SHORT COMMUNICATION

Detection of Chandipura Virus from Sand Flies in the Genus *Sergentomyia* (Diptera: Phlebotomidae) at Karimnagar District, Andhra Pradesh, India

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ABSTRACT A total of 191 adult sand flies belonging to the genus *Sergentomyia* were collected from seven villages in Karimnagar and Warangal districts of Andhra Pradesh State, India, after an outbreak of encephalitis due to Chandipura virus (CHPV). Fifteen pools, each containing two specimens, were tested by reverse transcriptase-polymerase chain reaction and sequencing. One pool of *Sergentomyia* from Kolanur village in Karimnagar District was positive for CHPV.

KEY WORDS Chandipura virus, sand flies, RT-PCR, *Sergentomyia*

AN OUTBREAK OF ACUTE ENCEPHALITIS was reported among children in several districts of Andhra Pradesh state of India between June and September 2003 (Rao et al. 2004). To determine the possible vector species involved in the epidemic, sand fly collections were made in the highly affected villages of Warangal and Karimnagar districts during July 2003 and tested for the presence of Chandipura virus (CHPV) by reverse transcriptase-polymerase chain reaction (RT-PCR). The present report describes the detection of CHPV RNA in *Sergentomiya* collected during the outbreak.

Materials and Methods

Sand Fly Collection. Sand flies were collected in seven villages in Karimnagar (18.28°N and 79.06°E) and Warangal districts (17.58°N and 79.40°E) of Andhra Pradesh state, India. The ecogeographical features of this area have been reported (Rao et al. 2004). Adult sand flies were collected from diurnal resting sites, including human dwellings, cattle sheds, and tree holes. Sand flies were held in small 15-cm^3 Barraud cages during transport from the site of collection to National Institute of Virology, Pune, India, where they were held for at least 24 h. before further processing. The sand flies were immobilized by chilling and then divided into pools according to genus and sex. Fifteen pools, each containing two sand fly specimens were subjected to RT-PCR and sequencing. The remaining specimens were used for taxonomic and biological studies.

Polymerase Chain Reaction and Sequencing. Each pool of sand flies was sonicated for 9 min by using 100 μl of sterile phosphate buffered saline (pH 7.2) as diluent (0.01 M phosphate buffer with 0.009% sodium chloride, 1% Chapso, and 1% phenylmethylsulfonyl fluoride. The suspension was centrifuged at 15,000 × *g* for 30 min at 4°C. The supernatant was used for RNA extraction using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA was reverse transcribed and cDNA was amplified using the primers of the most conserved region of the G gene of CHPV (Rao et al. 2004): CHAND-G-F2 425 5′ GTC TTG TGG TTA TGC TTC TGT 3′ 445, CHAND-G-R5 771 5′ TTC CGT TCC GAC CGC AAT AACT 3′ 750, CHAND-G-F5 541 5′ GAG AAT GCG ACC AGT CTT AT 3′ 560, and CHAND-G-R6 744 5′ TGC AAG TTC GAG ACC TTC CAT 3′ 724.

The expected size of the PCR product after nested PCR was 204 bp. cDNA was synthesized using CHAND-G-F2 primers with AMV reverse transcriptase (Promega, Madison, WI) for 1 h at 42°C. DNA amplification was carried out using primers CHAND-GF2 and GF5 by denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min for 35 cycles and final extension at 72°C for 1 min. The first PCR product was subjected to nested PCR by using R5 and R6 primer with the same reaction parameters. The PCR products were visualized on 2% agarose gels. Negative controls were included between each two samples and subjected to the entire PCR protocol. Pre- and postamplifications were performed in two different laboratory setups. PCR products were purified using Wizard PCR preps DNA purification kit (Promega) according to manufacturer's instructions and subsequently sequenced using Big Dye Terminator cycle sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA) and an automatic Sequencer (ABI PRISM 310 Genetic Analyzer, Applied Biosystems).

