

# Chikungunya, an epidemic arbovirosis

Gilles Pialoux, Bernard-Alex Gaüzère, Stéphane Jauréguiberry, Michel Strobel

Chikungunya is an arboviral disease transmitted by aedes mosquitoes. The virus was first isolated in 1953 in Tanzania. Chikungunya virus is a member of the genus Alphavirus and the family Togaviridae. The disease typically consists of an acute illness characterised by fever, rash, and incapacitating arthralgia. The word chikungunya, used for both the virus and the disease, means “to walk bent over” in some east African languages, and refers to the effect of the joint pains that characterise this dengue-like infection. Chikungunya is a specifically tropical disease, but it is geographically restricted and outbreaks are relatively uncommon. It is only occasionally observed in travellers and military personnel. More than 266 000 people have been infected during the ongoing outbreak in Réunion, in which *Aedes albopictus* is the presumed vector. In the ongoing Indian outbreak, in which *Aedes aegypti* is the presumed vector, 1 400 000 cases of chikungunya were reported during 2006. The reasons for the re-emergence of chikungunya on the Indian subcontinent, and for its unprecedented incidence rate in the Indian Ocean region, are unclear. Plausible explanations include increased tourism, chikungunya virus introduction into a naive population, and viral mutation.

## Introduction

Chikungunya is a viral disease transmitted by *Aedes* mosquitoes. The disease typically consists of an acute illness with fever, skin rash, and incapacitating arthralgia.<sup>1</sup> The latter distinguishes chikungunya virus from dengue, which otherwise shares the same vectors, symptoms, and geographical distribution.<sup>2,3</sup> The word chikungunya, which is used for both the virus and the disease, means “to walk bent over” in the African dialect Swahili or Makonde, and refers to the effect of the incapacitating arthralgia.<sup>4</sup>

Chikungunya is a specifically tropical disease. It is relatively uncommon and poorly documented.<sup>3,5</sup> A chikungunya outbreak is currently ongoing in Réunion (Indian Ocean), where about 266 000 of the 775 000 inhabitants have reported symptoms of the disease. The probable vector is *Aedes albopictus*, a mosquito species endemic to Réunion and other islands in the Indian Ocean (figure 1A).<sup>4</sup> In India, where the main vector is *Aedes aegypti* (figure 1B),<sup>6</sup> 1 400 000 cases were reported during 2006.<sup>7</sup> The last outbreak of the infection in India occurred in 1973.

## Epidemiology and natural history

### Chikungunya virus

Chikungunya virus, an arbovirus belonging to the genus Alphavirus (Togaviridae family), has a single-stranded RNA genome, a 60–70 nm diameter capsid and a phospholipid envelope. It is sensitive to desiccation and to temperatures above 58°C.<sup>8,9</sup> The Alphavirus group comprises 28 viruses, six of which can cause human joint disorders—namely chikungunya virus, o'nyong-nyong virus (central Africa), Ross River and Barmah Forest viruses (Australia and the Pacific), Sindbis virus (cosmopolitan), and Mayaro virus (South America, French Guyana).<sup>9</sup> These alphaviruses share certain antigenic determinants.<sup>9,10</sup> Three lineages with distinct genotypic and antigenic characteristics have been identified: isolates from the ongoing Indian Ocean outbreak represent a distinct clade within a large east, central, and southern African phylogroup distinct from

the Asian phylogroup and from the west African phylogroup.<sup>5</sup> The current Indian epidemic is caused by the central/east African genotype. Chikungunya virus strains isolated in India are closely related to strains isolated in Réunion (99·61% nucleotide sequence homology) and to strains isolated in Maharashtra province in 2000 (98·95% homology).<sup>6</sup> The viruses isolated in India from 1963 to 1973 belonged to the Asian genotype. All older isolates of the Asian genotype (India 1963–1973 and Thailand 1962–1978) cluster together, whereas later isolates from the Philippines (1985), Indonesia (1985), Thailand (1988, 1995–1996), and Malaysia (1988) form a distinct cluster.<sup>6</sup>

To date, ten complete chikungunya virus nucleotide sequences have been determined: two human isolates (Ross and S27) recovered during the 1952–1953 outbreak in Tanzania,<sup>8</sup> one strain (#37997) originally isolated from *Aedes furcifer* during the Senegal outbreak in 1983,<sup>11</sup> six during the recent outbreak in Réunion,<sup>12</sup> and one (formally designated LR2006-OPY1) from a traveller returning from an Indian Ocean island.<sup>13</sup>

Schuffenecker and colleagues<sup>12</sup> have reported the near-complete nucleotide sequences of chikungunya virus strains isolated from six patients in Réunion and the Seychelles, and partial (E1) sequences of isolates from 127 patients in Réunion, the Seychelles, Mayotte,

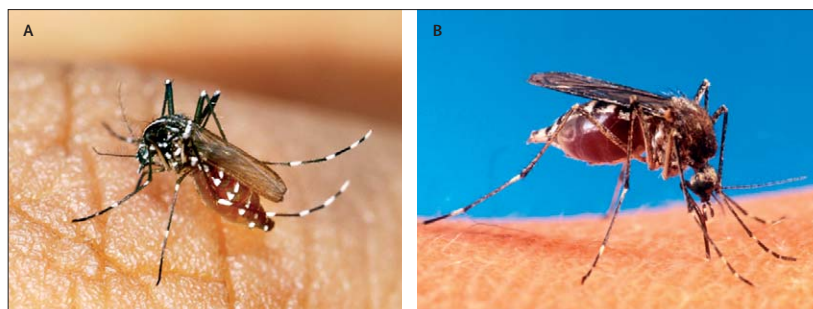
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Hôpital Tenon, Service des Maladies Infectieuses et Tropicales, Paris, France (Prof G Pialoux MD, S Jauréguiberry MD); CHD de Saint-Denis, La Réunion, France (BA Gaüzère MD); and Institut de Médecine Tropicale (IFMT), Vientiane, Laos (Prof M Strobel MD)

Correspondence to: Prof Gilles Pialoux, Service des Maladies Infectieuses et Tropicales, Hôpital Tenon, 4 rue de la Chine, 75970 Paris Cedex 20, France. Tel 33 1 56 01 74 17; fax 33 1 56 01 74 18; gilles.pialoux@tnn.aphp.fr

See [Reflection and Reaction](#) page 306

For more information on **chikungunya outbreaks** see <http://invs.sante.fr> and <http://www.searo.who.int/en/Section10/Section2246.htm>



**Figure 1: Mosquito vectors of chikungunya virus**

(A) Blood-gorged *A. albopictus* female feeding on a human host. *A. albopictus* is the primary chikungunya virus vector in the current Indian Ocean outbreak. (B) *A. aegypti* mosquito. *A. aegypti* is the primary chikungunya virus vector in Asian chikungunya outbreaks. Images from United States Department of Agriculture.

