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I'm your host, Dan Rutz, here to present an article published in Emerging Infectious Diseases, a public health journal fully online.

This report, "Chikungunya Outbreaks Caused by African Genotype, India" and the entire journal contents are available online from [www.cdc.gov/eid](http://www.cdc.gov/eid).

Chikungunya virus outbreaks have been documented in Africa and Southeast Asia. In India, the first Chikungunya virus outbreak was recorded in Calcutta and was followed by epidemics in 3 other cities in 1964; 4 more in 1965; and one additional Indian city in 1973. Recently, the virus has emerged in Southeast Asia and the Pacific region. Massive outbreaks have been reported from many islands in the Indian Ocean. *Aedes albopictus* is considered the vector in Reunion and other islands in the Indian Ocean, but *Aedes aegypti* is the main vector in Asia, including India. We investigated a large number of patients with fever with arthralgia, reported from October 2005 through March 2006, in many districts from Andhra Pradesh, Karnataka, and Maharashtra states.

Blood samples were collected from 1,938 suspected case-patients from the 3 states; serum was separated and transported to the laboratory on wet ice. Adult mosquitoes were collected from houses and sheds. Larval mosquitoes were collected from the affected areas by single-larva survey method. Adult household indexes and Breteau indexes were calculated for each area.

The C6/36 cell line was used for virus isolation. Immunoglobulin M antibodies to chikungunya virus and dengue virus were assayed by IgM capture ELISA. For chikungunya virus ELISA, brain suspensions from mice infected with the virus were the source of antigen, and monoclonal antibodies were the source of antibodies. Dengue/chikungunya virus IgM antibodies and negative control human sera were included for respective tests. Approval for use of mice for antigen preparation was obtained from the institutional ethical committee according to national guidelines.

Immunofluorescence assay was used to detect the virus in cell culture and in crushed heads of adult mosquitoes. A patient with the following was confirmed as having chikungunya virus infection: acute onset of moderate-to-high fever with joint pain of

varying severity; negative test results for malaria, typhoid, and tuberculosis; and positive results for IgM anti-chikungunya virus antibodies, seroconversion, or chikungunya virus isolation. We used the  $\chi^2$  test to compare proportions of cases in different age groups.

We studied chikungunya virus isolates obtained during current investigations and viruses isolated during earlier epidemics in India from 1963 to 2000). RNA was isolated by a commercially available viral RNA mini kit according to the manufacturer's instructions. An RNA polymerase was used for reverse transcription at 42° Celsius for 1 hour. Initially, *Alphavirus* genus-specific primers of 26 and 25 nucleotides produced a 472-base pair fragment (the NS4 gene). The second set of primers of 18 and 19 nucleotides amplified a 294-base pair product of E1 gene. For amplification, Platinum *Pfx* enzyme was used. Cycling conditions were 1 cycle at 94° Celsius for 5 minutes; then 35 cycles each of 94° Celsius for 1 minute, 50° Celsius for 1 minute, and 68° Celsius for 1.5 minutes; followed by final extension of 7 minutes at 68° Celsius. The PCR products were purified and sequenced.

Multiple alignments of nucleotide sequences were performed. The phylogenic status of the chikungunya virus isolates was assessed and reliability of different phylogenic groupings was evaluated with the bootstrap test (1,000 bootstrap replications) available in the software.

Acute onset of moderate-to-high fever in association with body ache, backache, and headache was recorded. Joint pain of varying severity occurred within 2 days of onset of fever and, in decreasing order of affliction, involved knees, ankles, wrists, hands, and feet. Joint pain was severe and incapacitating and lasted for weeks to months. Inflammation of joints and transient macular rash on earlobes, neck, trunk, and upper extremities were reported for a few patients. Hemorrhage did not occur. The cases were reported predominantly from rural areas; distribution was focal. Multiple cases were recorded in families. All ages and both sexes were affected; significantly more cases occurred in persons aged 15 years or older (that's 299 or 89.8% of 333). Cases were reported from 11 of 23 districts in Andhra Pradesh, 15 of 27 in Karnataka, and 16 of 35 in Maharashtra. Results of serologic testing and virus isolation are shown in the table.

