Updates on Chikungunya Epidemiology, Clinical Disease, and Diagnostics

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Abstract

Chikungunya virus (CHIKV) is an Aedes-borne alphavirus, historically found in Africa and Asia, where it caused sporadic outbreaks. In 2004, CHIKV reemerged in East Africa and spread globally to cause epidemics, including, for the first time, autochthonous transmission in Europe, the Middle East, and Oceania. The epidemic strains were of the East/Central/South African genotype. Strains of the Asian genotype of CHIKV continued to cause outbreaks in Asia and spread to Oceania and, in 2013, to the Americas. Acute disease, mainly comprising fever, rash, and arthralgia, was previously regarded as self-limiting; however, there is growing evidence of severe but rare manifestations, such as neurological disease. Furthermore, CHIKV appears to cause a significant burden of long-term morbidity due to persistent arthralgia. Diagnostic assays have advanced greatly in recent years, although there remains a need for simple, accurate, and affordable tests for the developing countries where CHIKV is most prevalent. This review focuses on recent important work on the epidemiology, clinical disease and diagnostics of CHIKV.

Key Words: Chikungunya—Epidemiology—Genotype—Signs and symptoms—Arthralgia—Molecular diagnostics—Serologic tests.

Introduction

Chikungunya virus (CHIKV) is a zoonotic virus endemic in Africa and Asia, where the mosquito vectors Aedes (Ae.) aegypti and Ae. albopictus are present. First described in Tanzania in 1952 (Robinson 1955), CHIKV caused sporadic outbreaks over the next 50 years in these two continents. Since 2004, the re-emergence of CHIKV and the unprecedented global scale of the resulting epidemic has brought much attention to this previously neglected virus. There have been several recent reviews on CHIKV (Thiberville et al. 2013b, Rougeron et al. 2015); therefore, this paper focuses on important findings in epidemiology, clinical disease, and diagnostics reported in the recent literature and during the Chikungunya 2013 conference (CHIKV2013).

Epidemiology

CHIKV likely originated in Africa, and phylogenetic analysis indicates three genotypes, West African, East/Central/South African (ECSA), and Asian. Prior to 2004, these were rarely found outside the geographic limits implied by their names. The epidemics subsequent to 2004 were mainly due to isolates forming a distinct clade within the ECSA lineage (Volk et al. 2010), likely originating in eastern Kenya before spreading to cause large outbreaks affecting millions in islands of the Indian Ocean, India, and Asia (for review, see Rougeron et al. 2015), as well as numerous imported cases in previously nonendemic regions, including Europe and the Americas. More recently, since 2013, the Asian genotype has also caused significant outbreaks in the Caribbean and the Americas. The outbreaks have been explosive, with high attack rates over a short period of time; in La Réunion Island, about 34% of the population (266,000 cases) was infected in little over a year (Renault et al. 2012), whereas in India, the annual peak incidence in 2006 was as high as 158 per 100,000 population (Muniaraj 2014). In the opening lecture of CHIKV2013, Ann Powers provided an overview of the epidemiology and spread of CHIKV in the last decade. An updated summary of all the countries where local transmission of CHIKV has been
reported is available (Centers for Disease Control and Prevention 2015).

CHIKV2013 was held in Malaysia, thus there was a regional focus, with researchers from Southeast Asia presenting the recent disease situations in their countries. I-Ching Sam explained that Malaysia experienced a nationwide outbreak affecting >15,000 people in 2008 and 2009, but that there are now only sporadic cases numbering <100 in each of the last 2 years. Similarly, Yong Poovorawan described how CHIKV spread from neighboring Malaysia to cause large epidemics affecting >50,000 people in southern Thailand in 2008–2009; since then, sporadic outbreaks have continued in northern regions (Wanlapakorn et al. 2014). Herman Kosasih provided important data from a prospective cohort study in Bandung, Indonesia, showing that apart from the explosive outbreaks for which it is well known, CHIKV also caused endemic transmission at a rate of 10.1/1,000 person years (Kosasih et al. 2013). Chanditha Hapuarachchi also showed a resurgence of CHIKV in Singapore in 2013, following a virus introduction event, with subsequent establishment of endemicity (International Society for Infectious Diseases 2013). Amado Tandoc and Szalay AbuBakar presented data on the sustained spread of Asian CHIKV throughout the Philippines from 2011 to 2013, with over 1600 cases reported in 2013 (International Society for Infectious Diseases 2013).

