Chikungunya Fever

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CASE REPRESENTATION

At the end of August 2007, the health district authority of Ravenna, a town in north-eastern Italy, informed the Istituto Superiore di Sanità (the Italian National Institute of Health) about an unusual event: a fever outbreak that they imagined to be “pappataci fever” (i.e., Toscana virus infection, which usually causes sporadic cases of fever or mild meningoencephalitis), which appeared to be localized in two small villages. The large number of cases urged a site visit to investigate the nature of the outbreak.

The clinical investigation highlighted that the patients were affected by high fever and joint pain. Headache and muscle pains were also commonly reported, whereas about half of them presented a macular skin rash, sometimes accompanied by itching. Joint pain was particularly severe, and some patients complained of persistence of pain that needed treatment for at least 1 month. The entomological investigation did not show the presence of pappataci, whereas a large number of Aedes albopictus mosquitoes (the so-called “tiger mosquito”) were identified. Thus, on clinical and entomological grounds, chikungunya virus (CHIKV) or dengue virus (DENV) were considered as the presumptive causal agents of the illness. Serum samples were collected and analyzed, and the diagnosis of CHIK fever (CHIKF) was confirmed by the use of PCR and/or serological assays (IgM against CHIKV). The epidemiological investigation revealed that the epidemic apparently started about 10 days after a few hours’ visit of a viremic person (the index case: a man coming from Kerala, India) to his relatives living in one of the originally affected villages, Castiglione di Cervia. This village, together with the contiguous village of Castiglione di Ravenna, represents the area where most of the cases subsequently occurred. The man developed high fever during the evening; Aedes albopictus mosquitoes, after biting the infected man, amplified the virus and the chain of transmission was initiated.
Then, during the course of the epidemic, several minor outbreaks occurred outside the initially affected area: small clusters of cases occurred in neighboring towns, within a radius of few kilometers, whereas other clusters were detected at an appreciable distance, up to 75 km from the epicenter of the epidemic.

The implementation of vector control measures and the decline of mosquito activity at the beginning of the cold season allowed outbreak control.

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1. WHY THIS CASE WAS SIGNIFICANTLY IMPORTANT AS AN EMERGING INFECTION

CHIKF is a viral disease which is endemic in Africa and causes recurrent epidemics in Asia. In recent years, a large epidemic ravaged Indian Ocean islands and the Indian subcontinent. During the epidemic, smaller outbreaks and sporadic autochthonous cases of CHIKF have been identified in temperate areas, such as north-eastern Italy and Mediterranean France, expanding the geographical range of detection of the virus.

2. WHAT IS THE CAUSATIVE AGENT?

CHIKF is caused by the CHIKV, an arbovirus (i.e., arthropod-borne virus) belonging to the alphavirus genus of the *Togaviridae* family. CHIKV has a single-stranded RNA genome, a 60–70 nm diameter capsid, and a phospholipid envelope. The virus contains four non-structural proteins (nsP1–4) and structural proteins (C, E3, E2, 6 K, and E1). The virus is sensitive to desiccation and to temperature above 58°C (Figure 12.1).

Alphaviruses are subdivided into those associated with polyarthritis and rash (predominantly Old World strains) and those associated with

![CHIKV characteristics and genome structure.](image)
mengoencephalitis (predominantly New World strains). CHIKV is a member of the Old World group, along with other viruses that may cause human diseases, such as the o’nyong nyong virus (Central Africa, similar to CHIKV), Ross River and Barmah Forest viruses (Australia and the Pacific), Sindbis virus (cosmopolitan), and Mayaro virus (South America).

Three different genotypes of CHIKV have been identified: the western African, the east-central-south African (ECSA), and the Asian genotype. Evolutionary studies have suggested an African origin for CHIKV. The Asian lineage exhibits a peculiar pattern of spread, with successive epidemics detected along an eastward path. The old Asian lineage, which caused epidemics in the 1950s, was a variant of the ECSA strain that probably split into two clades: an Indian lineage, which likely went extinct, and a South-East Asian lineage. The old Asian genotype has not been identified during the last epidemic in India, consistently with lack of sustainability of the human—mosquito cycle at a local scale in the absence of continued importation.

