

## VENEREAL TRANSMISSION OF CHANDIPURA VIRUS BY *PHLEBOTOMUS PAPATASI* (SCOPOLI)

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**Abstract.** Experiments were conducted in the laboratory on *Phlebotomus papatasi* to determine the possible role of males in maintaining or sustaining the Chandipura virus (CHPV) activity in nature. This study indicated that infected males are capable of passing on the virus to female sand flies while mating. The infection rate was found to be 12.5% in uninfected females when mated with infected males. The occurrence of venereal transmission of this virus may have epidemiologic importance in the natural cycle of CHPV.

### INTRODUCTION

Several studies have been conducted earlier to show that venereal transmission of arboviruses by its arthropod vectors might serve as one of the mechanism for horizontal transmission of arboviruses.<sup>1–5</sup> Studies carried out by Rosen<sup>6</sup> have shown that males of *Aedes aegypti* could venereally transmit all the four-dengue viruses to the females of that species. Subsequently, Tu and others<sup>7</sup> showed convincingly with ultrastructural studies on reproductive organ of male *Ae. aegypti* (L.) that the males infected transovarially with dengue virus can efficiently pass on the virus to their female mates by venereal route. Recently, Mavale and others<sup>8</sup> showed the venereal transmission of Chandipura virus by *Ae. aegypti*.

This study reports the venereal transmission of CHPV by males of *Phlebotomus papatasi* (Scopoli), a species in which transovarial transmission of Chandipura virus has been shown.<sup>9</sup>

### MATERIALS AND METHODS

**Sand flies rearing and the virus strain.** The National Institute of Virology (NIV) strain of *Ph. papatasi* was used. This colony was established from the sand flies collected by hand aspirator in the cattle sheds and human dwellings from the nearby villages of Pune town. The colony was maintained in specially designed plastic pots with plaster of paris at the bottom and was maintained at 28°C to 30°C temperature and 14:10 (L:D) photoperiod in the insectary of the Entomology department of NIV. Larvae were fed on a diet comprised of sand, cow dung, and goat liver powder (60:35:5). The adults were maintained between 28°C to 30°C at 95% relative humidity and provided with glucose as the sources of carbohydrate. The adults from the colony were periodically tested for the presence of the arboviruses including CHPV using IFA technique. The CHPV (strain 653514) isolated from the serum specimen collected from a human case during the acute phase of illness in Nagpur in 1965 was used. It had undergone three intracerebral passages in Swiss albino mice. The virus pool had a titer of 7.5 log LD<sub>50</sub>/0.2 mL by intracerebral route.

**Sand flies infection and mating.** Emerged virgin males and females were kept separately, and males were inoculated intrathoracically (ITI) with ~0.2 μL of virus suspension (4.2

log/0.2 μL MID<sub>50</sub>), following the method described by Rosen and Gubler.<sup>10</sup> All the inoculated male sand flies were held on 10% glucose solution at the insectary.

The head squashes of some (N = 10) inoculated males were examined on the second post-infection day by indirect immunofluorescence technique (IFAT) as described by Dhanda and Ilkal<sup>11</sup> using CHPV hyper-immune serum raised in mice. The remaining males were allowed to mate with virgin females of same age in a ratio of 1:3 (M:F). Surviving females (~90%) were pooled on the fourth day after the release of males. The pools consisting of 12 to 14 females were kept in three different cages and maintained on 10% glucose in an insectary with controlled temperature and humidity. The head squashes of the females from each cage were examined on the sixth day of release for detection of CHPV antigen by IFAT.

The pools of female bodies (N = 3) were triturated in bovine albumin phosphate saline (BAPS) and centrifuged at 10,000g for 1 hour at 4°C. The supernatant was checked for presence of virus genetic material by reverse transcriptase-polymerase chain reaction (RT-PCR) as described by Geervarghese and others.<sup>12</sup>

### RESULTS

A total of 56 males and 102 females of the F<sub>2</sub> generation were obtained from the colony. Of 56 males, 25 males were inoculated. On the second day after infection, randomly selected 10 males were checked for CHPV antigen; 100% individuals were found positive (10/10) for CHPV antigen. Males (N = 15) were allowed to mate with virgin females (N = 45) from the second day onward for 3 days. The overall infection rate among the females was 12%, whereas in three different cages, it ranged from 7% to 17% (Table 1). The minimum infection rate among the pools of bodies of these females was 10%.

### DISCUSSION

Venereal transmission is considered as one of the modes of maintenance of the virus in nature. This phenomenon has been studied in several vector species and most convincingly proved in the mosquito vectors for *Bunyavirus*,<sup>1</sup> *Alphavirus*,<sup>2</sup> and *Flavivirus*.<sup>3,4,7,13</sup> Ciufolini and others<sup>5</sup> showed venereal infection of Toscana virus and Aribia in *Ph. perniciosus* (Newstead) females when mated with transovarially infected males. This is the first report of experimental venereal transmission of CHPV in *Ph. papatasi*.

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TABLE 1

CHPV virus infection of *Ph. papatasi* females after exposure to infected males

Cages	No. of head squash of <i>Ph. papatasi</i> female positive/tested	Percent CHPV positivity	PCR positivity (pools of bodies positive/pools tested)
1	2/14	14.3	1/5
2	1/14	7.1	1/5
3	2/12	16.7	2/4
Total	5/40	12.5	

A few isolations of CHPV have been reported from wild-caught *Phlebotomine* sand flies in India. Therefore, *Phlebotomine* sand flies are considered as one of the vectors of the CHPV.<sup>14</sup> Among the *Phlebotomine* sand flies, *Ph. papatasi* is one of the most dominant anthropophagic and domiciliary species prevalent in several parts of India. The vertical transmission phenomenon of CHPV in *Ph. papatasi* has been already established,<sup>9</sup> which indicated that the males of *Ph. papatasi* can get infected with CHPV through vertical transmission which has already been reported by Tesh and Modi in 1983.<sup>9</sup> These vertically infected males can transfer the CHPV to females by venereal transmission (horizontal transmission). Our studies have shown that ~12% of previously uninfected females could get infection after mating with CHPV-infected males. It is possible that the resultant infected female sand flies may start a fresh infection cycle of the virus within the focus and aid in overall virus perseverance in the ecology of disease.

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