

Review

Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus

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Introduction

Arthropod-borne viruses (arboviruses) are the causative agents of some of the most important emerging infectious diseases and are responsible for significant global public health problems (Gubler, 2001). Of these viruses, the genus *Alphavirus* belongs in the family *Togaviridae*, and the species in this genus cause diseases ranging from mild febrile illness to severe polyarthritis to encephalitis. One alphavirus, chikungunya virus (CHIKV), caused recent outbreaks associated with severe morbidity. Traditionally, CHIKV causes a febrile illness similar to that seen in dengue virus infections. The hallmark feature of CHIKV disease is a debilitating and prolonged arthralgic syndrome that primarily affects the peripheral small joints. While the acute febrile phase of the illness normally resolves within a few days, the pain associated with CHIKV infection of the joints typically persists for weeks or months causing serious economic and social impact on both the individual and the affected communities. During the 2005–2007 explosive epidemics on the Indian Ocean islands and in India, anecdotal cases of CHIKV-associated deaths, encephalitis and neonatal infections were reported. Here, we review outbreaks of CHIKV disease and present a comprehensive analysis and comparison of clinical presentation, virus–vector associations and viral genetics to assess the possibility of further expansion and re-emergence of CHIKV illness.

Chikungunya virus

The genus *Alphavirus* consists of 29 distinct species that cause encephalitis, febrile illness with arthralgia or are not known to cause disease in humans. Electron microscopy studies of CHIKV in green monkey kidney (Vero) cells demonstrated a characteristic alphavirus morphology (Fig. 1; Simizu *et al.*, 1984), while serological cross-reactivity further defined the virus and grouped CHIKV within the Semliki Forest virus (SFV) antigenic serocomplex (Table 1;

Weaver *et al.*, 2005). Like all alphaviruses, CHIKV has a genome consisting of a linear, positive-sense, single-stranded RNA molecule of approximately 11.8 kb (Fig. 2). The non-structural proteins, required for viral replication, are encoded in the 5' two thirds of the genome, while the structural genes are collinear with the 3' one third. The structural proteins are produced by translation of an mRNA that is generated from an internal, subgenomic promoter immediately downstream of the non-structural open reading frame. The 5' end of the genome has a 7-methylguanosine cap, while the 3' end is polyadenylated. The structural gene products are generated by translation of a subgenomic mRNA 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 (Simizu *et al.*, 1984; Weaver *et al.*, 2005). E1 and E2 are post-translationally modified in the

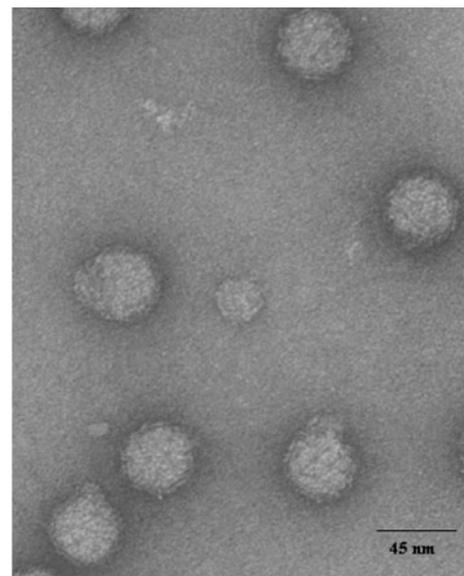


Fig. 1. Electron micrograph of purified CHIKV virions. CHIKV virions exhibit typical alphavirus structure. In thin sections, the virus shows a roughly spherical shape with a diameter of 42 nm composed of a 25–30 nm core (reproduced from Simizu *et al.*, 1984).

Published online ahead of print on 31 May 2007 as DOI 10.1099/vir.0.82858-0.

A supplementary table showing the number of globally reported cases of chikungunya virus from 2005 to 2007 is available with the online version of this paper.

Table 1. *Alphavirus* species and antigenic relationships

Antigenic complex	Species*†
Barmah Forest	Barmah Forest virus (BFV)
Eastern equine encephalitis	Eastern equine encephalitis virus (EEEV)
Middelburg	Middelburg virus (MIDV)
Ndumu	Ndumu virus (NDUV)
Semliki Forest	Bebaru virus (BEBV)
	Chikungunya virus (CHIKV)
	Getah virus (GETV)
	Mayaro virus (MAYV)
	O'nyong nyong virus (ONNV)
	Ross River virus (RRV)
	Semliki Forest virus (SFV)
	Una virus (UNAV)
Venezuelan equine encephalitis	Venezuelan equine encephalitis virus (VEEV)
	Mosso das Pedras virus (MDPV)
	Everglades virus (EVEV)
	Mucambo virus (MUCV)
	Tonate virus (TONV)
	Pixuna virus (PIXV)
	Cabassou virus (CABV)
	Rio Negro virus (RNV)
Western equine encephalitis	Aura virus (AURAV)
	Sindbis virus (SINV)
	Whataroa virus (WHAV)
	Fort Morgan virus (FMV)
	Highlands J virus (HJV)
	Western equine encephalitis virus (WEEV)
Unclassified	Trocar virus (TROV)
	Salmon pancreas disease virus (SPDV)
	Southern elephant seal virus (SESV)

*Species classification as in the most recent report of the International Committee on the Taxonomy of Viruses (ICTV); antigenic complexes as distinguished by the Subcommittee on Inter-Relationships Among Catalogued Arboviruses.

†ICTV abbreviations are shown in parentheses.

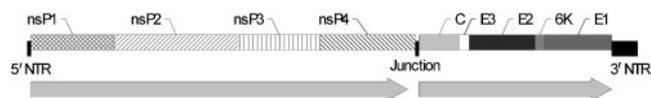


Fig. 2. Genomic organization of the first publicly available CHIKV genome (Ross strain, GenBank accession no. AF490259). The four non-structural proteins (nsP1–4) are translated as a single polyprotein directly from the positive-sense RNA genome. The structural proteins (C, E3, E2, 6K and E1) are translated from a subgenomic RNA (26S) transcribed from a separate promoter within the non-translated (NTR) junction region. 5' and 3' NTR regions flank the coding region.

endoplasmic reticulum and Golgi apparatus before being transported to the plasma membrane (Schlesinger & Schlesinger, 1986) where they maintain a close association with each other, forming a trimeric heterodimer spike structure (Anthony & Brown, 1991; Paredes *et al.*, 1993). As virion formation proceeds, the cytoplasmic nucleocapsids are trafficked to the cell membrane where they bind to the surface glycoproteins before budding from the cell (Garoff & Simons, 1974; Ekstrom *et al.*, 1994; Garoff *et al.*, 1998).

An important development in the study of alphaviruses has been the construction of full-length cDNA clones containing the entire viral genome. The resulting *in vitro* transcribed RNA can be transfected into cells to generate infectious virus. Several CHIKV infectious clones have been constructed. An isolate representative of the West African clade of CHIKV was used for the construction of one clone (strain 37997 from Senegal; A. M. Powers and others, unpublished results; Vanlandingham *et al.*, 2005). The prototype CHIKV strain from the Central/East African clade (Ross) was selected for another (C. H. Logue & G. J. Atkins, unpublished results) and strains from the 2005–2006 outbreaks have been identified for the construction of other clones. These clones provide tools to study synthesis and intracellular transport of membrane proteins, analysis of factors influencing viral pathogenesis, cell tropism and the identification of domains essential for the replication of RNA (C. H. Logue & A. M. Powers, unpublished data).