Madagascar, and Mauritius.<sup>12</sup> Interestingly, these authors detected a mutation at residue 226 of the membrane fusion glycoprotein E1 (E1-A226 V), which was absent from the strains isolated during the first months of the ongoing outbreak in Réunion, but was found in more than 90% of isolates after September, 2005.<sup>14</sup> This change could be related to virus adaptation to the mosquito vector species. Together with the lack of herd immunity, this might explain the abrupt and escalating nature of the Réunion outbreak. Other chikungunya isolates are currently being sequenced to provide spatiotemporal data on the viruses isolated from different locations (Réunion, Mayotte, Mauritius, Seychelles, and Madagascar) and to find out whether any molecular signatures could be associated with unusual clinical forms (Schuffenecker I, Centre National de Référence des Arbovirus, Institut Pasteur, Lyon, France, personal communication). A similar genetic change has been described in the Semliki Forest virus model.<sup>12</sup>

The vast scale and sudden emergence of the Réunion outbreak underlines how little we know on the biology of chikungunya virus.<sup>4,12,15,16</sup> It is also conceivable that chikungunya virus never disappeared entirely from the Indian subcontinent, and that the current outbreak is because of a simple resurgence.<sup>7</sup>

### Vectors

In Asia and the Indian Ocean region the main chikungunya virus vectors are *A. aegypti* and *A. albopictus*.<sup>17,18</sup> A larger range of *Aedes* species (*A. furcifer*, *Aedes vittatus*, *Aedes fulgens*, *Aedes luteocephalus*, *Aedes dalzieli*, *Aedes vigilax*, *Aedes camptorhynchites*) transmit the virus in Africa, and *Culex annulirostris*, *Mansonia uniformis*, and anopheles mosquitoes have also occasionally been incriminated.<sup>19–21</sup>

*A. albopictus* has a wide geographical distribution, is particularly resilient, and can survive in both rural and urban environments. The mosquito's eggs are highly resistant and can remain viable throughout the dry season, giving rise to larvae and adults the following rainy season. Originating from Asia, and initially sylvatic, *A. albopictus* has shown a remarkable capacity to adapt to human beings and to urbanisation, allowing it to supersede *A. aegypti* in many places (including China, the Seychelles, and Hawaii), and to become a secondary but important vector of dengue and other arboviruses.<sup>22</sup> It seems that most new introductions of *A. albopictus* have been caused by vegetative eggs contained in timber and tyres exported from Asia throughout the world. *A. albopictus* is therefore both rural and urban, zoophilic and anthropophilic, thriving both in the natural environment, and in habitations and their immediate periphery.<sup>23–26</sup> *A. albopictus* is relatively long-lived (4–8 weeks) and has a flight radius of 400–600 m. It is aggressive, silent, and diurnal, meaning that bednets are ineffective. The adult female

appears to transmit the virus vertically to her eggs, although this remains to be documented in the Indian Ocean outbreak.<sup>23</sup>

*A. albopictus* has long been present in the Indian Ocean region,<sup>4,22</sup> and was probably involved in the dengue epidemics that hit Réunion in 1977–78 and 2004.<sup>18</sup> It is very difficult to avoid *A. albopictus* bites and to control this mosquito species in tropical, human-modified ecosystems with modern infrastructures, irrigation, and massive solid waste production.<sup>22</sup>

In India, the dominant carrier of chikungunya virus is *A. aegypti*, which breeds mainly in stored fresh water in urban and semi-urban environments.<sup>6</sup>

Chikungunya virus is maintained in the human population by a human-mosquito-human transmission cycle that differs from the sylvatic transmission cycle described on the African continent. The cycle in Réunion probably follows a dengue-like model of outbreak dynamics, characterised by the absence of an animal reservoir and the ability to spread rapidly among human beings via domestic and peridomestic mosquitoes.<sup>13</sup>

Chikungunya virus has been imported to Europe and the USA by infected travellers returning from areas with high incidence rates, and *A. albopictus* has been introduced into several European countries (Belgium, Bosnia, Croatia, France, Greece, the Netherlands, Serbia, Spain, and Switzerland) and also to Central America, Brazil, and the USA.<sup>13,27</sup> *A. albopictus* has been imported through the trade in used tyres and in ornamental plants.<sup>22</sup> Some authors have suggested that if viraemic patients were to arrive in southern Europe during the summer they could cause a European outbreak.<sup>13,23</sup>

### Reservoirs

Human beings serve as the chikungunya virus reservoir during epidemic periods. Outside these periods the main reservoirs are monkeys, rodents, birds, and other unidentified vertebrates. Outbreaks might occur in monkeys when herd immunity is low; the animals develop viraemia but no pronounced physical manifestations.<sup>28,29</sup>

### Geographical range

Since the first recorded chikungunya epidemic, which occurred in Tanzania in 1952–53,<sup>1</sup> human chikungunya virus infection has been documented in Burma, Thailand, Cambodia, Vietnam, India, Sri Lanka, and the Philippines.<sup>30,31</sup> Epidemics were reported in the Philippines in 1954, 1956, and 1968,<sup>32</sup> and in south Sumatra, Java, Timor, Sulawesi, and the Moluccas Islands between 1982 and 1985.<sup>30,33</sup> 25 outbreaks were reported in Indonesia between 1999 and 2003, based on clinical observation (13 outbreaks) or serological diagnosis (12 outbreaks).<sup>31,34,35</sup> Chikungunya occurs in west Africa, from Senegal<sup>36</sup> to Cameroon,<sup>37</sup> Democratic Republic of

the Congo,<sup>38,39</sup> Nigeria,<sup>40,41</sup> Angola,<sup>42</sup> Uganda,<sup>43</sup> Guinea,<sup>44</sup> Malawi,<sup>45</sup> Central African Republic,<sup>46</sup> and Burundi,<sup>47</sup> and also in southern Africa.<sup>48,49</sup> Chikungunya is well known on the Indian subcontinent. Since the virus was first isolated in Calcutta in 1963 there have been several reports of chikungunya virus infection in India.<sup>7,50</sup> Since December, 2005, it is estimated that more than 1 400 000 cases have occurred in India.<sup>7,51</sup>

Neither Europe nor the Americas have had outbreaks of chikungunya virus so far. Imported cases have been diagnosed in the French West Indies and Guyana—where *A. aegypti* is endemic—among travellers returning from Réunion (figure 2).<sup>52,53</sup> Imported cases have also been reported in Germany, Switzerland, Italy, Norway,<sup>54</sup> China, and French Guyana,<sup>31</sup> and also among US Peace Corps volunteers in the Philippines.<sup>15</sup>

### Epidemics

As with all arboviruses, chikungunya virus outbreaks begin during the rainy season when vector density peaks. Available data suggest that chikungunya virus can be both endemic and epidemic.<sup>5</sup> Schematically, the endemic form appears to affect mainly rural Africa, with a wide range of vector species and reservoirs, high-level and continuous transmission to largely immune populations, and small rural outbreaks or sporadic cases.<sup>36,55</sup> In this situation, chikungunya virus may be discovered fortuitously during serological surveys of other arboviruses,<sup>55</sup> since there is a high degree of cross-reactivity between tests for chikungunya virus and o'nyong-nyong virus.<sup>9,37,56</sup>

