

Genomic evolution and phenotypic distinctions of Chikungunya viruses causing the Indian Ocean outbreak

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Abstract

In our current global community with the rapid movement of products and people across and between continents, the emergence of a human pathogen can have devastating consequences. One dramatic example of this has been the emergence of Chikungunya virus (CHIKV), which causes a severe, prolonged, and debilitating arthralgic disease. This virus emerged in a large outbreak on the east coast of Africa in 2004; over the subsequent seven years, CHIKV has spread across the Indian Ocean, the Indian subcontinent, Southeast Asia and even reached Europe, leaving more than two million people affected. Because CHIKV has a small genome, currently available tools to analyze complete viral genomes have provided scientists with unique opportunities to understand the epidemiology, pathogenesis and transmission of the virus. The most commonly used application of these cutting edge tools has been to track the movement of the virus over time and space. While this is an important concept for identifying areas that remain at risk for outbreaks, these postgenomic era tools can also be applied to the highly significant tasks of understanding how viral microevolutionary changes can affect both invertebrate transmission and vertebrate virulence. Significant alterations in the patterns of CHIKV movement have already been identified using microevolutionary studies. These approaches now need to be further expanded to aid in expanding vaccine, therapeutic and control options. This review will highlight some of the most significant recent research developments obtained using these cutting edge approaches for CHIKV.

Keywords: Chikungunya virus, microevolution, emerging human pathogen

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Introduction

Viruses transmitted by invertebrate vectors (arboviruses) are the causative agents of some of the most important emerging infectious diseases. Within the past decade alone, Rift Valley fever virus, West Nile virus, bluetongue viruses, yellow fever virus and Chikungunya virus have been responsible for massive outbreaks in both human and animal populations throughout the world.^{1–5} This last pathogen, Chikungunya virus (CHIKV), initiated an outbreak in 2004 that has lasted for over seven years, has moved from a starting point of coastal Kenya to the islands of the Indian Ocean, to India and Southeast Asia, resulting in over two million human cases of illness throughout its course.^{6,7} During this rapid and widespread movement of the outbreak, the virus was introduced into greater than 20 non-endemic countries by viremic travelers (Figure 1). Significantly, in 2007 in Italy, the virus established autochthonous transmission;⁸ this was the first documentation of localized transmission in an area outside the historical range of the African or Asian continents.

Furthermore, the virus has continued to re-surface during the course of the single outbreak, sometimes in areas that had never historically reported CHIKV activity.⁹

Since the first identification of CHIKV, there have been over 44 documented outbreaks of CHIKV fever but activity is typically self-limiting. An ongoing and continually moving epidemic is unprecedented and the reasons for the shift in epidemiological patterns require investigation. There are multiple factors that are involved in the emergence of any pathogen to cause an epidemic, including ecological factors, population immunity levels, social parameters, vector/host associations and genetics factors – both viral and host. While the interactions of all these parameters are both complex and challenging to understand, the relative simplicity of the alphaviral genomes provides them with an advantage and makes them amenable to more fine scale characterization than many of the other factors. Complete alphaviral genomes can be easily sequenced within a matter of days, providing a wealth of data for comparison in efforts to elucidate the role of

