Chikungunya: a re-emerging virus

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In the past decade, chikungunya—a virus transmitted by *Aedes* spp mosquitoes—has re-emerged in Africa, southern and southeastern Asia, and the Indian Ocean Islands as the cause of large outbreaks of human disease. The disease is characterised by fever, headache, myalgia, rash, and both acute and persistent arthralgia. The disease can cause severe morbidity and, since 2005, fatality. The virus is endemic to tropical regions, but the spread of *Aedes albopictus* into Europe and the Americas coupled with high viraemia in infected travellers returning from endemic areas increases the risk that this virus could establish itself in new endemic regions. This Seminar focuses on the re-emergence of this disease, the clinical manifestations, pathogenesis of virus-induced arthralgia, diagnostic techniques, and various treatment modalities.

Introduction

Chikungunya virus (CHIKV) is an enzootic virus found in tropical and subtropical regions of Africa, in the Indian Ocean Islands, and in south and southeast Asia. The virus was first isolated from a febrile patient during an outbreak on the Makonde Plateau in the southern province of Tanzania (formerly Tanganyika) in 1952–53.[1] The name chikungunya, which is used to describe both the virus and the disease, is derived from a Swahili or Makonde word Kun qunwala, meaning "to become contorted" or "that which bends up". The disease is characterised by fever, headache, myalgia, rash, and joint pain. Although most symptoms resolve, some patients have joint pain that can continue for years and can be so severe that they adopt a bent or stooping posture.[1]

The virus is a member of the Togaviridae family, belonging to the genus Alphavirus, which is composed of various serocomplexes that are grouped together on the basis of antigenic properties.[2] CHIKV belongs to the Semliki Forest antigenic complex along with other mosquito-borne alphaviruses such as Ross River virus, Mayaro, o'nyong-nyong virus, Getah, Bebaru, and Semliki Forest viruses. CHIKV is believed to have originated in Africa, where two genetically distinct lineages have been identified, the west African lineage and the east, central, and southern African lineage, which includes an Asian genotype.[3] Before 2000, large outbreaks of CHIKV were rare, but since 2000 outbreaks have become more frequent and emerging genetic evidence suggests possible mechanisms for evolutionary adaptation of the virus to the mosquito vector.[4,5]

In Africa, the virus is maintained in a sylvatic transmission cycle between non-human primates, small mammals (eg, bats and monkeys), and *Aedes* mosquitoes.[6] Serological evidence was used to confirm the role of non-human primates as the hosts in CHIKV transmission cycles and virus isolation studies provided evidence that *Aedes* mosquitoes are the main vectors.[7] During epidemics, CHIKV can circulate between human beings and mosquitoes without the need for animal reservoirs. On the basis of isolation frequency in Africa, *Aedes furcifer-taylori*, *Aedes africanus*, *Aedes luteocephalus*, and *Aedes aegypti* were identified as the most common vectors. However, the virus has been associated with many other species, including *Aedes dalzieli*, *Aedes vigilax*, *Aedes camptorhynchites*, *Aedes vittatus*, and *Aedes fulgens*,[7] and reports from Nigeria and Uganda have implicated *Mansonia* spp mosquitoes as probable vectors.[8] Outbreaks of CHIKV in Africa are usually associated with heavy rainfall and subsequent spillover of the virus from an enzootic forest cycle to an epizootic savannah or woodland cycle. Rural outbreaks occur when mosquito populations increase in areas where populations of non-immune people are present. A different transmission cycle is seen in Asia, where the virus circulates between mosquitoes and people, resulting in urban epidemics with *A aegypti* and *Aedes albopictus* (Asian tiger mosquito; figure 1) as the main vectors. *A aegypti* was identified as the primary vector of the 2005–06 outbreak in India.[9] *A aegypti* are found in the tropics and subtropics whereas *A albopictus* have a wider distribution (also being found in temperate regions) and have been distributed to new regions via world trade where they have readily established endemicity, which could cause the spread of CHIKV to new geographical regions. *A albopictus* is established in the southeast of the USA and in the Caribbean, meaning outbreaks could occur in the Pacific and the Americas.