State governments of Andhra Pradesh, Karnataka, and Maharashtra have declared outbreaks of chikungunya virus. By mid-April, the declared numbers of fever cases associated with this outbreak were greater than 25,000 in Andhra Pradesh, greater than 65,000 in Maharashtra, and greater than 36,000 in Karnataka. In absence of active surveillance for this disease, these numbers may be underestimates.

The predominant mosquito species in the affected areas was *Aedes aegypti*. *Aedes albopictus* was either absent or present in negligible numbers. The population of *Aedes aegypti* was reasonably high in most of the localities; adult household indexes and Breteau indexes, respectively, were 10 to 60 and 13 to 75 in Andhra Pradesh, 20 to 70 and 40 to 200 in Karnataka, and 10 to 30 and 30 to 50 in Maharashtra. High density of *Aedes aegypti* populations in affected areas and 23 isolations or detections of chikungunya virus from adult mosquitoes indicate that this species is the main vector in India. Earlier outbreaks in India were mainly restricted to large cities; in contrast, the current outbreak is predominantly rural.

Anti-chikungunya virus IgM was detected in 33.5 to 41.9% of patients tested. The finding of antibodies to dengue virus in 0.9 to 9.9% of patients and to chikungunya virus and dengue virus in 0.4 to 4.3% of patients indicates that these viruses cocirculate in the area. Nine patients whose acute-phase serum sample was negative had anti-chikungunya virus IgM in the early convalescent-phase sample, collected during the second week of illness.

NS4-based phylogenetic analysis identified the Yawat isolate (2000) from Maharashtra as central/East African genotype, not Asian genotype as reported earlier. This finding led us to resequence all isolates in our repository. Phylogenetic analyses based on NS4 and E1 regions yielded identical results. The Indian viruses isolated from 1963 through 1973 belonged to the Asian genotype, whereas the current isolates from the 3 Indian states and the Yawat isolate belonged to the central/East African genotype. Within the Asian genotype, all older isolates (that's India from 1963 to 1973 and Thailand from 1962 to 1978) clustered together, whereas later isolates from the Philippines (1985), Indonesia (also 1985), Thailand (1988, 1995, and 1996), and Malaysia (1998) formed a distinct cluster. The sequence from the Reunion Islands, which represents a recent

outbreak of the disease (GenBank accession no. DQ443544), also grouped with the recent Indian isolates. Percentage nucleotide identity within earlier (1963 to 1973) and recent (2005 to 2006) Indian isolates was  $99.71\% \pm 0.16\%$  and  $99.94\% \pm 0.05\%$ , respectively, whereas percentage nucleotide identity between these isolates was  $96.11\% \pm 1.09\%$ . The 2005–2006 Indian isolates were  $98.61\% \pm 0.6\%$  and  $98.95\% \pm 0.57\%$  identical with the Reunion and Yawat isolates, respectively.

This report confirms chikungunya virus as the causative agent for large outbreaks of fever with arthralgia and arthritis in 3 Indian states. Thus, chikungunya fever has emerged in outbreak form after 32 years.

The current epidemic is caused by central/East African genotype of chikungunya virus. That the Yawat isolate is grouped with central/East African genotype suggests that this genotype had been introduced at least and possibly more than 5 years before the current outbreaks. In this context, determining the genotype of currently circulating strains in Southeast Asia and understanding the modes of transportation of this strain in India and the conditions favoring such large outbreaks would be worthwhile.

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The published version of this article includes 2 data tables, 2 figures, 15 references, and author acknowledgments. See the online or print version for complete documentation.

This report, Chikungunya Outbreaks Caused by African Genotype, India, is available in entirety online from [www.cdc.gov/eid](http://www.cdc.gov/eid)

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