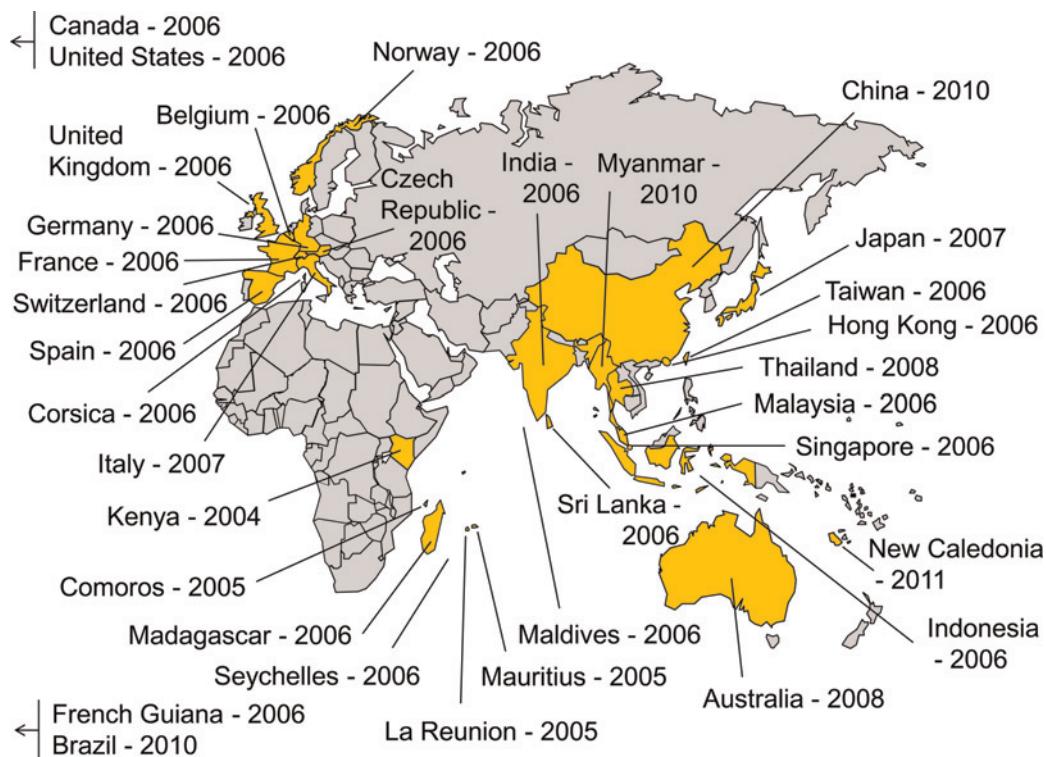


Figure 1 Countries with documented Chikungunya virus activity during the 2004–2011 outbreak. Year listed is the year activity was first confirmed. Not all countries affected have had ongoing or localized transmission; countries reporting only imported cases are also shaded

genetics and individual mutations in emergence events. Numerous efforts to evaluate the role of microevolution of the CHIKV genome have been undertaken during the ongoing epidemic. Highlights of these studies are presented here to provide a framework for understanding how viral genetic elements can participate in and influence epidemiological outcomes.

Chikungunya virus

Like all alphaviruses, CHIKV has a genome consisting of a linear, positive sense, single-stranded RNA molecule of approximately 11.8 kilobases.^{10,11} As such, the virus functions as a messenger RNA (mRNA) and begins replication and translation upon entry into a cell. The non-structural proteins, required for viral replication, are encoded in the 5' two-thirds of the genome, while the structural genes are colinear with the 3' one-third. The 5' end of the genome has a 7-methylguanosine cap and there is a polyadenylation signal at the 3' end. Upon cell entry, the non-structural proteins are synthesized and generate complexes involved in both genome replication and translation of proteins. The structural gene products are generated by translation of an abundant subgenomic message to produce a polyprotein that is processed to produce a capsid protein, two major envelope surface glycoproteins (E1 and E2) as well as two small peptides, E3 and 6K.¹⁰ The vast majority of partial genome sequences available for CHIKV span the E1 and 3' non-coding region.