The epidemic ECSA strains have continued to expand to countries and regions that have not previously reported CHIKV. Between 2009 and 2012 in endemic Asia, CHIKV outbreaks due to local transmission were confirmed for the first time in countries such as China (27 patients; Zheng et al. 2010), Bhutan (>200 patients; Wangchuk et al. 2013), and the Lao People’s Democratic Republic (197 patients; Soulaphy et al. 2013, and presented by Sivilay Xayaheuang during the conference). Local transmission of CHIKV was also seen in Bangladesh (39 patients; International Centre for Diarrhoeal Disease Research, Bangladesh 2009) and Brunei (one patient; Liew and Yung 2012), although the genotype was not reported.

Of great concern, local transmission has also been seen for the first time in regions previously considered nonendemic for CHIKV—Europe, the Middle East, Oceania, and the Americas. In Europe, where Ae. albopictus is present around the Mediterranean and Adriatic areas (Caminade et al. 2012), outbreaks occurred in northeast Italy in 2007 (>200 patients; Rezza et al. 2007) and southeast France in 2010 (two patients in Fréjus; Gould et al. 2010) and October, 2014 (11 patients in Montpellier; Institut de Veille Sanitaire 2014). In the Middle East, an outbreak affecting over 1500 people occurred in Yemen in 2010 (Zayed et al. 2012), and an autochthonous case was reported in neighboring Saudi Arabia (Hussain et al. 2013). The Oceania region, including Australia, New Zealand, and the subregions Melanesia, Micronesia, and Polynesia in the tropical Pacific Ocean, is known to have both Aedes vectors (Horwood et al. 2013a). Recent outbreaks have occurred in New Caledonia in 2011 (33 patients; Dupont-Rouzeyrol et al. 2012), Papua New Guinea in 2012 (>1500 patients; Horwood et al. 2013b), Yap in 2013 (>1000 patients), Tonga in 2014 (>3000 patients; International Society for Infectious Diseases 2014a), and French Polynesia in 2014 (>5000 patients; International Society for Infectious Diseases 2014b).

The global spread of the epidemic ECSA strains was facilitated by an A226V mutation in the E1 protein and second-step mutations that sequentially adapted the virus to Ae. albopictus (Tsetsarkin et al. 2014). However, an epistatic interaction between E1-226V and E1-98T, specific to the Asian lineage, limits the ability of Asian strains to adapt to Ae. albopictus by this E1-A226V mutation (Tsetsarkin et al. 2011). This may explain the apparent replacement of Asian strains by ECSA strains in some previously Asian genotype-endemic countries where Ae. albopictus is present, like Malaysia (Sam et al. 2009). Yet in others, the Asian genotype continues to circulate predominantly, such as in Indonesia (Kosasih et al. 2013) and the Philippines in 2013 (Lanciotti and Valadere, 2014).

In December, 2013, CHIKV was reported to cause autochthonous outbreaks for the first time in the Americas, Saint Martin Island, and the French West Indies (Leparc-Goffart et al. 2014). At the time of writing (March, 2015), there are over 1,280,000 suspected cases, 26,000 confirmed cases, and 184 associated deaths as the disease spread to >50 countries/territories in the Caribbean and the Americas, including the United States and Brazil (Pan American Health Organization 2015). This development is of major public health concern because both Ae. aegypti and Ae. albopictus are widespread in parts of the Americas, with dengue already occurring at high incidence, particularly in Central and South America. The Caribbean is also visited by many tourists and provides a further route of spread to currently nonendemic areas in Europe and North America, as was seen during the Indian Ocean island outbreaks.

