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## Chandipura virus encephalitis outbreak among children in Nagpur division, Maharashtra, 2007

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**Background & objectives:** An outbreak of acute encephalitis syndrome (AES) among children from Nagpur division, Maharashtra was investigated to confirm the aetiology and to describe clinico-epidemiological features.

**Methods:** AES cases among children <15 yr, from Nagpur division, hospitalized between June-September 2007, were investigated. Serum and cerebrospinal fluid (CSF) were tested for IgM antibodies against Chandipura virus (CHPV) and Japanese encephalitis virus (JEV) and for CHPV RNA by RT-PCR. Partial N gene sequences were used for phylogenetic analysis. Virus isolations were attempted in rhabdomyosarcoma (RD) cell line. Sandflies were collected, pooled and tested for CHPV RNA by RT-PCR.

**Results:** A total of 78 AES cases were recorded in children <15 yr of age. Case fatality ratio was 43.6 per cent. Male to female ratio was 1:1.2. Chandipura (CHP) was confirmed in 39 cases. CHPV RNA was detected in both CSF and serum specimens of 2 cases and in serum of 22 cases. Phylogenetic analysis showed 99.98 – 100 per cent nucleotide identity in the sequences studied. Anti-CHPV IgM antibodies were detected in CSF of 2 cases and in serum of 8 cases. Seroconversion to anti-CHPV IgM antibodies was observed in 5 cases. Clinical manifestations of CHP cases (n=38) were fever (100%), convulsion (76.3%), altered sensorium (34.2%), headache (23.7%), vomiting (44.7%) and diarrhoea (23.7%). CHPV RNA was detected in one of two pools of sandflies from affected locality.

**Interpretation & conclusions:** Chandipura virus was confirmed as the aetiological agent of this acute encephalitis outbreak with high case-fatality among children.

**Key words** Acute encephalitis syndrome - Chandipura virus - children - outbreak - sandfly

Chandipura virus (CHPV) is a vesiculovirus in the *Rhabdoviridae* family. Vesiculoviruses were isolated in 1965 in the Chandipura (Nagpur) region of India in two adult patients with febrile illness during an outbreak of febrile illness caused by chikungunya

and dengue viruses<sup>1</sup>. Human serum samples collected from Nagpur in year 1957, 1958 and 1965 showed seropositivity for CHPV by neutralization test<sup>1</sup>. Cases clinically diagnosed as viral encephalitis from Raipur in central India in 1980 showed CHPV aetiology,

confirmed by isolation of CHPV virus from the acute sera<sup>2</sup>.

CHPV was incriminated as the aetiological agent of large-scale encephalitis outbreaks in children (9 months to 15 yr of age) in various districts of Andhra Pradesh in 2003 with high case fatality rate (CFR) of 55.6 per cent<sup>3</sup>. In retrospective analysis of 104 hospitalized children including 21 confirmed CHPV cases, rapid evolution of signs and symptoms was noted with brainstem involvement<sup>4</sup>. In a focal outbreak in eastern districts of Gujarat between June and July 2004, CHPV aetiology in 11 of 20 encephalitis cases indicated its importance as an encephalitis causing virus in endemic areas in India<sup>5</sup>. In the Nagpur region of Maharashtra in 2003, 33 encephalitis cases were confirmed as Chandipura with case fatality rate (CFR) of 41 per cent<sup>6,7</sup>. In 2005, in an outbreak in Bhandara and Nagpur districts, 7 of 21 cases were confirmed as Chandipura<sup>8</sup>. In a hospital-based surveillance of acute encephalitis among children from endemic areas of North Telangana region of Andhra Pradesh between May 2005 and April 2006, CHPV aetiology was identified in 25 of 52 cases with seasonality in late summer and early monsoon<sup>9</sup>.

An outbreak of acute encephalitis syndrome (AES) in children was reported from Nagpur division, Maharashtra, between June and September 2007. We report here the findings of the investigations carried out to confirm the aetiology and to describe clinico-epidemiological features.

### Material & Methods

Nagpur division in Maharashtra State (Fig. 1) has six districts, located between 78° and 81° N and 18.5° and 21.5° E. Earlier, Japanese encephalitis (JE) cases were also reported from this area<sup>10</sup>. JE vaccination campaign was carried out in Nagpur and Bhandara districts during July and August 2007.

A case was defined as a hospitalized case, age <15 yr, with the acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures)<sup>11</sup>, and negative for malaria, tuberculosis and other common bacterial causes<sup>9</sup>.