CHIKV genetic studies

Due to the small size of these genomes combined with advances in rapid and deep sequencing methodologies, over 130 complete CHIKV genomes have been reported to sequence repositories as of the end of 2010. A further 1100 partial genomes contribute to the database of CHIKV sequence. Phylogenetic analysis of CHIKV virus sequences originally identified three distinct clades separated primarily by geography¹² designated the West African, Central/East African and Asian CHIKV clades. Specific clusters of CHIKV activity have been examined genetically subsequent to this first broad look at the phylogenetic relationships among CHIKV strains to evaluate patterns of microevolution. One particularly detailed study involved a set of Indian isolates spanning a 37-year window. These analyses were designed to reveal if any particular strain was associated predominantly with outbreaks¹³ compared with non-outbreak isolates; this information would be particularly valuable epidemiologically. Unfortunately, from a public health perspective, the study concluded that virtually all Indian strains were monophyletic showing little genetic variation. Another study of strains obtained from the Democratic Republic of Congo during an urban outbreak in 1999–2000 demonstrated the close genetic relationship of these isolates with other strains from Central Africa.¹⁴ As would be expected, these studies confirmed that when an outbreak occurs in a given region, the sequence of the virus associated with the epidemic is genetically aligned with other known geographically related strains. Furthermore, all isolations during a particular outbreak

were practically identical. However, in contrast to the smaller number of isolates obtained and limited sequenced data collected in previous epidemics, the ease of culturing virus and rapid full-genome sequencing available during the recent outbreak has allowed more detailed sequence analysis revealing that genetic variation during an outbreak is more extensive than previously believed. No additional genotypes have been identified as a result of the significant volume of sequence produced during the epidemic period, but the significant volume of individual variants (of multiple genotypes) may have highly variable pathogenic outcomes.^{15–18}

Very early during the ongoing epidemic, genetic analyses were underway to examine the role of the viral genome in the emergence and spread of the outbreak. Sequence of viruses isolated from coastal Kenya demonstrated, as would be expected, that the viruses belonged to the Central/East African genotype.⁶ Additionally, the 2004 isolates were less than 3% divergent from their closest, characterized, ancestral strain and based on genetic similarity data; the most likely ancestor is related to a Ugandan-like strain from the 1980s.¹⁹ Strains isolated just a few months later in Comoros revealed that this 2005 activity was a continuation of the Kenyan outbreak rather than an independent emergence event as the sequences of the genomes were virtually identical.⁶ Because the alphaviruses are typical of RNA viruses in that they lack replicase proof-reading function, there was considerable variability among the sequences from isolates within each individual outbreak. For example, isolates from Kenya obtained within weeks of each other still exhibited over two dozen unique nucleotide mutations. Similarly, in Comoros, nine amino-acid mutations separated isolates obtained less than 12 weeks apart. During this early activity, the epidemiology of these outbreaks involving over 260,000 cases was similar to past epidemics; thus, extensive genetic characterizations were not performed to examine the implications of each mutation identified.

A year later, the emergence of CHIKV in La Reunion was accompanied by a change in the epidemiological pattern of transmission that warranted more detailed genetic analyses in explaining the scope of the outbreak.¹⁵ There were cases identified in La Reunion as early as March of 2005 but the epidemic was not significant until the onset of the rainy season in December of 2005. With the increased level of activity, atypical presentations (neurological manifestations, neonatal complications, hepatic disease) were noted as was a low level of mortality.^{20–22} Neither was known to widely occur with Chikungunya fever although rarely atypical patterns have been described in previous large urban outbreaks. A detailed viral genetic study of approximately 130 patient isolates revealed a number of point mutations that could be mapped over the course of the year-long outbreak on the island.¹⁵ The authors identified subsets of mutations associated only with cerebrospinal fluid as well as changes documented only later during the outbreak. The unique changes led to the speculation that they were possibly more associated with severe or neuroinvasive disease or that they led to the increase in the scope of the outbreak during the final months, respectively. This is a

significant point as some mutations were rarely detected at the outset of the outbreak but were present in greater than 80% of the isolates obtained during peak transmission. It was postulated that these mutations may have affected the epidemic similarly to what was documented with the equine avirulent Venezuelan equine encephalitis subtype ID viruses where only seven amino-acid changes generated epidemic forms of the virus responsible for large outbreaks.²³ Alternatively, the mutations may have impacted the transmissibility of the virus by mosquito vectors. Studies examining these options are further described below.

As the outbreak continued to expand both in number of cases and geography, further microevolutionary studies continued. The combined results of a number of these reports describe a method to trace the evolutionary path of the virus across time and space.²⁴ Genetic signatures often associated with a limited locale began to evolve; it was hoped that these signature variants would correlate with virulence or transmissibility patterns, thus aiding public health investigations of viral movement.