CHIKV strains, which caused a large epidemic since 2004, spreading from Kenya toward Indian Ocean islands, the Indian subcontinent, and South-East Asia, belong to the ECSA genotype. During the epidemic, two different lineages were identified, suggesting independent introductions of CHIKV strains from Kenya into the Indian Ocean islands and India. A viral variant, presenting a substitution of the amino acid alanine with valine in the position 226 of the E1 protein (one of the two major envelope surface glycoproteins) of CHIKV (A226V), selected during the course of the epidemic, originated in the Indian Ocean and became predominant in specific affected areas where *Aedes albopictus* was largely predominant compared to *Aedes aegypti*, such as La Réunion and the Kerala district in India, allowing an efficient replication and dissemination of the A226V variant of CHIKV.

While the 226 A strain was the only genotype observed during the first period of the outbreak (March to June 2005), the E1 A226V genotype started to be observed since September 2005. On La Réunion, the identification of A226V variant circulation preceded by at least 3 months the explosive epidemic peak of mid-December, suggesting an increase in viral transmission. In the Indian region of Kerala, CHIKV strains isolated in 2007 presented the A226V mutation, which had not been found in strains isolated in Kerala and in other Indian regions in 2006. Thus, a single amino acid substitution may have influenced vector specificity, increasing the fitness of CHIKV for specific vector species and consequently CHIKV transmission. The same variant caused the outbreak propagated by *Aedes albopictus* in north-eastern Italy.

### 3. WHAT IS THE FREQUENCY OF THE DISEASE?

CHIKV was first isolated in the Newala district of Tanzania in 1952/1953. Since its identification, sporadic cases and a number of outbreaks have been reported in several African countries, on the Indian subcontinent, and in
South-East Asia.\textsuperscript{14,2} In the last few years, CHIKV has re-emerged, causing a series of large outbreaks, which started in Kenya in 2004, and ravaged the Comoros Islands, the island of La Réunion, and other islands in the south-west Indian Ocean in early 2005, which were followed by an epidemic in the Indian subcontinent in 2005/2006.\textsuperscript{15,16} CHIKV caused an outbreak in the north-east of Italy in the summer of 2007.\textsuperscript{1} Two autochthonous cases of CHIKF were also identified in Mediterranean France in the summer of 2010\textsuperscript{17} (Figure 12.2).

4. HOW IS THE VIRUS TRANSMITTED?

CHIKV is transmitted to humans by the bite of \textit{Aedes} spp. (i.e., \textit{Aedes aegypti} and \textit{Aedes albopictus}) mosquitoes. Although \textit{Aedes aegypti} is the main vector of CHIKV, \textit{Aedes albopictus} (the “tiger” mosquito) is becoming important in the emergence of CHIKV in temperate areas where \textit{Aedes aegypti} is not detected.

The virus appears to be enzootic across tropical areas of Africa and Asia. In west and central Africa, CHIKV is maintained in a sylvatic cycle involving wild non-human primates and forest-dwelling \textit{Aedes} spp. mosquitoes. However, there is little information on the vertebrate hosts involved in viral maintenance.\textsuperscript{18} Both humans and wild primates throughout the humid forests and the semi-arid savannahs of Africa have significant levels of antibodies against CHIKV, with small-scale outbreaks following a 3–4-year cyclical
pattern consequent to the repopulation of susceptible, non-immune, wild primates. In rural regions, outbreaks are more likely in the heavy rainfall season, when sylvatic mosquito density tends to increase. In Asia, the predominant vector is the urban, peri-domestic, anthropophilic *Aedes aegypti* mosquito, which is responsible for large-scale outbreaks characterized by long inter-epidemic periods, which may last several decades. As reported in Chapter 2, since a vertebrate reservoir or a sylvan transmission cycle has not been identified in Asia, the virus is presumed to persist in a human/mosquito/human cycle. However, this is still uncertain, since outbreaks of CHIKV fever in Asia have not been necessarily associated with outbreaks in Africa, which suggests an independent evolution of an African ancestor of CHIKV in Asia and the possibility of a sylvatic cycle maintaining autochthonous virus genotypes in the Asian continent (Figure 12.3).

As already mentioned, although *Aedes aegypti* is the main vector of CHIKV, *Aedes albopictus* is playing an important role in the spread of the infection outside the area of activity of *Aedes aegypti* (Figure 12.4).