Many historical epidemics that were reported to be caused by dengue virus could have been caused by CHIKV.[10] Virological or serological confirmations of CHIKV have been reported in countries in west, east, central, and southern Africa. Cases of naturally acquired human infection and virus isolation have been reported in Tanzania,[1] Senegal,[11] Guinea,[12] Nigeria,[13] Cameroon,[14,15] Central African Republic,[16] Gabon,[17] Democratic Republic of the Congo,[18] Uganda,[19] Kenya,[20] Angola,[21] southern Africa,[22–24] and Madagascar.[25] Serological evidence alone

Search strategy and selection criteria

We searched PubMed for papers written in English with the search term "chikungunya". We then selected all articles that focused on clinical manifestations of the disease, virus-induced pathology, animal models, vaccines and therapies, and diagnosis. Relevant review articles and book chapters were used and bibliographies of selected articles were reviewed for other relevant references.

has been reported from Sierra Leone,[22] Liberia,[23] Benin,[26] Malawi,[27] Burundi,[28] and Sudan.[29]

The earliest confirmation of an outbreak in Asia was from the Philippines in 1954, with subsequent outbreaks in 1956 and 1968. During the 1970s, outbreaks occurred frequently in southern and southeast Asia but subsequently decreased in incidence, and virus activity seemed to have ceased in many areas, with small localised outbreaks only. Outbreaks and sporadic cases have been confirmed in Thailand, Sri Lanka, Vietnam, Pakistan, Cambodia, Laos, Burma, the Philippines, and India.[30–34] Between 1982 and 1985, the virus spread into Indonesia and was identified in south Sumatra, Java, Kalimantan, Sulawesi, Timor, Nusatengarra, Mollucas Islands, and Irian Jaya. In 1998 the first outbreak in Malaysia was recorded.

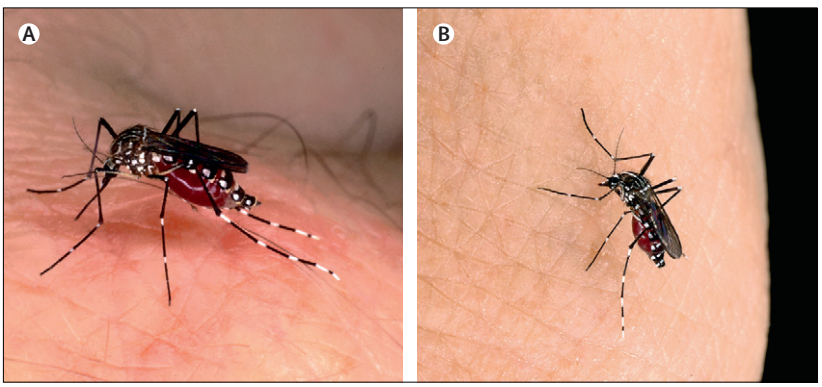

Figure 1: Aedes aegypti (A) and *Aedes albopictus* (B) mosquitoes

Re-emergence of chikungunya virus

After several decades of absence, CHIKV has re-emerged in both Africa and Asia, causing large outbreaks and has become a substantial public health concern (figure 2). In 2000, an urban epidemic of CHIKV was described in Kinshasa, Democratic Republic of the Congo, after an absence of 39 years[18] and, in 2001–03, the virus re-emerged in Indonesia after an absence of 20 years.[36] In 2004 the virus emerged in Lamu and Mombasa (coastal towns of Kenya) and by January, 2005, it had spread to the Comoros Islands (Grande Comore, Moheli, Anjouan, and Mayotte), where cases continued to occur until May, 2005.[37] 215 000 residents in Grande Comore, which has a population of about 341 000, were estimated to have been infected.[37] In March and April cases were reported in neighbouring islands Seychelles and Mauritius.[38] By May, 2005, local cases had been identified in Reunion, where new cases were recorded until early 2007.[39] By April, 2006, an estimated 244 000 cases (a third of the Reunion population) were recorded in Reunion with 203 deaths associated with the infection and an overall attack rate of 35%.[39] The scarcity of *A aegypti* in Reunion and the surrounding islands during this period and the abundance of *A albopictus* suggested that *A albopictus* was the main vector. Sporadic cases were subsequently confirmed in 2009 and an outbreak with 100 confirmed cases occurred in 2010, suggesting active transmission was occurring, possibly the result of reintroduction from Madagascar.[40,41] The virus was identified in the Maldives for the first time in December, 2006, and in Singapore in January, 2008. Genetic analysis of CHIKV isolates from Kenya and the Indian Ocean Islands confirmed that an African genotype was circulating. These epidemics in the past decade might be caused in part by changes in the virus genome that could affect the interaction of CHIKV with mammalian hosts or mosquitoes. The CHIKV strain circulating during the 2005–06 Indian Ocean epidemic seemed to have acquired a mutation in the envelope glycoprotein E1-A226V, which alters vector specificity, allowing the virus to adapt well to replicating in *A albopictus* and probably contributing to the size of the outbreak.[4] E1-A226V improves adaptation for dissemination by *A albopictus*[4] and is present in Reunion, India, and Cameroon. Furthermore, changes in glycoproteins E1 and E2 could also play a part in the adaptation of the virus in mosquitoes in central Africa.[4]