By contrast, the epidemic form of chikungunya virus tends to be Asian and urban, and to be transmitted by two vectors (*A. aegypti* and *A. albopictus*) to populations with weak herd immunity. In these settings the disease is characterised by abrupt, massive epidemics with high attack rates (eg, 37% in India in 1978).<sup>57</sup> The epidemics peak then decline gradually as an increasing proportion of the population develops immunity.<sup>34</sup> This second scenario, which corresponds with the situation in Réunion, has previously been observed in Asia.<sup>30,58</sup> In the 1960s, epidemics occurred in several distinct rural and urban foci, followed by a lull of 20–30 years and re-emergence in the 1990s in Thailand,<sup>59,60</sup> Indonesia and the Philippines,<sup>34,35</sup> and India.<sup>57,61,62</sup> Bangkok, Jakarta, Rangoon, and Calcutta were greatly affected in the 1960s, with seroprevalence rates reaching 60–74% in adults.<sup>35,63,64</sup> Economic development does not protect populations from vector-borne diseases such as dengue or chikungunya. On the contrary, development can favour outbreaks by profoundly modifying the ecosystem. Malaysia, a rich country by comparison with other countries affected by chikungunya, had its first recorded chikungunya virus epidemic in 1998. *A. aegypti* is endemic in Malaysia, and the virus was probably introduced by migrant workers.<sup>19</sup> The same vectors can sometimes transmit several arboviruses, and confusing

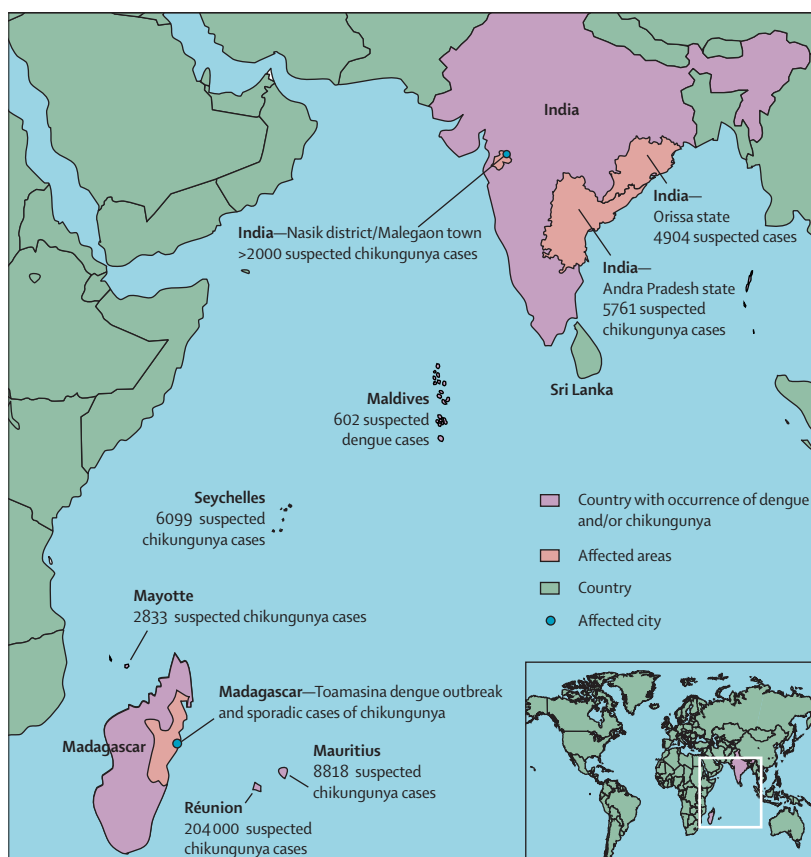


Figure 2: Chikungunya and dengue incidence in India and Indian Ocean islands (WHO) Status as of March 17, 2006. Data from WHO, <http://www.who.int>.

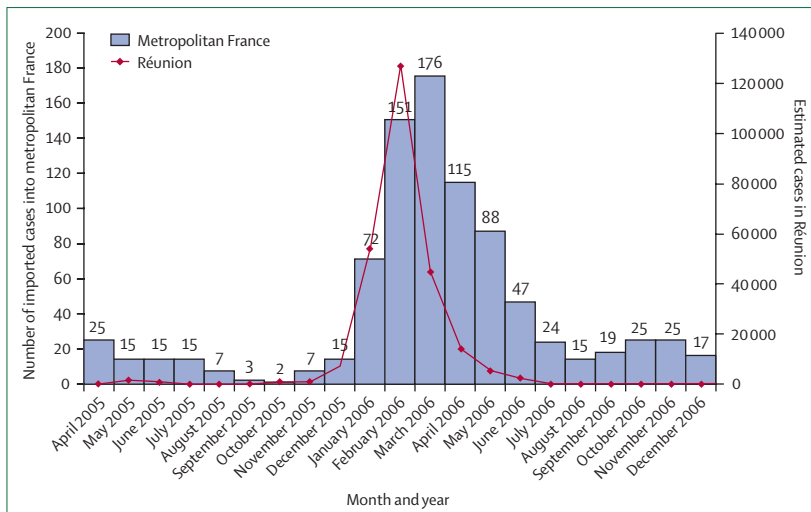
mixed epidemics have occasionally been described, such as yellow fever plus chikungunya virus,<sup>55</sup> dengue plus chikungunya virus,<sup>63</sup> or more recently *Plasmodium falciparum* malaria plus dengue type 1 and chikungunya on Madagascar's east coast.<sup>65</sup>

Chikungunya epidemics probably occurred before the virus was discovered, as suggested by descriptions of epidemic fevers accompanied by pronounced arthralgia.<sup>30</sup> They sometimes appear to have been confused with dengue, o'nyong-nyong, or Sindbis virus infection.<sup>66</sup>

### Recent outbreaks: India and Indian Ocean islands

The ongoing epidemic in the Indian Ocean region probably emerged first in Kenya (Lamu and Mombasa; July, 2004), before reaching the Comoros (January, 2005) and Seychelles (March, 2005), followed by Mauritius. Systematic studies showed a prevalence of 75% of the population in the Kenya (Lamu) outbreak, 63% in the Comoros, and 26% in Mayotte (2006).<sup>14,31,52,67,68</sup>

The virus reached Réunion (figure 2) in March–April, 2005, and around 266 000 cases had been diagnosed by Feb 19, 2007. For the first time, a substantial number of



**Figure 3: Chikungunya cases in Réunion and imported cases into metropolitan France, April 2005–December 2006**

Weekly notifications based on an estimated mathematical extrapolation (<http://www.invs.sante.fr> and reference 52) and imported cases in France.