Significantly, the advanced sequencing and bioinformatics tools utilized during the recent outbreaks also allowed researchers to confirm that the Asian genotype virus is still present and undergoing active transmission.^{18,25} This is important as many of these detections have been imported cases rather than as part of large local foci of activity. This may suggest that the Asian genotype strains are less virulent and genetic signatures associated with this genotype should be examined for elements responsible for reduced pathogenicity.

Mosquito susceptibility is altered with CHIKV genetic changes

While the genetic tracing of microevolutionary patterns can aid epidemiologically, it remains highly important to determine if, and how, these individual mutations affect disease progression or the ability of the virus to be transmitted. Fortunately, one such genetic element has been well characterized for recent strains of CHIKV. One of the genetic analyses identified a single nucleotide change resulting in an alanine (Ala) to valine (Val) substitution at E1 glycoprotein position 226.¹⁵ This conservative change altered the ability of the virus to infect and disseminate within one of the urban transmission cycle vectors.²⁶ As background, maintenance of CHIKV in West and Central Africa occurs in a sylvatic cycle involving wild non-human primates and forest-dwelling *Aedes* spp. mosquitoes.^{27,28} The outbreaks in these primarily rural regions typically tended to be of smaller scale and dependent upon the ecological factors that influence sylvatic mosquito densities.²⁹ In contrast to those mosquitoes associated with the sylvatic cycle, *Ae. aegypti* has been found to be the most significant vector in the urban cycles documented in Asia.^{30–32} Urban outbreaks during 2004–2005 in East Africa and Comoros were also associated with the presence of *Ae. aegypti* mosquitoes.³³ A second mosquito sometimes associated with human CHIKV cases is *Aedes albopictus*. This is the species that was

incriminated in La Reunion island where *Ae. aegypti* are rare and *Ae. albopictus* is the predominant vector.²⁶

Because the number of cases increased so dramatically with the onset of the rainy season in La Reunion, there was speculation that changes in vectorial capacity had occurred. The viral genetic change identified at E1 226 was of particular interest because this site in the protein fusion domain had previously been shown to be associated with growth in cholesterol-depleted insect cells. Thus, mosquito infection studies using isolates obtained early during the outbreak were compared with those obtained during the peak and contained a Val substitute for the original Ala.²⁶ These elegant studies indicated there was a difference in how the virus infected and disseminated within *Ae. albopictus*. The Val variant was found to be more suited to transmission by *Ae. albopictus* than the Ala early outbreak variant. This observation was later confirmed using infectious clone derived viruses where the only difference was the presence or absence of the alanine at E1-226.³⁴ This was the first evidence that a naturally occurring point-mutant variant detected during the course of a CHIKV epidemic had likely impacted the scale and epidemiology of the outbreak. Curiously, this particular adaptive mutation was speculated to have emerged independently at least three times during the ongoing outbreak.^{17,35} This is an unusual situation and is suggestive of widespread high rates of transmission combined with abundant populations of the relevant mosquito species. Interestingly, further studies of this point mutation revealed that the genetic backbone of the virus influenced the effect of this mutation in various mosquitoes.³⁶ This need for additional differences in genetic elements supports the concept of infrequent positive selection requiring the involvement of multiple viral elements.

Role of CHIKV genetic changes in vertebrate virulence

While laboratory studies of mosquito vectors have identified microevolutionary changes associated with altered vector susceptibility, to date, no genetic evidence has explained the unusual clinical presentations observed initially in La Reunion. As noted above, atypical manifestations resulting from CHIKV infection have included encephalopathy (particularly in neonates), meningo-encephalitis, severe vesicular rashes, hepatitis and ocular diseases. However, one positive outcome resulting from the prevalence of these uncharacteristic symptoms has been the increased level of research into the understudied field of CHIKV pathogenesis. Like those investigations involving mosquito transmission or epidemiological tracking of the virus, genomic and bioinformatic tools have been instrumental in determining the role of various vertebrate components in CHIKV virulence patterns.