It is of interest that the outbreaks in New Caledonia and the Americas were due to the Asian genotype, showing that it is not only the epidemic ECSA lineage that may invade new areas. Only Ae. aegypti vectors, and not Ae. albopictus, are present in New Caledonia and the Caribbean islands, which were the first to be affected in the spread to the Americas (Dupont-Rouzeyrol et al. 2012, Leparc-Goffart et al. 2014). Could the relative proportions of these two Aedes vectors in a given location then determine the most likely genotype to circulate? ECSA strains have clearly been shown to be better adapted to Ae. albopictus than the Asian strains (Tsetsarkin et al. 2011), whereas there is evidence that Ae. aegypti may have greater competence for Asian strains over ECSA strains (Sam et al. 2012, Vega-Rúa et al. 2014). It is likely that vector competence for different CHIKV genotypes varies widely between countries.

CHIKV has high attack rates, induces long-term neutralizing antibodies (Nitapattana et al. 2014), and has not been consistently shown to transmit transovarially in mosquitoes. These factors may partly explain why, historically, interepidemic periods lasted many years (Pavri 1986). Modern improvements in diagnosis, awareness, and surveillance will enable better understanding of the long-term epidemiology of CHIKV. In the years following the epidemics in the Indian Ocean islands, surveillance there has revealed only sporadic cases and limited outbreaks in previously unaffected areas within countries (Renault et al. 2012). The main concern over the next few years would be larger epidemics in newly affected regions such as the Americas, Europe, and Oceania. These regions contain countries with naïve populations and well-established Aedes vectors, and some have relatively poor diagnostic and public health infrastructure. Continued
surveillance and sharing of data will enable tracking of disease spread. In the absence of an available licensed vaccine and antivirals (for review, see Ahola et al. 2014), vector control is the only means of control available, but previous experiences in containing dengue are not encouraging.

Clinical Disease

The classical symptoms of fever, rash, arthralgia, myalgia, and headache appear after a short incubation period of 2–10 days. Fever is generally the first symptom to appear and does so abruptly. Other symptoms and signs then follow within a few days. Arthralgia or arthritis usually affects more than one joint, with the most commonly affected being the knees, ankles, hands, and wrists (Gérardin et al. 2013). Joint symptoms may be less pronounced and less frequent in children (Sebastian et al. 2009). The rash is usually maculopapular and may be itchy. Other less common symptoms include diarrhea, vomiting, hemorrhage, and, as presented during CHIKV2013 by Emilie Javelle and Padmamalini Mahendradas, inflammation of the ear (Javelle et al. 2014) and ocular disease, most commonly anterior uveitis (Mahendradas et al. 2013). Infants below the age of 1 year may present quite differently than older children, with febrile seizures, peripheral cyanosis, pedal edema, and vesiculo-bullous skin lesions that eventually peel (Valamparampil et al. 2009, Nkoghe et al. 2012). Unusual and severe manifestations occur rarely, such as neurological disease (meningo-encephalitis, Guillain-Barré syndrome), myocarditis, and multiorgan failure; these may be fatal, particularly in neonates and elderly patients with co-morbidities (Lemant et al. 2008). Increases in monthly crude death rates of 16.7–57.0% have been temporally linked to peak incidence months during outbreaks in Réunion (Josseran et al. 2006), Mauritius (Beeson et al. 2008), and Ahmedabad, India (Mavalankar et al. 2008), although the lack of laboratory confirmation and postmortem studies make it difficult to directly attribute deaths to CHIKV. This has allowed an estimate of a CHIKV case fatality rate of 1 in 1000 patients (Josseran et al. 2006).

