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Review

# Chikungunya virus infections in Brazil: learning from the recent outbreaks and disseminating this knowledge to prevent arboviral outbreaks elsewhere

**Danillo Lucas Alves Esposito, Marcus Vinicius Gomes da Silva and Benedito Antônio Lopes da Fonseca***

Department of Internal Medicine, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

## ABSTRACT

Chikungunya virus (CHIKV) is an arbovirus transmitted by *Aedes* sp. mosquitoes that results in a disease characterized mainly by polyarthralgia and high fever. The virus was first detected in Brazil in 2014 and since then, thousands of cases were reported in the country resulting in a considerable amount of deaths associated with the virus. In this review, we summarize all aspects of chikungunya virus infection and the characteristics of the recent outbreak in Brazil, discuss some complications triggered by this infection, as well as the available diagnostic options and some alternatives for treatment of the disease, and prevention of future epidemics of chikungunya virus. The dissemination of knowledge acquired by studying such diseases is important to devise strategies to face outbreaks occurring elsewhere.

**KEYWORDS:** chikungunya virus, outbreak, mayaro, diagnosis, arbovirus.

## INTRODUCTION

Chikungunya disease results from infection by an arbovirus (***ar***thropod-***bo***rne ***virus***) belonging to the genus *Alphavirus* (*Togaviridae* family), and is transmitted to humans mainly by the bite of the female *Aedes* mosquito (*Ae. aegypti* and *Ae. albopictus*). The virus is usually found in nature in either a sylvatic or enzootic cycle maintained mainly by non-human primates as its host, but in the last decade it has established an urban cycle (human-mosquito-human) in several countries. Like other alphaviruses, chikungunya virus (CHIKV) is a spherical, enveloped virus of approximately 70 nm, with a genome that is composed by a single-stranded positive-sense RNA [1].

The infection process begins with the virus entering a susceptible cell through the interaction of cellular receptors and viral-encoded envelope E2 protein. Within the endosome, the acidic environment induces a breakdown of the E2-E1 dimer, exposing the fusion loop located in the distal region of the E1 protein and determining the fusion of the viral and endosomal membranes, allowing the capsid to penetrate into the cytoplasm, which is rapidly unfolded, releasing viral RNA in the cytoplasm. The RNA genome is transcribed into 7 proteins (and two peptides) from subgenomic RNAs expressing two open-reading frames: one for the non-structural genes (nsP1-4) and another for structural genes (capsid, envelope proteins E3-E2, 6k linker and E1) [2]. Translation of virus RNA begins with the synthesis of non-structural proteins, as an early stage of viral replication, followed by the generation of a subgenomic RNAs that express the structural protein genes, which will be the constituents of the virus particle. The resulting polyproteins are cleaved by host and virus proteases. Structural

*Corresponding author: baldfons@fmrp.usp.br

proteins are expressed in membranous structures of the cytoplasm (viral factories), where the capsid assembly takes place. The maturation of envelope proteins occurs in the endoplasmic reticulum and Golgi complex as they are exported to the cell membrane where the viral particles are assembled by budding from host cell membrane [2, 3].

After the establishment of infection in a susceptible host, the virus goes through an incubation period of 2-4 days that ends with the symptomatic phase of the disease. The symptoms are characterized by a high fever (above 39 ºC), which starts usually on the first day of disease onset, myalgia, conjunctivitis, joint edema, non-pruritic maculopapular rash and arthralgia (present in almost 90% of patients and very characteristic of this disease) [4]. This is referred to as the acute phase of the disease and coincides with a viremic period that lasts about 5 days. With the exception of arthralgia, which may persist for months, the symptoms of the disease disappear a few days after the disease onset. The majority of cases of chikungunya are usually symptomatic, with few reports of asymptomatic cases.

**Epidemiology and recent outbreak in Brazil**

CHIKV, first isolated in 1952 in Tanzania, is typically found in tropical and subtropical countries of the world. The word chikungunya comes from the Makonde dialect (or Kimakonde) meaning “that which bends up”, with reference to the posture of patients due to intense arthralgia [5]. The virus has a single serotype, but four genotypic variants – Asian, West African, East/Central/South African (ECSA) and the Indian Ocean Lineage (IOL), which is a new variant that emerged on Réunion Island [6]. It is of interest to note that IOL acquired a mutation (E1-226A) that gives it a selective advantage to replicate in mosquitoes [6].

