7.

45. Prashant S, Kumar AS, Basheeruddin DD, Chowdhary TN, Madhu B. Cutaneous manifestations in patients suspected of chikungunya disease. *Indian J Dermatol.*2009;54:128-31.
46. Nimmannitya S, Halstead SB, Cohen SN, Margiotta MR. Dengue and chikungunya virus infection in man in Thailand, 1962-1964.I. Observations on hospitalized patients with hemorrhagic fever. *Am J Trop Med Hyg.*1969;18:954-71.
47. Renault P, Solet JL, Sissoko D, Balleydier E, Larrieu S, Filleul L, et al. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005-2006. *AmJ Trop Med Hyg.*2007;77:727-31.
48. Carey DE, Myers RM, DeRanitz CM, Jadhav M, Reuben R. The 1964 chikungunya epidemic at Vellore, South India, including observations on concurrent dengue. *Trans R Soc Trop Med Hyg.*1969;63:434-45.
49. Economopoulou A, Dominguez M, Helynck B, Sissoko D, Wichmann O, Quenel P, et al. Atypical Chikungunya virus infections: clinical manifestations, mortality and risk factors for severe disease during the 2005-2006 outbreak on Réunion. *Epidemiol Infect.* 2009;137:534-41.
50. Lemant J, Boisson V, Winer A, Thibault L, André H, Tixier F, et al. Serious acute chikungunya virus infection requiring intensive care during the Reunion Island outbreak in 2005-2006. *Crit Care Med.*2008;36:2536-41.
51. Gérardin P, Barau G, Michault A, Bintner M, Randrianaivo H, Choker G, et al. Multidisciplinary prospective study of mother-to-child chikungunya virus infections on the island of La Réunion. *PLoS Med.*2008;5:e60.
52. Ramful D, Carbonnier M, Pasquet M, Bouhmani B, Ghazouani J, Noormahomed T, et al. Mother-to-child transmission of Chikungunya virus infection. *Pediatr Infect Dis J.*2007;26:811-15.
53. World Health Organization. Regional office for South-East Asia. Guidelines for prevention and control of Chikungunya fever. c2009-[cited 2012 Jul 15]. Available from: [http://www.searo.who.int/Link Files/Publication\\_SEA-CD-182.pdf](http://www.searo.who.int/LinkFiles/Publication_SEA-CD-182.pdf)
54. Lanciotti RS, Kosoy OL, Laven JJ, Panella AJ, Velez JO, Lambert AJ, et al. Chikungunya virus in US travelers returning from India, 2006. *Emerg Infect Dis* 2007; 13:764–7.
55. Shah KV, Gibbs CJ Jr, Banerjee G. Virological investigation of the epidemic of haemorrhagic fever in Calcutta: Isolation of three strains of Chikungunya virus. *Indian J Med Res* 1964;52:676-83.
56. Pastorino B, Muyembe–Tamfum JJ, Bessaud M, Tock H, Durand JP, Peyrefitte CN. Epidemic resurgence of chikungunya virus in Democratic Republic of the Congo: identification of a new central African strain. *J Med Virol*2004;74: 277-82.