In countries where both Aedes vectors are present, and where diagnostic capabilities may be limited, it may be hard to distinguish CHIKV and dengue infection, particularly in children, because symptoms and signs may overlap (Chipwaza et al. 2014). Comparative studies have shown that features such as myalgia, arthralgia, and rash are particularly associated with CHIKV, whereas low platelets may indicate dengue (Lee et al. 2012, Mohd Zim et al. 2013).

As presented by Patrick Gérardin during the conference, it was further observed that pregnant mothers infected within a few days of delivery transmitted CHIKV to their babies, resulting in severe neonatal disease, most commonly encephalopathy with subsequent neurodisability (Gérardin et al. 2008, Shenoy and Pradeep 2012). However, no congenital infection was seen in mothers infected earlier during pregnancy, suggesting that the placenta provides an effective antepartum barrier to CHIKV and that maternal–fetal transmission is mainly due to fetal contact with viremic maternal blood during labor (Ramful et al. 2014).

A proportion of infected patients remain asymptomatic, and estimated rates range from 5% to 28% (Ayu et al. 2010, Dupuis-Maguiraga et al. 2012). These patients nevertheless remain a public health concern because they represent an undetected, potential source of transmission. During an epidemic, asymptomatic viremic donors pose a threat to blood transfusion safety because their median viral loads are similar to those of symptomatic cases and are at levels known to be sufficient to transmit CHIKV to animals (Appassakij et al. 2013). During the conference, Hatsadee Appassakij presented the application of a probabilistic model to estimate the risk of transfusion-associated CHIKV in Thailand. When combined with other measures, such as screening at-risk donors and quarantine of at-risk blood products, potential transfusion-associated CHIKV was mitigated (Appassakij et al. 2014).

During CHIKV2013, Carlos Dommar described an agent-based model representing individuals to study spatio-temporal evolution of CHIKV. He showed that subclinical patients continue to spread the disease during an outbreak, even if restriction of movement of symptomatic cases is implemented (Dommar et al. 2014). It would, however, be difficult to enforce isolation of asymptomatic contacts of proven cases during an outbreak. In a further model of an outbreak in a Cambodian village, failure to incorporate asymptomatic cases and cases with unknown onset dates into the epidemic curve led to underestimation of the basic reproduction number, which could negatively impact implementation of control measures (Robinson et al. 2014). Although asymptomatic patients are neglected during outbreaks because they do not need health care, these studies demonstrate the importance of their identification.

Considerable evidence now exists that CHIKV leads to persistent rheumatic diseases in a proportion of adult patients for months to years after infection, although this is far less common in children (Sebastian et al. 2009). These musculoskeletal disorders include arthralgia, inflammatory arthritis, tenosynovitis, enthesisitis, and exacerbation of existing joint disease (Waymouth et al. 2013). Reported rates of persistent arthralgia range from 4.1% to 75.4% in different countries in Africa, Asia, and Europe, although studies used varying protocols and definitions (Manimunda et al. 2010, Chopra et al. 2012, Moro et al. 2012, Nkoghe et al. 2012, Gérardin et al. 2013, Mohd Zim et al. 2013). These different rates are likely due to multiple factors, including the genetic susceptibility of the affected populations, cultural attitudes to pain, and health care practices. Some of these studies used telephone interviews or questionnaires that relied on participants’ self-reported symptoms. Symptoms of persistent arthralgia may be relapsing or remitting, often affect multiple joints, and are associated with functional loss impairing activities of daily living, reduced quality of life, and symptoms of asthenia, depression, and anxiety (Couturier et al. 2012).