History of outbreaks

CHIKV was first isolated from the serum of a febrile human in Tanzania (formerly Tanganyika) in 1953 during an epidemic of dengue-like illness (Robinson, 1955; Ross, 1956). While the initial assessment was that the outbreak was due to a dengue virus, serological and antigenic characterization of the isolates indicated that it was an alphavirus closely related to Mayaro and SFV (Casals & Whitman, 1957; Spence & Thomas, 1959). Retrospective case reviews have suggested that CHIKV epidemics occurred as early as 1779 but were frequently documented inaccurately as dengue outbreaks (Carey, 1971). Between the 1960s and 1990s, the virus was isolated repeatedly from numerous countries in Central and Southern Africa including Sudan, Uganda, Democratic Republic of Congo (DRC, formerly Zaire), the Central African Republic (CAR), Malawi, Zimbabwe, Kenya and South Africa. CHIKV has also been isolated in western African countries including Senegal, Benin, the Republic of Guinea, Côte d'Ivoire and Nigeria (Table 2).

In South East Asia, frequent outbreaks were reported from the 1960s through to 2003 in India, Malaysia, Indonesia, Cambodia, Vietnam, Myanmar, Pakistan and Thailand (Chastel, 1963; Jadhav *et al.*, 1965; Myers *et al.*, 1965; Munasinghe *et al.*, 1966; Halstead *et al.*, 1969a, b, c, d; Nimmannitya *et al.*, 1969; Thaug *et al.*, 1975; Marchette *et al.*, 1978; Thein *et al.*, 1992; Thaikruea *et al.*, 1997; Mourya *et al.*, 2001; Kit, 2002; Laras *et al.*, 2005; Parola

Table 2. Chronological order of documented outbreaks of CHIKV

Those countries in italics represent cases involving individuals from those countries but infected elsewhere and do not represent epidemics nor endemicity in those countries.

Year of outbreak(s)	Country	Genotype
1952	Tanzania	Central/East African
1958, 1971, 1985	Uganda	Central/East African
1960, 1999–2000	DRC	Central/East African
1960, 1962–1964, 1988, 1991–1993, 1995*	Thailand	Asian
1961, 1963	Zimbabwe	Central/East African
1963	Cambodia	Asian
1963–1965, 1973, 2006*	India	Asian & Central/East African*
1965, 1967	Vietnam	Asian
1965–1969, 1998–1999, 2006*	Malaysia	Asian
1966, 1982, 1996–1997	Senegal	West African
1967, 2006*	<i>Taiwan</i>	Asian & Central/East African
1969, 1974–1975, 1980	Nigeria	West African
1970	South Africa	Central/East African
1970	Kenya	Central/East African
1973	Burma	Asian
1973, 1980, 1983–1984, 1998–1999*, 2000–2005*	Indonesia	Asian
1980–1982	Burundi	Central/East African
1982	Gabon	Central/East African
1983	Pakistan	Asian
1985–1986	Philippines/USA	Asian
1987–1989	Malawi	Central/East African
1990, 2006	<i>Australia</i>	Asian & Central/East African
1992	Guinea	West African
2003*	Timor	Asian
1999–2000	CAR	Central/East African
2004, 2005	Kenya	Central/East African
2005*, 2006*	Comoros	Central/East African
2005*, 2006*	Réunion	Central/East African
2005*	Seychelles	Central/East African
2005*, 2006*	Mauritius	Central/East African
2006*	Mayotte	Central/East African
2006*	Madagascar	Central/East African
2006	Cameroon	Central/East African
2006*	<i>Canada</i>	Central/East African
2006*	<i>Hong Kong</i>	Central/East African
2006*	<i>UK</i>	Central/East African
2006*	<i>Belgium</i>	Central/East African
2006*	<i>Czech Republic</i>	Central/East African
2006*	<i>Germany</i>	Central/East African
2006*	<i>Norway</i>	Central/East African
2006*	<i>Switzerland</i>	Central/East African
2006*	<i>France</i>	Central/East African
2006*	<i>Italy</i>	Central/East African
2006*	<i>Corsica</i>	Central/East African
2006*	<i>Sri Lanka</i>	Central/East African
2006*	<i>Singapore</i>	Central/East African
2006*	<i>USA</i>	Central/East African
2007*	<i>Spain</i>	Central/East African
2007*	<i>Japan</i>	Central/East African
2007*	<i>Gabon</i>	Central/East African

*Data sourced from the International Society for Infectious Disease's Program for Monitoring Emerging Diseases ProMED-mail, Eurosurveillance, CDC-MMWR and WHO; these data are based on a compilation of reported, suspected and confirmed cases of CHIKV infection.

et al., 2006). Numerous large cities in South East Asia including Calcutta and Bangkok have been identified as particularly active sites of transmission and disease (Pavri, 1964; Sarkar *et al.*, 1965b; Halstead *et al.*, 1969d; Burke *et al.*, 1985).

Beginning in 1986, CHIKV outbreaks resurged with major disease clusters documented in Senegal in 1986 and 1996/1997 (Diallo *et al.*, 1999), Ivory Coast in 1996/1997 (Thonnon *et al.*, 1999), DRC during 1998–2000 (Nur *et al.*, 1999; Pastorino *et al.*, 2004), Indonesia in 2003 (Porter *et al.*, 2004; Laras *et al.*, 2005), Kenya in 2004, Comoros in 2005 (Sergon *et al.*, 2007), the Seychelles, Mauritius, Madagascar and Réunion islands during 2005–2006 and in India in 2006/2007 (Ravi, 2006; Saxena *et al.*, 2006). Outbreaks occurred almost continuously during 2004–2007 with hundreds of thousands of reported cases

and new geographical areas involved (Supplementary Table S1 available in JGV Online). Cases were reported in Europe (UK, Belgium, Germany, Czech Republic, Norway, Italy, Spain and France), Hong Kong, Canada, Taiwan, Sri Lanka and the USA; these were directly associated with the return of tourists from India and affected islands of the Indian Ocean (Warner *et al.*, 2006; Supplementary Table S1).

