Chikungunya
Chapter 8 - Clinical Review for IDCNA
Emerging and Re-Emerging Infectious Diseases

Title:

Chikungunya – Epidemiology, Pathogenesis, Clinical Features, Management and Prevention

Running Title: Chikungunya

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KEYPOINTS

- Chikungunya is a disabling and debilitating zoonotic disease of humans caused by the Chikungunya virus (CHIKV) which is transmitted by infected *Aedes spp* mosquitoes which sustain sylvatic and human rural and urban CHIK cycles.

- Chikungunya is listed on the WHO Blueprint priority pathogens since over the past 5 years an alarming and unprecedented magnitude of spread has occurred with cases reported from over 100 countries, across Asia, Africa, Europe and the Americas.

- The incubation period of between 1 and 12 days, is followed by symptoms similar to Dengue, Zika, parvovirus, enterovirus, malaria, with an abrupt onset of high fever, nausea, polyarthralgia, myalgia, widespread skin rash, and conjunctivitis.

- Serious complications include myocarditis, uveitis, retinitis, hepatitis, acute renal disease, severe bullous lesions, meningoencephalitis, Guillain-Barré syndrome, myelitis, and cranial nerve palsies. Severe disease occurs in: neonates exposed during pregnancy, the elderly, and those with co-morbid diabetes, renal, liver and heart disease

- Treatment is supportive and there is no specific antiviral treatment and no effective vaccines.

SUMMARY (163 words)

Chikungunya is a disabling and debilitating zoonotic disease of humans caused by the Chikungunya virus (CHIKV) which is transmitted by infected *Aedes spp* mosquitoes which sustain sylvatic and human rural and urban CHIK cycles. The past 5 years has witnessed an alarming global increase and spread of CHIV to over 100 countries across Asia, Europe, Africa and the Americas. CHIKV is listed on the WHO Blueprint priority pathogens. The incubation period of between 1 and 12 days, is followed by symptoms similar to Dengue, Zika, parvovirus, enterovirus, malaria and other febrile infections, with a sudden onset onset of high fever, nausea, polyarthralgia, myalgia, widespread skin rash, and conjunctivitis. Occasional serious complications include: myocarditis, uveitis, retinitis, hepatitis, acute renal disease, severe bullous lesions, meningoencephalitis, Guillain-Barré syndrome, myelitis, and cranial nerve palsies. Severe disease occurs in neonates exposed intrapartum, the elderly, and those with co-morbid diabetes, renal, liver and heart disease. Treatment is supportive and there is no specific antiviral treatment and no effective vaccines.

INTRODUCTION

Chikungunya (CHIK), is a disabling and debilitating zoonotic disease of humans caused by the Chikungunya virus (CHIKV) which is transmitted by CHIKV-infected *Aedes spp* mosquitoes (**Fig 1**). The primary CHIKV reservoir hosts are non-human primates [1]. Chikungunya is listed on the WHO Blueprint priority pathogens (https://www.who.int/blueprint/en/) since over the past 5 years an alarming and unprecedented magnitude of spread has occurred with cases reported from over 100 countries across the Americas, Africa, Europe and Asia, affecting millions of people are occurring (**Fig. 2**).

HISTORICAL

CHIKV most likely originated in East and central Africa where the virus is endemic to a sylvatic cycle between mosquitoes and non-human primates living in forests. In 1952 CHIK was described during an outbreak on the Makonde plateau in southern Tanzania on the border with Mozambique [2]. CHIKV was isolated from the serum of a febrile patient during an outbreak of an exanthematous febrile disease. Soon after in 1953 CHIKV was isolated in mosquitoes of the *Aedes aegypti* (*Ae. Aegypti*) and the virus was placed in arbovirus group A [3]. There are remarkable similarities between the clinical syndromes caused by Dengue virus and CHIKV [4]. Prior to the discovery of CHIK cases were mostly diagnosed and treated as malaria or Dengue cases. The actual name 'Chikungunya' is derived from the Makonde tribe (Kimakonde language) meaning "*that which bends up*" or "to become contorted" which describes the stooped bent posture of patients with Chikungunya who developed joint pain (Virusnet.com). The virus is maintained in a complex sylvatic and rural cycle, progressing to an urban cycle every 5 to 20 years, causing global pandemics (**Fig.3**). Following discovery of CHIK in Tanzania CHIK it was identified in Uganda and subsequently in many other sub-Saharan African countries [5] with global spread over the ensuing years (**Fig.2**). These CHIKV strains where grouped in a single lineage and named after the geographical location as East, Central and South Africa (ECSA) CHIKV lineage. A different monophyletic group was identified during outbreaks in Asia from 1958 to 1973 and called Asian CHIKV lineage [6,7]. These different geographical genotypes exhibit differences in their transmission cycles: in

Asia, the CHIKV appears to be maintained in an urban cycle with *Ae. aegypti* mosquito vectors, while CHIKV transmission in Africa involves a sylvatic cycle, primarily with *Ae.furcifer* and *Ae. africanus* mosquitoes. A distinct CHIKV clade, the West Africa lineage, was isolated from West Africa and this has been circulating over the past 30 years. The 2 major enzootic CHIKV lineages in Africa were introduced from eastern Africa into Asia between 1920–1950. The more recent Indian Ocean and Indian epidemic CHIKV strains emerged independently from the mainland of East Africa [8]. This lineage has been called Indian Ocean Lineage (IOL) and has been repeatedly associated with outbreak from 2005-2014 [8,9].

EPIDEMIOLOGY

The Chikungunya Virus

Phylogenetic analysis has shown four different genotypes of CHIKV based on geographical regions. The west African genotype (Senegal and Nigeria), The East/ Central/South African (ECSA) genotype, the Asian genotype and the Indian Ocean Lineage (IOL) genotype [6,7,10,11]. CHIKV is an enveloped, spherical, single-stranded positive-sense RNA alphavirus belonging to the family Togaviridae. It has a genome size of ~12 kb and it consists of two open reading frames cleaved into four non-structural proteins (nsP1, nsP2, nsP3, and nsP4) [12] and five structural proteins (C, E3, E2, 6 K, and E1). [13]. E1 and E2 are surface glycoproteins, 439 and 423 amino acid-long, respectively [14]. E1 and E2 carry the major viral epitopes and participate in the attachment and the entry of the virus into target cells, where E2 is responsible for receptor binding, and E1–for membrane fusion [15]. E3 consists of 64 amino acids that are required for E3-E2-6K-E1 or E3-E2-TF polyprotein translocation into the endoplasmic reticulum for virus spike formation [16]. The 6K protein is a cation-selective ion channel that causes increased cell permeability to monovalent cations and virion budding during infection [17]. Transframe protein TF is produced as a result of C-terminal extension of 6K protein in the −1 frame [18]. It retains ion-channel activity similar to that of 6K and appears to be important for the virus particle assembly and release [19]. Although the non-structural proteins nsP1-nsP4 are primarily associated with the viral replication process [20,21], they have additional functions during the viral infection, just

like in other alphaviruses [22]. Of note is that non-structural proteins are not packaged into the final virions, and hence the structural proteins (mainly surface glycoproteins E2 and E1) are the key targets of the host humoral immune response and of most CHIK vaccines [23].