One study that utilized the large volume of genetic data available for recent CHIKV outbreak strains revealed that certain regions of the CHIKV genome were undergoing mild positive selective pressure postulated to be caused by vertebrate immune factors.³⁷ The authors identified a

number of 'antigenic switches' (changes in expression of genes at a specific site) that were suggested to eliminate binding to HLA molecules. In particular, there were significant HLA class I-restricted recognition patterns identified within the CHIKV E1 and E2 genes that had undergone substantial levels of mutation. Curiously, the domains where these mutations reside have been reported to be important for vector infectivity and may have been involved in the adaptation of the virus to *Ae. albopictus*. Furthermore, particular sets of mutations were associated with strains from distinct geographic regions; those isolates from the Indian Ocean outbreak clustered together with a distinct immunological signature. This early study on the influence of human immune response to CHIKV infection demonstrated that vertebrate immunity could play a major role in the viral evolution. The study also suggested the possibility that distinct human populations could exhibit genetic traits that could influence disease severity or presentation.

Building upon the idea that vertebrate immune factors could influence CHIKV outcome, several recent studies have examined the profile of inflammatory cytokines and chemokines in relation to disease severity. One such study which examined patients with either high or low viral loads during the acute phase of infection demonstrated that high levels of viremia were associated with higher levels of proinflammatory cytokines, chronic arthralgia and more severe disease.³⁸ A similar study documented that patients with high viral loads, high levels of IFN- α mRNA in peripheral blood mononuclear cells, and elevated levels of circulating interleukin-12 tended to have chronic symptoms in contrast to patients with lower levels.³⁹ While these studies have not yet tied specific genetic mutations with distinct phenotypic markers such as disease severity or persistence, these findings and others of a similar nature are important discoveries related to the disease. Certainly, the next step is determining how viral genetics affect these vertebrate immunomodulatory factors and overall patterns of disease.

While it would be ideal to evaluate the role of CHIKV genetics in human infections, there is often a lack of viral genomic data available from these patients. Thus, a suitable animal model is necessary. One limitation in the development of understanding the interactions of specific CHIKV genotypes with their vertebrate hosts has been the lack of an appropriate and widely available animal model that mirrors the human disease patterns. Recent development of non-human primate,^{40,41} and more importantly, common mouse models,⁴²⁻⁴⁴ particularly those that induce the characteristic CHIKV arthritis and tenosynovitis, have expanded the capacity to examine how variations in the CHIKV genome affect immunological response and disease outcome. While these studies are still early in their progression, one model has revealed differences in levels of inflammatory mediators and foot swelling when comparing strains from distinct CHIKV genotypes.⁴² Further studies are clearly warranted and are certain to be forthcoming with the combined use of viral genomics, infectious clone technology and new vertebrate models of disease.

Summary

CHIKV is a significant human pathogen that has been causing outbreaks for several hundred years. However, it is only with the large scope of recent outbreaks combined with a plethora of genomic and bioinformatic tools that transmission and epidemiological patterns associated with microevolution of the virus have been identifiable. Several potential research areas have been of particular focus based on viral genetic studies including vector capacity, viral evolution and virulence potential. This research is exceptionally important as the virus continues its expansion into new ecological niches where different mosquito populations and naïve populations with unique genetic characteristics may greatly affect the potential establishment and transmission of the virus. Given that CHIKV is vectored by two species of mosquitoes that are distributed throughout the tropics and reach into many subtropical latitudes, realistically, it is only a matter of time before the virus may then have the capacity to expand its enzootic range to include Europe, the Southern US, the Caribbean and Latin America. When this eventuality occurs, having information regarding mutations of significance in pathogenesis or transmission based on microevolutionary studies of the genome performed now, may aid in public health surveillance and diagnostic efforts in the future.

Disclaimer: The findings and conclusions in this report are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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