At the time of this review, the most recent CHIKV activity was reported in the Mannar district of Sri Lanka, possibly as a result of introduction by refugees from southern India. This outbreak came only weeks after reports of numerous infected individuals in several states in southern India including Andhra Pradesh, Karnataka and Maharashtra. A map of the current global distribution of CHIKV can be seen in Fig. 3, while a table of all reported cases (suspected and confirmed) can be seen in Supplementary Table S1

Geographical Distribution of Chikungunya Virus

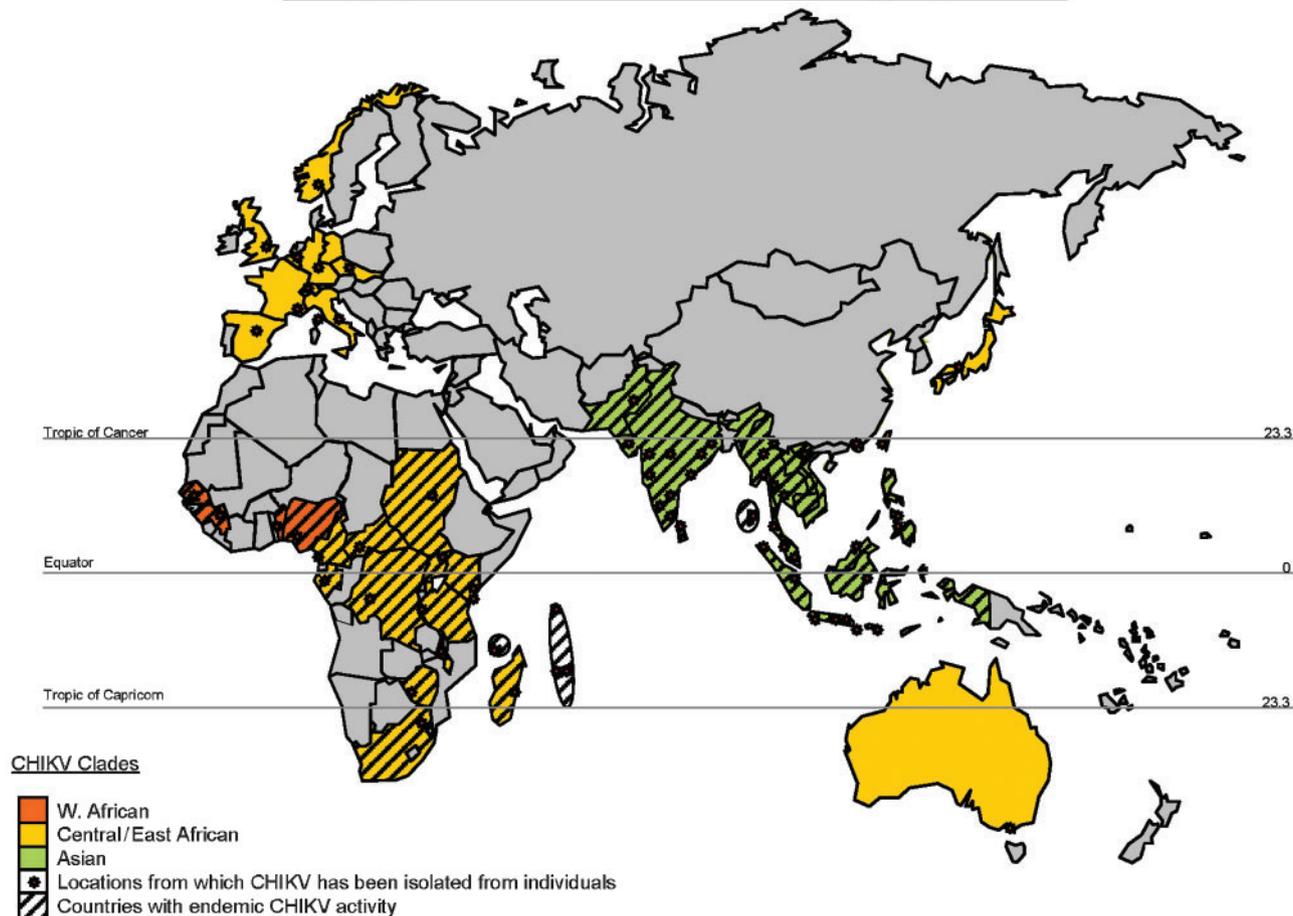


Fig. 3. Worldwide distribution of CHIKV. Shading of countries indicates the predominate (or only) genotype reported to have been identified in a given country. India is shaded in green (Asian genotype) as outbreaks from 1963 to 1965 and 1973 were confirmed to have been caused by members of the Asian clade; however, reports from India during 2005–2007 indicate this outbreak was caused by the same CHIKV strains detected during the Indian Ocean outbreaks (Central/East African genotype). Japan, Australia and parts of Europe are shaded in yellow as the strains isolated from the respective imported cases were members of the East/Central African clade. Red shading indicates the presence of the West African CHIKV genotype. Asterisk indicates a location from which CHIKV was isolated.

(available in JGV Online). When observing the global distribution of CHIKV it is apparent that all outbreaks have occurred within the 23rd latitude parallels. At present, CHIKV is endemic in 23 countries and phylogenetic analysis of viral sequences has identified three distinct clades separated primarily by geography (Powers *et al.*, 2000). These include the West African, Central/East African and Asian CHIKV clades; these will be described in more detail later.

CHIKV has caused numerous well-documented outbreaks in both Africa and South East Asia (Fig. 3, Table 2). However, the number of individuals infected with CHIKV during outbreaks, particularly the 2005–2007 epidemics in the Indian Ocean islands and India, is difficult to confidently ascertain due to the lack of published numbers of laboratory-confirmed cases. The scope of the 2005–2007 outbreaks is certainly of unprecedented magnitude but without serosurveys, clinical studies with laboratory diagnosis or other official reporting mechanisms, the absolute number of affected individuals can only be broadly estimated (Supplementary Table S1). For example, as of November 3 2006, the Indian government's official press information bureau reported a total of 1695 CHIKV cases with no deaths (http://pib.nic.in/release/rel_print_page1.asp?relid=21734); however, over 1.3 million cases are estimated to have occurred based on the number of individuals reporting clinically compatible illness (Supplementary Table S1).

In all documented outbreaks, morbidity has been significant with extensive incapacitation being universal, although unexpectedly, there were reports in the 2005–2006 outbreaks of deaths associated with CHIKV infection (Josseran *et al.*, 2006). However, as infection by no means infers a causal relationship with mortality, reported deaths may not necessarily have been caused directly by the virus even though CHIKV infection was confirmed in some of the fatalities. Much of the mortality has been estimated based upon crude death rates (CDR); this method is a useful screening tool but must be interpreted with caution in the absence of detailed cause-of-death studies of individual cases (G. L. Campbell, personal communication). In an epidemic where over a million individuals are infected, a background mortality rate would not be unexpected. These deaths may have arisen from circumstances such as co-infection with other pathogens, infection of individuals with compromised immune systems or the presence of a more virulent variant. For example, the very young and old may be risk groups that fall into the category of individuals with weakened immune systems, particularly those with underlying medical conditions. To date, only a very small number of peer-reviewed publications address CHIKV-associated mortality, and at the time of this review, no death has been solely and conclusively attributed to CHIKV infection. Additional case studies and research projects will undoubtedly provide a clearer clinical picture and any possible virus-associated mortality patterns.