Re-emergence of CHIKV was reported in India during 2005–06 after a 32-year hiatus of viral activity.[42,43] The outbreak started in the coastal towns of Andhra Pradesh and Karnataka and spread to other districts, resulting in an estimated 1·3 million cases in 13 different states.[43] The outbreaks that occurred in the Indian Ocean Islands and subsequently in India have been attributed to a strain that is closely related to east African strains of CHIKV. However despite a 99·9% nucleotide similarity between isolates from the Indian outbreak and isolates from the Indian Ocean Islands, the mutation proposed to be associated with adaptation to *A albopictus* was not present in strains circulating during the 2005–06 Indian outbreak.[42] Further evidence from a mosquito isolate from Maharashtra obtained in 2000 suggested that the genotype switch from Asian to African had occurred before the explosive 2005 outbreak.[43]

CHIKV infections have been confirmed in travellers returning to Europe, Australia, the UK, and the USA from endemic regions in the Indian Ocean Islands and Asia.[44–50] Of particular concern was the local transmission of CHIKV in 2007 in northern Italy, which resulted in an estimated 254 locally acquired infections.[51] *A albopictus* is found in various countries in Europe and both *A albopictus* and *A aegypti* are well established species in southeastern regions of the USA. Returning travellers with high viraemia could be a source of virus for local *A albopictus* populations and there is a risk that CHIKV could establish endemicity in parts of Europe.

Clinical findings

CHIKV infection has several similarities with dengue fever infection. The incubation period ranges from 1 day to 12 days, with an average of 2–4 days, and is followed by sudden onset of chikungunya fever, which is

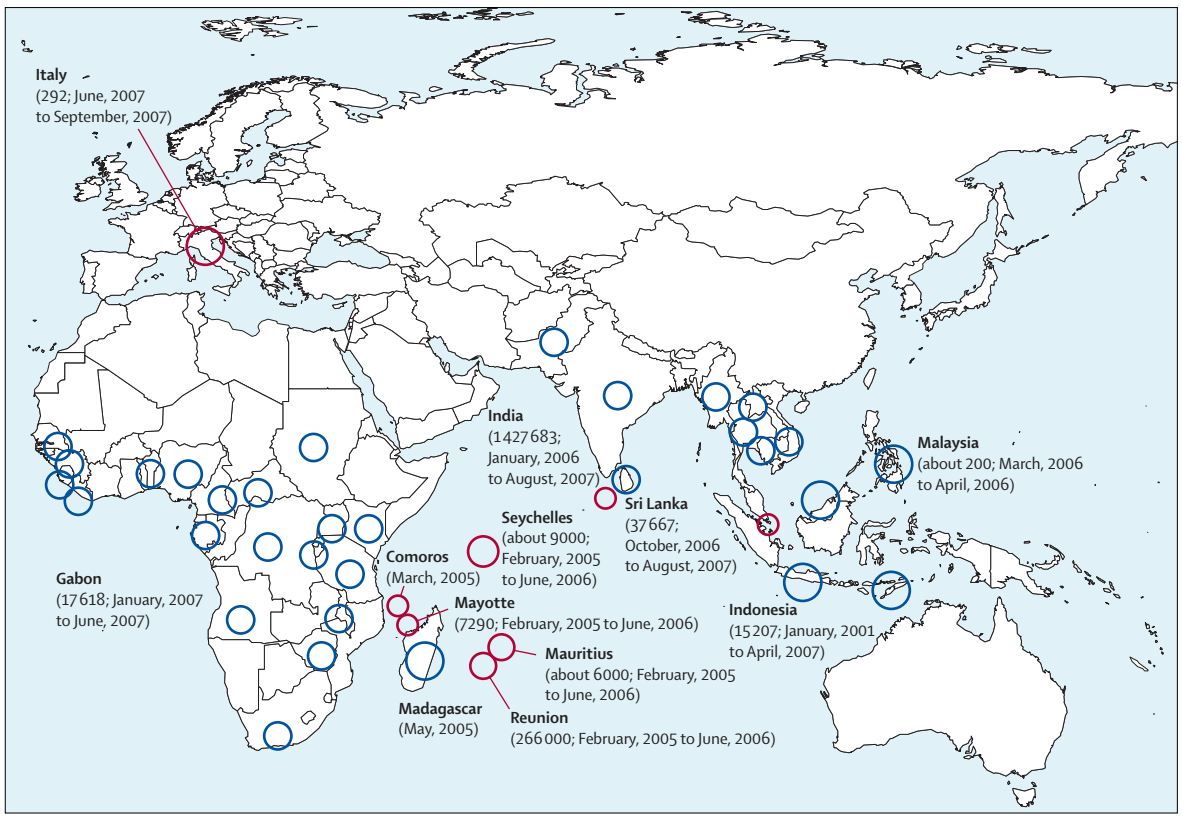

Figure 2: **Global distribution of chikungunya virus**
Blue circles indicate historical distribution of chikungunya virus in countries where the virus has been identified by virus isolation or serological evidence. Red circles indicate global re-emergence within the past decade. The number of cases, when available, is given in parentheses, along with dates of outbreak. Data from ref 35.