Global distribution, transmission and CHIKV outbreaks

Human cases of CHIK have been reported from all continents affecting males and females of all age groups. The CHIKV circulates between mosquitoes and naive human hosts in cyclical form similar to Dengue viruses. Until 5 years ago the majority of CHIK cases were reported from Africa, Asia, Europe, and the Indian and Pacific Oceans. International travel led to spread of CHIKV to Europe and focused global attention [24]. The first local transmission of CHIKV in the Americas was identified in Caribbean countries and territories in 2013 and subsequently rapidly spread throughout the Americas [25] **(Fig.2)**. To date human cases of CHIK have been found in over 100 countries (**Table 1**).

Large outbreaks have been reported in Comoros in 2005 with approximately 215000 infections [26] and in Reunion Island between March 2005 and April 2006 with 255.000 cases [27]. The spread of CHIKV to the island of the Indian Ocean, India and Southeast Asia after a large outbreak in Kenya in 2004, has been key factor in focusing global attention. Selected outbreaks in various countries is listed in **Table 2**. CHIKV subsequently spread beyond its original tropical locations in Africa and the Indian subcontinent becoming a serious emerging issue in temperate regions of Europe, and the Americas with autochthonous small outbreaks occurring as the consequence of spill over from endemic tropical areas, in continental Europe: Italy in 2007, and in France in 2010, in 2014, and eventually in 2017 [28-31]. In 2017, a major outbreak in Italy were concentrated around three main foci (Anzio, Rome, Guardavalle Marina) in two different regions, Lazio (Anzio, Rome) and Calabria (Guardavalle Marina), in Central and Southern Italy [32]. Phylogenetic analysis showed that the CHIKV from the Lazio outbreak belonged to the East, Central, and South Africa (ECSA) clade, and clusters within the IOL [33].

In Africa, CHIKV epidemics have been reported from Central African Republic, Guinea, Burundi, Angola, Uganda, Malawi, Nigeria, Democratic Republic of the Congo,South Africa, and Nigeria. In June 2004 in an outbreak that occurred in Lamu Atoll, Kenya and spread to Mauritius, Seychelles, Comoros, and La Réunion Island almost half a million cases were

reported. Several other epidemics occurred in all southwestern Indian Ocean islands except Madagascar during the years 2005 to 2007. In 2011, a CHIKV epidemic in the Democratic Republic of the Congo (317 cases), Pool (460 cases), and Brazzaville (7014 cases).

Mosquito Vectors

In Africa, CHIKV circulates in an enzootic cycle between forest dwelling *Aedes spp*. mosquitoes (*Ae. furcifer, Ae. taylori, Ae. africanus, Ae. luteocephalus*) and is maintained in non-human primates and other vertebrate reservoirs such as rodents and bats (**Fig.1, Fig.3**) [34,35,1]. The primary CHIKV reservoir hosts are non-human primates, and the 5 to 10-year periodicity of CHIKV transmission may depend on oscillations in monkey herd immunity [1]. In Senegal, enzootic strains of CHIKV have been isolated from diverse species of mosquito including: *Ae. Diceromyia furcifer, Ae. (Diceromyia) taylori, Ae. (Stegomyia) luteocephalus, Ae. (Stegomyia) africanus and Ae. (Stegomyia) neoafricanus* [1]. Sporadic spillover of enzootic CHIKV into urban inter-human transmission cycles is amplified by the involvement of anthropophilic mosquito species such as: Ae. *(Stegomyia) aegepti and Ae. (Stegomyia) albopictus* [36].

The behavior and ecology of *Ae. Aegypti* makes it an ideal vector during epidemic cycles due to its anthropophilic nature. Moreover, adult females often take several blood meals during a single gonotrophic cycle and artificial containers are preferred larval sites [37]. *Ae. albopictus* mosquitoes are both zoophilic and anthropophilic and are active throughout the day. In Asia, CHIKV is maintained in an urban transmission cycle vectored by the mosquito *Ae. aegypti* and *Ae. albopictus*. [38]). *Ae. albopictus*, the Asian tiger mosquito, was discovered in 1894 in India and is endemic to Southeast Asia. *Ae. albopictus* mosquitoes have successfully colonized all five continents throughout both temperate and tropical regions [39]. It has successfully spread due to its ability to thrive in arid and cold conditions, undergo periods of adult diapause, and overwinter by laying desiccation-resistant eggs. Although these mosquitoes do not have a specific ecological niche, distinct

temperate and tropical populations have arisen. CHIKV circulation typically coincides with periods of heavy rains and increased mosquito densities [40]. The urbanization and human migration from rural to urban areas has led to the introduction of *Ae. aegypti* and *Ae. albopictus* living around human habitations, sustaining CHIKV transmission in a mosquito-human cycle.

CHIKV Mutations and infectivity

The E1-A226V mutation has resulted in a dramatic increase in the infectivity of CHIKV, and the transmission of CHIKV has spread to Europe and the Americas facilitated by widespread distribution of the *Aedes spp* vectors. The non-synonymous mutation in the E1 glycoprotein of the viral envelope (E1-A226V) was identified in the 90% of the isolates during the outbreak in Reunion island in 2006 [41]. This mutation is important for viral fitness in the *Ae. Albopictus* although the mutation does not affect the viral replication in *Ae. Aegypti* [42,43]. This adapted variant has been involved in outbreaks in North-Eastern Italy in 2007 and in the South-Eastern France in 2014 and 2017 where *Ae. albopictus* is widespread [29,44,45]. Thus, although *Ae. aegypti* was widely recognized as the main urban vector of CHIKV in tropical areas, *Ae. albopictus* is considered able to transmit CHIKV in temperate climate areas too. Mosquito studies highlighted the role of *Ae. albopictus* as CHIKV vector during the European outbreaks [26, 27], and experimental infection confirmed a high susceptibility of local European *Ae. albopictus* populations to the mutated ECSA CHIKV strain. Other less widespread mutations are thought to increase initial infection in *Ae. Albopictus* midgut cells, all of them in the IOL lienage. E2-K252Q, E2-K233E, E2/E3-R198Q/S18F, E2-L210Q.All these affects initial infection of the *Ae. albopitus* midgut cells with major effect on infection of *Ae. aegypti* [46]. Of note, the same mutations are predicted to affect CHIKV Asian lineages circulating in the Americas due to a so called 'founder effect and resultant epistasis'. The amino acid mutation A98T in E1 protein in the Asian lineage completely prevents penetrance for *Ae. albopictus* infection [8,47].

