# A REVIEW ON CHIKUNGUNYA VIRUS

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**ABSTRACT**
Mosquitoes transmit numerous arboviruses including dengue and chikungunya virus (CHIKV). Chikungunya is a re-emerging arthropod-borne viral disease caused by Chikungunya virus (CHIKV) belonging to the Togaviridae family of genus Alphavirus. It is a virus with a single stranded, positive sense RNA, as its genome. It is maintained in a sylvatic and urban cycle involving humans and the mosquito species Aedes aegypti and Aedes albopictus. It has a major health impact on humans as it causes fever, rashes, arthralgia and myalgia. Polyarthralgia is the most important feature of CHIKV infection which primarily affects the small joints of the wrists and fingers along with the large joints like shoulders and knees. Currently, there are no vaccines or treatment regimens available for CHIKV infection. The molecular mechanism underlying the chronic polyarthralgia observed in patients is not well understood. The abundance of bacteria from the Enterobacteriaceae family increased with CHIKV infection whereas the abundance of known insect endosymbionts like Wolbachia and Blattabacterium decreased. In this review we have summarized the CHIKV organization, replication, epidemiology, clinical manifestations and pathogenesis with emphasis on the arthralgia.
**Keywords** Aedes albopictus, Bacterial community, Wolbachia, Chikungunya, microarray, quantitative PCR.

## INTRODUCTION
The ability of RNA viruses to emerge and cause human disease often reflects their ability to exploit new ecologic contacts and rapidly adapt to new amplification hosts or vectors. These adaptations can lead to the expansion of viral ecological niches and often facilitate introductions into new geographic ranges. Numerous examples have demonstrated that this emergence process often relies on the acquisition of only one or a few adaptive mutations that allow RNA viruses to overcome host-specific barriers.[1-4]

Chikungunya virus (CHIKV: Togaviridae: Alphavirus) is an envelope, single-stranded, positive-sense RNA virus transmitted by mosquito vectors. CHIKV is endemic to both Africa and Asia, although transmission cycles differ considerably on these continents. CHIKV is primarily maintained in Africa via a zoonotic sylvatic cycle that relies on nonhuman primates as reservoir hosts and arboreal, primatophilic Aedes (Stegomyia) spp. mosquitoes (e.g., Aedes furcifer and Aedes africanus).[5] In contrast, in Asia humans serve as the primary hosts of CHIKV, with Aedes aegypti traditionally serving as the primary vector in most urban epidemics.[6] Phylogenetic studies and historical analyses have indicated that major epidemics in India and Southeast Asia resulted from the introduction of CHIKV from Africa beginning as early as the 18th century and continuing into the 21st century.[7] Although there is no evidence that CHIKV persisted in Asia following a well-documented outbreak in 1788, an introduction that occurred around 1950 or earlier resulted in an endemic Asian lineage of CHIKV that still persists there. Among several factors associated with the recent emergence and spread of CHIKV, a prominent role has been attributed to the adaptation of the emerging IOL strains to the mosquito, Aedes albopictus, previously considered to be only a secondary vector. Unlike past epidemics mediated by Ae. aegypti, Ae. albopictus has served as the primary CHIKV vector during the majority of recent outbreaks, including those on several islands of the western Indian Ocean, parts of India, Singapore, Malaysia, Thailand, Sri Lanka, Gabon, and Italy.[9,11] Phylogenetic and epidemiologic studies indicate that CHIK outbreaks in regions highly infested with Ae. albopictus were always associated with an alanine-to-valine substitution in the E1 envelope glycoprotein (E1-A226V), which was selected convergently by different CHIKV lineages.[8,9,10]

### Clinical Presentation Of CHIKV Infection
CHIKV infection in humans usually causes a nonlethal, self-limiting, febrile illness. Until recently, much of our knowledge of human disease has been descriptive. Patients developed viremias that could exceed $10^9$ mouse $LD_{50}$/ml and last for 4 days, and displayed nonfatal symptoms of variable severity that could persist for many months.[12,13] Chikungunya fever is usually characterized by high fever, arthralgia, and rash. During the acute phase, patients can experience painful and disabling arthritis that can last for 7 days. During some outbreaks, two-thirds of patients have had to be hospitalized.[14] Unfortunately, after the acute phase, polyarthritis can be recurrent and may persist for up to several years after infection. Other symptoms of CHIKV infection can include retro-orbital pain, neurological and hemorrhagic manifestations, and myocarditis. In the most recent epidemics, many nontypical symptoms were reported for the first time, including lethal hepatitis, encephalitis, maternal-fetal transmission, and an increased death rate.