For more information on the chikungunya outbreak in Réunion see <http://www.invs.sante.fr> and <http://www.orstrun.net>

For more information on the European Centre for Disease Prevention and Control report on chikungunya see <http://www.ecdc.eu.int>

For more information on the chikungunya outbreak in India see <http://mohfw.nic.in>, <http://who.int> and <http://www.hinduonnet.com>

deaths (254) were attributed, directly or indirectly, to chikungunya virus (data from Institut de Veille Sanitaire). The epidemic peaked with 46 000 new cases in week 6 of 2006 (figure 3), after which it gradually declined. Women (68%) were more often affected than men, and adults more affected than children (74% of victims were over 30 years of age).<sup>68</sup>

From February, 2006, to Oct 10, 2006, the WHO regional office for southeast Asia and the National Vector Borne Disease Control Programme of India reported that 151 districts located in ten states/provinces of India had been hit by chikungunya fever. About 1·36 million suspected cases have been reported in the south of this country, where 539 million people live. Some provinces have reported attack rates as high as 45%. By comparison, in 1973, the attack rate in Maharashtra province was 37·5%. Andhra Pradesh was the first Indian province to report suspected cases in December, 2005, and is also one of the worst affected (more than 80 000 suspected cases).<sup>7</sup> As in the Réunion outbreak, neurological complications such as meningoencephalitis have been reported but, to our knowledge, no deaths have been attributed to chikungunya virus infection. Additionally, 23 isolations or detections of chikungunya virus in adult *A aegypti* mosquitoes have been reported in affected areas of India.<sup>6</sup>

Antibodies to dengue virus were found in 0·9–9·9% of chikungunya patients in India and to both chikungunya virus and dengue virus in 0·4–4·3% of patients, confirming that the two viruses co-circulate in this country.<sup>7</sup>

The resurgence of dengue and chikungunya in India highlights a steady deterioration of public-health services, and the inability to control mosquitoes. The

Indian chikungunya outbreak seems to have followed the outbreak in the Indian Ocean islands, and may be related to the heavy tourist traffic between the two regions.<sup>31,53</sup> In a serosurvey done in Calcutta City 10 years ago, 4·37% of 379 samples tested positive for chikungunya virus. The highest rates were observed in the 51–55 year age-group, and no cases of seropositivity were detected in the youngest group.<sup>7</sup> Studies are underway to determine the genomic structure and virulence of Indian chikungunya virus isolates and to explain the re-emergence of chikungunya virus in this country.<sup>51</sup>

The Indian and Indian Ocean outbreaks are caused by an African strain of chikungunya virus. The Kenyan outbreak in 2004–2006 followed several months of unusually warm and dry conditions along the Indian Ocean coastline,<sup>69</sup> and such conditions may have favoured chikungunya virus transmission in the Indian Ocean islands.

### Chikungunya virus in travellers

The Indian Ocean islands, India, and Malaysia are popular tourist destinations. According to the World Tourism Organization, an estimated 1474218 people travelled from Madagascar, Mauritius, Mayotte, Réunion, and the Seychelles to European countries in 2004.<sup>13,27</sup> The outbreak in the Indian Ocean islands has substantially dented the region's tourist industry.<sup>23</sup>

Among travellers returning from the tropics, febrile arthralgia with exanthema may be caused by a variety of viral and bacterial infections, or adverse drug reactions.<sup>53</sup>

Chikungunya and dengue are among the most difficult diseases to distinguish, especially because simultaneous co-infection can occur.<sup>5,13,53,70</sup> In the only published study comparing the symptoms of chikungunya and dengue, done in Thailand, chikungunya symptom onset was more abrupt, fever was shorter lived, and rash, conjunctival injection, and arthralgia were more frequent than in dengue.<sup>53,71</sup>

### Clinical manifestations

#### Common chikungunya virus infection

After infection with chikungunya virus, there is a silent incubation period lasting 2–4 days on average (range 1–12 days).<sup>19</sup> Clinical onset is abrupt (see table), with high fever, headache, back pain, myalgia, and arthralgia; the latter can be intense, affecting mainly the extremities (ankles, wrists, phalanges) but also the large joints.<sup>1,19,51,53,68</sup> Skin involvement is present in about 40–50% of cases, and consists of (1) a pruriginous maculopapular rash predominating on the thorax, (2) facial oedema, or (3) in children, a bullous rash with pronounced sloughing, and (4) localised petechiae and gingivorrhagia (mainly in children).<sup>72,73</sup> Haemorrhagic fever has been reported in chikungunya virus-infected patients in Thailand.<sup>71</sup>

The table shows the frequency of signs and symptoms during the ongoing Réunion epidemic and the 1998

	Malaysia 1998 (%)	Réunion 2005–Feb 2006 (%)
Skin rash	50	39
Myalgia	50	60
Headache, spinal pain	50, 50	70, NR
Arthralgia (all types)	78	100
Large joints	18	NR
Fever	100	100
Number of reported cases	51	504

NR=not reported. Data for Malaysia from Lam and colleagues (2001)<sup>19</sup> and data for Réunion from <http://www.invs.sante.fr>.

**Table: Frequency of clinical manifestations during the 1998 Malaysian epidemic and the 2005 Réunion epidemic**

Malaysian epidemic.<sup>19</sup> Symptoms generally resolve within 7–10 days, except for joint stiffness and pain.<sup>17,72</sup>

#### Arthralgia and arthritis

Erratic, relapsing, and incapacitating arthralgia is the hallmark of chikungunya, although it rarely affects children. It may persist for several months. There are few precise descriptions of chikungunya virus-associated joint disorders, and the underlying mechanism is unknown.<sup>17,72–74</sup>

More data are available on the arthritis caused by related alphaviruses, and especially Ross River virus infection, which has been described as an epidemic polyarthritis.<sup>17,58,66,75,76</sup> Arthralgia/arthritis appear to affect 73–80% of patients with serologically confirmed chikungunya virus or o'nyong nyong virus infection,<sup>73,74</sup> and can persist in about 33% of patients for 4 months,<sup>74</sup> 15% for 20 months,<sup>73</sup> and 10% for 3–5 years.<sup>72</sup> Radiological findings are normal, and biological markers of inflammation are normal or moderately elevated.<sup>73,74</sup>

#### Severe forms affecting adults in Réunion

Chikungunya is not generally considered to be a life-threatening disease.<sup>59</sup> As recently discussed by Stephen Higgs,<sup>23</sup> by the summer of 2006 some 155 deaths might have been directly or indirectly caused by the disease in the ongoing Réunion outbreak. By March 14, 2006, the French health authorities had collated 96 serologically documented cases in Réunion residents who required intensive care; 79 of these patients were adults, and the male/female sex ratio was 1.4. 228 patients with a mean age of 78 years died.<sup>68</sup> The possible link between chikungunya virus infection and multiorgan failure is under investigation.<sup>31,52</sup> The maximum estimated incidence of severe chikungunya virus infection is 34 cases per 200 000 population (less than 0.02%).<sup>68</sup> The reasons why these severe clinical manifestations of chikungunya had not previously been described are unclear. It may be noteworthy, however, that this is the first time that chikungunya has occurred in a country largely free of other tropical diseases (except for dengue) and that has European health standards. As

mentioned above, the possible clinical repercussions of mutations found in chikungunya virus strains isolated in Réunion remain to be determined.<sup>12</sup>

Anecdotal reports of severe chikungunya virus are found in the literature.<sup>77,78</sup> Neurological complications such as meningoencephalitis were reported in a few patients during the first Indian outbreak in 1973, and during the ongoing Indian outbreak.<sup>7,79</sup> The neurovirulence and neuroinvasiveness of several other alphaviruses is well established, and chikungunya virus has been isolated from two children with clinical signs of encephalitis and meningitis.<sup>11</sup>

#### Chikungunya in pregnancy and in newborn babies

Among 3066 women that delivered children in Réunion hospitals during 2006, 159 reported symptoms of chikungunya virus (124 during the pregnancy, 35 around the time of delivery), and chikungunya virus infection was confirmed by RT-PCR or serology in 151 (95%) of these women (data from Observatoire Régional de la Santé de La Réunion).

