Short Communication

Molecular Evidence of Chandipura Virus from Sergentomyia species of Sandflies in Gujarat, India

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SUMMARY: The spread and establishment of Chandipura virus (CHPV) infection in India has raised serious epidemiological concerns. The virus interface with the vertebrate hosts (including humans) and vector competence are the important parameters of disease prevalence. Interestingly, in the present study, a highly zoophilic species of the sandfly Sergentomyia was found to be a potential vector of CHPV in Gujarat. This is probably the first report from India of male sandflies testing positive for CHPV in RT-PCR analysis. These findings signify vertical transmission of the virus among sandflies and have epidemiological significance. Health Officers from Gujarat referred 9 pools comprising 277 adult sandflies from disease-affected and unaffected areas to the National Institute of Virology, Pune. The pools were subjected to RT-PCR analysis and sequencing. Of the 9, 2 female and one male pool tested positive for CHPV. Phylogenetic analysis showed similarity of the new sandfly-borne CHPV strains with the human strain from Andhra Pradesh (AP) 2003. The present study highlights the possible role of Sergentomyia spp. in the transmission of CHPV in India.

Chandipura virus (CHPV) belongs to the family Rhabdoviridae and causes encephalitis in humans. It was first identified in 1965 in Chandipura village, Nagpur, in Maharashtra state, India (1). It remained largely ignored until the acute encephalitis epidemic of AP state in 2003. Since then, it has become a leading cause of acute encephalitis in the pediatric population of India judging by various viral encephalitis outbreaks in AP, Gujarat, and Maharashtra and recently in Orissa and Bihar. All these outbreaks have been characterized by high mortality rates ranging from 28% to 78.3% (2–5) and National Institute of Virology (NIV), unpublished data. In India, CHPV was first isolated from sandflies belonging to the genus Phlebotomus (6). In the recent past, the role of Sergentomyia in the transmission of CHPV from the endemic regions of AP and Maharashtra has been established (7–8). Transovarial transmission of CHPV in the laboratory has also been reported (9). These data have raised concerns about the role of various vectors capable of transmitting this virus.

This study presents molecular evidence of the characterization and detection of CHPV in sandflies obtained from 3 districts of Gujarat in 2015. Human CHPV cases were also reported during the same period. To our knowledge, this is perhaps the first report on detection of CHPV in sandflies from this state.

The study’s protocol was approved by the Institutional Biosafety Committee. A small CHPV outbreak was reported during June–August 2015 in Gujarat where 4 districts were involved (10). During the same period, NIV received 9 pools comprising 277 adult sandflies from Health Officers of Gujarat (Fig. 1). Among them, 100 male and 114 female sandflies could be recovered and processed. Table 1 shows details of the sandflies received. The sandfly specimens were chilled and identified on wet ice using the taxonomic key of Lewis (11). Individual pools of the sandflies were prepared based on genus identification and gender. Immediately after segregation, pools of male individual sandflies [02–32] and female individual sandflies [05–27] were ground up separately using Knotes pestles (SIGMA-ALDRICH, St. Louis, MO, USA), centrifuged, passed through a 0.22 μm cellulose acetate filter (Costar, Corning Inc., Corning, NY, USA), and used for virus isolation and RT-PCR. RT-PCR–positive sandfly suspensions were subjected to virus isolation as described earlier (8) and did not yield any isolate.

RNA extraction and RT-PCR were carried out on each freshly prepared sandfly suspension followed by phylogenetic analysis as reported earlier (10). The dataset comprised 2 sequences from this study representing a partial G gene sequence of CHPV (accession numbers KX639161 and KX639160) along with 11 sequences from GenBank, which included one sequence from the sandfly (Senegal, 1978) and 6 Indian sequences from earlier NIV studies. Indian sequences consisted of human strains from states AP (2003), Maharashtra (2010, 2012), and Gujarat (2012, 2014, and 2015).

All the sandfly pools belonged to Sergentomyia spp. Of the 9, 2 female (AA46363, AA46364; Vadodara) and one male sandfly pool (AA46366, Panchmahal) tested positive for CHPV in RT-PCR.

Phylogenetic analysis revealed 2 groups of Indian CHPV strains. Group 1 consisted of human CHPV strains from AP (2003) and from Maharashtra and Gujarat (2010, 2012, and 2014). Group 2 consisted of strains of CHPV from sandflies (Gujarat 2015) along with CHPV strains from Gujarat (2015), strain CIN0360 (AP, 2003), and the
oldest prototype virus of 1965 (Fig. 2). At the nucleotide (nt) level, the 2 sandfly strains of 2015 were 99.91% identical with the Group 2 strains but 96.26% identical with the Group 1 strains. At the amino acid (aa) level, the percentage identity with these groups was 99.73% and 99.02%, respectively. The sandfly strain from Nigeria was the most distinct strain in the phylogenetic tree showing less than 79% and 95% identity at the nt and aa level, respectively, with the remaining strains.