MODES OF CHIKV TRANSMISSION

There are several ways in which CHIKV is acquired by humans [48]:

Transmission by mosquito bites: CHIKV is transmitted to people through the bites of the mosquitoes *Ae aegypti*, *Ae. albopictus* and *Ae. polynesienses* the same mosquitoes which transmit the Dengue virus. These mosquitos breed in or near human habitations and prefer to feed on humans during the daytime in shady areas, and early in the evenings. Horizontal transmission in *Aedes spp* can occur and contribute to the maintenance of CHIKV cycles [49]. Vertical transmission is rare but has been observed under natural and experimental conditions [50-52]. In Africa, CHIKV circulates primarily in an enzootic cycle, with occasional spillover infections of humans.

Infection with more than one arbovirus: Co-infection with Dengue virus and CHIKV, [53], and of CHIKV with Dengue virus, Zika virus, Yellow fever virus and West Nile virus have been described [54].

Iatrogenic transmission:

Blood and blood products associated transfusion: To date, no studies on transfusion-transmitted CHIKF from viremic donors to recipients has been published. Increasing concern is being recognized that CHIKV might be transmitted via transfusions given its high-level viremia high attack rate during outbreaks and a significant proportion of asymptomatic infections. Viremic asymptomatic [55] CHIKV-infected cases have shown high potential as disseminators of transfusion-associated CHIKF, since CHIKV levels capable of inducing CHIKF were found in the blood of asymptomatic cases during 2009 epidemic in Songkhla, Thailand [55]. As with other arboviruses, several factors will determine the impact of CHIKV on transfusion medicine: 1) prevalence of viremia among blood donors, 2) proportion of components derived from viraemic donations that transmit infection to recipients, 3) clinical impact on infected transfusion recipients, 4) availability of measures to reduce transfusion transmission when required, and 5) the cost and disruption incurred by those measures. Several models have been applied to estimate the risk of transfusion-associated HIKV transmission. Model results from La Réunion Island [56] and Thailand [55] indicate a significant short-term risk of transfusion-associated CHIKV transmission during the large outbreaks, while the Italian model suggests a small, but quantifiable risk that may exceed accepted safety standards during smaller, focal outbreaks [57] that may occur in temperate areas.

Transplant associated transmission: Very few cases of CHIKV infection in Solid organ transplant (SOT) recipients have been reported [58-60]. In a case series from Brazil 13 SOT recipients (nine kidney, four liver) infected with CHIKV showed similar clinical presentation to immunocompetent hosts, including chronic joint symptoms in 46%. However, there were no complications or death and these transplant patients experienced no apparent damage to the graft. Additionally, infectious CHIKV can be isolated from corneal grafts from both symptomatic and asymptomatic donors, though no corneal transplant transmitted cases have been reported to date [61]. SOT recipients who travel to or live in CHIKV endemic areas are under high risk of acquiring the disease.

Maternal-foetal transmission: Maternal-foetal transmission has been reported. Intrapartum contamination without actual placental infection has been well documented for chikungunya virus, which, is not able to infect the placenta. [62; 63] CHIKV is not transmitted to the fetus in the absence of placental breaches, which allow a transfer of maternal blood to the fetal circulation [62]. A recent meta-analysis [64] estimated a pooled mother-to-child transmission risk across the analyzed cohorts of 15.5%. with the highest risk among infections in the intrapartum period (+ 2 days from delivery). During intrapartum viremia, the vertical transmission rate of CHIKV is reported as 48.7%. Mothers who have a high viral load in their placenta are more likely to transmit the virus [62]. There is no evidence of CHIKV transmission via breast milk to infants [65].

Sexual transmission: CHIKV has been detected in the semen after 30 days from symptom's onset [66], indicating possible sexual transmission. No evidence of sexual transmission between humans has been reported so far.

PATHOGENESIS

During the first week of infection, chikungunya virus can be found in the blood and passed from an infected person to a mosquito through mosquito bites. CHIKV has certain cell types being particularly susceptible to infection, these include human epithelial and endothelial cells, primary fibroblasts, and monocyte-derived macrophages [67]. Lymphoid and monocytoid cells, primary lymphocytes and monocytes, and monocyte-derived dendritic cells did not demonstrate CHIKV replication [68]. The human skin is the first site of viral replication, mainly in the dermal fibroblasts, takes place. From here the virus enters lymph

lymph nodes and the circulatory system disseminating to all organs[69]. During the acute and subacute phases CHIKV reaches muscle and joint compartments. Primary muscle fibroblasts and skeletal muscle fibroblast and both found permissive [63, 70].

'Chikungunya clinical syndrome' is characterized by arthralgia which usually is symmetric and affects distal synovial joints more than proximal [71]. In patients during acute and persistent arthralgia CHIKV RNA and proteins have been found in synovial tissue and fluids with synovial fibroblasts and macrophages susceptible to the infection [63,71,72]. Infected macrophages are the preferred site for viral replication contributing to the viral persistence and the chronic symptoms [71]. The presence of elevated levels of cartilage bioproducts in urine [73] and low plasma levels of Hepatocyte Growth Factor (HGF) in chronic patients [74] indicates connective tissue alteration and cartilage damage. CHIKV replicates actively and persists in the osteoblasts [72, 75]. Bone loss is a characteristic of the CHIK-associated arthritis. The pathogenesis of persistent symptoms post-CHIK infection is still unclear. CHIKV proteins have been detected in macrophages and muscle cells tissue of patients with relapse of chronic pain, suggesting that low replicative viruses or non-replicative CHIKV debris may persist. A persistent immune activation has been detected in mouse models [76,77]. Immune and inflammatory responses to CHIKV infection may also contribute to pathogenesis [78, 79].

Neurological manifestations have been often reported in several outbreaks with a growing number of cases with neurological complications after the re-emergence of CHIKV in the Indian Ocean in 2005. The virus has been frequently isolate from the cerebrospinal fluid (CSF) [80]. The target cells for CHIKV in the human brain remain unknown. The *in vitro* infection of human cells have demonstrated the susceptibility of neuroblastoma cells [81] microglial cells [82] and glial cells, such as astrocytes [83] showing signs of apoptosis. Nevertheless, it is still unclear if the pathogenesis of the nervous system is directly connected with the infection of the neurons and glial cells or is indirectly connected triggering the immune mediated effects.