### Mouse Models Of CHIKV
As with dengue fever, the lack of a good animal model replicating human chikungunya fever symptoms and pathology has been a major obstacle for understanding the course of infection, spectrum of disease severity, and persistence of symptoms. A mouse model for RRV, predating the recent work with CHIKV, is characterized by hind limb dragging due to the destruction of the skeletal muscle.[15] Multiple CHIKV mouse models have been proposed, but they are either unable to provide a good working background for vaccine-related testing or unsuccessful in reproducing true

human-like symptoms. Subcutaneous injections of CHIKV in young outbred mice, although they induce disease similar in symptomology to CHIKV infection in humans, are not good platforms for the study of vaccine efficacy.[16] Intranasal inoculation of CHIKV into inbred mice results in neurological involvement and high mortality,[17] symptoms not seen in human CHIKV infection. Other models using immunodeficient mice, including IFN-deficient mice, partially mimic human disease, but are not good models for vaccine testing.[18]

## Signs And Symptoms

The incubation period of chikungunya disease is from two to five days. Its symptoms include a fever up to 40 °C (104 °F), a petechial or maculopapular rash of the trunk and occasionally the limbs, and arthralgia or arthritis affecting multiple joints.[19] Other nonspecific symptoms can include headache, conjunctivitis, and slight photophobia. Typically, the fever lasts for two days and then ends abruptly. However, other symptoms—namely joint pain, intense headache, insomnia and an extreme degree of prostration—last for a variable period; usually for about five to seven days. Patients have complained of joint pains for much longer time periods; some as long as two years, depending on their age.[20, 21]

## Pathophysiology

Human epithelial and endothelial cells, primarily fibroblasts and monocyte-derived macrophages, are susceptible to infection. Lymphoid and monocytoid cells, primary lymphocytes and monocytes and monocyte-derived dendritic cells are not susceptible to infection. Viral entry occurs through pH-dependent endocytosis. Infection is cytopathic and associated with the induction of apoptosis in the infected cell. Infection is highly sensitive to the antiviral activity of type I and II interferon.

## Prevention

The most effective means of prevention are protection against contact with the disease-carrying mosquitoes and mosquito control. These include using insect repellents with substances such as DEET (N,N-diethyl-meta-toluamide; also known as N,N'-diethyl-3-methylbenzamide or NNDB), icaridin (also known as picaridin and KBR3023), PMD (p-menthane-3,8-diol, a substance derived from the lemon eucalyptus tree), or IR3535. Wearing bite-proof long sleeves and trousers (pants) also offers protection. In addition, garments can be treated with pyrethroids, a class of insecticides that often has repellent properties. Vaporized pyrethroids (for example in mosquito coils) are also insect repellents. Securing screens on windows and doors will help to keep mosquitoes out of the house. In the case of the day-active Aedes aegypti and Aedes albopictus, however, this will have only a limited effect, since many contacts between the vector and the host occurs outside.

## Vaccine Development

A virus-like particle-based vaccine has protected monkeys from chikungunya virus infection, and passive immunization from these monkeys protected immunodeficient mice against exposure to a dose of virus that would otherwise be lethal, demonstrating the humoral response was highly protective.[22] A DNA vaccine candidate is also being tested. The vaccine cassette was designed based on CHIKV capsid and envelope specific consensus sequences with several modifications, including codon optimization, RNA optimization, the addition of a Kozak sequence, and a substituted immunoglobulin E leader sequence. These constructs induced humoral and cellular immune responses in mice.[23]

## Diagnosis

Common laboratory tests for chikungunya include RT-PCR, virus isolation, and serological tests.

Virus isolation provides the most definitive diagnosis, but takes one to two weeks for completion and must be carried out in biosafety level 3 laboratories. The technique involves exposing specific cell lines to samples from whole blood and identifying chikungunya virus-specific responses.

RT-PCR using nested primer pairs is used to amplify several chikungunya-specific genes from whole blood. Results can be determined in one to two days.