After its isolation, the virus has been reported in several parts of the world, such as Africa, Europe, Asia and the Americas. The first chikungunya fever epidemic took place in the African continent, from where it was imported to the Asian continent in the early 1960s and to India in the 1960s and 1970s [7]. In 2004, a major outbreak of the disease broke out in Kenya (East Africa), spreading then to Southeast Asia and India [5]. Between 2005 and 2006, the Réunion Island, a French Indian Ocean District, experienced a large outbreak of chikungunya disease, with about 224,000 cases and 203 deaths related to this infection [8].

In Brazil, the first cases of chikungunya infection date from 2010 and all three cases were imported from travelers who were infected abroad. In August 2010, a 41-year-old male from Rio de Janeiro returned from Indonesia with high fever and polyarthralgia with the presence of IgM (Immunoglobulin M) antibodies against CHIKV, but negative to all other arboviruses [9]. Again in August 2010, a 55-year-old man who had also been to Indonesia presented with symptoms comparable to chikungunya fever, also tested positive for CHIKV antibodies. Although other people traveling in the same flight also presented fever and arthralgia, no data on their diagnosis is available. In October 2010, another diagnosis of chikungunya was made in a 25-year-old woman returning from India and presenting with fever, skin rash, malaise and joint pain, and was positive for CHIKV IgM antibodies 10 days after the onset of symptoms [10].

The first autochthonous chikungunya cases in the Americas were reported in late 2013, mainly in the Caribbean islands of Saint Martin, Martinique, and Guadeloupe. Those cases created a big concern in the Americas due to the wide geographic distribution of the main transmission vector for this infection, the *Aedes* mosquito [11]. After the first reported cases in the Caribbean islands, it did not take long for the virus to arrive in Brazil. In the same year, a major epidemic began to spread throughout the country, with cases reported mainly in the north and northeast regions of Brazil [12, 13]. In September 2014, in the city of Oiapoque, Amapá state, in the extreme north of Brazil, the first case of autochthonous transmission of CHIKV was reported. The CHIKV outbreak that occurred in Oiapoque was caused by the Asian variant of the virus, which probably entered the country from the Caribbean Islands, where the same variant was circulating. At the same time, another epidemic of the virus was occurring in the northeast of Brazil (Feira de Santana – Bahia state), but caused by a different variant, the East/Central/South African (ECSA), resulting in thousands of reported cases. It is believed that the index case-patient for this outbreak was a Brazilian citizen returning from Angola, as all epidemiological investigations detected

a higher incidence of the disease in the neighborhood where this patient lived compared to other regions of the city where the virus was initially detected [14]. Therefore, it is possible that at least two variants of CHIKV are currently circulating in Brazil.

According to the Brazilian Ministry of Health, on its September 2017 Epidemiological Bulletin, three years after its introduction in Brazil more than 474,000 cases of CHIKV infection had been reported with autochthonous cases in all regions of the country. The incidence of cases by region (number of cases per 100 thousand inhabitants) shows that, in 2016 and early 2017, the regions with the highest incidence were the Northeast and the Northern regions, respectively. In 2017, the Northern and Midwestern regions experienced an increase in the number of reported cases, more than double the cases in comparison to 2016. The region with the lowest number of cases was the Southern region [15], probably due to the cooler climate and lower infestation of *Aedes* mosquitoes. However, the total number of reported cases of chikungunya and others arboviruses decreased in 2017, especially those caused by Zika and dengue viruses (a drop of 95.7 and 85% respectively). Also, a fewer percentage of CHIKV cases (38%) was reported in 2017 compared to the previous year [15], but the decrease was not as intense as for other arboviruses, probably due to the higher spread of this infection to other parts of the countries compared to other viruses transmitted by the *Aedes* mosquitoes, since this vector is widely distributed in Brazil. In addition, the global importance of the outbreak caused by the Zika virus, with its associated neurological complications (microcephaly in neonates and Guillain-Barré syndrome in adults), as well as a more pronounced vector control policy implemented by the country may have contributed to the low number of reported cases.