CLINICAL FEATURES

Incubation period:

The incubation period can vary from 1 to 12 days (average 2 to 7 days). CHIKV infection causes high levels of viremia, which usually last for 4-6 days after the onset of symptoms. Chikungunya infection is symptomatic in the majority of children and adults who are infected with less than 15% having an asymptomatic sero-conversion [56].

Symptoms:

The symptoms (**Table 3**) are similar to those of other arboviruses such as Dengue and other common causes of febrile illnesses in the tropics, thus the accurate diagnosis is challenging. During the acute phase of illness, the intensity of the clinical symptoms correlates with the viremia during the acute infection, usually lasting 1 week when anti-CHIKV IgM antibodies appear [84]. Following the onset of fever, intense myalgia and arthralgia occur. These symptoms can be very severe and disabling causing much morbidity. The disabling poly-arthralgia is a key symptom for differential diagnosis with an 80% positive predictive value greater than 80% [85]. The joint pain is usually symmetric in both the arms and legs; the large joints are almost invariably symptomatic. Other common signs are nausea, fatigue, headache, back pain, and skin rash (50% cases). The skin lesions are characterized by a macular or maculopapular transitory eruption often in the body extremities, palms, soles of the feed, torso and face [84,86]. Gastrointestinal tract involvement can manifest with nausea, vomiting and abdominal pain [87]. Ocular manifestations can occur during the acute phase with photophobia, retro-orbital pain and conjunctivitis [88].

The acute phase is usually followed by a post-acute stage, usually from the 4^{th} week to the end of the 3^{rd} month [89]. This phase is characterized by the persistence of the initial inflammatory events, including inflammatory arthralgia, arthritis (synovitis with or without effusion), tenosynovitis, bursitis, which slowly regress. It often associates decompensation of pre-existing degenerative or traumatic arthropathy (sometimes unknown) such as osteoarthritis or sometimes-calcific tendinitis, and local events such as reactionary edema, entrapment syndromes, joint stiff-ness, or neuropathic pain.

The chronic stage (after the 3rd month) is defined by the absence of return to the pre-existing condition more than 3 months after the onset of CHIK. The chronic phase can last a few months to several years (more than 6 years for a small group of infected patients in the Reunion Island symptoms. The observed clinical symptoms are the same as in the post-acute

stage. It is common to observe painful rebounds on joints too strongly used considering their post-CHIK inflammatory condition. The diagnostic approach is to qualify the nosology of each patient according to the presence or absence of inflammatory symptoms (arthritis, enthesitis, tenosynovitis, inflammatory arthralgia) and the number of joints involved (polyarticular if ≥ 4 joints)

In the infected newborns, symptoms generally develop on days 3–7 of life with fever, rash, and peripheral edema. Pathology typically reveals a bicytopenia, increased prothrombin time, and AST. The presentation is subsequently complicated by seizures, hemorrhagic syndrome, hemodynamic disorders, displayed by both infants, and myocardial dysfunction [62,90]. Neonatal symptoms range from mild presentation (43%) to severe infection with encephalitis (53%) [62] that requires intensive care. Fever and acute respiratory distress have also been reported. [91] Neurological complications can have severe effects on postnatal neurological development, such as lower development quotient at age of two years, moderate to severe global neurodevelopmental delays [92].

Atypical features and complications

Complications of the cardiovascular, renal respiratory, hepatic gastrointestinal and adrenal systems are associated with the infection and referred as atypical features (**Table 4**). As reported during the La Reunion outbreak in 2005 [1993], reported an proportion of atypical cases of 0.3%. Atypical cases were defined as patients with clinical presentation of fever, arthralgia and other atypical sign. The median age of the cases was 70 years. Of the 610 atypical cases, 546 (89%) had underlying medical conditions, 479 (78%) were on medication prior to hospitalization. However, the involvement of the central nervous system is the most common complication of CHIK infection. Neurological disease following chikungunya virus infection was first reported during an outbreak in 1964 in Madras, India [94]. In 2 studies investigating manifestations of chikungunya in patients requiring intensive care, a neurological disorder was the primary issue in 61% [95] and 79% [96] of CHIKV-infected patients. 65 Given the large spectrum of neurological disease and scarce epidemiological data, estimating the incidence of neurological disease amongst all systemically symptomatic chikungunya infections is difficult. In one study from the 2006 Indian outbreak, 18 (4.4%) of 405 suspected chikungunya cases attending the recruiting hospital over 3 months developed

neurological complications [94]. An epidemiological study of the 2005 to 2006 Réunion Island outbreak found approximately 0.3% of all chikungunya infections resulted in “atypical” cases [93] of which 24.1% of the adults presented with abnormal neurology. Thus, approximately 0.1% (1 case per 1000) of all chikungunya infections developed neurological disease. It has been observed that severe complications of chikungunya typically arise in patients with comorbidities. Studies from La Reunion and from India, show that underlying diseases play a role in neurological disorders and other complication but are not an indispensable requisite. Age has been reported as a significant risk factor for severe manifestations in the elderly (>65 years) [93; 94] and in infants [97].

A recent systematic review [80] describe the frequency of reported neurological syndromes and diseases. The most frequent are encephalopathy and encephalitis, myelopathy and myelitis, Guillan-Barrè syndrome, acute disseminated encephalomyelitis, neonatal hypotonia, neuro-ocular disease. Other manifestations are described less frequently such as behavioral changes, seizure with and without fever, stroke, cerebellitis, meningism, third nerve palsy, encephalopathy and bilateral total ophthalmoplegia.

Mortality

Whilst morbidity is debilitating, the CHIKV mortality rates are low. As with most viral illnesses, people at a higher risk for more severe disease include the newborn, elderly, and those with co-morbid illnesses such as diabetes, heart disease, chronic liver and kidney disease and HIV. An increased mortality has been observed in the last epidemics probably due to neurological disorders mainly in neonates, immunocompromised and elderly [58, 66,98]. In Europe the case fatality rate was 2.5 per 1,000 clinical cases [32], lower than the one reported in the 2007 outbreak in Italy (0.5%) but consistent with those reported from la Reunion (1 death per 1,000 clinical cases) [29,99].

LABORATORY DIAGNOSIS

Several Laboratory tests are available for diagnosing CHIK utilizing serum or plasma to detect virus, viral nucleic acid, or virus-specific immunoglobulin (Ig) IgM and neutralizing antibodies. Given the high viral load during viremia CHIKV viral RNA can be detected during the first 5-8 days of illness using commercial tests with high sensitivity and specificity. The

choice between the types of tests is dictated by the timing of the sampling with respect to the beginning of the symptomatology and the volume of the samples available.