57. Pastorino B, Bessaud M, Grandadam M, Murri S, Tolou HJ, Peyrefitte CN. Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African chikungunya viruses. *J Virol Methods*. 2005; 124:65-71.
58. Pfeffer M, Linssen B, Parke MD, Kinney RM. Specific detection of chikungunya virus using a RT-PCR/nested PCR combination. *J Vet Med B Infect Dis Vet Public Health*. 2002;49:49-54.
59. Lakshmi V, Neeraja M, Subbalaxmi MV, Parida MM, Dash PK, Santhosh SR, et al. Clinical features and molecular diagnosis of Chikungunya fever from South India. *Clin Infect Dis* 2008;46:1436-42.
60. Kashyap RS, Morey SH, Ramteke SS, Chandak NH, Parida M, Rao PV, et al. Diagnosis of chikungunya fever in an Indian population by an indirect enzyme-linked immunosorbent assay protocol based on an antigen detection assay: a Prospective cohort study. *Clin Vaccine Immunol*,2010;17(2):291–7.
61. Shukla J, Khan M, Tiwari M, Sannarangaiah S, Sharma S, Rao PV, et al. Development and evaluation of antigen capture ELISA for early clinical diagnosis of chikungunya. *Diagn Microbiol Infect Dis*.2009;65(2):142–9.
62. Hunderkar SL, Thakare JP, Gokhle MD, Barde PV, Argade SV, MouryaDT. Development of monoclonal antibody based antigen capture ELISA to detect chikungunya virus antigen in mosquitoes. *Indian J Med Res*. 2002;115:144-48.
63. Thein S, La Linn M, Aaskov J, Aung MM, Aye M, Zaw A Myint A. Development of a simple indirect enzyme-linked immunosorbent assay for the detection of immunoglobulin M antibody in serum from patients following an outbreak of chikungunya virus infection in Yangon, Myanmar. *Trans R Soc Trop Med Hyg*. 1992;86:438-42.
64. Sam IC and AbuBakar S (2006) Chikungunya virus infection. *Med J Malaysia*. 2006;61:264-9.
65. Blackburn NK, Besselaar TG, Gibson G. Antigenic relationship between chikungunya virus strains and o'nyongnyong virus using monoclonal antibodies. *Res Virol*.1995;146:69-73.
66. Litzba N, Schuffenecker I, Zeller H, Drosten C, Emmerich P, Charrel R, et al. Evaluation of the first commercial chikungunya virus indirect immunofluorescence test. *J Virol Methods* 2008; 149:175–9.
67. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institutes of Health. *Arboviruses and Related Zoonotic Viruses In: Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition*. Washington DC: US Government Printing Office, 2007.

68. Johnson AJ, Noga AJ, Kosoy O, Lanciotti RS, Johnson AA, Biggerstaff BJ. Duplex microsphere-based immunoassay for detection of anti-West Nile virus and anti- St. Louis encephalitis virus immunoglobulin M antibodies. *ClinDiagn Lab Immunol* 2005;12(5):566–74.
69. Hunsperger EA, Yoksan S, Buchy P et al. Evaluation of commercially available diagnostic tests for the detection of dengue virus NS1 antigen and anti-dengue virus IgM antibody. *PLoS Neglected Tropical Diseases*. 2014;8(10):Article ID e3171.
70. Mohan A. Chikungunya fever: clinical manifestations & management. *Indian J Med Res*. 2006;124:471-4.
71. Briolant S, Garin D, Scaramozzino N, Jouan A, Crance JM. In vitro inhibition of Chikungunya and Semliki Forest viruses replication by antiviral compounds: synergistic effect of interferon-alpha and ribavirin combination. *Antiviral Res*. 2004;61:111-7.
72. De Lamballerie X, Ninove L, Charrel RN. Antiviral treatment of chikungunya virus infection. *Infect Disord Drug Targets*. 2009;9:101-4.
73. Dunman PM, Nesin M. Passive immunization as prophylaxis: when and where will this work? *Curr Opin Pharmacol*. 2003;3:486-96.
74. Couderc T, Khandoudi N, Grandadam M, Visse C, Gangneux N, Bagot S, et al. Prophylaxis and therapy for Chikungunya virus infection. *J Infect Dis*. 2009;200:516-23.
75. Levitt NH, Ramsburg HH, Hasty SE, Repik PM, Cole FE, Lupton HW. Development of an attenuated strain of chikungunya virus for use in vaccine production. *Vaccine*. 1986;4:157-62.
76. Edelman R, Tacket CO, Wasserman SS, Bodison SA, Perry JG, Mangiafico JA. Phase II safety and immunogenicity study of live chikungunya virus vaccine TSI-GSD-218. *Am J Trop Med Hyg*. 2000;62:681-5.
77. Wang E, Volkova E, Adams AP, Forrester N, Xiao SY, Frolov I, et al. Chimeric alphavirus vaccine candidates for chikungunya. *Vaccine*. 2008;26:5030-9.