CHIKV transmission cycles

CHIKV appears to be enzootic across tropical regions of Africa and Asia. CHIKV in West and Central Africa is believed to be maintained in a sylvatic cycle involving wild non-human primates and forest-dwelling *Aedes* spp. mosquitoes. The virus has been isolated from sylvatic mosquito species in several countries including Senegal, Côte d'Ivoire, Central African Republic and South Africa. The mosquito species involved vary geographically and with ecological conditions; however, the major species involved in sylvatic cycles are *Ae. furcifer*, *Ae. taylori*, *Ae. luteocephalus*, *Ae. africanus* and *Ae. neoafricanus* (McIntosh *et al.*, 1977; Jupp & McIntosh, 1988; Diallo *et al.*, 1999). The principal vectors during epidemics in these geographical regions appear to be members of the *Ae. furcifer-taylori* group (Jupp & McIntosh, 1990). In these primarily rural regions, the outbreaks have tended to be of smaller scale and appear to be heavily dependent upon the sylvatic mosquito densities that increase with periods of heavy rainfall (Lumsden, 1955), a phenomenon also seen in Ahmadabad in western India in 2006.

While forest-dwelling mosquito species are primary vectors in West and Central Africa, the urban mosquito *Ae. aegypti* has been found to be the most significant vector in Asia with virtually all Asian mosquito isolates coming from this species. These are urban and peridomestic, anthropophilic mosquitoes that maintain close associations with humans and thus, are likely responsible for regional large outbreaks. Urban or large outbreaks during 2004–2005 in East Africa and Comoros were also associated with the presence of *Ae. aegypti* mosquitoes (R. Sang & A. M. Powers, unpublished data). To complement the field data, numerous laboratory studies have examined distinct populations of *Ae. aegypti* to understand the variable susceptibilities of this species in the transmission of CHIKV (Soekiman, 1987; Banerjee *et al.*, 1988; Mourya *et al.*, 1994). Understanding mosquito population/virus infection dynamics will be even more important as CHIKV continues to spread into new areas.

In addition to *Ae. aegypti*, other common peridomestic species, including *Ae. albopictus*, *Ae. vittatus* and *Anopheles stephensi* have been found in abundance in CHIKV endemic areas and have been assessed for their vectorial capacity (Soekiman *et al.*, 1986a, b; Mourya, 1987; Mourya & Banerjee, 1987; Turell *et al.*, 1992; Yadav *et al.*, 2003b). Because all three species were found to be competent vectors in the laboratory, their role as primary vectors in urban outbreaks of CHIKV disease should be considered (Reiter *et al.*, 2006). Mosquitoes such as *Ae. albopictus* that have a broad distribution could introduce CHIKV into many new ecological niches. The virus may then have the capacity to expand its enzootic range to include Europe, the southern United States, the Caribbean and Latin America.

While numerous field and laboratory studies have been conducted on mosquito vectors involved in the transmission of CHIKV, there is far less information on the vertebrate hosts involved in viral maintenance. Most of the speculation regarding potential vertebrate reservoirs has

been derived from serosurveys and laboratory animal studies that have demonstrated the presence of CHIKV-specific antibodies (McIntosh, 1961; Paul & Singh, 1968; Bedekar & Pavri, 1969; Johnson *et al.*, 1977). Both humans and wild non-human primates throughout the humid forests and semi-arid savannahs of Africa have been found to have significant antibody levels against CHIKV. It is postulated that the appearance of CHIKV epidemics follows a 3–4 year cyclical pattern that coincides with the repopulation of susceptible, non-immune, wild primates (Jupp & McIntosh, 1988). To date, a vertebrate reservoir or sylvan transmission cycle for CHIKV has only been identified in Africa; in Asia, the virus is presumed to exist in a human-mosquito-human cycle.

Clinical presentations of CHIKV

A classical triad of signs for CHIKV infection from every documented epidemic includes fever, arthralgia and a rash that may or may not be accompanied by other indicators of the disease (Deller & Russell, 1967; McGill, 1995; Adebajo, 1996). CHIKV illness typically begins with a sudden onset of fever reaching as high as 104 °F (Deller & Russell, 1967) that may last up to 10 days. The fever almost always precedes the rash and joint pain and has occasionally been reported as biphasic with recurrence noted on the fourth or fifth day of illness (Robinson, 1955; Jadhav *et al.*, 1965). No reports of biphasic fever were noted during the 2005–2007 outbreaks. In past outbreaks, cases of febrile convulsions in young children were also reported (Moore *et al.*, 1974). The non-pruritic rash is typically maculopapular and erythematous in character, is visible starting 2–5 days post-infection, may last up to 10 days, and is distributed primarily on the face, limbs and trunk of the body.

Perhaps the most significant manifestation of CHIKV illness is the severe joint pain that occurs with virtually every clinical case (Deller & Russell, 1968; Fourie & Morrison, 1979). The name itself indicates the degree of discomfort caused: ‘chikungunya’ in Makonde means ‘that which bends up’ or ‘to dry up or become contorted’. The arthralgia is most commonly symmetrical and peripheral being noted in the ankles, toes, fingers, elbows, wrists and knees. The joints exhibit extreme tenderness and swelling with patients frequently reporting incapacitating pain that lasts for weeks or months (Fig. 4). Paresthesia of the skin over the affected joints is common, suggesting neurological involvement, but no evidence of neurological sequelae was documented in the early outbreaks. Most infections completely resolve within weeks or months but there have been documented cases of CHIKV-induced arthralgia persisting for several years with up to 12% of patients with CHIKV disease developing chronic joint problems (Brighton *et al.*, 1983; McGill, 1995; Calisher, 1999). In a rare instance, the rheumatic manifestations resulted in joint destruction before resolution after 15 years (Brighton & Simson, 1984).

Other common symptoms reported with CHIKV infection include headache, retro-orbital pain, photophobia, lumbar