The role of sandflies as vectors of CHPV has been established earlier. Three of 9 pools tested positive for CHPV in RT-PCR. The findings are consistent with the earlier studies where CHPV RNA has been detected in *Sergentomyia* from AP and the Nagpur region of Maharashtra. 

Fig. 1. (Color online) CHPV activity in different districts of Gujarat.

Fig. 2. (Color online) Phylogenetic analysis of CHPV strains from sandflies (2015). Phylogenetic analysis of partial G gene of Chandipura virus; bootstrap values are indicated at each node. Accession no of CHPV strains from Sandfly (Gujarat 2015) are KX639160 (Strain No AA6364) and KX639161 (Strain No AA46366) are shown in red font. Accession no of CHPV strains from Humans (Gujarat 2015) are KX639154 (Strain No 1511379) and KX639155 (Strain No 1511315) are shown in blue font. CHPV, Chandipura virus; AP, Andhra Pradesh.
Chandipura virus in Sergentomyia spp. in Gujarat

Table 1. Details of the Sandfly pools referred from Gujarat in July 2015

| Pool No. | District     | Location of sandfly collection | Sandflies received | Sandflies recovered | Male | Female |
|----------|--------------|--------------------------------|--------------------|--------------------|------|--------|
| 1        | Dahod        | Patient’s House                | 12                 | 08                 | 03   | 05     |
| 2        | Panchmahal   | Adjoining house of patient     | 35                 | 29                 | 18   | 11     |
| 3        | Panchmahal   | Patient’s House                | 15                 | 15                 | 08   | 07     |
| 4        | Panchmahal   | Wild pool from field           | 50                 | 45                 | 32   | 13     |
| 5        | Panchmahal   | Wild pool from field           | 38                 | 19                 | 07   | 12     |
| 6        | Varodara     | Adjoining house of death case  | 35                 | 30                 | 12   | 18     |
| 7        | Varodara     | Adjoining house of death case  | 17                 | 14                 | 05   | 09     |
| 8        | Varodara     | House dwelling                 | 35                 | 14                 | 02   | 12     |
| 9        | Vadodara     | House dwelling                 | 40                 | 40                 | 13   | 27     |
| Total    |              |                                | 277                | 214                | 100  | 114    |

Maharashtra (7–8). The virus was also isolated from Vero E6 cells during a transmission season of CHPV in Nagpur (8). This finding is strong evidence of Sergentomyia spp. emerging as an important vector of CHPV transmission.

The phylogenetic analysis indicated the important role of Sergentomyia spp. in the transmission of CHPV to humans, given the 99.9% identity of CHPV strains from the sandfly with human strains from Gujarat isolated during the same period. It was also evident that the strains similar to the oldest strain of 1965 are still in circulation.

CHPV has been isolated from Phlebotomine sandflies from West Africa (12). In India, this species has been collected in human dwellings and cowsheds in Aurangabad, Maharashtra (6). Due to vector control programs, there has been a significant decrease in the number of these sandflies in residential housing.

During the same period, in Vadodara city, CHPV was detected simultaneously in both human cases and in sandfly pools. The virus-positive sandfly pools were found in houses adjacent to those of encephalitis death cases. This result is consistent with an earlier report where a positive pool was obtained during the outbreak in AP (2). The reason could be the presence of Sergentomyia in and around the houses; this evidence indicates their possible role in the transmission of CHPV as reported earlier (8). On the other hand, in Panchmahal, although the sandfly pools were virus-positive, human CHPV cases were not reported. This finding could be due to high seropositivity for CHPV in the population or may indicate the probable zoonotic nature of the virus in this area (13).

This is probably the first report from India of male sandflies testing positive for the CHPV genome. Positive male sandflies have been reported in Pakistan and Iran (14). Similarly, Schmidt et al. (1971) isolated 2 strains of sandfly viruses from male sandflies in Egypt (15). This finding signifies vertical transmission of the virus among sandflies, which facilitates the establishment and spread of the disease (9).

The recent findings of CHPV encephalitis in a high-altitude region of Orissa state have epidemiological importance. The presence of both Phlebotomus and Sergentomyia sandflies in this area and an environment conducive to breeding point to their role in the emergence of the infection (5).

The ecological features of Sergentomyia species, the virus-vector interactions, and the interplay with the susceptible human population all these factors contribute to the spread and establishment of the disease. These results underline an urgent need not only to initiate vector control programs but also to conduct in-depth studies on the association of CHPV with potential vectors.

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Conflict of interest None to declare.

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