***Serological tests*:**

CHIKV IGM antibodies normally develop toward the end of the first week of illness and to definitively rule out the diagnosis, convalescent-phase samples should be obtained from patients whose acute-phase samples test negative. Serological diagnosis of CHIK is made by by detecting CHIKV-specific IgM in serum samples for 5-7 days after symptom onset, or by demonstrating a four-fold increase (or seroconversion) of CHIK-specific IgG antibody titers in a pair of serum samples at least 15 days apart from each other (acute and convalescent phase of the disease). IgM antibodies specific for CHIKV may persist for up to one year, particularly in patients with long-term arthralgia, but typically persist for 3-4 months. The specific CHIKV-IgG is can be detected for many years after initial infection. Serological cross-reactions and false positive tests have been reported due to infection with closely related alphaviruses belonging to the Semliki forest virus. The Enzyme-linked immunosorbent assay (ELISA), may confirm the presence of IgM and IgG anti-chikungunya antibodies. IgM antibody levels are highest 3 to 5 weeks after the onset of illness and persist for about 2 months. Samples collected during the first week after the onset of symptoms should be tested by both serological and virological methods (RT-PCR).

***Molecular diagnostic tests*:**

PCR Detection of specific CHIKV viral RNA can be detected by RT-PCR in serum or plasma/EDTA samples obtained from patients during the acute phase of infection (typically ≤ 7 days after the onset of symptoms). CHIKV infection causes high levels of viremia, which usually last for 4-6 days after the onset of symptoms. This is a very favorable situation for diagnosis. Real-time RT-PCR is the ideal test for the diagnosis of CHIKV infections in the acute phase of infection. RT-PCR can typically be performed within the first 7 days of symptom onset to confirm CHIKV infection. The virus may be isolated from the blood during the first few days of infection. Various reverse transcriptase–polymerase chain reaction (RT–PCR) methods are available but are of variable sensitivity. Some are suited to clinical diagnosis. RT–PCR products from clinical samples may also be used for genotyping of the virus, allowing comparisons with virus samples from various geographical sources.

Viral culture may detect virus in the first 3 days of illness; however, CHIKV should be handled under biosafety level (BSL) 3 conditions.

TREATMENT

Supportive treatment:

There is no effective antiviral treatment and thus treatment of CHIK is supportive and symptomatic. It should be adapted to the clinical context and risk groups aimed at controlling fever and pain, treating dehydration, organ support, and preventing iatrogenic complications and functional impairment. Infection control procedures should be instituted to reduce risk of iatrogenic infection to healthcare and laboratory workers.

Analgesics:

Analgesia based on acetaminophen therapy is preferred. Using NSAIDs and salicylates is not recommended in the 14 days after onset of the disease because of the risk of bleeding complications related to dengue fever unless this diagnosis is ruled out, and Reye's syndrome induced by aspirin. Using analgesics (weak opioids) is required if acetaminophen is not effective: tramadol alone or in combination with acetaminophen.

The treatment of the post-acute stage should be based on primarily based on analgesics (stage 1 and 2, ant neuropathic drugs) and non-steroidal anti-inflammatory drugs (NSAIDs).

NSAIDS:

No NSAID class has demonstrated superiority of effectiveness on post-CHIK symptoms. A local anti-inflammatory therapy (topical or infiltration) should be prescribed in case of tenosynovitis, bursitis, tunnel syndrome, capsulitis, or synovitis inadequately controlled by oral treatment, so as to limit the therapeutic excess. The risk of drug toxicity by overdose(self-medication) or drug interaction is high for acetaminophen as well as for other analgesics, anti-inflammatory drugs, long-term treatments, and traditional medicines used for self-medication [89].

Use of steroids:

The use of corticosteroids is not recommended. Steroids may also cause severe rebound of arthritis and tenosynovitis. Systemic corticosteroids should be used only for very inflammatory polyarticular presentations, especially when associated with tenosynovitis, active synovitis, or in case of resistance or contra-indication to NSAIDs.

Newer therapies:

Off-label use of other FDA-approved drugs in a therapeutic manner has been proposed and is under consideration. In animal models of CHIKV infection, prophylaxis with CHIKV IgG or CHIKV-specific monoclonal antibodies was found to be protective [100], suggesting that antibody-based therapies may be a promising disease prevention strategy for individuals who are at risk for severe CHIKV infection. In cell-based screens of compounds against CHIKV infection, a number of drugs with antiviral activity have been identified, some of which target distinct steps in the CHIKV replication cycle. These include chloroquine [101] and chlorpromazine [102], which affect virus entry. Harringtonine and homoharringtonine [103] have been found to affect viral protein translation. Others, including trigocherriolide A [104], ribavirin [105], interferon-alpha [106], apigenin and silybin [102], affect virus replication. More extensive preclinical evaluation of these, and other identified drugs, in animal models of CHIKV disease are necessary before they are proposed for use in humans.

PREVENTION

At present, there is no effective vaccine against CHIKV infection. The proximity of mosquito vector breeding sites to human habitation is a significant risk factor for chikungunya. Prevention and control rely on reducing the number of natural and artificial water-filled container habitats that support breeding of the mosquitoes. This requires mobilization of affected communities.

Vector control and breeding reservoirs:

Vector control is dependent on reducing the number of natural and artificial water-filled container habitats that support breeding of mosquitoes. Prevent accumulation of stagnant water. Change the water in vases once a week. Avoid using saucers underneath flower pots. Cover water containers tightly. Ensure air-conditioner drip trays are free of stagnant water. Put all used cans and bottles into covered dustbins. During outbreaks, insecticides may be

sprayed to kill flying mosquitoes, applied to surfaces in and around containers where the mosquitoes land, and used to treat water in containers to kill the immature larvae.

Clothing and insect repellants:

For protection during CHIK outbreaks, clothing which minimizes skin exposure to the day-biting vectors is advised. Repellents can be applied to exposed skin or to clothing in strict accordance with product label instructions. Repellents should contain DEET (N, N-diethyl-3-methylbenzamide), IR3535 (3-[N-acetyl-N-butyl]-aminopropionic acid ethyl ester) or icaridin (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester). For those who sleep during the daytime, particularly young children, or sick or older people, insecticide-treated mosquito nets afford good protection. Mosquito coils or other insecticide vaporizers may also reduce indoor biting.