Among the 35 women who were ill at delivery, 30 delivered an infected newborn baby. As of March 16, 2006, 35 biologically documented cases of neonatal chikungunya virus infection had been notified in Réunion, of which 27 were severe.<sup>80</sup> Neonatal chikungunya virus infection had never previously been reported. The possible risks of embryopathy, fetopathy, and late sequelae are unknown, and prospective follow-up of these “chikungunya virus babies” is therefore warranted. These cases are reminiscent of neonatal infection by dengue virus.<sup>81</sup> Recently, Robillard and colleagues<sup>82</sup> reported details of the first ten cases of maternofetal chikungunya virus transmission and Lenglet and colleagues<sup>83</sup> reported 16 cases of newborn babies presenting symptoms of, and confirmed diagnosis for, neonatal chikungunya.

#### Chikungunya virus in children

At the beginning of the Réunion epidemic the attack rate was proportional to age. However, in January, 2006, the attack rate in children approached that observed in adults. During week 11 of 2006, more than 18% of all children with suspected chikungunya virus infection had been hospitalised, compared with 40% of adults (data from Observatoire Régional de la Santé de La Réunion).

A number of differential diagnoses must be considered in infants less than 3 months old, and lumbar puncture is often justified (after assessing the bleeding risk). Bullous rash seems frequent in children (figure 4), and chikungunya virus may be found by PCR in blister fluid (figure 5).

#### Biological diagnosis of chikungunya virus infection

Virus isolation is based on inoculation of mosquito cell cultures, mosquitoes, mammalian cell cultures, or mice. Two main diagnostic methods are available, namely RT-PCR and serology (IgM or IgG) (figure 6).

For more information on chikungunya virus in pregnancy see <http://www.orsrun.net>

For more information on chikungunya virus in children see <http://www.orsrun.net>



Figure 4: Rash and bullous cutaneous manifestations frequently observed in children with chikungunya virus



Figure 5: Chikungunya virus can be detected by PCR in blister fluid

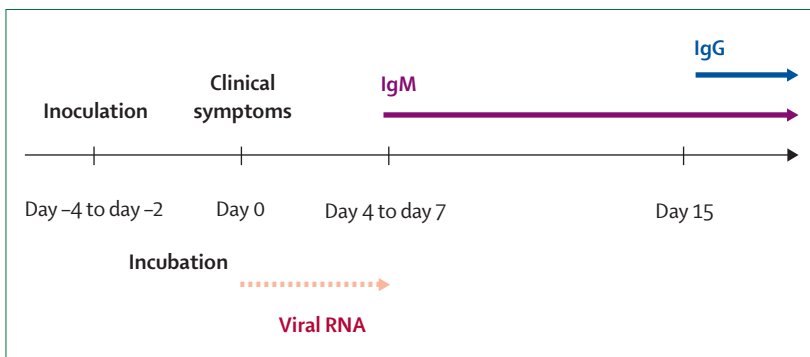


Figure 6: Biological diagnosis of chikungunya

RT-PCR is useful during the initial viraemic phase (day 0 to day 7),<sup>39,84,85</sup> but classic serological methods are simpler (haemagglutination inhibition, complement binding, immunofluorescence, and ELISA).<sup>86,87</sup> IgM is detectable after an average of 2 days by ELISA immunofluorescent assay (1–12 days) and persists for several weeks to 3 months.<sup>88</sup> IgG is detected in convalescent samples and persists for years. The sensitivity and specificity of these tests are poorly established, and so is the possibility of false-positive

For more information on the US Army Medical Research Institute candidate vaccine see <http://www.amb-usa.fr>

**Panel: Classification of the Semliki Forest antigenic complex within the Alphavirus genus (adapted from Strauss and Strauss, 1994<sup>9</sup>)**

**Semliki Forest group**

Semliki Forest (Africa, Eurasia); Middelburg (Africa); chikungunya (Africa, Asia, India, Indian Ocean); o'nyong-nyong (Africa); Ross River (Australia, Oceania); Barmah Forest (Australia); Getah (Australia, Asia); Sagiyama (Japan); Bebaru (Malaysia); Mayaro (South America); Una (South America)

reactions resulting from cross-reactivity with dengue or other arboviruses such as o'nyong-nyong virus.<sup>89</sup> Serologically, chikungunya virus is most closely related to o'nyong-nyong virus and is a member of the Semliki Forest antigenic complex (panel).

Individual serological testing is not particularly useful, except when faced with atypical or severe forms, or in travellers returning from an epidemic zone.<sup>90</sup> Parola and colleagues<sup>13</sup> analysed sera from four infected travellers returning from Indian Ocean islands by using a quantitative real-time RT-PCR-based method.<sup>13</sup> They detected a viral load of 10<sup>9</sup> copies per mL in one case. Such high levels of viraemia are uncommon in arthropod-borne diseases such as dengue fever and West Nile disease.<sup>91</sup>

PCR, antigen detection, and viral culture are also used to detect chikungunya virus in mosquitoes during epidemiological studies, and also to assess vector competence (the capacity to be infected by and transmit the virus).

**Specific immunity and vaccination**

Chikungunya virus infection seems to elicit long-lasting protective immunity. Experiments in animal models have shown cross-protection among chikungunya virus and other alphaviruses.<sup>92,93</sup>

There is currently no commercial vaccine for chikungunya virus, although some candidate vaccines have been tested in human beings.<sup>92,94</sup> In the latest trials, conducted by the US Army Medical Research Institute, very satisfactory seroconversion rates (98% on day 28) and neutralising antibody titres were obtained, persisting in 85% of cases at 1 year. Arthralgia occurred in 8% of the 59 volunteers.<sup>92</sup> These American vaccine trials were interrupted in 2003, even though the candidate vaccine appeared safe in human beings, when other infectious disease research efforts were prioritised to counter potential terrorist use of biological agents. On Sept 6, 2006, the US Army Medical Research and Materiel Command signed a Material Transfer Agreement with the French National Institute of Health and Medical Research focusing on the records of previous clinical studies. A phase III trial of the US Army candidate vaccine is in preparation (US Embassy in France; press release, Sept 14, 2006). The candidate vaccine is a live

vaccine (TSI-GSD-218) based on chikungunya virus strain 15561 isolated from a patient in Thailand and attenuated by serial passage in MRC-5 cells.<sup>94</sup>

## Prevention

Pending vaccine development, the only effective preventive measures consist of individual protection against mosquito bites and vector control. Control of both adult and larval mosquito populations uses the same model as for dengue and has been relatively effective in many countries and settings. Mosquito control is the best available method for preventing chikungunya. Breeding sites must be removed, destroyed, frequently emptied, and cleaned or treated with insecticides.<sup>65</sup> Large-scale prevention campaigns using DDT have been effective against *A aegypti* but not *A albopictus*. Control of *A aegypti* has rarely been achieved and never sustained.<sup>25</sup> Recent data show the different degrees of insecticide resistance in *A albopictus* and *A aegypti*.<sup>95</sup> However, vector control is an endless, costly, and labour-intensive measure and is not always well accepted by local populations, whose cooperation is crucial. Bednets should be used in hospitals and day-care facilities.<sup>96</sup> Surveillance is also important for early identification of outbreaks. For example, in India, a multidisciplinary team was deployed in February, 2006, to assist local health authorities in improving public-health measures, including strengthening of arbovirus surveillance, clinical management of cases, vector control, and social mobilisation.<sup>65</sup>