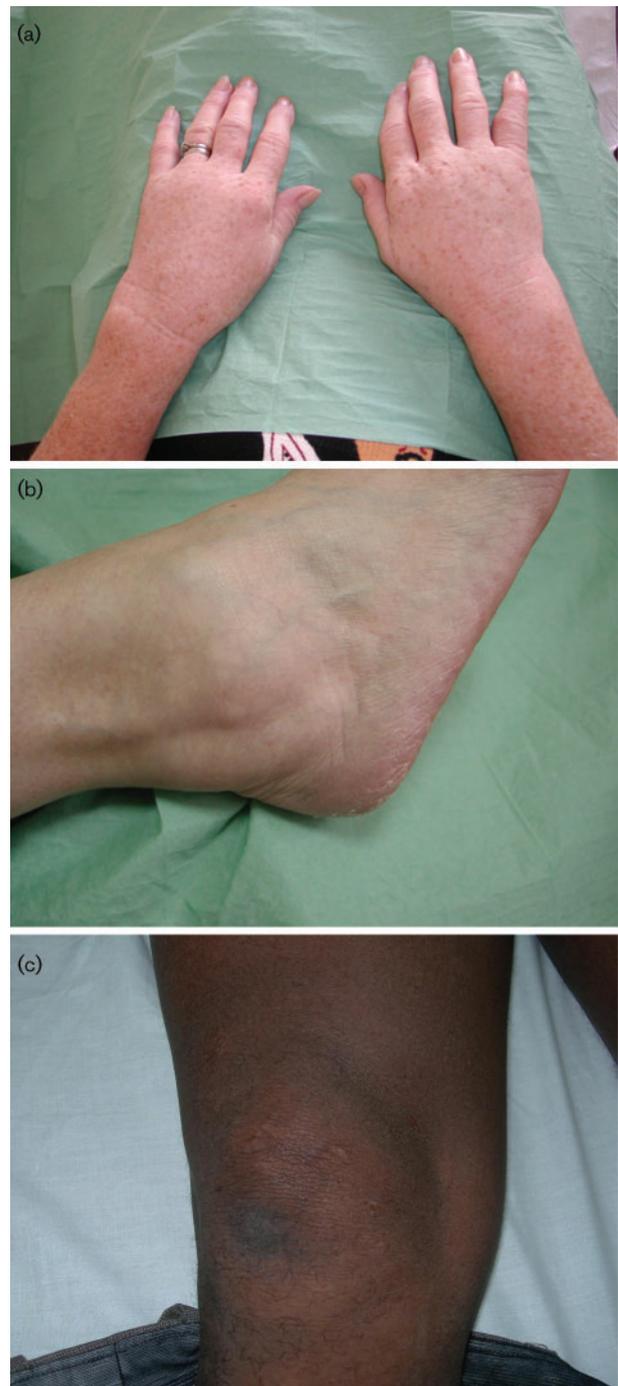


Fig. 4. Chikungunya-associated rheumatism. Second stage: severe relapse with polyarthritis and multiple tenosynovitis of wrists and fingers (a), hypertrophic tenosynovitis of one ankle (b) and swelling observed in the right knee of a CHIKV-infected patient from Mauritius (c). Photos generously provided by Dr F. Simon, Laveran Military Hospital, Marseille, France (a, b) and Dr Adil Fakim (LRCP & LRCS), Mauritius (c).

back pain, chills, weakness, malaise, nausea, vomiting and myalgia (Brighton, 1981; McGill, 1995; Calisher, 1999). Some combination of these symptoms is generally reported

by all patients but their presence and frequency are variable. Additionally, signs including conjunctivitis, pharyngitis and lymphadenopathy may be observed. Unfortunately, the symptoms of CHIKV infection are quite similar to those caused by many other infectious agents in the endemic areas. One particular difficulty in identifying CHIKV infection is its overlapping distribution with dengue viruses. It has been postulated that many cases of dengue virus infection are misdiagnosed and that the incidence of CHIKV infection is much higher than reported (Myers & Carey, 1967; Carey, 1971).

Atypical clinical presentations associated with CHIKV infection

Occasionally, unusual CHIKV clinical presentation has been observed. For example, one individual with serological evidence of CHIKV infection during the outbreak in

Sri Lanka in 1972 revealed evidence of myocarditis after acute febrile illness (Obeyesekere & Hermon, 1972, 1973). Presentation included sinus tachycardia, ventricular ectopics, cardiomegaly and abnormal electrocardiograms. Signs of congestive heart failure were documented several months after initial illness. Atypical clinical features have been of particular note in the 2005–2007 outbreaks, where descriptions of cases from Réunion Island have included neurological involvement in adults, fetuses and neonates (Schuffenecker *et al.*, 2006; Robillard *et al.*, 2006). Laboratory studies have also shown the potential for neurological involvement. Neuropathological studies in BALB/c mice intranasally infected with the prototype strain (Ross) revealed that CHIKV, like SFV, infects neurons and causes neuronal necrosis by 5 days post-infection (Fig. 5; C. H. Logue & B. J. Sheahan, unpublished findings). Further investigation of the neuropathogenesis of CHIKV in mice is ongoing to evaluate the possibility that the strains from the 2005–2007 outbreak and specific viral elements associated with these strains have enhanced neurovirulence characteristics (C. H. Logue & A. M. Powers, unpublished data).

There has also been infrequent documentation of haemorrhagic manifestations including haematemesis and melaena due to CHIKV infection in South East Asia (Sarkar *et al.*, 1965a). During the 1963–1964 outbreak in Calcutta, haemorrhagic manifestations of various grades of severity were documented (Sarkar *et al.*, 1965a). Eleven patients exhibited haemorrhagic symptoms; however, of the seven patients showing seropositivity to CHIKV, two exhibited a simultaneous rise in dengue virus antibodies and all survived. CHIKV was isolated from the remaining four patients; two of these died. However, there was no serological data to support CHIKV as the direct cause of the mortality. As in these early case reports, the potential complications of CHIKV co-infection with another virus infection such as dengue-2 virus may explain the recent deaths associated with CHIKV infection in Réunion and India. Haemorrhagic fever has also been noted in the Philippines, Bangkok and Réunion (Hammon *et al.*, 1960; Halstead *et al.*, 1969c; Nimmannitya *et al.*, 1969; Robillard *et al.*, 2006). While clinical cases with mortality, neurological disease or intrauterine transmission with possible teratology are not typical, they serve as a reminder that this virus may have diverse and perhaps evolving patterns of virulence that should be monitored as a public health prevention measure.

Clinically, laboratory parameters for CHIKV infections can also be quite variable and frequently do not aid in diagnosis. Results can include a normal haematocrit (varying from 40 to 50%), thrombocytopenia or alternatively, no obvious platelet deficiencies, normal urinalysis and low to normal white blood cell counts of 4000–7000, although leukocytosis has been documented (Jadhav *et al.*, 1965; Deller & Russell, 1968). The erythrocyte sedimentation rate may be slightly elevated, which is suggestive of rheumatoid arthritis and may confound diagnosis of isolated CHIKV

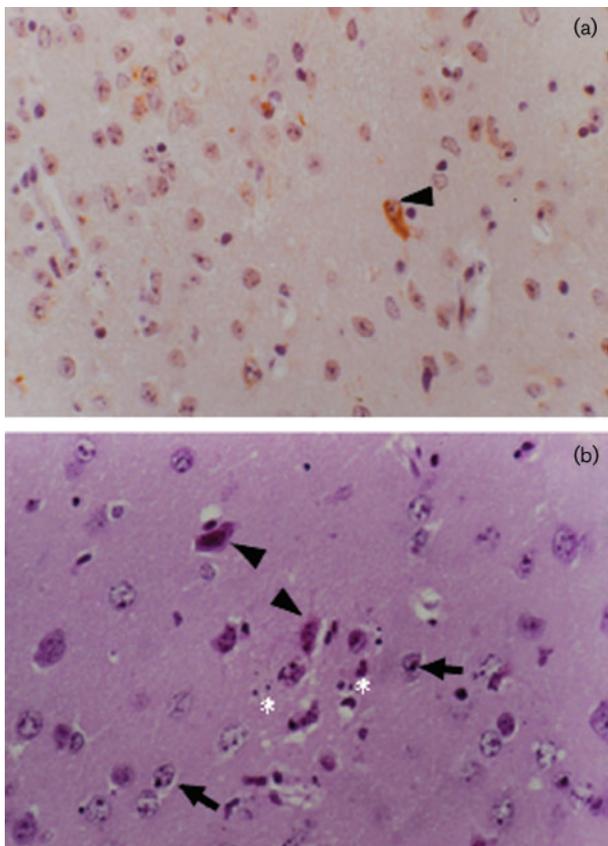


Fig. 5. Immunohistochemical labelling (a) and haematoxylin and eosin staining (b) on brain sections from a BALB/c mouse following intranasal infection with the prototype strain of CHIKV (Ross) at 5 days p.i. Viral antigen in a neuron (arrowhead) in the anterior olfactory nucleus labelled with rabbit anti-SFV, $\times 400$ (a). Intact neurons (arrows), necrotic neurons with contracted nuclei and cytoplasmic eosinophilia (arrowheads) and nuclear debris (asterisks) in the pyriform cortex, $\times 400$ (b). Photographs kindly provided by Professor Brian J. Sheahan, University College Dublin, Ireland.

infections. Overall, diagnosis is dependent upon isolation of virus or confirmation of virus-specific antibodies using acute and convalescent sera. Diagnosis is facilitated during large epidemics, while small rural outbreaks or individual cases are often never diagnosed.