Miscellaneous advice to travelers:

Basic precautions should be taken by people travelling to risk areas and these include use of repellents, wearing long sleeves and pants and ensuring rooms are fitted with screens to prevent mosquitoes from entering bedrooms. General measures on preventing mosquito-borne diseases include wearing loose, light-coloured, long-sleeved tops and trousers, and use of insect repellent on exposed parts of the body and clothing. Avoid using fragrant cosmetics or skin care products. Treating clothing, tents, bed nets with permethrin (an insecticide). Travelers who return from affected areas and feel unwell, e.g. run a fever, should be advised to seek medical advice promptly, and provide travel details to doctor.

Vaccines

After the reemergence of CHIKV in 2004 and its rapid expansion throughout the Indian Ocean and Southeast Asia, and unexpected autochthonous transmission in Europe in 2007 and later in 2017, there has been renewed interest in developing a vaccine against CHIKV. Several approaches have been used for the development of CHIKV vaccines, including non-infectious [107] and infectious DNA vaccines [108], virus-like particles (VLP), and inactivated virus. Live attenuated vaccines under development include rationally attenuated alphavirus chimeras [109] and deletion mutants [110]; a vesicular stomatitis-vectored vaccine [111]; and an internal ribosome entry site-modified CHIKV strain [112]. To date, three CHIKV vaccines have progressed to clinical trials. The strain 181/clone25, developed by the U.S.

Army in the 1980s [113], proved highly immunogenic but mildly reactogenic in Phase II trials [114]. A VLP vaccine produced by expression of the CHIKV structural proteins in vertebrate cells demonstrated efficacy in preclinical studies to mice or Rhesus macaques [Akahata et al.,2010]. Phase 1 clinical studies showed strong immunogenicity after 2–3 doses [115]. Currently this vaccine is not licensed to a commercial partner. A CHIKF vaccine in advanced stages of clinical development employs an attenuated measles virus strain as a vector to express the CHIKV structural proteins [116]. In a Phase 1 trial, this vaccine was well tolerated and induced neutralizing antibodies in 44% of volunteers receiving a single low-dose, 92% receiving a medium-dose group, and 90% receiving a high-dose. A booster raised seroconversion to 100%, and immunogenicity was not affected by pre-existing anti-measles immunity. This vaccine is now in a Phase 2 trial [117].

CONCLUSIONS

Several challenges are involved in the development of tools and strategies for prevention of zoonotic and re-emerging infections with epidemic potential, including CHIK (**Table 5**). The early identification of human cases, developing rapid point of care diagnostics, effective treatments and vaccines. The establishment of more effective collaborative research networks involving different disciplines, such has medical entomologists, virologists, veterinarians, Infectious diseases clinicians, social science experts, anthropologists, community leaders and policy makers is required to enable more effective definition of host reservoirs, improving outbreak response and control activities. Given the unpredictability and paucity of CHIK and other zoonotic outbreaks, public health response and preparedness should be ready to perform research immediately during an outbreak, allowing for evaluation of existing and newer diagnostics, treatments and vaccines. These are being addressed through increasing research capacity during inter-epidemic periods with an integrated 'One Human-environmental-Animal Health' [119;120] to assist in rapid investigations of zoonotic outbreaks and developing local capacity to improve national public health institutions.

LEGENDS TO FIGURES

FIGURE 1:

Mosquito vector of Chikungunya - Female *Aedes aegypti* mosquito

A closeup lateral view of the female, *Aedes aegypti* mosquito, from a left lateral perspective, feeding on the human host with distended abdomen filled with host blood

FIGURE 2.

World map showing Countries and territories reporting Chikungunya

FIGURE 3:

The sylvatic, rural and urban transmission cycles of Chikungunya virus.

(Modified from reference [118].)

A) The sylvatic cycle of Chikungunya virus occurs between non-human primates, rodents and possibly bats and forest-dwelling Aedes species (*Ae. albopictus*, *Ae. furcifer*, *Ae africanus*, *Ae. taylori*).

B) The rural cycle is established when rural populations are bitten by CHIKV-infected forest-dwelling Aedes spp mosquitoes.

C) The urban transmission cycle occurs when CHIKV-infected humans living in large urban areas are bitten by urban *Aedes mosquitoes* (*Ae. aegypti* and *Ae. albopictus*)

LEGENDS TO TABLES

TABLE 1: List of Countries reporting Human Chikungunya cases

TABLE 2: Major Global outbreaks of Chikungunya

TABLE 3: Clinical features of Chikungunya: Symptoms and Signs

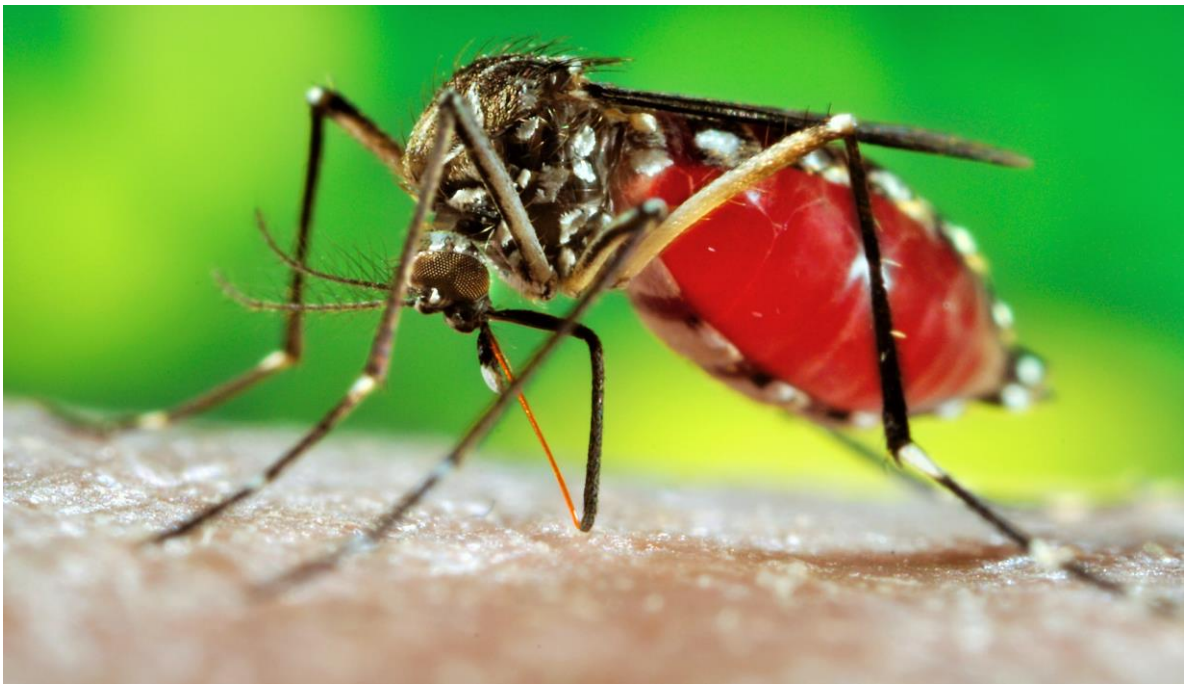
TABLE 4: Clinical features of Chikungunya: Complications

TABLE 5: Addressing knowledge gaps and strengthening Public health surveillance and vector control

FIGURE 1

Mosquito vector of Chikungunya - Female *Aedes aegypti* mosquito

A closeup lateral view of the female, *Aedes aegypti* mosquito, from a left lateral perspective, feeding on the human host with distended abdomen filled with host blood.