## Treatment

There is currently no effective antiviral treatment for chikungunya. Treatment is therefore purely symptomatic and is based on non-salicylate analgesics and non-steroidal anti-inflammatory drugs. Synergistic efficacy was reported between interferon- $\alpha$  and ribavirin on chikungunya virus in vitro.<sup>97</sup> A trial in southern Africa failed to confirm the efficacy of chloroquine on arthralgia.<sup>98</sup>

## Conclusion

Several lessons can be drawn from the ongoing outbreaks of chikungunya in India and the Indian Ocean islands. First, clinical manifestations are highly variable and may be more severe than previously reported. Second, economic development does not protect countries from vector-borne diseases (eg, West Nile virus in the USA, and dengue fever in Rio or Singapore); on the contrary, modern lifestyles may amplify an epidemic through travel, population ageing, and production of solid waste that can shelter aedes mosquitoes. Third, the chikungunya outbreaks highlight the importance of monitoring vector-borne and zoonotic diseases. These outbreaks have provided an opportunity to improve our previously poor knowledge of the biology, epidemiology, dynamics, and immunology of chikungunya virus infection. Genome sequencing may lead to new therapeutic and preventive measures.<sup>12</sup>

## Search strategy and selection criteria

Data for this Review were collected by searching the English and French literature from 1955–2007 through PubMed, Current Contents, and the reference lists of relevant articles. The key words were “arboviruses”, “alphavirus”, “chikungunya”, “*Aedes albopictus*”, “India outbreak”, “*Aedes aegypti*”, and “Reunion Island outbreak”. We also searched comprehensive and authoritative websites such as <http://www.chikungunya.re>, <http://www.medicinetropicale.com>, <http://www.invs.sante.fr>, <http://www.who.int>, and <http://www.orsrun.net>. Abstracts were not considered.

## Conflicts of interest

We declare that we have no conflicts of interest.

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## References

- Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952–53. I. Clinical features. *Trans R Soc Trop Med Hyg* 1955; **49**: 28–32.
- Carey DE. Chikungunya and dengue: a case of mistaken identity? *J Hist Med Allied Sci* 1971; **26**: 243–62.
- Deller JJ, Russell PK. Chikungunya disease. *Am J Trop Med Hyg* 1968; **17**: 107–11.
- Enserink M. Infectious diseases. Massive outbreak draws fresh attention to little-known virus. *Science* 2006; **311**: 1085.
- Powers AM, Brault AC, Tesh RB, Weaver SC. Re-emergence of chikungunya and o'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. *J Gen Virol* 2000; **81**: 471–79.
- Yergolkar P, Tandale B, Arankalle V, et al. Chikungunya outbreaks caused by African genotype, India. *Emerg Infect Dis* 2006; **12**: 1580–83.
- Ravi V. Re-emergence of chikungunya virus in India. *Indian J Med Microbiol* 2006; **24**: 83–84.
- Khan AH, Morita K, Parquet Md Mdel C, Hasebe F, Mathenge EG, Igarashi A. Complete nucleotide sequence of chikungunya virus and evidence for an internal polyadenylation site. *J Gen Virol* 2002; **83**: 3075–84.
- Strauss JH, Strauss EG. The alphaviruses: gene expression, replication, and evolution. *Microbiol Rev* 1994; **58**: 491–562.
- Yadav P, Shouche YS, Munot HP, Mishra AC, Mourya DT. Genotyping of chikungunya virus isolates from India during 1996–2000 by reverse transcription-polymerase chain reaction. *Acta Virol* 2003; **47**: 125–57.
- Vanlandingham DL, Tsatsarkin K, Hong C, et al. Development and characterization of a double subgenomic chikungunya virus infectious clone to express heterologous genes in *Aedes aegypti* mosquitoes. *Insect Biochem Mol Biol* 2005; **35**: 1162–70.
- Schuffenecker I, Iteman I, Michault A, et al. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. *PLoS Med* 2006; **3**: e263.
- Parola P, De Lamballerie X, Jourdan J, et al. Novel chikungunya virus variant in travelers returning from Indian Ocean Islands. *Emerg Infect Dis* 2006; **12**: 1493–98.
- Bonn D. How did chikungunya reach the Indian Ocean? *Lancet Infect Dis* 2006; **6**: 543.
- CDC. Chikungunya fever among US Peace Corps volunteers—Republic of the Philippines. *MMWR Morb Mortal Wkly Rep* 1986; **35**: 573–74.
- Mishra B, Ratho RK. Chikungunya re-emergence: possible mechanisms. *Lancet* 2006; **368**: 918.
- Jeandel P, Josse R, Durand JP. Exotic viral arthritis: role of alphavirus. *Med Trop* 2004; **64**: 81–88 (in French).