Immunological factors

There has been little research on the immunological response of CHIKV-infected individuals; however, several papers elucidating the molecular and cellular aspects of Ross River virus (RRV; see Table 1) disease development may also be applicable to CHIKV infections. RRV causes epidemic polyarthritides generating a pattern of illness including rheumatic symptoms, rash and fever similar to that seen with CHIKV (Harley *et al.*, 2001). Also, like CHIKV disease, the painful arthralgia may persist for several months. Numerous components of the immune system have been demonstrated to be involved in RRV pathogenesis including T cells, chemokines secreted by macrophages and viral-specific antibodies; these may likely be involved in the CHIKV disease process as well.

In RRV infections, CD8⁺ T cells are the major cell type associated with epidemic polyarthritides patients who quickly recover in contrast to the CD4⁺ cells that predominate in the synovial fluid of patients with chronic disease (Fraser & Becker, 1984). As demonstrated that activated CD8⁺ T cells can eliminate virus, treatment of productively and persistently infected *in vitro* macrophages with CD8⁺ T cells generated by vaccination of mice with RRV capsid protein, resulted in complete clearance of the infection (Linn *et al.*, 1998). This suggests that a defective cell-mediated immune response (CMI) where CD8⁺ T cells are absent or inactive may play a role in chronic disease and viral persistence (Fraser, 1986). Macrophages are a second immune cell type that has been found to be infected by and responsible for RRV disease. The proposed models of pathogenesis due to these cells have postulated the over-secretion of toxic chemokines or apoptosis as causes of cell/tissue destruction and associated clinical symptoms (Lidbury *et al.*, 2000; Mateo *et al.*, 2000; Mahalingam *et al.*, 2003). Finally, virus-specific antibodies may be involved in pathogenesis by an antibody-dependent enhancement (ADE) mechanism similar to that suggested for dengue viruses (Rulli *et al.*, 2005). While a clear association between an ADE infection mechanism and clinical RRV disease has not been documented, it has been shown that subneutralizing levels of RRV-specific IgG significantly increased cellular infection and that ADE-RRV infection resulted in the elimination of production of two known antiviral factors *in vitro* (Rulli *et al.*, 2005; Lidbury & Mahalingam, 2000).

Due to the similarities between RRV and CHIKV, preliminary experiments have been undertaken to identify possible mechanisms of disease pathogenesis. Intranasal infection of mice with CHIKV has resulted in neuronal necrosis (Fig. 5) and further experiments should reveal

if immune modulation plays a role in cell destruction. Cell tropism of CHIKV in the murine brain may also show the relationship between infected cell type and neurological signs, such as infection of the oligodendrocytes and observation of demyelination in the olfactory bulbs as seen with SFV (C. H. Logue, B. J. Sheahan & G. J. Atkins, unpublished data). Additional immunological studies assessing the likelihood of cross-protection against CHIKV infection due to previous alphavirus infection should be undertaken. Laboratory studies have shown that both antibody-mediated (Wust *et al.*, 1987) and cell-mediated cross-protection (Latif *et al.*, 1979) could occur between alphaviruses in different antigenic complexes. The presence of cross-protective CHIKV antibodies or CMI could lead to a decrease in severe disease, while at the same time complicating serological diagnosis. This may be of particular interest as CHIKV expands its geographical range and perhaps moves to areas where encephalitic alphaviruses are present.

Molecular epidemiology

The scope and magnitude of the 2005–2007 CHIKV outbreaks has led to speculation that the virus has mutated to a more virulent form. This phenomenon has been documented with the equine avirulent, Venezuelan equine encephalitis subtype ID viruses, where as few as 7 aa changes can generate epidemic forms of the virus responsible for large outbreaks (Greene *et al.*, 2005). Genetic sequencing has confirmed the close relationships of viruses within the Semliki Forest antigenic serocomplex where the CHIK viral species form a monophyletic clade excluding other members of the genus *Alphavirus* (Fig. 6). Molecular genetic assessment of the genomes may lead to identification of viral elements involved in or responsible for the 2005–2007 activity.

From one of the earliest genetic studies of CHIKV, two distinct CHIKV lineages were delineated; one contained all of the available isolates from West Africa and the second comprised all South and East African strains as well as isolates from Asia. Within this second lineage, Asian strains grouped together in a genotype distinct from the African groups. Additionally, paraphyletic grouping of the African sequences in phylogenetic trees corroborated historical evidence that CHIKV originated in Africa and subsequently was introduced into Asia (Powers *et al.*, 2000; Fig. 6).

Subsequent to this first broad look at the phylogenetic relationships among CHIKV strains, several reports have been published that have examined specific clusters of CHIKV activity. For example, a set of isolates obtained from India spanning a 37-year-window was designed to reveal if any particular strain was circulating predominantly during outbreaks (Yadav *et al.*, 2003a, b). With virtually no exception (prior to 2005), the Indian strains examined formed a monophyletic clade, indicating little genetic variation. Similar studies of strains obtained from the DRC during an urban outbreak in 1999–2000 showed, not unexpectedly, that the isolates were most closely related

