

Short Communication

Chikungunya Virus Isolated in Lakshadweep Islands in the Indian Ocean: Evidence of the Central/East African Genotype

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SUMMARY: Virological investigation was carried out to determine the etiology of suspected Chikungunya fever among humans reported in the Lakshadweep islands in the Indian Ocean. Three out of 23 acute sera samples showed cytopathological changes in Vero cell lines. Further, indirect immunofluorescence antibody test and reverse transcriptase-polymerase chain reaction studies demonstrated the Chikungunya virus etiology during the episode. E1 gene sequence analysis has confirmed the involvement of the Central/East African genotype of the Chikungunya virus.

Chikungunya (CHIK) is a self-limiting febrile infection caused by a mosquito-borne alpha virus (family *Togaviridae*). The virus was first recognized in Tanzania in 1952-1953 and since then has been reported in several countries in mainland Africa and Southeast Asia, as well as in distantly located diverse islands in the Indian Ocean (1). Recently, apart from cases occurring in Mauritius, Maldives, Sri Lanka, and the Andaman and Nicobar islands, the Chikungunya virus (CHIKV) caused a massive outbreak involving approximately 255,000 human cases occurred in the French territory of the Reunion Islands in the Indian Ocean in 2005-2006 (2). India experienced its first CHIK fever outbreak from 1963 through the mid-1970s, and the infection re-emerged in 2005-2006 characterized by prolonged arthritis and varied hemorrhagic manifestations (3).

In October 2006, an outbreak of suspected CHIK fever cases erupted in the Lakshadweep archipelago in the Indian Ocean, mainly on Andrott and Kalpeni islands (Fig. 1). Lakshadweep archipelago (population 60,595; latitude 10°N, longitude 73°E), a Union Territory of India in the Indian Ocean, is a group of 10 islands situated west of Kerala State.

Thirty-five sera samples were collected from suspected human cases and transported to the laboratory on wet ice. A confluent monolayer of Vero cells was grown in 24-well culture plates (NUNC, Roskilde, Denmark) in D-MEM supplemented with 10% fetal bovine serum (FBS) and penicillin (100 unit/ml), streptomycin (100 µg/ml), and nystatin (20 µg/ml). The cell culture medium and reagents were procured from Sigma (St. Louis, Mo., USA). Twenty-three (71.9%) acute sera were processed for virus isolation (4). The sera were diluted (1:10) in D-MEM supplemented with penicillin, streptomycin, and nystatin. One hundred microliters of the filtered sera (using 0.2 µ disposable syringe filters [Sartorius, Goettingen, Germany]) was inoculated in the confluent monolayer of Vero cells and incubated for adsorption at 37°C for 1 h. After adsorption, the cells were fed

with fresh D-MEM supplemented with 2% FBS. Inoculated cells were kept in a CO₂ incubator supplying 95% air and 5% CO₂. Appropriate cell controls were maintained in a similar manner. The cells were observed daily with an inverted microscope for any cytopathic effects (CPE). Three samples exhibited CPE (Fig. 2). The cells showing CPE were subjected to an indirect fluorescence antibody (IFA) test (5) employing

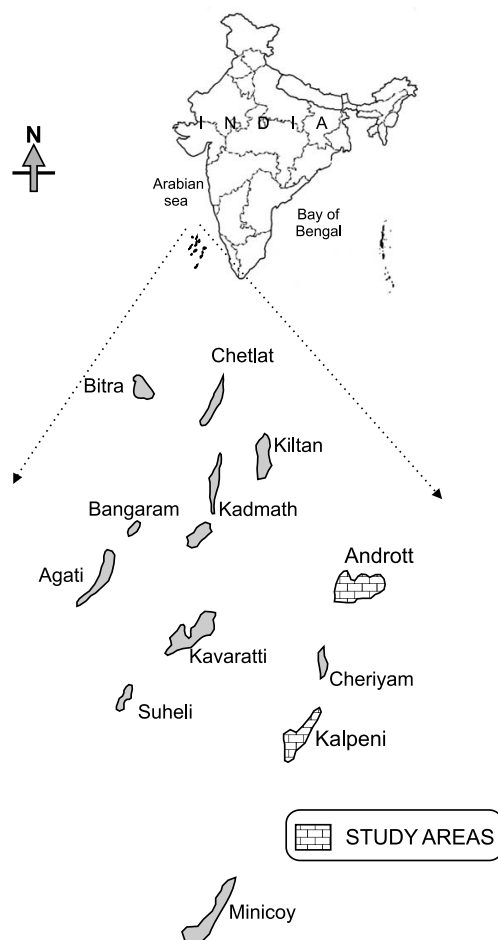


Fig. 1. Map of the Lakshadweep archipelago showing the study areas.