**Severe and adverse outcomes associated with CHIKV infections**

The classic symptoms of CHIKV disease are abrupt fever and joint pain, but other occasional symptoms may occur including myalgia, headache, nausea, and maculopapular rash [16]. In fact, the debilitating joint impairment is a clinical feature that can distinguish CHIKV from other arbovirus infections and, although some patients recover completely after a few days of disease onset, in other patients joint pain may persist for a long time, even years after infection. During the outbreak that occurred in the Réunion Island-France, between 2005 and 2006, several other symptoms were associated with CHIKV infections, such as severe hepatitis, respiratory and cardiac problems, renal failure, meningoencephalitis, Guillain-Barré Syndrome and other neurological disorders [8, 17]. Besides that, several deaths were reported due to CHIKV infection, with a high mortality rate in that region (1.2% of overall confirmed cases) and an increased incidence of mortality rate related with age [8]. Rajapakse *et al.* have also described a series of severe complications caused by CHIKV infection, such as neurological (meningoencephalitis, Guillain-Barré syndrome, neuropathies), cardiovascular (myocarditis, arrhythmias), and renal (nephritis) diseases among other health impairments that could influence the mortality rate due to the disease [18].

In Brazil, due to the association between microcephalic babies and Zika virus infections, which led to a global awareness about these infections, such CHIKV severe outcomes may have been overlooked. However, according to official epidemiological bulletins [15], chikungunya infections had a higher mortality rate when compared to other arbovirus infections (0.1% of overall suspected cases compared to 0.06% and 0.02% from dengue and Zika suspected cases, respectively) in the same period (2015-2017). Although these numbers are smaller than those observed on the Réunion Island outbreak, they are worrisome. On the other hand, we have to be cautious analyzing the difference between both outbreaks because the mortality data from Réunion Island were calculated taking into consideration the confirmed cases while in Brazil they were calculated based on suspected cases and up to this date, nearly half of the suspected cases do not have laboratory confirmation.

There are several reports of complications after CHIKV infection in Brazil and Latin America, such as vertical transmission of the virus [19], neonates with neurological involvement [20], deaths [21], encephalitis [22-25], meningoencephalitis [26], prolonged joint disease and myositis [27, 28], and other neuropathies, such as Carpal Tunnel Syndrome [29] and Guillain-Barré Syndrome [22].

**Advances in diagnosis, treatment and prophylactic measures**

The correct diagnosis of arbovirus infections represents a major challenge in countries where more than one of those pathogens co-circulate, especially when they produce similar outcomes and are close phylogenetically. For CHIKV, which has a short viremia, the recommendation for a specific laboratory diagnosis is the use of RT-PCR (Reverse Transcription Polymerase Chain Reaction) during the acute phase of infection (up to 5 days after the onset of symptoms) and serological tests of great sensitivity for the convalescent phase, usually an ELISA or a similar technique.

The sensitivity of the commercial tests for CHIKV diagnosis was evaluated by CDC (Centers for Disease Control and Prevention) and summarized by Johnson *et al*. [30], showing that about 80% of the commercial tests for serological detection of CHIKV did not present good sensitivity to diagnose the infection. In addition, RT-PCR protocols that evaluate the presence of the genetic material during the acute phase of the disease may show variation according to the viral copy number in the patient's serum, compromising the sensitivity of the test. Also, it is important to consider the use of a RT-PCR protocol that detects the mutation characteristic of the Indian Ocean lineage, since *Aedes albopictus* may play a more important role on the transmission of this lineage that it is for other *Aedes*-transmitted virus.

In Brazil, the Ministry of Health issued guidelines for the diagnosis and treatment of this disease, and the main clinical criteria for evaluation of chikungunya patients is through clinical presentation and associated outcomes, which can differentiate it from other arboviral infections, especially dengue and Zika. Physicians must be aware that viral isolation and RT-PCR are recommended for the laboratory diagnosis in the acute phase and serological assays (ELISA or Point-of-Care lateral flow tests) for the chronic phase of the disease. Aiming at the correct diagnosis of chikungunya, the Brazilian National Agency of Sanitary Surveillance (ANVISA – Agência Nacional de Vigilância Sanitária) licensed 12 diagnostic kits for the detection of acute and chronic phases of the infection (Table 1). According to the Brazilian guidelines, the gold standard for CHIKV diagnosis is viral isolation and PRNT (Plaque Reduction Neutralization Test), although it requires viral handling in a BSL-3 laboratory [31]. Indeed, many reported cases of CHIKV disease have only clinical-epidemiological confirmation, with a low percentage of laboratory evaluation, due to the lack of reliable tests.