characterised by high fever and severe arthralgia and myalgia, together with headaches, photophobia, and a skin rash.[50–54] Asymptomatic infections are rare; roughly 3–25% of people with serological evidence of infection have no obvious symptoms.[53,55]

Polyarthralgia is a feature of most cases of CHIKV fever[1,50–52] and is the most disabling symptom. Nearly all patients with CHIKV infection have arthralgia that is usually symmetrical and almost always affects more than one joint.[56–58] Fingers, wrists, ankles, elbows, toes, and knees are most often affected. Swelling is a common feature but there are usually few other signs of joint inflammation.[52] Joints that are already damaged by underlying disorders such as osteoarthritis are particularly susceptible. The acute signs and symptoms of CHIKV infection usually resolve within 1–2 weeks, but the arthralgia can persist for months or years.

Most patients have partial improvement in arthralgia 1–2 weeks after acute onset of disease.[56,59] At this stage, some patients recover fully, but many patients have persistent arthralgia that lasts for months or years. Progression to persistent arthralgia has been analysed in several studies.[56–60] Although the design of each of these studies varies substantially, including timeframe and population of patients, the overall conclusion is consistent—a substantial proportion of those infected with CHIKV have persistent arthralgia. For example, in a retrospective cohort study, Sissoko and colleagues[57] noted that 15 months after the initial infection 57% of individuals reported persistence or episodes of recurrence. Joint symptoms can persist for years; an early study of serologically proven CHIKV infection noted that 12% of patients described residual joint symptoms such as stiffness, swelling, and pain 3 years after initial infection.[59] In a prospective study of 21 patients with chikungunya infection from Reunion who were also diagnosed with rheumatoid arthritis, only four patients had normal radiographs of their hands and feet 12 months after infection, and most patients showed signs of erosive arthritis and joint space narrowing.[61] Destructive arthritis and increased expression of inflammatory mediators have been reported in a patient with persistent IgM antibodies 2 years after infection.[62] The likelihood of developing persistent arthralgia is highly dependent on age. In the study by Sissoko, individuals older than 45 years were significantly more likely to show persistence. Similar results were obtained in a longitudinal follow-up study of 203 patients with confirmed CHIKV infection, in which the likelihood of developing persistent arthralgia was associated with age.[58] Other factors that increase the

likelihood of persistent arthralgia included underlying disorders and the severity of pain at disease onset.[57] Children are also at risk for severe manifestations of the disease with common clinical features such as abrupt onset high-grade fever, skin rashes, and swollen ankles or wrists (Stéphanie Robin and Duksha Ramful, Centre Hospitalier Regional, Saint-Denis, Reunion Island, personal communication; figure 3).[63]

The chronic arthralgic phase is normally characterised by fluctuations in intensity and relapses, usually affecting the same joint sites that were affected during the acute phase. The chronic phase is generally less severe than the acute phase, but many patients still have a pronounced reduction in movement and quality of life.

Skin lesions are normally present during acute CHIKV infections, most often affecting a patient's torso, limbs, and face.[64,65] The incidence varies between different studies but is usually reported as affecting about 50% of patients. The most common manifestation is a transient maculopapular rash in patients' legs and arms that lasts for 2–3 days (figure 4), with pruritus accompanying the rash in some cases.[50,56] Several additional skin presentations have been described, including aphthous-like ulcers, vesiculobullous lesions with desquamation, and vasculitic lesions.[63–65]

Diarrhoea, vomiting, and abdominal pain were reported in about half of hospital-referred patients during the 2005–06 Reunion outbreak.[52] Similar symptoms have been reported in other outbreaks although the incidence was mostly lower than that in the Reunion outbreak.[66–68]

CHIKV is not generally thought to be a neurotropic virus, but much evidence exists of neurological involvement in CHIKV infection.[69] Although some early studies reported neurological involvement in chikungunya infection,[68,70] most reports of this complication are from the outbreaks within the past decade in Reunion and India. In adults who need to be admitted to hospital during acute CHIKV infection, neurological presentations include encephalopathy, acute flaccid paralysis, and Guillain-Barré syndrome.[71–73] In an epidemiological study in Reunion, neurological involvement was reported in about 25% of patients with atypical CHIKV infection (<0·1% of all patients admitted to hospital), with encephalitis, malaise, and meningoencephalitis reported most often.[74] Most of these individuals also had underlying disorders, including stroke, epilepsy, hypertension, and diabetes mellitus.