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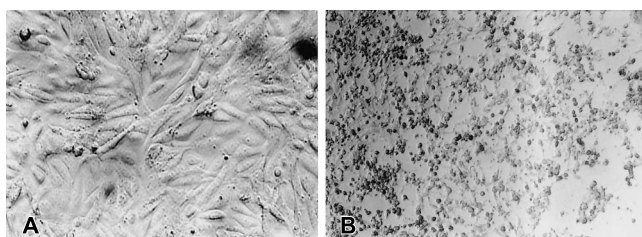


Fig. 2. Showing virus infected and uninfected Vero cells. (A) Uninfected Vero cells. (B) Virus infected Vero cells showing cytopathic effects.

CHIKV monoclonal antibody (kindly gifted by Dr. Ananda Nisalak, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand). The anti-human fluorescein isothiocyanate (FITC) conjugate used was procured from Dako Netherlands (Glostrup, Denmark). The stained control and inoculated cells were observed and documented in an UV microscope (Axioskop-40; Carl-Zeiss, Oberkochen, Germany).

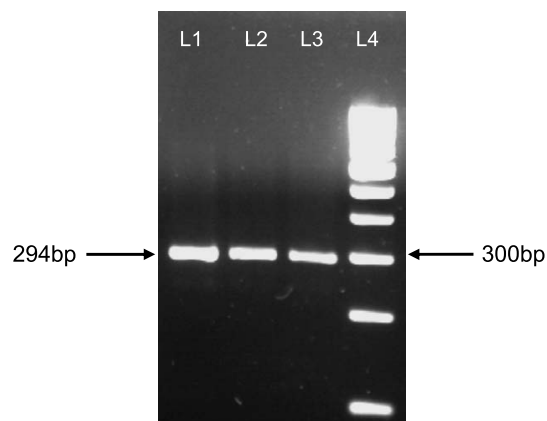


Fig. 3. Gel photograph showing 294-bp fragment of E1 gene of Chikungunya virus by reverse transcriptase-polymerase chain reaction. Line 1-3, 294-bp amplicon of E1 gene from clinical samples; line 4, molecular weight marker.