- 18 Zeller HG. Dengue, arbovirus and migrations in the Indian Ocean. *Bull Soc Pathol Exot* 1998; **91**: 56–60.
- 19 Lam SK, Chua KB, Hooi PS, et al. Chikungunya infection—an emerging disease in Malaysia. *Southeast Asian J Trop Med Public Health* 2001; **32**: 447–51.
- 20 Jupp PG, McIntosh BM. *Aedes fuscifer* and other mosquitoes as vectors of chikungunya virus at Mica, northeastern Transvaal, South Africa. *J Am Mosq Control Assoc* 1990; **6**: 415–20.
- 21 Jupp PG, McIntosh BM, Dos Santos I, DeMoor P. Laboratory vector studies on six mosquito and one tick species with chikungunya virus. *Trans R Soc Trop Med Hyg* 1981; **75**: 15–19.
- 22 Knudsen AB. Global distribution and continuing spread of *Aedes albopictus*. *Parassitologia* 1995; **37**: 91–97.
- 23 Higgs S. The 2005–2006 chikungunya epidemic in the Indian Ocean. *Vector Borne Zoonotic Dis* 2006; **6**: 115–16.
- 24 Pages F, Corbel V, Paupy C. *Aedes albopictus*: chronical of a spreading vector. *Med Trop* 2006; **66**: 226–28 (in French).
- 25 Reiter P, Fontenille D, Paupy C. *Aedes albopictus* as an epidemic vector of chikungunya virus: another emerging problem? *Lancet Infect Dis* 2006; **6**: 463–64.
- 26 Rohani A, Yulfi H, Zamree I, Lee HL. Rapid detection of chikungunya virus in laboratory infected *Aedes aegypti* by reverse-transcriptase-polymerase chain reaction (RT-PCR). *Trop Biomed* 2005; **22**: 149–54.
- 27 Depoortere E, Coulombier D. Chikungunya risk assessment for Europe: recommendations for action. *Euro Surveill* 2006; **11**: E060511.2.
- 28 Inoue S, Morita K, Matias RR, et al. Distribution of three arbovirus antibodies among monkeys (*Macaca fascicularis*) in the Philippines. *J Med Primatol* 2003; **32**: 89–94.
- 29 Wolfe ND, Kilbourn AM, Karesh WB, et al. Sylvatic transmission of arboviruses among Bornean orangutans. *Am J Trop Med Hyg* 2001; **64**: 310–16.
- 30 Mackenzie JS, Chua KB, Daniels PW, et al. Emerging viral diseases of southeast Asia and the western Pacific. *Emerg Infect Dis* 2001; **7**: 497–504.
- 31 Ligon BL. Reemergence of an unusual disease: the chikungunya epidemic. *Semin Pediatr Infect Dis* 2006; **17**: 99–104.
- 32 Macasaet F. Further observation on chikungunya fever. *J Philipp Med Assoc* 1970; **46**: 235–42.
- 33 Gratz NG. Critical review of the vector status of *Aedes albopictus*. *Med Vet Entomol* 2004; **18**: 215–27.
- 34 Laras K, Sukri NC, Larasati RP, et al. Tracking the re-emergence of epidemic chikungunya virus in Indonesia. *Trans R Soc Trop Med Hyg* 2005; **99**: 128–41.
- 35 Porter KR, Tan R, Istary Y, et al. A serological study of chikungunya virus transmission in Yogyakarta, Indonesia: evidence for the first outbreak since 1982. *Southeast Asian J Trop Med Public Health* 2004; **35**: 408–15.
- 36 Diallo M, Thonnon J, Traore-Lamizana M, Fontenille D. Vectors of chikungunya virus in Senegal: current data and transmission cycles. *Am J Trop Med Hyg* 1999; **60**: 281–86.
- 37 Kuniholm MH, Wolfe ND, Huang CY, et al. Seroprevalence and distribution of Flaviviridae, Togaviridae, and Bunyaviridae arboviral infections in rural Cameroonian adults. *Am J Trop Med Hyg* 2006; **74**: 1078–83.
- 38 Muyembe-Tamfum JJ, Peyrefitte CN, Yogoletto R, et al. Epidemic of chikungunya virus in 1999 and 2000 in the Democratic Republic of the Congo. *Med Trop* 2003; **63**: 637–38 (in French).
- 39 Pastorino B, Muyembe-Tamfum JJ, Bessaud M, et al. Epidemic resurgence of chikungunya virus in Democratic Republic of the Congo: identification of a new central African strain. *J Med Virol* 2004; **74**: 277–82.
- 40 Fagbami A. Epidemiological investigations on arbovirus infections at Igbo-Ora, Nigeria. *Trop Geogr Med* 1997; **29**: 187–91.
- 41 Moore DL, Reddy S, Akinkugbe FM, et al. An epidemic of chikungunya fever at Ibadan, Nigeria, 1969. *Ann Trop Med Parasitol* 1974; **68**: 59–68.
- 42 Filipe AF, Pinto MR. Arbovirus studies in Luanda, Angola. 2. Virological and serological studies during an outbreak of dengue-like disease caused by the chikungunya virus. *Bull World Health Organ* 1973; **49**: 37–40.
- 43 Kalunda M, Lwanga-Ssozi C, Lule M, Mukuye A. Isolation of chikungunya and pongola viruses from patients in Uganda. *Trans R Soc Trop Med Hyg* 1985; **79**: 567.
- 44 Ivanov AP, Ivanova OE, Lomonosov NN, Pozdnyakov SV, Konstantinov OK, Bah MA. Serological investigations of chikungunya virus in the Republic of Guinea. *Ann Soc Belg Med Trop* 1992; **72**: 73–74.
- 45 van den Bosch C, Lloyd G. Chikungunya fever as a risk factor for endemic Burkitt's lymphoma in Malawi. *Trans R Soc Trop Med Hyg* 2000; **94**: 704–05.
- 46 Guilherme JM, Gonella-Legall C, Legall F, Nakoume E, Vincent J. Seroprevalence of five arboviruses in Zebu cattle in the Central African Republic. *Trans R Soc Trop Med Hyg* 1996; **90**: 31–33.
- 47 Rodhain F, Carteron B, Laroche R, Hannoun C. Human arbovirus infections in Burundi: results of a seroepidemiologic survey, 1980–1982. *Bull Soc Pathol Exot* 1987; **80**: 155–61 (in French).
- 48 McIntosh BM. Antibody against chikungunya virus in wild primates in southern Africa. *S Afr J Med Sci* 1970; **35**: 65–74.
- 49 McIntosh BM, Harwin RM, Paterson HE, Westwater ML. An epidemic of chikungunya in south-eastern southern Rhodesia. *Cent Afr J Med* 1963; **43**: 351–59.
- 50 Rao TR. Immunological surveys of arbovirus infections in south-east Asia, with special reference to dengue, chikungunya, and Kyasanur Forest disease. *Bull World Health Organ* 1971; **44**: 585–91.
- 51 Saxena S, Singh M, Mishra N, Lakshmi V. Resurgence of chikungunya virus in India: an emerging threat. *Euro Surveill* 2006; **11**: E060810.2.
- 52 Cordel H, Quatresous I, Paquet C, Couturier E. Imported cases of chikungunya in metropolitan France, April 2005–February 2006. *Euro Surveill* 2006; **11**: E060420.3.
- 53 Hochedez P, Jaureguiberry S, Debruyne M, et al. Chikungunya infection in travelers. *Emerg Infect Dis* 2006; **12**: 1565–67.
- 54 Pfeffer M, Loscher T. Cases of chikungunya imported into Europe. *Euro Surveill* 2006; **11**: E060316.2.
- 55 Thonnon J, Spiegel A, Diallo M, Diallo A, Fontenille D. Chikungunya virus outbreak in Senegal in 1996 and 1997. *Bull Soc Pathol Exot* 1999; **92**: 79–82.
- 56 Kiwanuka N, Sanders EJ, Rwaguma EB, et al. O'nyong-nyong fever in south-central Uganda, 1996–1997: clinical features and validation of a clinical case definition for surveillance purposes. *Clin Infect Dis* 1999; **29**: 1243–50.
- 57 Paddidri VS, Gnanaswar TT. Epidemiological investigations of chikungunya epidemic at Barsi, Maharashtra state, India. *J Hyg Epidemiol Microbiol Immunol* 1979; **23**: 445–51.
- 58 Mackenzie JS, Smith DW. Mosquito-borne viruses and epidemic polyarthritides. *Med J Aust* 1996; **164**: 90–93.
- 59 Fukunaga T, Rojanasuphot S, Pisuthipornkul S, Wungkorbkiat S, Thammanichanon A. Seroepidemiologic study of arbovirus infections in the north-east and south of Thailand. *Biken J* 1974; **17**: 169–82.
- 60 Thaikruea L, Charearnsook O, Reanphumkarnkit S, et al. Chikungunya in Thailand: a re-emerging disease? *Southeast Asian J Trop Med Public Health* 1997; **28**: 359–64.
- 61 Mourya DT, Thakare JR, Gokhale MD, et al. Isolation of chikungunya virus from *Aedes aegypti* mosquitoes collected in the town of Yawat, Pune District, Maharashtra State, India. *Acta Virol* 2001; **45**: 305–59.
- 62 Pavri K. Disappearance of chikungunya virus from India and south east Asia. *Trans R Soc Trop Med Hyg* 1986; **80**: 491.
- 63 Neogi DK, Bhattacharya N, Mukherjee KK, et al. Serosurvey of chikungunya antibody in Calcutta metropolis. *J Commun Dis* 1995; **27**: 19–22.
- 64 Thauung U, Ming CK, Swe T, Thein S. Epidemiological features of dengue and chikungunya infections in Burma. *Southeast Asian J Trop Med Public Health* 1975; **6**: 276–83.
- 65 WHO. Outbreak news. Chikungunya and dengue, south-west Indian Ocean. *Wkly Epidemiol Rec* 2006; **81**: 106–08.
- 66 Harley D, Sleight A, Ritchie S. Ross River virus transmission, infection, and disease: a cross-disciplinary review. *Clin Microbiol Rev* 2001; **14**: 909–32.
- 67 Chastel C. Chikungunya virus: its recent spread to the southern Indian Ocean and Reunion Island (2005–2006). *Bull Acad Natl Med* 2005; **189**: 1827–35.
- 68 Quatresous I. E-alert 27 January: chikungunya outbreak in Reunion, a French overseas department. *Euro Surveill* 2006; **11**: E060202.1.
- 69 Epstein PR. Chikungunya fever resurgence and global warming. *Am J Trop Med Hyg* 2007; **76**: 403–04.