In children, neurological complications were a common cause of hospitalisation for CHIKV infection, with encephalitis, febrile seizures, and acute encephalopathies the most common manifestations.[75] In the 2005–06 Reunion epidemic, vertical (mother-to-child) transmission was described for the first time, and encephalopathy was the most common severe clinical manifestation in newborn babies infected by this route.[76]

In epidemics that have occurred since 2005, mortality associated with CHIKV infection has been described, with a case-fatality rate of about 1 in 1000.[52,54,76–78] Most of the deaths have occurred in neonates and elderly people, and in adults with underlying disorders. In patients with CHIKV infection, the common causes of death are heart failure, multiple organ failure, hepatitis, and encephalitis.[74] In most cases, a direct causal link between death and CHIKV infection has not been shown.

A wide range of additional clinical manifestations are associated with acute CHIKV infection. Conjunctivitis has been recorded in a few patients.[51,56] Additional severe clinical manifestations include cardiovascular disorders, pneumonia, pre-renal failure, and respiratory failure.[74] Haemorrhagic manifestations are rare, which is an important distinction from dengue fever.[52] In addition to arthralgia, fatigue is the other main persistent symptom of CHIKV infection, and can last for months or even years after initial infection.[58,79]

As discussed above, although atypical CHIKV-induced disease, which can include encephalitis and multi-organ failure resulting in death, is certainly a major aspect of CHIKV's effect on public health, the most common and distinguishing feature of CHIKV infection is severe debilitating arthralgia. Although the mechanisms underlying CHIKV-induced arthralgia are poorly

Figure 3: **Severe manifestations of chikungunya in an infant**
Hyperalgesic infant aged 7 months with maculopapular rash presented with oedema of hands and feet. Other presentations include swollen ankles or wrists, without typical inflammatory joints.

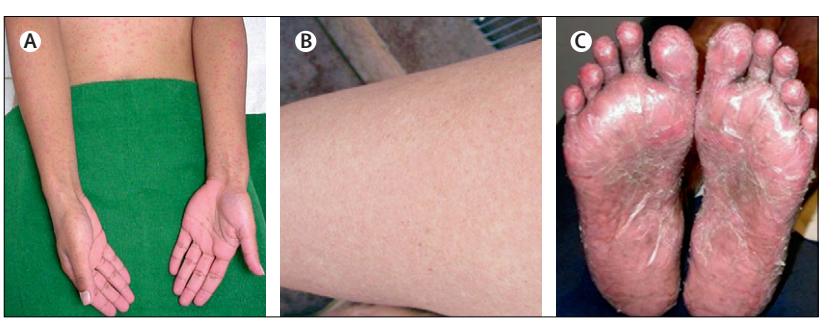

Figure 4: **Typical rashes with chikungunya virus infection**
Maculopapular rash, petechial spots and erythroderma of arms (A), legs (B), and feet (C).

understood, evidence from human beings and animal models suggests that the host inflammatory response plays some part in CHIKV-induced disease. As discussed above, soft tissue swelling is a common symptom of acute CHIKV infection, although joint effusions are present in only a small subset of patients with CHIKV during the acute phase of disease.[52,58] Furthermore, a study by Ng and colleagues,[80] suggested that the pro-inflammatory cytokines interleukin-1 and interleukin-6 were up-regulated in individuals with severe CHIKV-induced disease. Although the sample size was small, these results suggest that increased inflammatory cytokine expression is associated with CHIKV-disease severity, raising the possibility that dysregulation of the host inflammatory response could contribute to CHIKV-induced disease. Unlike Ross River virus, in which viral antigen and RNA are present in synovial samples and inflammatory cells have been isolated from joints,[81] the paucity of synovial samples from people acutely infected with CHIKV has prevented the determination of whether inflammatory cells are present in the joints of individuals with acute CHIKV. However, Ozden and colleagues[82] provided evidence for inflammatory cell infiltration and viral replication within muscle during CHIKV-induced myositis, suggesting that inflammatory cells are present in CHIKV-infected tissues. Studies with newly developed non-human primate and mouse models also suggest that CHIKV replicates to high concentrations in joint tissues, and that this viral replication leads to the recruitment of inflammatory cells, with monocytes, macrophages, and natural killer cells being the major inflammatory cell types.[83,84] Studies with the mouse model by Gardner and colleagues[83] showed that CHIKV infection led to swelling of the foot, and was substantially reduced by macrophage depletion, suggesting that components of the host inflammatory response contribute to CHIKV-induced disease. Although these results need to be validated in people infected with CHIKV, they do suggest that interfering with the host inflammatory response could be a reasonable approach for the development of treatments for CHIKV-induced polyarthritis.