Quality of life may be impaired over a broad range of aspects, from physical (function, pain, general health) to mental (emotional and mental health, social functioning) components (Manimoutou et al. 2012). A few follow-up studies have also included clinical examination, which confirmed joint disease; in Italy, 33.3% of patients with arthralgia were diagnosed with arthritis and 9.8% with tenosynovitis (Moro et al. 2012); in India and La Réunion, over half of patients with arthralgia had clinically detectable joint swelling (Manimunda et al. 2010, Schilte et al. 2013). A small percentage of patients have evidence of chronic inflammatory erosive arthritis, which resembles rheumatoid arthritis and induces a similar inflammatory
response (Gasque et al. 2015), although rheumatoid factor may be negative. Imaging of some of these affected patients revealed bony erosions, joint effusions, and synovial thickening (Bouquillard and Combe 2009, Manimunda et al. 2010).

The persistent arthralgia may be due to continuing local inflammation in response to persistent viral antigen in the joints. In a synovial biopsy from a patient with chronic arthralgia, synovial hyperplasia, vascular proliferation, and infiltration by macrophages, natural killer (NK) cells and CD4 cells were seen, with CHIKV antigen and RNA detected in the perivascular macrophages (Houra et al. 2010). A recent study using next-generation sequencing found that amino acid diversity of the virus at the acute stage of infection was associated with persistent arthralgia at 300 days; the authors suggested that increased intrahost genetic diversity of CHIKV improves viral fitness and may increase the chances of virus persistence (Thiberville et al. 2013a). The most consistently identified clinical risk factor for developing persistent arthralgia in most studies is older age; others include longer duration of acute symptoms (Couturier et al. 2012), involvement of more than six joints during acute disease (Gérardin et al. 2013), history of existing joint disease (Moro et al. 2012), and delayed appearance of neutralizing immunoglobulin G (IgG) (Kam et al. 2012c).

It is increasingly appreciated that the impact of CHIKV disease extends beyond the acute, intense phase, to the considerable clinical burden of long-term morbidity. The economic cost of persistent arthralgia has been rarely studied, but in a study from La Réunion, the annual cost has been estimated at €34 million, or €250 per patient (Schilte et al. 2013). Efforts are required to understand the pathogenesis of persistent arthralgia and find early diagnostic indicators and treatments.

Diagnostics

Several diagnostic tests have been developed so far to detect CHIKV infections in the acute or later stage of the disease. Although nucleic acid amplification or antigen detection can only be used during the viremic phase of the disease, serological tests are necessary to identify previous infections or to determine the immune status of a patient. Despite the numerous diagnostic assays already described (for review, see Parida et al. 2008), continuous efforts are being made to expand and improve CHIKV diagnostics.

Molecular diagnostics

Molecular diagnostic tests used for detection of CHIKV encompass reverse transcriptase (RT)-PCR assays, in particular real-time RT-PCRs amplifying fragments in the nsP1, nsP2, nsP3, nsP4, or E1 regions of the CHIKV genome (Pastorino et al. 2005, Carletti et al. 2007, Edwards et al. 2007, Lanciotti et al. 2007, Laurent et al. 2007, Parida et al. 2007, Panning et al. 2009). In addition to labeled probes, SYBR Green is used for real-time quantification of the amplified PCR products (Ho et al. 2010, Ummul Haninah et al. 2010). For the described assays, the limits of detection range between 0.5 and 1.5 log10 RNA copies/reaction. Although the detection of positive-strand RNA is commonly used for diagnostic purposes, assays to detect negative-strand CHIKV RNA have also been developed. These include strand-specific quantitative RT-PCRs for nsP1 (Plaskon et al. 2009) and nsP3 (Chiam et al. 2013), which use a tagged-primer system to improve PCR specificity and accuracy. In comparison to detection of positive-strand RNA, the detection limit for negative-strand RNA assays is slightly decreased to 3 log10 RNA copies (Chiam et al. 2013).

While there is an ever-present threat of CHIKV spreading to developed countries in the Americas and Europe, CHIKV still predominantly occurs in resource-limited countries. Loop-mediated isothermal amplification (LAMP) represents a cheaper alternative amplification method, albeit with lower sensitivity compared to real-time RT-PCR. Monitoring by turbidity as well as observation of color change after adding SYBR Green has been described for detection of CHIKV (Parida et al. 2007, Reddy et al. 2012). Visualization is done with the naked eye without requiring any sophisticated equipment, thus LAMP is especially useful in developing countries or in field studies.