Several attempts to improve techniques to detect both virus [32] and antibody have recently been developed [33], including new technologies such as multiplex detection of chikungunya, dengue and Zika using a smartphone-based platform [34]. Reaching a rapid diagnosis may help the management of the disease in the acute and chronic phases of the disease. The improvement of CHIKV diagnostic has allowed the identification of viruses in selected biological fluids such as semen, urine, saliva [35, 36] and breast milk [37]. However, detecting this virus in these biological fluids does not mean they are infectious since the transmission of CHIKV by these fluids has not been established.

There is no antiviral therapy specific for chikungunya virus, and treatment is directed only at symptom relief. The use of non-steroidal anti-inflammatory drugs, such as ibuprofen during the acute phase of the infection, as well as acetylsalicylic acid and corticosteroids, which can cause renal complications and bleeding is not recommended. Hydration, physical therapy and bed rest for the patient, as well as the use of potent analgesics if pain is a debilitating clinical symptom are suggested. In the chronic phase, non-steroidal anti-inflammatory and corticosteroids may be given. Other approaches such as the use of chloroquine, methotrexate and monoclonal antibodies against TNF-α must be cautiously used, especially the latter in countries with a high prevalence of tuberculosis. A guide to the treatment of acute and chronic presentation for both adults and children can be found in Brito *et al*. [38].

Since there is no effective treatment against the virus, prevention is the best approach to avoid the spread of CHIKV infection. Intensive vector control policies have been in place for decades in Brazil and were reinforced in 2017. These measures and probably climate changes may have contributed to the decrease in the number of CHIKV cases, as well as for other arboviruses circulating in Brazil. A number of different approaches has been used for vaccine development and improvement in vaccine production has been

**Table 1.** Chikungunya virus detection kits approved by National Agency of Sanitary Surveillance (ANVISA) in Brazil showing their sensitivity and specificity.

| Test name | Manufacturers' name | Detection stage | Methodology | Sensitivity | Specificity |
|---|---|---|---|---|---|
| IF Chikungunya IgM | Euroimmun | Chronic phase | Indirect Immunofluorescence | 96.7% | 95.7% |
| IF Chikungunya IgG | Euroimmun | Chronic phase | Indirect Immunofluorescence | 96.7% | 100% |
| ELISA Anti-Chikungunya IgM | Euroimmun | Chronic phase | ELISA | 98.1% | 98.9% |
| ELISA Anti-Chikungunya IgG | Euroimmun | Chronic phase | ELISA | 100% | 100% |
| Bio Gene Chikungunya PCR | Quibasa | Acute phase | Reverse Transcription PCR | 99.9% | 99.9% |
| Chikungunya IgG/IgM ECO Test | Eco Diagnóstica | Chronic phase | Lateral Flow Immunoassay | >99.9% | IgM: 97.7%<br>IgG: 99.6% |
| QuickProfile IgG/IgM Combo Test Card | Lumiquick | Chronic phase | Lateral Flow Immunoassay | IgM: 92.3%<br>IgG: 100% | IgM: 94.1%<br>IgG: 96.4% |
| Teste Rápido Chikungunya IgM | Bahiafarma | Chronic phase | Lateral Flow Immunoassay | 94% | 95% |
| Kit Teste Rápido Chikungunya | OrangeLife | Chronic phase | Lateral Flow Immunoassay | 98.5% | 99.5% |
| Kit NAT Diferencial Dengue-Chikungunya-Zika virus | Fiocruz | Acute phase | Reverse Transcription PCR | Not Informed | Not Informed |
| Chikungunya IgM – XG-CVM-MB | Biometrix | Chronic phase | ELISA | >98% | >98% |
| Chikungunya IgG: XG-CVG-MB | Biometrix | Chronic phase | ELISA | >98% | >98% |

achieved in the last few years, with the developments of new vaccine candidates. A new vaccine using chimeric recombinant viruses [39] has shown great protection against the CHIKV virus challenge and could be the first certified vaccine against the virus. Schwameis *et al.* have reviewed the current state of art of chikungunya vaccine development [40].