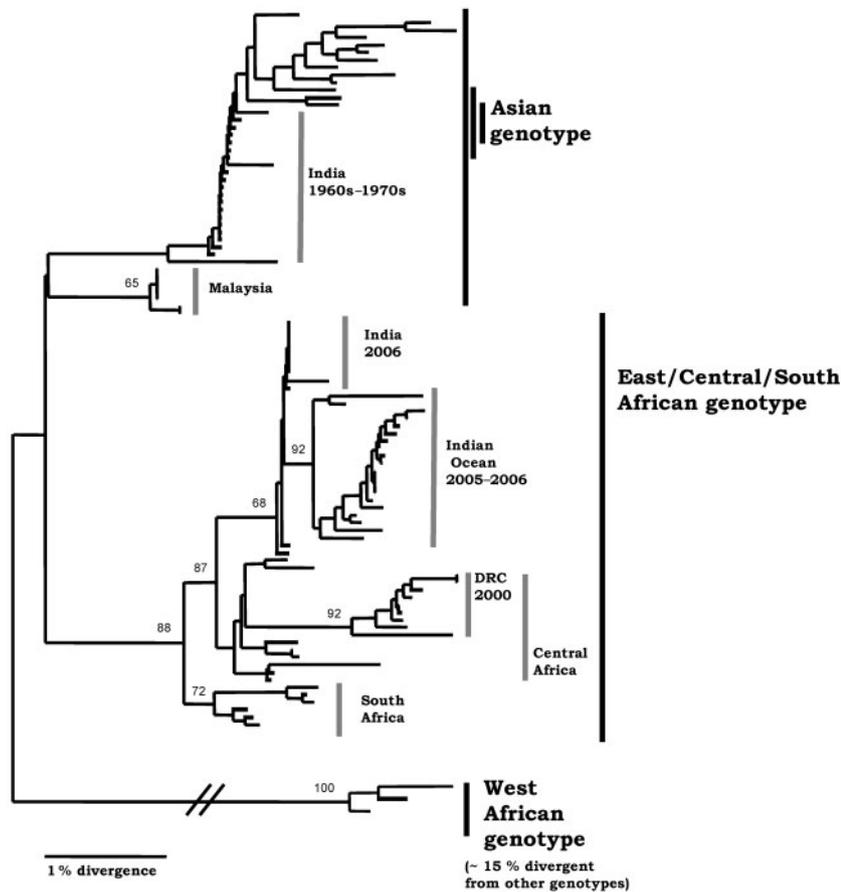


Fig. 6. Phylogram of 99 CHIKV E1 sequences demonstrating the main genotypes and close relationships among the lineages from each genotype based upon geography and time of the outbreak. Numbers at nodes indicate bootstrap support of 1000 replications.

to other strains from Central Africa from previous years (Pastorino *et al.*, 2004; Peyrefitte *et al.*, 2007). These studies have 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 strains based upon geography and that all isolations during a particular outbreak are practically identical. Fig. 6 clearly demonstrates the geographical and temporal clustering of all available CHIKV E1 sequences (using 1168 nt from each of 99 strains), including strains from the DRC, the Indian Ocean islands, India and representatives of all historically available strains.

It is the high level of conservation of this RNA virus within a particular outbreak that has been of considerable interest during the progression of the 2005–2007 epidemics. Genetic studies have described the molecular characterization of partial E1 gene or complete genome sequences from samples collected in Réunion and other Indian Ocean islands (Parola *et al.*, 2006; Schuffenecker *et al.*, 2006). As observed in previous studies of samples collected during a single outbreak, isolate sequences showed only infrequent changes representing expected levels of genetic drift associated with an RNA genome. However, selected mutations were identified that may have an association with samples collected from patients demonstrating more severe illness. The number and distribution of amino acid differences

that may have potential significance are noted in Table 3; these are changes uniquely associated with a specific CHIKV genotype. Of particular note is the fact that no single amino acid change throughout the entire genome is uniquely associated with the Central/East African genotype from which the lineage causing the 2005–2007 epidemic evolved. There are two changes, one in the E3 gene and one in the E2 glycoprotein sequence that demonstrate difference among all three genotypes; however, neither change alters the charge or hydrophobicity of the amino acid incorporated so the biological relevance of these changes can only be speculated upon until reverse genetic laboratory studies can assess the role of individual viral genetic elements.

While it is tempting to assign virulence patterns to these infrequent genetic changes, it is important to emphasize the need to verify the phenotypic and/or biological changes accompanying any genotypic differences. This is of particular importance when there are numerous samples from a short-time period available as it is often difficult to definitively track movement of any individual mutation through a population. Infectious clones allow the introduction of point mutations from which clear empirical evidence of virulence differences can be ascertained. Furthermore, as more sequences are collected and analysed, the percentage of the genome and gene regions assessed will be of importance, particularly when the strains closely cluster temporally. For

Table 3. Number of amino acids that are uniquely associated with specific CHIKV clades and shared by all members of that genotype based upon 16 full-length genomes

Genotype	Gene	No. amino acids uniquely associated with given genotype
Asian	nsP1	7
Asian	nsP2	2
Asian	nsP3	8
Asian	nsP4	5
Asian	Capsid	3
Asian	E3	2
Asian	E2	7
Asian	6K	1
Asian	E1	5
Central/East/South African	nsP2	2
Central/East/South African	nsP3	6
Central/East/South African	nsP4	3
Central/East/South African	Capsid	1
Central/East/South African	E3	1
Central/East/South African	E2	1
Central/East/South African	E1	2
Western	nsP1	9
Western	nsP2	14
Western	nsP3	40
Western	nsP4	18
Western	Capsid	8
Western	E3	4
Western	E2	21
Western	6K	3
Western	E1	11

example, upon looking at approximately 1.1 kb of the E1 gene from all available CHIKV sequences, only 251 characters are parsimony informative. Furthermore, datasets that include smaller fragments of the E1 gene (approx. 300 nt) are significantly less robust with resulting bootstrap values on major clades dropping from over 90 (when using sequences of 1168 nt) to 60 or less. Because conclusions concerning viral evolution, epidemiology and virulence are drawn from such analyses, it is imperative to have sufficient sequencing data collected for useful and accurate interpretations.

Vaccine and antiviral development

Due to the significant infection rates during outbreaks, the extensive geographical distribution of this virus, and the severe morbidity associated with clinical disease, a CHIKV vaccine is highly desirable. Given the numerous documented cases of laboratory acquired infections (Biosafety in Microbiological and Biomedical Laboratories 5th Edition), a vaccine would also be beneficial to personnel with occupational risk. However, while there has been extensive work in vaccinology for several other alphaviruses (Rayner *et al.*, 2002; Nalca *et al.*, 2003; Johnston & Davis, 2004), the

history of vaccine development for CHIKV is short and none of these efforts have yet resulted in a licensed vaccine.

The most extensive work performed in the development of a human CHIKV vaccine was initiated by investigators at Walter Reed (USA). Virus from the original outbreak (Mason & Haddow, 1957) was formalin inactivated and potency tests using this product generated variable results depending upon dose, route of inoculation and vaccine concentration. One of the preparations harvested from green monkey kidney cells (GMKC) was found to induce high levels of antibodies, it was protective against intracranial challenge with homologous virus, it produced no detectable viraemia and it resulted in good protection in monkeys after challenge with four strains of CHIKV (Harrison *et al.*, 1967).

The continuation of work by these investigators focused on safety and immunogenicity by looking for immune response, adverse events and possible side effects of the formalin-inactivated product. In this phase, the investigators changed from the highly passaged original outbreak strain to an isolate obtained directly from the serum of an infected patient in Thailand (designated 15561). Potency tests were conducted in mice and were followed up with a human volunteer study. No adverse events were noted in any volunteers and all subjects developed significant levels of neutralizing antibody by day 42 (Harrison *et al.*, 1971).

The development of a second generation CHIKV vaccine began using the 15561 strain as seed material to generate vaccine CHIK 181/clone 25 resulting from a series of 18 plaque-to-plaque passages in MRC-5 cells of the starting virus, which had been passaged 11 times in GMKC culture (Levitt *et al.*, 1986). The CHIK 181/clone 25 strain exhibited small plaques, it was temperature sensitive, it had decreased virulence for suckling mice and it showed a reduced level of viraemia in monkeys. All of these characteristics have been associated with attenuation in other host systems (Eckels *et al.*, 1980; Halstead *et al.*, 1984), suggesting that 181/25 would have reduced virulence as well. In one monkey model study, all the animals developed neutralizing antibody and were completely aviraemic upon challenge, indicating strong protective immunity (Levitt *et al.*, 1986).