More recently, as a further alternative for the field, a microfluidic lab-on-chip integrating multiplex molecular amplification and DNA microarray hybridization has been developed for the simultaneous detection of 26 globally important tropical pathogens such as CHIKV, Dengue virus (DENV), and other arboviruses (Tan et al. 2014). Such diagnostic capacity provides an effective and rapid means to establish the presence of defined potential pathogens.

Serological assays

The majority of epidemiological surveys described are based on IgM and/or IgG enzyme-linked immunosorbent assays (ELISAs), demonstrating the importance of this type of assay. There are commercial IgG- and IgM-capture ELISA assays now available. Besides ELISAs based on native inactivated CHIKV antigen, recombinant structural proteins such as capsid (C), E2, and E1 have been used (Kowalzik et al. 2008, Chua et al. 2014). An ELISA based on CHIKV Env p62-E1 protein antigen, in which the ectodomains of CHIKV-115 P62 and E1 are joined with a glycine serine linker to form a soluble protein, was recently established (Voss et al. 2010). The latter ELISA is independent of the propagation of infectious CHIKV and was successfully used to evaluate CHIKV vaccine candidates (Garcia-Arriaza et al. 2014, Hallengård et al. 2014a, b). Antibodies against a linear B-cell epitope in the amino-terminus of E2 (designated E2EP3) are detectable in early and late convalescent CHIKV infection, making an E2EP3-based ELISA a useful assay for serological diagnosis (Kam et al. 2012a, b).

Further serological tests for detection of CHIKV antibodies include indirect immunofluorescence and immunoblot assays. A commercial indirect immunofluorescence test based on strain LR2006-OPY1 (GenBank acc. no. DQ443544) from La Réunion is available (Litzba et al. 2008). Immunoblot analyses have been described based on bacterially expressed CHIKV proteins, namely C, E2, and E1. Validation using serum samples previously tested positive in indirect immunofluorescence analysis showed that a mixture of E2 and C proteins seemed to be best for detecting human anti-CHIKV antibody-positive serum samples (Kowalzik et al. 2008). However, the performance of several of the available commercial assays in independent evaluation (Prat et al. 2014) and in clinical settings has been disappointing (Yap et al. 2010, Blacksell et al. 2011).
To be able to determine the immune status of a patient, it is necessary to determine the level of neutralizing antibodies. Neutralization (NT) assays based on infectious CHIKV have been used either with a plaque reduction assay or an immuno-fluorescence-based cell infection assay (Warter et al. 2011, Kam et al. 2012b). Furthermore, a pseudotyped lentiviral vector–based NT assay was established for CHIKV infection. The CHIKV-pseudotyped lentiviral vector was prepared by co-transfection with plasmids encoding the CHIKV glycoproteins, packaging elements, and a firefly luciferase reporter for readout (Kishishita et al. 2013). This setup allows the NT assays to be performed in a biosafety level 2 facility. Similarly, Beate Kümerer presented a recently developed assays to be performed in a biosafety level 2 facility. Similarly, Beate Kümerer presented a recently developed assay to be performed in a biosafety level 2 facility. Au enthralling story of this specimen is that of an affordable, simple, and reliable assay to be used in new areas, especially those with endemic vectors, is a major concern, and continued global monitoring is mandatory. Further research is needed to understand the factors governing the emergence and transmission of both the ECSA and Asian strains. Meanwhile, in countries that have already suffered large outbreaks, attention is focused on the long-term burden of persistent arthralgia in a proportion of patients. The early identification of patients at risk and treatment options for those with arthralgia are key areas for study. Although numerous methods for laboratory diagnosis have been developed in recent years, a significant unmet need is that of an affordable, simple, and reliable assay to be used in developing countries where CHIKV is mainly endemic.

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