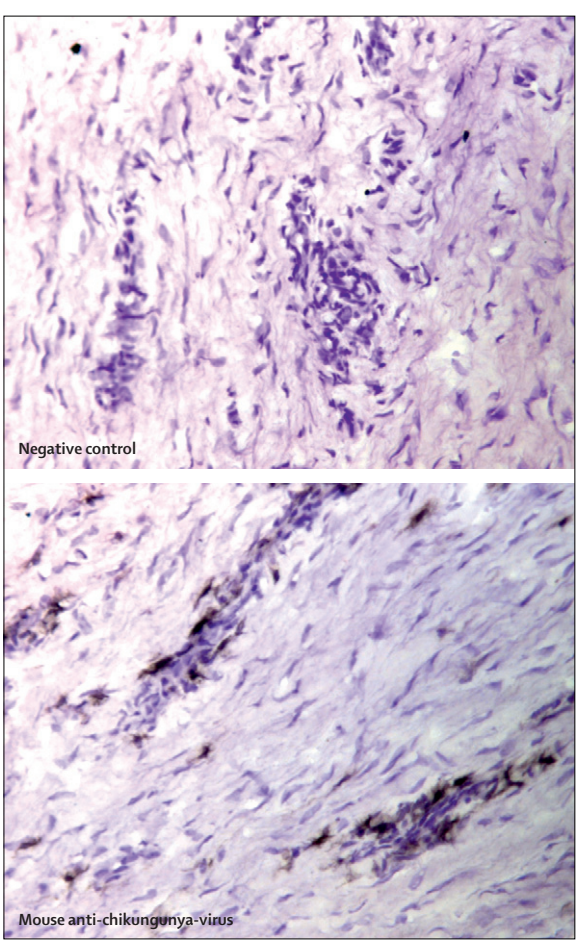

Figure 5: **Immunoperoxidase (brown) staining of synovial tissue of a patient 18 months after chikungunya virus infection**
Nuclei are counterstained in blue with haematoxylin (×200 magnification).

Although CHIKV-induced arthritis and arthralgia are acute and self-limiting in most individuals, a subset of patients develop chronic joint pain for months or even years after the initial onset of disease, with elderly people being more heavily predisposed to developing chronic disease.[57] The chronic nature of alphavirus-induced arthralgias and destructive arthritis has led to the suggestion that chronic joint pain is caused by viral persistence.[62] In support of this suggestion, people with chronic CHIKV-induced arthralgia often have persistent virus-specific IgM.[60,62] Because persistence of antigen-specific IgM is thought to be caused by continued exposure to antigen, it has been taken as indirect evidence for viral persistence. However, direct evidence for viral persistence had been absent until Hoarau and colleagues[85] provided evidence for persistence of viral antigen and RNA in synovial tissue from a patient with chronic arthralgia for 18 months after CHIKV infection (figure 5). Consistent with this finding, in a study of non-human primates, Labadie and colleagues[84] recorded evidence for viral persistence in lymphoid tissues. However, the nature of CHIKV persistence in human beings and how this persistence relates to chronic disease has not been established.

Diagnosis

Chikungunya infection is diagnosed on the basis of clinical, epidemiological, and laboratory criteria. An acute onset of fever and severe arthralgia or arthritis that is not explained by other medical disorders is considered as a possible CHIKV case. The case becomes probable if the patient has lived in or visited epidemic areas (figure 6).[86] However, laboratory confirmation is crucial, because the case should be distinguished from various disorders that have similar clinical manifestations, such as dengue fever, other alphaviruses and arthritic diseases, and endemic malaria. The interpretation of laboratory findings is

dependent on knowledge of the kinetics of viraemia and antibody responses in human beings. Confirmation of the diagnosis in the acute phase of illness consists of detection of viral nucleic acid in serum samples by reverse-transcriptase PCR (RT-PCR), isolation of the virus, or detection of an antibody response.[87–97] In samples obtained later, the diagnosis is confirmed by the presence of an immune response. The RT-PCR with conventional thermocycling or real-time PCR constitutes a rapid and sensitive technique for diagnosing chikungunya infection during the early stages of illness before an antibody response is evident. Real-time RT-PCR assays with either SYBR green or Taqman probe-based technology targeting a region of the non-structural protein (*nsp1*) gene or the envelope (*E*) gene and real-time loop-mediated isothermal amplification (RT-LAMP) assays have been used as diagnostic methods.[48,87,88,90,91,93,94]