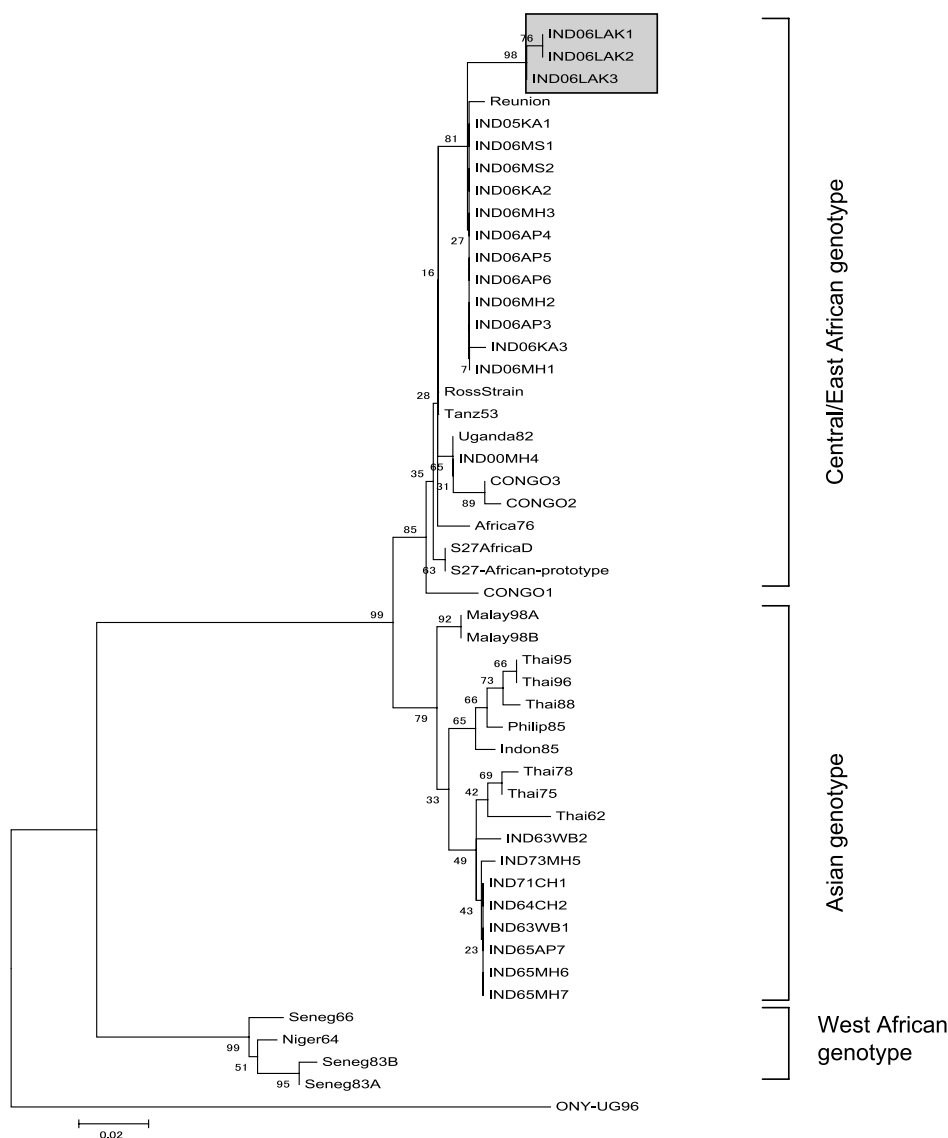


Fig. 4. Phylogenetic analysis of partial E1 gene. The following sequences were obtained from GenBank database: ROSS (AF490259); S27AfricaD (NC-004162); Tanz53 (AF192905); Africa76 (AF192903); CONGO1 (AY549583); CONGO2 (AY549581); CONGO3 (AY549579); Uganda82 (AF192907); Thai95 (AF192897); Thai96 (AF192900); Thai88 (AF192896); Thai62 (AF192908); Thai75 (AF192898); Thai78 (AF192899); Philip85 (AF192895); Indon85 (AF192894); Malay98A (AF394210); Malay98B (AF394211); Seneg83A (AY726732); ONY-UG96 (AF079456); Seneg83B (AF192892); Niger64 (AF192893); Seneg66 (AF192891); S27 African prototype (AF369024.2); Reunion (LR2006_OPY1).

Total RNA was extracted from the tissue culture fluid (TCF) of the IFA-positive samples using TRIzol (Gibco-BRL, Rockville, Md., USA). The reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out using a one Tube RT-PCR kit (RobusT RT-PCR kit; Finnzymes, Espoo, Finland) targeting the immunodominant virus 'E1' protein coding gene with specific primers (CHIK/E1-S and CHIK/E1-C) (6). RT-PCR was performed in the Eppendorf (Hamburg, Germany) thermal cycler. The amplified product was visualized in 1.2% agarose gel and documented in a gel documentation system. The RT-PCR products were custom-sequenced (MWG-Bio Tech Pvt. Ltd., Bangalore, India). The sequences of the three isolates, viz., IND06LAK1, IND06LAK2, and IND06LAK3, were submitted to GenBank, and the accession numbers are EU148606, EU148607, and EU148608, respectively. The BLAST program was used for the database search. The partial gene sequences were aligned using ClustalW (<http://www.ebi.ac.uk/Tools/clustalw/index.html>) and analyzed using phylogenetic software (MEGA 4.0) (7).

The present study confirms the involvement of the Central/East African genotype strain as the etiological agent of the CHIK fever outbreak in the Lakshadweep islands. An IFA test on the CPE-positive cells also showed virus-specific fluorescence. The RT-PCR amplified the 294 bp of the E1 gene fragment (Fig. 3). The BLAST analysis revealed that the three sequences were found to possess 99% sequence identity with the recent CHIKV isolates recovered from India (8,9). The E1 gene-region-based phylogenetic analysis showed that the CHIKV isolates belonged to the Central/East African genotype (Fig. 4), which is comparable with that of the outbreak on the Indian mainland in 2005-2006 but very different from that of the 1963-1973 outbreak (8). The CHIKV isolates of the Lakshadweep islands have been grouped in

the same cluster along with the Reunion, Yawat (IND00MH4), and CONGO (1, 2, and 3) isolates, some of the other Indian isolates (IND06KA2, IND06KA3, IND06MS2, IND06MS1, IND06MH1, IND06MH2, IND06MH3, IND06AP3, IND06AP4, IND06AP5, and IND06AP6), and the Ross, Tanzanian, and African strains. Thus, it is evident that CHIKV isolates from the Lakshadweep islands belonging to the Central/East African lineage carry great epidemiologic significance in regions bordering the Indian Ocean.

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