## Conclusions and future perspectives

There are a lot of speculations on what to expect from CHIKV infection in the coming years and although this is still an open question, investment in research in all scientific fields such as vaccination, diagnosis, cellular and molecular aspects of infection, as well as improvement in clinical care is necessary for facing new outbreaks of this virus, in Brazil and other countries. Therefore, it is important to report strategies implemented by different countries in order to identify those that have the biggest impact in controlling arthropod-borne viral infections. Prevention of outbreaks through vector control must be in place due to the high morbidity associated with CHIKV infections and if successfully implemented, these measures will certainly decrease the impact of outbreaks caused by other arboviruses. For instance, as it was true for Brazil, it is possible that the magnitude of the Zika virus outbreak that occurred in almost every country in the Americas and Caribbean have pushed the governments of these countries to implement public health measures that resulted in the decrease of these viral infections in 2017 (Table 2). Another possibility for the decline in the number of cases is herd immunity because Zika and chikungunya virus infections induce long-lasting immunity against all lineages and, due to the immense magnitude of the outbreaks that occurred in the above-mentioned regions, a high percentage of the population became immune

**Table 2.** Reported and confirmed cases of dengue, Zika and chikungunya in 2016 and 2017 according to data from the Pan American Health Organization/World Health Organization*.

| Disease | American region | Number of autochthonous cases | | | |
|---|---|---|---|---|---|
| | | 2016 | | 2017 | |
| | | Reported | Confirmed | Reported | Confirmed |
| Dengue | North America | 764 | 764 | 190 | 190 |
| | Central America and Mexico | 286,131 | 26,497 | 136,794 | 15,006 |
| | Caribbean | 90,268 | 3,810 | 4,416 | 1,419 |
| | South America | 1,961,685 | 402,645 | 341,808 | 44,345 |
| | | | | | |
| Zika | North America | 0 | 217 | 0 | 226 |
| | Central America and Mexico | 53,858 | 12,826 | 63,484 | 19,472 |
| | Caribbean | 105,851 | 41,365 | 113,786 | 48,979 |
| | South America | 374,844 | 123,206 | 405,874 | 154,659 |
| | | | | | |
| Chikungunya | North America | 3 | 0 | 0 | 0 |
| | Central America and Mexico | 33,675 | 1,623 | 1,963 | 38 |
| | Caribbean | 3,793 | 242 | 135 | 74 |
| | South America | 313,863 | 150,904 | 58,171 | 28,865 |

*: Data shown on the table refer to cases reported to the Pan American Health Organization in 2016 (Dengue up to February 6, 2017; Zika up to December 29, 2016; and chikungunya up to January 27, 2017) and 2017 (Dengue up to October 27, 2017; Zika up to December 21, 2017; and chikungunya up to July 7, 2017).

to these diseases. However, herd immunity may not explain the decline in dengue cases since there is no cross-immunity against all four serotypes and the number of dengue cases was reduced by more than 50%. According to the data from the Pan American Health Organization (PAHO) shown on Table 2, there was an impressive decline in the number of cases of dengue and chikungunya in all regions where these diseases are highly prevalent. The same decrease was not observed for Zika, but the increase in the number of Zika virus infections reported to PAHO might represent either the cases that occurred in 2016 but were only confirmed in 2017 or the increased awareness of this disease by physicians on countries of these regions, resulting in higher number of reported cases of Zika. Thus, investigation, analyses and reporting of the possible reasons for the decline or increase in the number of cases are extremely important to increase the awareness of public health officials from other countries and to implement measures to prevent or control possible outbreaks.

Other research topics deserve special attention, such as the co-infection of CHIKV with other arboviruses due to the possible complications in the patient's clinical manifestations. Another important issue is the development of assays for the correct diagnosis of these infections, as many suspected cases are not confirmed due to the lack of laboratory evaluation. In addition, serologic cross-reactions among flaviviruses (dengue, yellow fever and Zika viruses) and alphaviruses (chikungunya and mayaro viruses) are often present. As an example, many suspected chikungunya cases might be the result of mayaro virus infection, another endemic alphavirus in Brazil, and possibly in all Latin America, which is probably misdiagnosed due to their similar disease onset and antibody cross-reactivity in serological tests. In addition, research on the pathophysiology of the complications caused by CHIKV, especially the Guillain-Barré Syndrome, which has shown an increased incidence in recent years and may also be associated with other virus infections such as Zika virus, is urgently needed, since the factors that contribute to the development of this disease are still unknown.

Finally, learning from strategies implemented by different countries resulting in an improvement in the diagnosis and prevention of CHIKV infections may potentiate the decrease in the number of cases of other arbovirus infections, a finding similar to what was observed in the year 2017, and this epidemiologic situation could be reproduced in the years to come.

## CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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