CHIKV can be isolated with mosquito or mammalian cell cultures, or by intracerebral inoculation of 1-day-old mice. CHIKV will produce cytopathic effects in various mammalian cell lines, although Vero cells are used most often. The presence of early antibody seems to prevent isolation of the virus, hence virus isolation has been shown to be successful largely in antibody-negative samples obtained on or before day 2 of illness.[96] RT-PCR can detect viral nucleic acid in samples obtained from 1 day before onset of symptoms up to and including day 7, even in the presence of a detectable antibody response.[89,96] Antigen capture ELISA have been described for detection of antigen in serum samples and cerebrospinal fluid obtained as early as day 2 after onset. Antigen detection assays can be used in laboratories with less-sophisticated equipment and cost considerations.[98]

Indirect immunofluorescence and ELISA are rapid and sensitive techniques for detection of an immune response to chikungunya and can distinguish between IgG and IgM antibodies. A specific IgM antibody response is usually detectable between 2 days and 7 days after onset of fever with ELISA and immunofluorescence, although it has been reported as early as day 1 with a lateral flow rapid test.[49,95–97] Similarly, an IgG antibody response has been reported as early as day 2 after onset,[96] although it is more frequently detected from days 5 to 6.[49,96] IgG antibody persists for years, whereas IgM antibody activity usually decreases to undetectable levels by month 3–4 after infection, although persistence of specific IgM antibodies within 24 months after infection has been reported.[62,96] Various in-house ELISA techniques that use whole antigen or recombinant capsid or envelop antigens have been described.[99] Commercial serological assays are available and results obtained from a comparison of the assays suggested that the sensitivity for detection of an early antibody response before day 5 is dependent on the strain of the virus used for the assay or the source of the antigen and that recombinant antigens might be too specific with regard to mutations.[95,97,99] With the exception of o'nyong-nyong virus, serological tests can be used to readily distinguish CHIKV infections from flavivirus infections and other alphaviruses, such as Mayaro and Ross River viruses, which are also geographically distinct from CHIKV, O'nyong-nyong virus (which is found in east and central Africa) was initially thought to have emerged from CHIKV as a consequence of mutations but is distinct from CHIKV, forming a separate genetic lineage and having antigenic and biological differences.[3,100]

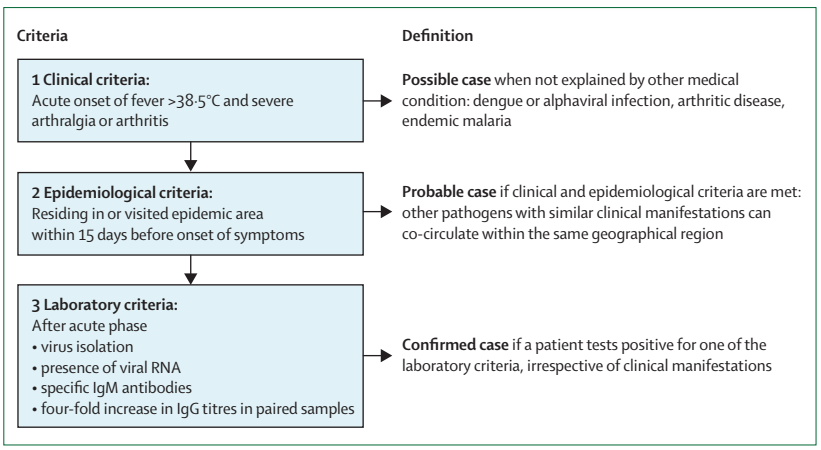

Figure 6: **Diagnostic criteria for chikungunya virus fever**

Vaccines and treatments

The only recommended treatments for CHIKV-induced arthralgia are non-steroidal anti-inflammatory drugs. However, the re-emergence of CHIKV has led to the assessment of several potential treatments, including CHIKV antibodies, ribavirin, and chloroquine. Passive transfer of CHIKV immune serum protects against CHIKV-induced lethality in mouse models, suggesting that monoclonal antibody treatment could have value against CHIKV.[83,101,102] This type of approach is likely to be most effective as a prophylactic treatment, because immune serum is only protective when provided before or immediately after CHIKV challenge.[101]