Serological diagnosis requires a larger amount of blood than the other methods, and uses an ELISA assay to measure chikungunya-specific IgM levels. Results require two to three days, and false positives can occur with infection via other related viruses, such as o'nyong'nyong virus and Semliki Forest virus.

## Epidemiology

Blood samples were collected from 1,938 suspected case-patients from the 3 states; serum was separated and transported to the laboratory on wet ice. Adult mosquitoes were collected from houses and sheds. Larval mosquitoes were collected from the affected areas by single-larva survey method. Adult household indexes and Breteau indexes were calculated for each area. The C6/36 cell line was used for virus isolation. Immunoglobulin M antibodies to chikungunya virus and dengue virus were assayed by IgM capture ELISA. For chikungunya virus ELISA, brain suspensions from mice infected with the virus were the source of antigen, and monoclonal antibodies were the source of antibodies. Dengue/chikungunya virus IgM antibodies and negative control human sera were included for respective tests. Approval for use of mice for antigen preparation was obtained from the institutional ethical committee according to national guidelines. Immunofluorescence assay was used to detect the virus in cell culture and in crushed heads of adult mosquitoes. A patient with the following was confirmed as having chikungunya virus infection: acute onset of moderate-to-high fever with joint pain of varying severity; negative test results for malaria, typhoid, and tuberculosis; and positive results for IgM anti-chikungunya virus antibodies, chikungunya virus isolation.

We used the $\chi^2$ test to compare proportions of cases in different age groups. We studied chikungunya virus isolates obtained during current investigations and viruses isolated during earlier epidemics in India from 1963 to 2000). RNA was isolated by a commercially available viral RNA mini kit according to the manufacturer's instructions. An RNA polymerase was used for reverse transcription at 42° Celsius for 1 hour. Initially, Alphavirus genus–specific primers of 26 and 25 nucleotides produced a 472-base pair fragment (the NS4 gene). The second set of primers of 18 and 19 nucleotides amplified a 294-base pair product of E1 gene. Acute onset of moderate-to-high fever in association with body ache, backache, and headache was recorded. Joint pain of varying severity occurred within 2 days of onset of fever and, in decreasing order of affliction, involved knees, ankles, wrists, hands, and feet. Joint pain was severe and incapacitating and lasted for weeks to months. Inflammation of joints and transient macular rash on earlobes, neck, trunk,

and upper extremities were reported for a few patients. Hemorrhage did not occur. The cases were reported predominantly from rural areas; distribution was focal. Multiple cases were recorded in families. All ages and both sexes were affected; significantly more cases occurred in persons aged 15 years or older (that's 299 or 89.8% of 333). Cases were reported from 11 of 23 districts in Andhra Pradesh, 15 of 27 in Karnataka, and 16 of 35 in Maharashtra. State governments of Andhra Pradesh, Karnataka, and Maharashtra have declared outbreaks of chikungunya virus. By mid-April, the declared numbers of fever cases associated with this outbreak were greater than 25,000 in Andhra Pradesh, greater than 65,000 in Maharashtra, and greater than 36,000 in Karnataka. In absence of active surveillance for this disease, these numbers may be underestimates. The predominant mosquito species in the affected areas was Aedes aegypti. Aedes albopictus was either absent or present in negligible numbers. The population of Aedes aegypti was reasonably high in most of the localities; adult household indexes and Breteau indexes, respectively, were 10 to 60 and 13 to 75 in Andhra Pradesh, 20 to 70 and 40 to 200 in Karnataka, and 10 to 30 and 30 to 50 in Maharashtra. High density of Aedes aegypti populations in affected areas and 23 isolations or detections of chikungunya virus from adult mosquitoes indicate that this species is the main vector in India. Earlier outbreaks in India were mainly restricted to large cities; in contrast, the current outbreak is predominantly rural.

Anti-chikungunya virus IgM was detected in 33.5 to 41.9% of patients tested. The finding of antibodies to dengue virus in 0.9 to 9.9% of patients and to chikungunya virus and dengue virus in 0.4 to 4.3% of patients indicates that these viruses cocirculate in the area. Nine patients whose acute-phase serum sample was negative had anti-chikungunya virus IgM in the early convalescent-phase sample, collected during the second week of illness.

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