Additional safety testing included examining the potential of the vaccine to be transmitted by mosquitoes (Turell & Malinoski, 1992). As this was a live virus, the possible loss of vaccine characteristics during replication of the virus in the mosquito was of concern. The vaccine was examined in both *Ae. aegypti* and *Ae. albopictus*, the two mosquitoes known to be competent urban vectors of the virus (Banerjee *et al.*, 1988; Turell *et al.*, 1992). While the vaccine was able to replicate in both species and could be transmitted by the mosquitoes that received the virus via intrathoracic inoculation, there was no evidence of reversion to virulence. Based upon the low viraemias the vaccine strain generated in test subjects, it was postulated that it would be unlikely for either mosquito vector to become infected from feeding upon a vaccinated human (Turell & Malinoski, 1992).

The promising results generated by these studies led to the submission of an investigational new drug application for the CHIK 181/clone 25 vaccine to provide a route for the initiation of human clinical trials. Both phase 1 and 2 clinical trials were undertaken. In naïve recipients, 98% developed CHIKV-specific neutralizing antibodies with 85% of participants maintaining neutralizing-antibody activity at 1 year. Only a few adverse events were noted with five recipients in the vaccine group developing transient joint pain (Edelman *et al.*, 2000). Unfortunately, limited resources and lack of commercial potential (prior to the 2005–2007 outbreaks) restricted the scope of additional testing; however, overall, the vaccine appeared to be promising with further safety, immunogenicity and duration of immunity studies being warranted. The latest development in vaccine production was a Material Transfer Agreement on September 6th 2006 signed by the United States Army Medical Research Institute for Infectious Diseases (USAMRIID) and the French National Institute of Health and Medical Research (Inserm) Transfert, Inserm's technology-transfer organization. This agreement allowed the transfer of records of previous clinical studies and supplies of the vaccine and seed stock from which it was made for further development of the vaccine, including additional clinical trials in affected areas. (Press Release – Embassy of the United States of America, Paris, France, September 14th 2006).

As CHIKV characteristically induces a non-fatal, self-limiting disease, treatment has historically been entirely supportive for the symptoms. With the primary signs including fever and joint pain with swelling, analgesics, antipyretics and anti-inflammatory agents are the most appropriate treatment. Commonly, these agents would include aspirin but due to the reports of possible haemorrhagic manifestations, other options (Tesh, 1982) including acetaminophen, ibuprofen, steroid therapy and non-steroidal agents such as indomethacin are preferred over aspirin. Unfortunately, some of these therapies can have serious side effects; therefore, the need for prolonged use should be a consideration in treatment regimens. For chronic cases of arthritis due to CHIKV, chloroquine phosphate was shown in one study to provide relief to patients that had limited response to non-steroidal anti-inflammatory drugs (Brighton, 1984). In addition to this drug therapy, actions including bed rest and fluids are recommended.

The lack of specific treatment for CHIKV infection has resulted in several laboratory studies to identify antiviral agents effective against this virus. Compounds including ribavirin, sulfated polysaccharides (iotacarrageenan, fucoidan and dextran sulfate), 6-azauridine, glycyrrhizin and interferon- α have been evaluated for their ability to inhibit replication of CHIKV in cell culture. With the exception of the polysaccharides, all were found to have both potent and selective antiviral activity (Briolant *et al.*, 2004). Additionally, these four compounds have demonstrated efficacy in the treatment of other diseases including

haemorrhagic fever viruses, herpesviruses and encephalitic alphaviruses warranting additional testing for their use against CHIKV (Deneau & Farber, 1975; Andrei & De Clercq, 1993; Grieder & Vogel, 1999; van Rossum *et al.*, 1999; Ryman *et al.*, 2000). In particular, multi-drug therapies may prove useful as each treatment has distinct pharmacological actions that may produce synergistic effects as well as reduce the likelihood of resistance. The scope of the outbreaks between 2005 and 2007 has sparked much needed interest by researchers in the pharmaceutical industry in developing viable antiviral agents against these arthralgic alphaviruses.

Summary

CHIKV has been responsible for significant human morbidity for (probably) several hundred years; yet in spite of its prevalence, CHIKV epidemiology and mechanisms of virulence and pathogenesis are poorly understood. The 2005–2007 epidemic of CHIKV disease, like the West Nile virus (WNV) epidemics in North America that began in 1999, has served as a reminder that laboratory and field research combined with epidemiological preparedness are essential for timely and appropriate public health response and control measures.

Curiously, it was the sheer magnitude of the 2005–2007 CHIKV outbreaks that brought this virus into the awareness of both the scientific community and the general public. The public has been introduced to the virus through numerous (and not always accurate) anecdotal and media reports of widespread death and devastation due to the virus. The outbreaks have even inspired the writing of songs about the virus and the recognition of 'Keelamma – the chikungunya goddess'. Unfortunately, much of the information published or reported about CHIKV includes only suspected cases and 'positive cases' that have not been laboratory confirmed, which has led to artificial epidemiological data.

Scientifically, the large scope of the outbreaks has provided opportunities to accurately document transmission and epidemiological patterns associated with movement of the virus. Furthermore, because of the magnitude of the outbreak, 'unusual' patterns of illness and routes of transmission (similar to what was seen during the North American WNV epidemics) have been identified, leading to a resurgence of public health response capacity combined with an interest in expanding basic and applied scientific knowledge of the virus. Several potential research areas have been of particular focus including vector capacity, viral evolution and virulence potential. For example, CHIKV replicates in a number of mosquito vectors that are also common in the USA, Europe and South America and the virus could replicate to sufficient levels in humans to initiate localized transmission cycles leading to European epidemics during the summer months or establishment of the virus in the southern United States, the Caribbean or Latin America. Concomitant with the possibility of expansion into new ecological niches is the

recognition that different mosquito populations may greatly affect the potential establishment and transmission of the virus; further vector competence studies of mosquitoes in areas where the virus is not enzootic but may be introduced by viraemic travellers are warranted to assess future risk to naïve populations.

Whether or not the virus moves to the New World, it is still a significant burden on the already overstretched hospitals, health systems and communities of the affected regions where attack rates reach as high as 70% (Halstead *et al.*, 1969d; Paddidri & Gnaneswar, 1979; Jupp & McIntosh, 1988). The economic pressures due to CHIKV infections are noted in the state and national laboratories, local businesses affected by absenteeism due to the incapacitating symptoms, and on individual families whose members are unable to work for weeks or months. Therefore, laboratory research aimed at the development of vaccine candidates, antiviral strategies and commercially available diagnostic kits is needed. To reach these goals, several investigations into further characterizing CHIKV are already ongoing including murine studies investigating cell tropism and neurovirulence determinants, transmissibility studies in mosquitoes using chimeric viruses, and virulence/pathogenesis studies investigating the outcomes due to CHIKV strain variation (A. M. Powers, C. H. Logue, J. P. Ledermann, B. J. Sheahan & G. J. Atkins, unpublished results). Hopefully, these efforts will lead to advances in public health capacity for prevention of future arboviral outbreaks combined with rapid control of outbreaks that do occur.

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