Ribavirin has antiviral activity against several RNA viruses, although its activity is less pronounced against alphaviruses.[103] However, Ravichandran and Manian[104] suggested that ribavirin treatment had a moderate beneficial effect in alleviating arthralgia and swelling associated with chronic CHIKV arthralgia, although their study consisted of a very small cohort of patients and additional studies are needed to find out whether ribavirin has any true benefit in the treatment of chronic CHIKV-induced disease. Briolant and colleagues[105] noted that ribavirin had some antiviral activity against CHIKV in cell culture, but interferon-α was more effective in inhibiting CHIKV replication in their study. This finding is consistent with several studies with other alphaviruses, including CHIKV,[83] where type I interferons showed potent antiviral activity, especially when given before infection.[106] Briolant

and colleagues[105] also noted that interferon-α and ribavirin showed a synergistic antiviral effect, suggesting that combination treatment with type I interferon-α and other antiviral drugs could have benefit for treatment of CHIKV.

Another potential treatment for CHIKV is chloroquine, which inhibits CHIKV infection in cell culture through its effects on endosomal acidification.[107,108] Chloroquine and its derivative, hydroxylchloroquine, also show anti-inflammatory activity, in part through their ability to interfere with endosomal toll-like receptor activation, and has a long history of use in treating chronic inflammatory diseases such as systemic lupus erythematosus and spondyloarthritis.[109] Chloroquine's anti-inflammatory activity has been discussed in reducing the inflammatory aspects of CHIKV-induced disease; however, because toll-like receptors have been suggested to contribute to CHIKV control in animal models,[110] chloroquine could interfere with protective host responses against CHIKV. Studies with chloroquine treatment of CHIKV-induced disease in human beings have given inconclusive results. The results from a small open clinical trial with patients with chronic CHIKV-induced arthralgia suggested that chloroquine treatment might have a beneficial effect.[111] By contrast, a study consisting of 27 patients treated with chloroquine during the acute stage of infection and 27 controls reported no beneficial effect on acute disease, and treated individuals showed a statistically significant increase in the incidence of chronic arthralgia.[108]

The explosive nature of CHIKV epidemics combined with the few available antiviral drugs for the treatment of CHIKV suggests that vaccination could be the most cost-effective means of protecting at-risk populations against CHIKV-induced disease. Epidemiological evidence suggests that CHIKV infection results in lifelong protective immunity and little evidence exists for viral antigenic variation resulting in immune escape. Furthermore, although the immune correlates of protection from CHIKV-induced disease are not completely understood, good evidence is provided by adoptive transfer studies that indicate that neutralisation by anti-CHIKV antibodies protect against CHIKV infection and are thought to be the major protective correlate of CHIKV vaccination.[83,101,102] A live-attenuated CHIKV vaccine was developed by the US Army and was assessed in phase 2 clinical trials. This vaccine was developed by serial passage of the virus in MRC-5 cells resulting in a virus (TSI-GSD-218), which carried several genetic changes. This vaccine elicited a robust anti-CHIKV neutralising antibody response in vaccinated individuals, and although a few vaccinated individuals developed transient arthralgia, the vaccine was thought to be generally safe and efficacious.[112] However, further assessment of this vaccine was discontinued by the US Army in 2000 because research priorities changed. Inactivated vaccines have been developed by several groups, including a detergent inactivated vaccine first reported in 1970.[113] In general, these vaccines will elicit neutralising antibody and protect mice from CHIKV replication and disease.[83,113] A formalin inactivated CHIKV vaccine was tested in a small cohort of human volunteers in the early 1970s, in which it elicited neutralising anti-CHIKV antibody responses and no observed adverse effects.[114] In addition to classic live-attenuated and inactivated vaccines, several other vaccination approaches, including DNA,[115] alphavirus chimeras,[116] and virus-like particle vaccines,[102] which might offer advantages over inactivated and live-attenuated CHIKV vaccines in terms of efficacy or safety, are in development. The chimeric alphavirus approach consists of the CHIKV structural protein coding region placed in combination with the replicase genes of another alphavirus, such as Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, or Sindbis virus. These chimeric viruses have several attractive attributes as vaccines, including the fact that the chimeras can be grown to high titres in culture, which reduces production costs, were highly attenuated in susceptible young mice when compared with wild-type CHIKV, and elicited a strong neutralising antibody response after a single immunization.[116] Another promising approach involves the generation of CHIKV virus-like particles, which are non-infectious and therefore inherently safe, but which elicited very strong neutralising anti-CHIKV responses and protected from viral replication in non-human primates.[102] Therefore, although additional work is needed to develop useful CHIKV vaccines, several of the approaches discussed above have promising safety and efficacy profiles in controlling CHIKV in animal models, suggesting that the development of safe and effective CHIKV vaccines should be readily achievable.

Contributors
All authors contributed equally.

Conflicts of interest
We declare that we have no conflicts of interest.

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