Results and Discussion

CHPV RNA was detected in one of 15 pools of sand flies from the village of Kolanur in Karimnagar district. The Chandipura (CHP) sequence showed 98.1% ho-

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CIN036514V  GGATTCCTGC TGATCAGACT AAGAAGAACA TTTGCGGCCA GTCATTTACC CCACT [ 55]
CIN0360R    ..........  ..........  ..........  ..........  ..........  ..... [ 55]
CIN0327R    ..........  ..........  ..........  ..........  ...C......  ..... [ 55]
CIN0331M    ..........  ..........  ..........  ....T.....  ...C......  ..... [ 55]
CIN0309R    ..........  ..........  ..........  ..........  ...C......  ..... [ 55]
CIN0318R    ..........  ..........  ..........  ..........  ...C......  ..... [ 55]
SANDFLY     ..........  ..........  ..........  ..........  ..........  ..... [ 55]

CIN036514V  AACTGTGACT GTTGCTTATG ACAAAACCAA AGAAATCACT GCAGGCGGAA TAGTT [110]
CIN0360R    ..........  ..........  ..........  ....G..G..  .....A.C..  ..... [110]
CIN0327R    G........G  ..........  .T........  .......G..  .......C..  ....C [110]
CIN0331M    G........G  ..........  .T........  .......G..  .......C..  ....C [110]
CIN0309R    .........G  ..........  T.........  .......G..  .....T.C..  ....C [110]
CIN0318R    G........G  ..........  .T........  .......G..  .......C..  ....C [110]
SANDFLY     ..........  ..........  ..........  ....G..G..  .....A.C..  ..... [110]

CIN036514V  TTTAAAAGCA AATATCACTC TCACATGGAA GGTGCTCGAA CTTGCA [156]
CIN0360R    ..........  ..........  ..........  ..........  ...... [156]
CIN0327R    .....G....  ..........  ..........  ..........  ...... [156]
CIN0331M    .....G....  ..........  ..........  ..........  ...... [156]
CIN0309R    .....G....  ..........  ..........  ..........  ...... [156]
CIN0318R    .....G....  ..........  ..........  ..........  ...... [156]
SANDFLY     ..........  ..........  ..........  ..........  ...... [156]
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Fig. 1. Partial G gene nucleotide sequence alignment of the sand fly-derived sequence with five sequences corresponding to clinical specimens reported previously (Rao et al. 2004, GeneBank accession nos. AY614717–AY614719, AY614721–AY614723).

mology with a 1965 CHPV isolate (Fig. 1). Percentage of nucleotide identity varied from 94.2 to 100% compared with sequences obtained from clinical specimens from five patients (Rao et al. 2004). CHP virus, which is a member of family Rhabdovirdae, was first isolated in India from patients with febrile illness and from a fatal case in a child with an encephaltic syndrome (Bhatt and Rodrigues 1967, Rodrigues et al. 1983). It also was isolated from a pool of 253 sand flies belonging to the genus *Phlebotomus* caught in human dwellings and cowsheds in Maharashtra states of India (Dhanda et al. 1970). Subsequently, transovarial transmission of CHPV was demonstrated in *Phlebotomus papatasi* (Scopoli) (Tesh and Modi 1993). Most visiculoviruses are transmitted by sand flies (Mattos et al. 2001); however, this is the first detection of CHPV from sand flies in the genus *Sergentomyia.* Sand flies were not identified to species during the current study; *S. punjabensis* (Annandale), *S. bailyi* (Scopoli), and *S. babu* (Sinton) are prevalent in the study areas (National Institute of Virology, unpublished data). The present sand fly-positive pool was obtained came from the house of a patient who was positive for CHPV during the outbreak (Rao et al. 2004). The present findings and an earlier report of isolations of CHPV from sand flies from both India and Africa (Didier et al. 1994) indicate the potential importance of sand flies as vectors of CHPV.

Acknowledgments

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