In Europe, *Aedes albopictus* was first detected in Albania in 1979 and established in the last decade in at least 12 countries, especially in southern Europe. Although shipment of used tires infested with mosquito eggs is the main modality of spread, *Aedes albopictus* infestation has been recently associated with the shipment of a commercial plant product known as “lucky bamboo” (*Dracaena* spp.), packaged in standing water, and introduced in Europe (i.e., Netherlands) from mainland south China. The presence of *Aedes albopictus* in Italy, with the risk that this mosquito could transmit viral infections, has long been recognized, well before the occurrence of the Italian outbreak. Two major tire retreading companies located in north-eastern Italy, importing used tires directly from the USA, were the source of mosquito...
entry to, and spread across, the country through internal trade of tires performed in collaboration with smaller companies. During its further spread, *Aedes albopictus* demonstrated a high degree of fitness and capacity to breed in a huge variety of disposable containers and ground water collection sites. In temperate areas, mosquito activity heavily declines during the cold season, thus transovarial transmission is a possible mechanism to augment the probability of virus overwintering. In La Réunion Island the virus has been isolated from field-collected *Aedes albopictus* females and in two out of 500 pools of larvae, demonstrating a low level of vertical transmission.

Finally, during the recent epidemic on La Réunion, mother-to-child transmission has been documented, with an estimated vertical transmission rate of 49% in pre-term deliveries in the context of intra-partum viremia. Severe illness, mainly consisting of encephalopathy, was observed in 53% of the cases.

5. WHICH FACTORS ARE INVOLVED IN DISEASE PATHOGENESIS? WHAT IS THE PATHOGENIC MECHANISM?

CHIKV is a member of the arthritogenic alphaviruses but sometimes it may cause meningoencephalitis. Unlike typical encephalogenic viruses, which infect neurons, CHIKV seems to infect stromal cells of the central nervous system, in particular the lining of the choroid plexus.

Following transmission, CHIKV replicates in the skin, and then disseminates to the liver and joints, presumably through the blood. Epithelial and endothelial cells, fibroblasts, and, to a lesser extent, macrophages, are susceptible to CHIKV infection and allow viral production. CHIKV produces a marked cytopathic effect in cell cultures, and virus replication is associated with induction of apoptosis in infected cells. Animal models show that the virus may be detected in muscle, joint, and skin fibroblasts, but also in
epithelial and endothelial layers of organs like the liver, skin, and brain, as it happens in mice at higher risk of severe diseases such as those lacking the type I IFN receptor.\textsuperscript{28} Non-human primate models also show persistent infection in splenic macrophages and endothelial cells lining the liver sinusoids. Samples from human patients affected by CHIKV myositic syndrome show viral antigen expression in skeletal muscle satellite cells but not in muscle fibers; fibroblasts may also be infected. The pathogenesis of the long-term severe debilitating arthralgia experienced by convalescent patients is still undefined, and the role of adaptive immune responses in the possible induction of autoimmunity caused by cross-reactivity between viral and host antigens. To this regard, the possibility that B and T cells responsive to CHIKV are implicated needs to be better explored.\textsuperscript{29} However, the increased inflammatory cytokine expression (i.e., up-regulation of the pro-inflammatory cytokines interleukin-1 and interleukin-6) appears to be associated with joint disease severity.\textsuperscript{30,31}

6. WHAT ARE THE CLINICAL MANIFESTATIONS?

The term CHIK means “to walk bent over,” in the African dialect of Makonde (or Shawili), and refers to the effects of the incapacitating arthralgia. The incubation period is short (i.e., 2–4 days on average, with a range of 1 to 12 days), and clinical onset is often abrupt. Patients affected by CHIK usually present with high fever, and severe and persistent joint pain; arthralgia/arthritis may affect about 70 to 80\% of the patients and is more often localized to the extremities (i.e., ankles, wrists, phalanges), but large joints may also be affected. Headache, back and muscle pains are also common, whereas a macular skin rash is present in more than 50\% of the cases, sometimes accompanied by itching. The frequency of the most common signs and symptoms of CHIKF are summarized in Table 12.1.\textsuperscript{1,2,32,33} Lymphopenia and hypocalcemia are the most prominent laboratory findings, while thrombocytopenia is rarely observed.\textsuperscript{34} In most cases, CHIKF is a relatively mild disease, and severe cases, especially with neurological complications (i.e., encephalopathy, acute flaccid paralysis, or Guillain–Barré syndrome) or hemorrhagic manifestations, are relatively rare.\textsuperscript{1,2,31} Encephalitis or meningoencephalitis is usually observed in older individuals with underlying disorders. Chronic joint pain, which may last for months or even years, is an important complication that occurs in more than one-third of the patients.