Outbreak investigations were initiated immediately after cerebrospinal fluid (CSF) and blood samples were sent to the National Institute of Virology, Pune, by Directorate of Health Services, Maharashtra.

Predesigned proforma was used to collect information from the cases. Clinical investigations included recording history, clinical findings and results of routine laboratory investigations, review of hospital records and collection of CSF and/or blood (serum) from patients. Acute CSF and/or serum specimens were tested for anti-JEV and anti-CHPV IgM antibodies using enzyme-linked immunosorbent assay (ELISA)<sup>9</sup>. Detection of CHPV RNA was done by RT-PCR in acute serum samples and CSF according to the method described earlier<sup>3</sup>. Isolation was attempted in rhabdomyosarcoma (RD) cell line<sup>5</sup>.

Viral RNA was isolated using QIAamp viral RNA Mini kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. SuperScript™ II reverse transcriptase was used for the cDNA synthesis and Platinum Pfx DNA polymerase was used for the amplification according to the manufacturer's instructions (Invitrogen, CA). Partial N gene was amplified using primers NF9 (5'TCA AAA TGA GTT CTC AAG TAT T 3') /GR7 (5'TCT CCT CTG CAT CCT TCG TGC 3'). Amplified fragment (527 bp) was visualized by ethidium bromide agarose gel staining, extracted from the gels and sequenced directly using Big Dye Terminator Cycle Sequencing kit (Applied Biosystems, CA). Phylogenetic analyses of nucleotide sequences were carried out in MEGA v.3.1 employing Neighbour-Joining method and p-distance algorithm<sup>12</sup>. The reliability of the different phylogenetic groupings was evaluated by taking a support of 1000 bootstrap replications. The isolates and sample used were from Maharashtra (Nagpur - 2007-1 CIN0728, Nagpur - 2007-2 CIN0724), Andhra Pradesh (Andhra Pradesh-2003 CIN0327, Warangal - 2007 CIN0755) and Gujarat (Vadodara - 2004 CIN0451) (Accession numbers GU 212856-58, GU 190711).

Sandflies were collected from houses and around the house of the cases. They were transported in plastic jars, identified, pooled, stored at -70°C until processed and tested for CHPV RNA by RT-PCR<sup>13</sup>.

Chi-square test was employed for comparing proportions.  $P < 0.05$  was considered as statistically significant.

### Results

A total of 78 cases were recorded. Case fatality ratio was 43.6 per cent. Majority of the cases were from Bhandara (23, 29.55%). No statistically significant difference was observed in various districts among confirmed Chandipura cases and cases with unknown

aetiology (Table I). Cases were scattered with usually only one case in a village (Fig. 1). Majority (61, 78.2%) of the cases occurred between June 16 and July 20, 2007 (Fig. 2). Age distribution showed preponderance in <5 yr of age (36, 46.1%). Male to female ratio was 1:1.2.

Fever was recorded in all of the 78 cases. Most of these cases (66/78, 84.6%) had fever less than 3 days at admission. Detailed clinical history was available for 76 cases (Table II). Other clinical manifestations in these 76 cases included convulsions (49, 64.5%), altered sensorium (31, 40.8%), headache (11, 14.5%), vomiting (32, 42.1%) and diarrhoea (17, 22.4%) (Table II). Majority (17 of 28, 60.7%) of the deaths occurred within 24 h of hospitalization. Hospital stay in 44 recovered cases ranged between 2 and 21 days (median- 7 days). Neurological sequelae were not observed in any of the cases at discharge and even at follow up done on 30 to 90 days (median- 73 days).

Clinical specimens included 18 CSF, 71 acute sera and 29 convalescent sera. All these specimens were negative for anti-JEV IgM antibodies. CHPV RNA was detected in both CSF and serum specimens of 2 cases. CHPV RNA was detected in serum sample of 22 cases, of which two yielded CHPV isolates. Anti-CHPV IgM antibodies were detected in CSF of 2 cases. Anti-CHPV IgM antibodies were detected in serum of 8 cases. Seroconversion to anti-CHPV IgM antibodies was detected in 5 cases. Thus, CHP was confirmed in 39 cases based on CHPV RNA in CSF (2), anti-CHPV IgM in CSF (2), CHPV isolate in serum (2), CHPV RNA in serum (20), anti-CHPV IgM in serum (8) and anti-CHPV IgM seroconversion (5). Phylogenetic analysis based on partial N gene sequences clearly showed that the CHP isolates recovered during 1965-2007 were closely related with present nucleotide identity (PNI) varying from 99.98 to 100 per cent (Fig. 3).