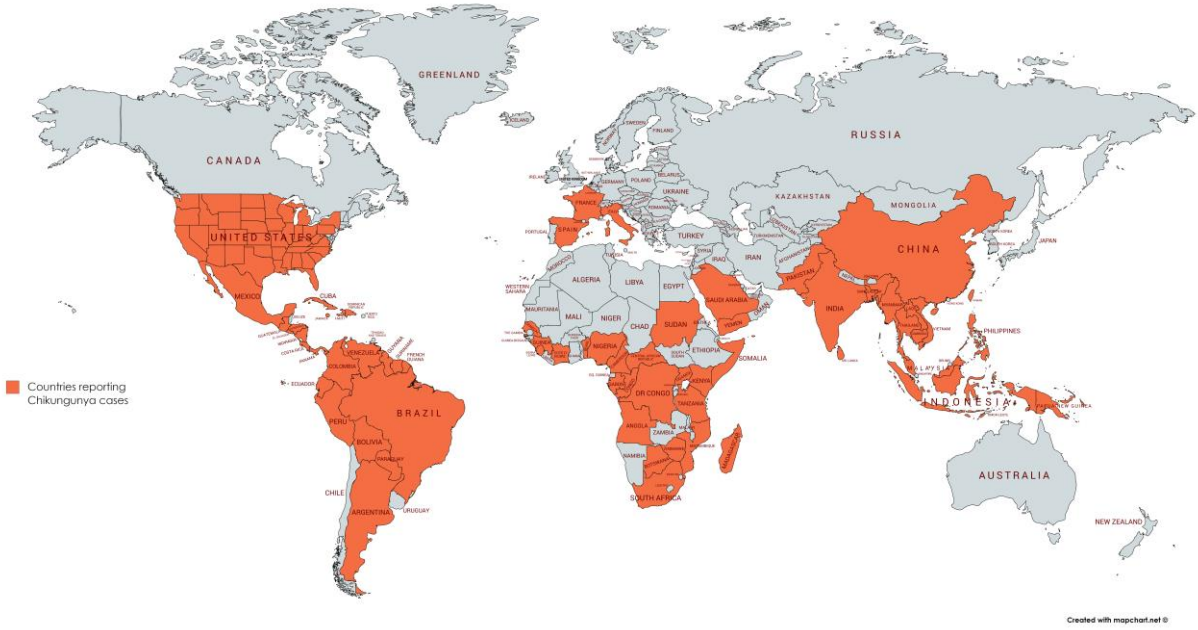


Courtesy of US-CDC, Public Health Image Library (PHIL)

https://phil.cdc.gov/Details.aspx?pid=9260

FIGURE 2

World Map showing countries and territories reporting Chikungunya



Data source https://www.cdc.gov/chikungunya/geo/index.html)

FIGURE 3:

The sylvatic, rural and urban transmission cycles of Chikungunya virus.

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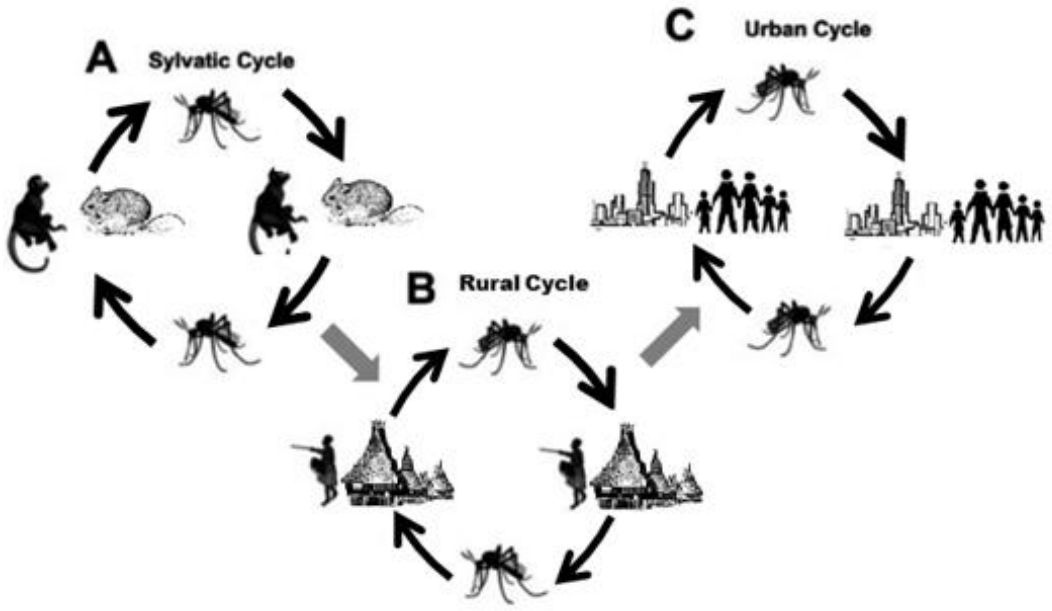

A) The sylvatic cycle of Chikungunya virus occurs between non-human primates, rodents and possibly bats and forest-dwelling *Aedes species* (*Ae. albopictus*, *Ae. furcifer*, *Ae africanus, Ae. taylori*).
B) The rural cycle is established when rural populations are bitten by CHIKV-infected forest-dwelling *Aedes spp* mosquitoes.
C) The urban transmission cycle occurs when CHIKV-infected humans living in large urban areas are bitten by urban *Aedes mosquitoes* (*Ae. aegypti and Ae. albopictus*) and CHIKV spread to other humans

Table 1. Countries where Chikungunya human infections have been reported (source https://www.cdc.gov/chikungunya/geo/index.html)