7. HOW DO YOU DIAGNOSE?

In case a person is suspected to be affected by CHIK fever, laboratory confirmation is needed, and both RT-PCR and serology (IgM or IgG, detected through HI, indirect fluorescent antibody (IFA), or enzyme-linked
immunoabsorbent assay (ELISA)) are to be used. RT-PCR is useful during the initial viremia phase (day 0 to 7), while IgM are detectable after an average of 2 days by ELISA or about 1 week by HI and persist for several weeks. IgG may be detected in convalescent sera. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) may detect higher levels of CHIK viremia compared with other arthropod-borne infections. A four-fold increase in IgG titers in paired samples is also confirmatory of CHIKV infection. Finally, viral culture may be used as a diagnostic tool.

8. HOW DO YOU DIFFERENTIATE THIS DISEASE FROM SIMILAR ENTITIES?

CHIKF should be suspected in individuals who have recently visited areas affected by outbreaks and who present with fever and joint pain, with or without skin rash. Occasionally, joint pain may not be present or be so mild and unspecific as to be unreported, particularly in aged patients. Furthermore, other arboviral diseases, such as dengue, whose geographical distribution in Africa and Asia largely overlaps with that of CHIK virus, is responsible of similar symptoms.

CHIKF may be misdiagnosed when one or more cases of a dengue-like syndrome occur in individuals who did not travel 2 weeks before fever onset. In this case, epidemiological (i.e., identification of index case and chain of transmission) and entomological (i.e., presence of a competent vector) investigation may corroborate clinical suspicion and guide lab diagnosis (Box 12.1).

9. WHAT IS THE THERAPEUTIC APPROACH?

There is no effective treatment for CHIKF. Specific antiviral drugs are not available and treatment is purely symptomatic. Ribavirin, which has antiviral activity against RNA viruses, is considered less active against alphaviruses.
whereas the only recommended treatments are non-steroidal anti-inflammatory drugs for joint pain.\textsuperscript{31}

10. WHAT ARE THE PREVENTIVE AND INFECTION CONTROL MEASURES?

Measures for controlling the population of *Aedes albopictus* are of primary importance (Box 12.2). They include the use of fast-acting insecticides (synergized pyrethroids) for 3 consecutive days, applied with a truck-mounted atomizer in public spaces and a backpack mist blower in private spaces. Anti-larval measures, using formulations of insect growth regulators and *Bacillus thuringiensis* var. *israeliensis* serotype H14, are also important. Other interventions involve house-to-house interventions to control or eliminate breeding places, and encouraging community participation. For each suspected case of infection, these control measures should be done within a radius of 100 m of the individual’s residence; for clusters, the control measures were extended within a 300-m radius of the most external case. Recommendations for mosquito control at community level and personal protective measures are summarized in Box 12.2.
With regard to other prevention tools, safe and effective vaccines are not available yet, but research and development is ongoing with promising results.\(^{31}\)

Finally, preparedness for unusual outbreaks in countries where the presence of competent vectors has been identified is key. Strengthening activities addressed toward surveillance and control of *Aedes albopictus* and early etiological identification of clusters of unusual human cases is essential to reduce the risk of outbreaks due to exotic viral agents in temperate climate countries.

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**Box 12.2 Fighting the “Tiger” Mosquito: Prevention and Outbreak Control Activities Should be Aimed at the Control of Competent Vectors**

**A. Prevention Activities**
- Vector control
  1. Monitoring of *Aedes* indices
  2. Source reduction
  3. Preventive larviciding
  4. Space spraying when necessary
  5. Personal protection
- Public awareness
- Health education
- Publicity
- Mass media
- Community participation
- Routine recording and reporting

**B. Outbreak Control Activities**
- Vector control
- Outbreak control activities
  1. Space spraying
  2. Thermal fogs
  3. Ultra low volume aerosols spray
  4. Source reduction
  5. Preventive larviciding on large-scale basis
- Personal protection
  1. Repellents
  2. Mosquito coils
  3. Window and door screens
- Health education
- Community participation
- Mass media anti-CHIKV campaign
- Cooperation between government agencies/public sectors
- Follow-up activities
- Routine recording and reporting
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