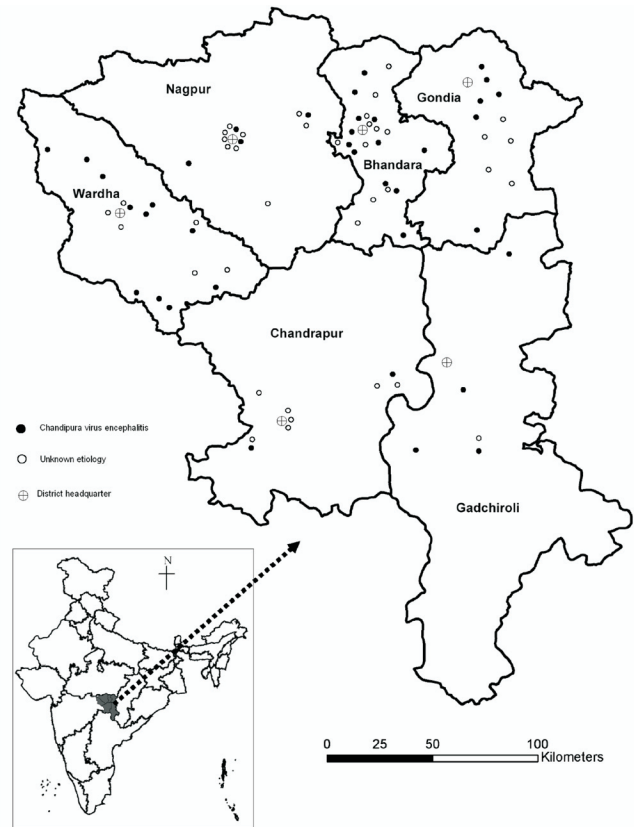
Among 39 CHP confirmed cases, there were 20 (51.3%) deaths. Male to female ratio was 1:1.2.

**Table I.** District-wise distribution of Chandipura virus encephalitis cases and with those of unknown aetiology

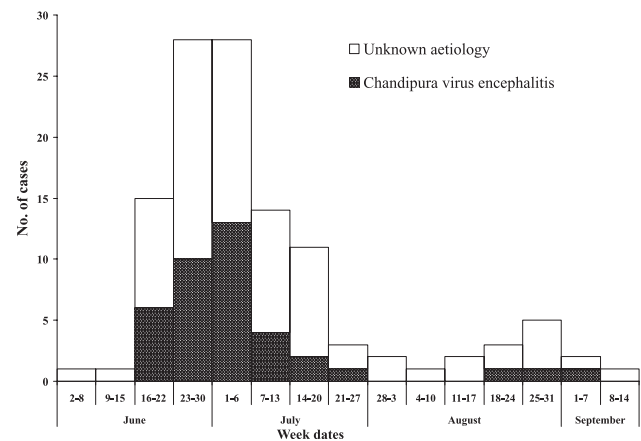
District name	Chandipura virus encephalitis, N=39	Unknown aetiology, N=39
Bhandara	12 (30.8)	11 (28.2)
Wardha	11 (28.2)	6 (15.4)
Nagpur	4 (10.2)	9 (23.1)
Gondia	6 (15.4)	5 (12.8)
Chandrapur	2 (5.1)	7 (17.9)
Gadchiroli	4 (10.2)	1 (2.6)

Figures in parentheses are percentages

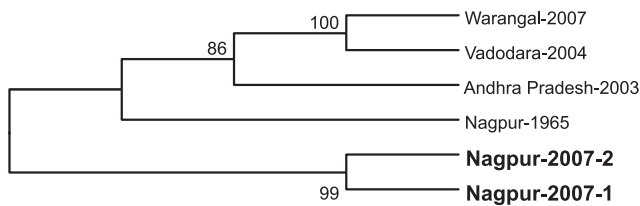
There were 15 (38.4%) cases aged <5 yr. Clinical features of Chandipura virus encephalitis cases are shown in Table II. Neurological manifestations included abnormal plantar reflex (6), abnormal deep tendon reflexes (10), sluggish pupillary reflex



**Fig. 1.** Place distribution of 78 acute encephalitis cases, with Chandipura aetiology (n=39), among children in Nagpur division, Maharashtra, India, 2007.



**Fig. 2.** Epidemic curve of 78 acute encephalitis cases with Chandipura aetiology (n=39), among children in Nagpur division, Maharashtra, India, 2007.



**Fig. 3.** Phylogenetic analysis of chandipura viruses based on partial N gene sequences. Figures at nodes represent per cent bootstrap support. Sequences generated during the study are presented in bold.