Continent	Country	
AFRICA	• Angola • Benin • Burundi • Cameroon • Central African Republic • Comoros • Cote d'Ivoire • Democratic Republic of the Congo • Djibouti • Equatorial Guinea • Gabon • Guinea • Kenya • Madagascar • Malawi	• Mauritius • Mayotte • Mozambique • Nigeria • Republic of the Congo • Reunion • Senegal • Seychelles • Sierra Leone • South Africa • Somalia • Sudan • Tanzania • Uganda • Zimbabwe
ASIA	• Bangladesh • Bhutan • Cambodia • China • India • Indonesia • Laos • Malaysia • Maldives • Myanmar (Burma)	• Nepal • Pakistan • Philippines • Saudi Arabia • Singapore • Sri Lanka • Thailand • Timor-Leste • Vietnam • Yemen
EUROPE	• France • Italy • Spain	
AMERICAS	• Anguilla • Antigua and Barbuda • Argentina • Aruba • Bahamas • Barbados • Belize • Bolivia • Brazil • British Virgin Islands • Cayman Islands • Colombia	• Haiti • Honduras Jamaica • Martinique • Mexico • Montserrat • Nicaragua • Panama • Paraguay • Peru • Puerto Rico • Saint Barthelemy • Saint Kitts and Nevis

	• Costa Rica • Cuba • Curacao • Dominica • Dominican Republic • Ecuador • El Salvador • French Guiana • Grenada • Guadeloupe • Guatemala • Guyana	• Saint Lucia • Saint Martin • Saint Vincent and the Grenadines • Sint Maarten • Suriname • Trinidad and Tobago • Turks and Caicos Islands • Venezuela • United States • US Virgin Islands
OCEANIA/PACIFIC ISLANDS	• American Samoa • Cook Islands • Federal States of Micronesia • Fiji • French Polynesia • Kiribati	• Marshall Islands • New Caledonia • Papua New Guinea • Samoa • Tokelau • Tonga

Table 2. Major global outbreaks of Chinkungunya

YEAR	COUNTRY
1954	United Republic of Tanzania
1999-2000	Democratic Republic of Congo
2005 to date	Islands of the Indian Ocean Maldives India Myanmar Thailand
2006-2007	India
2007	Gabon
2007	Italy
2011	Republic of Congo
2013	France
2014	Pacific Islands (Cook, Marshall etc)
2015	Americas
2016	Pakistan
2017	Italy
2017	Kenya
2019	Republic of Congo
2019	Democratic Republic of Congo

Table 3. Clinical features of Chikungunya: Symptoms and Signs

STAGE	SIGNS AND SYMPTOMS
Acute stage	**Common**
	Fever Macular to maculopaular rash edema of theface and extremities Benign bleeding (gingival bleeding, epistaxis) – in children Pruritus Myalgia Arthargia Rerorbital pain Headache Lynphadenopathy
	Less common
	Diarrhea, Vomiting , Abdominal pain Confusion Optical neuritis Oral or gingival ulceration Conjuntivitis
Post-acute stage	Inflammatory arthralgia, Joint stiff-ness Arthritis (synovitis with or without effusion) Tenosynovitis, Bursitis Decompensation of pre-existing degenerative or traumatic arthropathy Osteoarthritis or sometimes-calcific tendinitis Entrapment syndromes Neuropathic pain Severe asthenia Neuropsychological disorders
Chronic stage	**Joints:** Articular Arthritis, Synovitis, Degenerative osteoarthritis, Bursitis **Tendons:** Tendinitis, Enthesitis, Tenosynovitis Edema Neuropathic pain Stiffness Loss of physical fitness Postural hypotension Mood disorders

Table 4. Clinical features of Chikungunya: Complications

ORGAN/SYSTEM	COMPLICATION(S)
Nervous system	**Frequent**
	Encephalopathy and meningitis Myelopathy and myelitis Encephalomyelopathy Myeloneuropathy Encephalomyeloneuropathy Guillain-Barrè syndrome Acute disseminated encephalomyelitis Neonatal hypotonia Optic neuritis
	Less frequent
	Seizures Sensorineural hearing loss Stroke Cerebellitis Third nerve palsy Encephalopathy Behavioural changes Carpal tunnel syndrome Bilateral ophtalmoplegia
Cardiovascular system	NHeart failure Arrhythmias Myocarditis/pericarditis Blood pressure instability Acute myocardial infarction
Ocular	Conjunctivitis, Episcleritis, Non-granulomatous anterior uveitis, Granulomatous anterior uveitis Keratitis, Retinitis with vitritis, Bilateral retinitis, Multifocal choroiditis, Optic neuritis, Retrobulbar neuritis Exudative retinal detachment, Pan-uveitis
Other organ involvement	Pre-renal failure Exacerbation of chronic renal failure Pneumonia and Respiratory failure Hepatic insufficiency, Subacute hepatitis Bullous dermatosis Pancreatitis Syndrome of inappropriate antidiuretic hormone secretion Hypoadrenalism

TABLE 5

Chikungunya: addressing gaps in knowledge and strengthening public health preparedness

KNOWLEDGE GAPS / NEEDS	ACTIONS REQUIRED
Understanding the epidemiology and pathogenesis of CHIKV across geographical settings	Appropriately funded, well designed longitudinal and cross sectional clinical, pathogenesis, epidemiological, studies of sylvatic, rural and urban cycles (animal and human studies)
The spectrum of the vertebrate intermediate hosts	Evaluate the possibility of endemic circulation of Chikungunya virus by means of virus identification at the human-animal interface.
Prevalent mosquito vectors and their behaviour	Integrating entomological and human surveillance
Improved diagnostic, treatment, prognostic, prevention and surveillance tools	More investments into development and evaluation of: a) newer, affordable, field-friendly rapid diagnostic tests and sequencing platforms; b) new biomarkers of disease progression c) newer treatments (antivirals, immunotherapies and host-directed therapies) d) New vaccines
Defining the animal and environmental host reservoirs	Cross-sectional and longitudinal studies at the human-animal interface.
Customization of vector control measures adapted to local culture and norms in the context of the population behaviour	Close collaboration between medical and social science/anthropologists/animal-human-environmental sectors and local communities
Establish the co-circulation of other arboviruses	Appropriate use of serology and metagenomics analysis during outbreaks
Lack of understanding factors underlying pathogenesis of organ involvement and complications eg acute and persisting synovial pathology and arthritis; and of maternal-infant transmission and infection rate of new-borns.	Longitudinal cohort studies during outbreaks
New surveillance tools, early warning systems and real-time data management	The integration of different surveillance tools and the combination with entomological surveillance in a one health dedicated surveillance system should facilitate the detection, response and control of arboviruses spreading including CHIKV.

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