- 70 Myers RM, Carey DE. Concurrent isolation from patient of two arboviruses, chikungunya and dengue type 2. *Science* 1967; **157**: 1307–08.
- 71 Nimmannitya S, Halstead SB, Cohen SN, Margiotta MR. Dengue and chikungunya virus infection in man in Thailand, 1962–1964. I. Observations on hospitalized patients with hemorrhagic fever. *Am J Trop Med Hyg* 1969; **18**: 954–71.
- 72 Brighton SW, Prozesky OW, de la Harpe AL. Chikungunya virus infection. A retrospective study of 107 cases. *S Afr Med J* 1983; **63**: 313–15.
- 73 Fourie ED, Morrison JG. Rheumatoid arthritic syndrome after chikungunya fever. *S Afr Med J* 1979; **56**: 130–02.
- 74 Kennedy AC, Fleming J, Solomon L. Chikungunya viral arthropathy: a clinical description. *J Rheumatol* 1980; **7**: 231–36.
- 75 McGill PE. Viral infections: alpha-viral arthropathy. *Baillieres Clin Rheumatol* 1995; **9**: 145–50.
- 76 Suhrbier A, La Linn M. Clinical and pathologic aspects of arthritis due to Ross River virus and other alphaviruses. *Curr Opin Rheumatol* 2004; **16**: 374–79.
- 77 Mazaud R, Salaun JJ, Montabone H, Goube P, Bazillio R. Acute neurologic and sensorial disorders in dengue and chikungunya fever. *Bull Soc Pathol Exot Filiales* 1971; **64**: 22–30 (in French).
- 78 Sarkar JK, Chatterjee SN, Chakravarti SK, Mitra AC. Chikungunya virus infection with haemorrhagic manifestations. *Indian J Med Res* 1965; **53**: 921–25.
- 79 Chatterjee SN, Chakravarti SK, Mitra AC, Sarkar JK. Virological investigation of cases with neurological complications during the outbreak of haemorrhagic fever in Calcutta. *J Indian Med Assoc* 1965; **45**: 314–16.
- 80 Cordel H. Chikungunya outbreak on Reunion: update. *Euro Surveill* 2006; **11**: E060302.3.
- 81 Lum LC, Lam SK, Choy YS, George R, Harun F. Dengue encephalitis: a true entity? *Am J Trop Med Hyg* 1996; **54**: 256–59.
- 82 Robillard PY, Boumahni B, Gerardin P, et al. Vertical maternal fetal transmission of the chikungunya virus. Ten cases among 84 pregnant women. *Presse Med* 2006; **35**: 785–88 (in French).
- 83 Lenglet Y, Barau G, Robillard PY, et al. Chikungunya infection in pregnancy: Evidence for intrauterine infection in pregnant women and vertical transmission in the parturient. Survey of the Reunion Island outbreak. *J Gynecol Obstet Biol Reprod (Paris)* 2006; **35**: 578–83 (in French).
- 84 Pastorino B, Bessaud M, Grandadam M, Murri S, Tolou HJ, Peyrefitte CN. Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African chikungunya viruses. *J Virol Methods* 2005; **124**: 65–71.
- 85 Pfeffer M, Linssen B, Parke MD, Kinney RM. Specific detection of chikungunya virus using a RT-PCR/nested PCR combination. *J Vet Med B Infect Dis Vet Public Health* 2002; **49**: 49–54.
- 86 Hundekar SL, Thakare JP, Gokhale MD, Barde PV, Argade SV, Mourya DT. Development of monoclonal antibody based antigen capture ELISA to detect chikungunya virus antigen in mosquitoes. *Indian J Med Res* 2002; **115**: 144–48.
- 87 Thein S, La Linn M, Aaskov J, et al. Development of a simple indirect enzyme-linked immunosorbent assay for the detection of immunoglobulin M antibody in serum from patients following an outbreak of chikungunya virus infection in Yangon, Myanmar. *Trans R Soc Trop Med Hyg* 1992; **86**: 438–42.
- 88 Sam IC, AbuBakar S. Chikungunya virus infection. *Med J Malaysia* 2006; **61**: 264–69.
- 89 Blackburn NK, Besselaar TG, Gibson G. Antigenic relationship between chikungunya virus strains and o'nyong nyong virus using monoclonal antibodies. *Res Virol* 1995; **146**: 69–73.
- 90 Pile JC, Henchal EA, Christopher GW, Steele KE, Pavlin JA. Chikungunya in a North American traveler. *J Travel Med* 1999; **6**: 137–39.
- 91 Sudiro TM, Zivny J, Ishiko H, et al. Analysis of plasma viral RNA levels during acute dengue virus infection using quantitative competitor reverse transcription-polymerase chain reaction. *J Med Virol* 2001; **63**: 29–34.
- 92 Edelman R, Tacket CO, Wasserman SS, Bodison SA, Perry JG, Mangiafico JA. Phase II safety and immunogenicity study of live chikungunya virus vaccine TSI-GSD-218. *Am J Trop Med Hyg* 2000; **62**: 681–85.
- 93 Hearn HJ Jr, Rainey CT. Cross-protection in animals infected with Group A arboviruses. *J Immunol* 1963; **90**: 720–24.
- 94 Levitt NH, Ramsburg HH, Hasty SE, Repik PM, Cole FE, Lupton HW. Development of an attenuated strain of chikungunya virus for use in vaccine production. *Vaccine* 1986; **4**: 157–62.
- 95 Cui F, Raymond M, Qiao CL. Insecticide resistance in vector mosquitoes in China. *Pest Manag Sci* 2006; **62**: 1013–22.
- 96 Reiter P, Sprenger D. The used tire trade: a mechanism for the worldwide dispersal of container breeding mosquitoes. *J Am Mosq Control Assoc* 1987; **3**: 494–501.
- 97 Briolant S, Garin D, Scaramozzino N, Jouan A, Crance JM. In vitro inhibition of chikungunya and Semliki Forest viruses replication by antiviral compounds: synergistic effect of interferon-alpha and ribavirin combination. *Antiviral Res* 2004; **61**: 111–17.
- 98 Savarino A, Di Trani L, Donatelli I, Cauda R, Cassone A. New insights into the antiviral effects of chloroquine. *Lancet Infect Dis* 2006; **6**: 67–69.