**Table II.** Clinical features of Chandipura virus encephalitis cases compared with those of unknown aetiology

Clinical features	Chandipura virus encephalitis, N=38	Unknown aetiology, N= 38
Fever <3 days at admission	32 (84.2)	34 (89.5)
Altered sensorium	13 (34.2)	18 (47.4)
Seizures	29 (76.3)	20 (52.6)
Skin rash	4 (10.5)	0
GIT symptoms	9 (23.7)	8 (21.1)
Bleeding tendencies	4 (10.5)	2 (2.7)
Abnormal pupils	5 (13.6)	3 (7.9)
Shock	6 (15.8)	4 (10.5)
Headache	9 (23.7)	2 (5.3)

Figures in parentheses are percentages

(5), and hypertonia and hypotonia in one case each. Convulsions were generalized tonic clonic type with up rolling of eyeballs. Other manifestations included tachycardia (3), tachypnoea (8), severe breathlessness (3), crepitations (4), haemorrhages (4) and urinary incontinence (2). Bleeding tendencies were seen in 4 cases. Anaemia was observed in 14 cases and leucocytosis in 6 cases. Blood urea was raised in 2 cases. CSF (9 cases) was clear, colourless with white blood cell count between 0-5 cells/mm<sup>3</sup> and normal protein and glucose levels. No statistically significant difference was observed in clinical manifestations among confirmed Chandipura cases and cases with unknown aetiology (Table II).

Sandflies (n=189) were collected in 10.5 man-hours from 9 localities with Chandipura cases and were pooled in 20 pools. CHPV RNA was detected in one of 2 pools of sandflies collected from affected locality in Khangaon village, Wardha district.

### Discussion

Chandipura aetiology was confirmed in 39 (50%) cases among children in this outbreak. The confirmation

rate is similar to earlier outbreaks in Andhra Pradesh (28, 50.9%)<sup>3</sup>, Gujarat (11, 55%)<sup>5</sup> and Warangal, Andhra Pradesh (25, 48.1%)<sup>9</sup>. As indicated in earlier studies<sup>3,5</sup>, RNA detection was an important diagnostic tool. In this study, CHPV RNA was detected in serum samples of 24 cases. Similar observations on utility of RNA detection were made in other studies<sup>3,7,9</sup>. Seroconversion to anti-CHPV IgM antibodies in 5 cases in this study reaffirms the importance of convalescent sera as also noted in 3 cases in Warangal, Andhra Pradesh<sup>9</sup>.

Though 50 per cent of the cases were confirmed to be due to CHPV, possibility of time of sampling and transient viraemia may have prohibited CHP diagnosis in others. The samples were collected in hospitals and often cold chain is not strictly maintained during the storage and transport, leading to loss of viral RNA.

Case fatality ratio was similar to the earlier outbreak in the same area<sup>6-8</sup> and also other studies<sup>3,5</sup>. Majority of the CHP cases were confirmed in early monsoon season between June and July. Absence of clustering and age were similar to earlier reported outbreaks in the same area<sup>6-8</sup> and other outbreaks<sup>3,5</sup>.

Clinical findings were consistent with that of earlier studies<sup>3,5,8</sup>. Routine haematological, biochemical and cerebrospinal fluid analysis in most cases were within normal limits and similar to earlier study<sup>5</sup>. In the present study bleeding tendencies were seen in four cases. Similar bleeding tendencies in four patients and shock in three patients due to disseminated intravascular coagulopathy (DIC) were reported from Andhra Pradesh<sup>4</sup>. As also seen in earlier studies<sup>4,9</sup>, almost 85 per cent cases reported fever of <3 days duration at hospitalization and 60 per cent deaths occurred within 24 h. Clinical profile of this outbreak was similar to earlier studies<sup>3,8</sup> but was different from JE as the clinical course was shorter and progression to death was rapid. JE vaccination campaigns may have played a role in decreasing the incidence of JE in the area.

Phylogenetic analysis confirmed that CHPV did not mutate significantly during 1965-2007 in Nagpur area or elsewhere in the affected area.

In this study CHPV RNA was detected in one of the two pools of sandflies collected from affected locality in Khangaon village, Wardha district. Similar observations were reported from Karimnager, Andhra Pradesh during 2003 outbreak<sup>13</sup>.

In conclusion, Chandipura virus encephalitis outbreak was confirmed in Nagpur division with

seasonality in early monsoon. Detection of Chandipura viral RNA in one sandfly pool from an affected village suggests the role of sandflies as the vectors. Surveillance should be strengthened in the area for estimating the disease burden of Chandipura virus encephalitis.

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