

### Outbreak investigations

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## **Encephalitis**

### **Gorakhpur, UP**

An epidemic of encephalitis was reported from Gorakhpur district during September to October 2004 and as a part of the central team NIV investigated this epidemic. Laboratory investigations of 56 representative samples collected from patients confirmed the presence of Japanese Encephalitis Virus (JEV) IgM antibodies in 20 samples. Virus isolation attempts were carried out on 11 JEV IgM positive and four negative samples using peripheral blood mononuclear cells (PBMC) co-cultures as per standard protocol. Two blind passages were carried out in each case and virus growth assayed using JEV specific monoclonal antibodies (HS3 and HS4) in antigen capture ELISA assays.

### **Saharanpur, UP**

An outbreak of encephalitis with high mortality in children (age range 2 - 9 years) was investigated between Oct-Nov 2004 in Saharanpur, UP. The outbreak started in the last week of September and a total 156 cases with 115 deaths were reported till first week of December. These cases were scattered throughout the district and familial clustering was observed only in 2 cases. The clinical symptoms included acute fever for 3-4 days duration, vomiting, altered sensorium, convulsions (in a few cases) and coma leading to death. A total of 528 specimens comprising 330 serum samples (acute, recovered, contacts and control, 87 Cerebro Spinal Fluid (CSF), 48 Throat Swabs (TS), 21 Rectal Swabs (RS), 1 stool sample, 38 Urine specimens and 3 brain aspirates were collected during this period from acute cases, contacts and controls and recovered cases for etiology investigations. Tests for IgM antibodies to JEV and West Nile Viruses (WNV) were carried out in 286 serum and CSF specimens of which one tested positive for JEV, one for WN, and one for both JEV and WNV. 175 sera samples were tested for Chandipura IgM and were found to be negative. No etiologic agent could be identified for this outbreak.

### **Chhota Udepur, Gujarat**

An outbreak of encephalitis among children was investigated in Vadodra district of Gujarat State. 26 probable patients were recorded with a CFR of about 78.3%, death in majority case occurred with 24 hours of admission in hospital. After filling a detailed questionnaire, clinical samples of 20 available patients who met the case definition were taken. All samples tested negative for JE, Dengue (DEN) and WNV. Two out of twenty patients were positive for IgM antibodies against Chandipura virus (IgM anti CHPV) and neutralizing antibodies (Nabs). In addition, the only patient, whose convalescent sample was available

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on the 12th post onset day, tested IgM and Nab for CHPV positive in the second sample, although the acute phase sample was negative for both. Serological tests conducted in apparently healthy population showed 10.6% anti CHPV IgM and 65.3% Nab positivity in children. Age-wise analysis showed that children below 5 years of age had a significantly low frequency of Nabs (8/18,44%) as compared to those above 5 years (28/38,73.3%) ( $p < 0.05$ ). Among adults 4.5% were IgM-anti CHPV positive and 97.7% for Nabs.

Nine of the twenty serum samples were found positive for CHPV viral RNA by Polymerase Chain Reaction (PCR). Virus Isolation in mice, RD and PS cell lines yielded one isolate which was confirmed to be CHPV by PCR and sequencing. The phylogenetic status of the CHPV sequences obtained from the clinical samples and one isolate during the present outbreak is presented in figure 1. Overall, the percent nucleotide identity (PNI) between different CHPV isolates varied from 91.1-100%. Of the three major clusters identified, Six Vadodara sequences formed one cluster; Three Andhra Pradesh (AP) and Three Vadodara sequences grouped together in the second cluster whereas Two AP and the prototype 1965 strain isolated from Maharashtra state formed the third cluster. The PNI between groups was 96.7 (95-98.5, clusters 1 & 2), 93.8 (91.1-96.6, clusters 1 & 3) and 95.9 (94.4-97.8, clusters 1 & 2). Thus all the sequences were closely related to each other and the outbreak was not characterized by circulation of only one type of isolate.

Ninety-two human sera were tested by in vitro neutralization test in Vero cell culture for detection of Neutralizing (N) antibodies to CHPV virus. Approximately 70% of the sera were positive.

Twenty-one animal sera were tested by in vitro virus neutralization test in Vero cell culture to detect antibodies to CHPV virus. Two of the 15 cattle sera were positive at 1:5 dilutions. All six-goat sera were negative. The above laboratory tests confirmed that CHPV as the etiologic agent for the outbreak

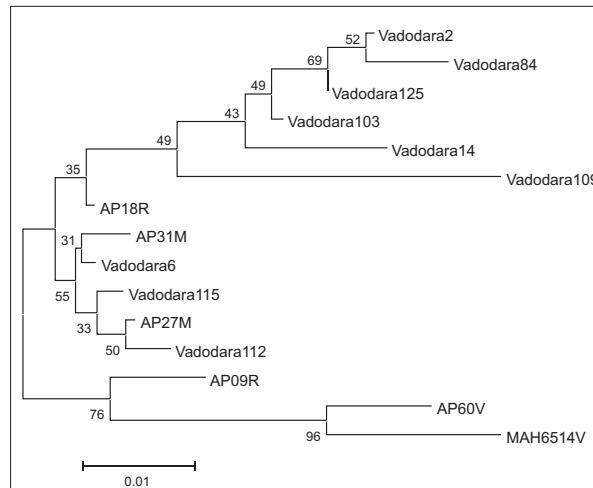


Figure 1: Phylogenetic tree of the Chandipura Virus strains isolated from Vadodara based on N gene sequences

### Siliguri, West Bengal.

An outbreak of febrile illness with altered sensorium was investigated in Siliguri, West Bengal between January and February 2001. A total of 66 patients, presenting with symptoms of fever and altered sensorium were recorded (Case Fatality Rate -75%). All the patients were adults and 45 of them were medical or paramedical personnel belonging to three different hospitals of Siliguri or individuals having hospital exposure. Blood samples of 17 and urine samples of six acute hospitalized patients were collected. Post mortem lung and brain aspirates from 2 patients was also collected. Nineten contacts of patients were interviewed and their blood samples collected. All serum samples were tested for IgM antibodies to JEV, WNV/ DEN, IgM against leptospira and hantavirus All samples were negative in the respective tests, except one serum sample which tested positive for IgM against leptospira. The lung aspirates tested for influenza and RSV by IFA were negative.

Earlier analysis of 4 sera from patients in 2001 at CDC indicated Nipah etiology and further analysis of additional clinical material was carried out at the CDC in May 2004. Nine of the seventeen patient sera were IgM-Nipah and IgG positive. One patient was IgG reactive in the absence of IgM (his blood sample was drawn one day post onset of illness). One of the nine contacts was IgG reactive in the absence of IgM, he was in contact with a patient 3 weeks prior to giving his blood sample. Inoculation and blind passages of 6 urine samples

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in Vero E6 cell line did not yield isolation of any virus. 5/6 urine samples were found positive for Nipah virus RNA by RT-PCR based on primers from N gene primers. Sequence analysis (150 nucleotides) showed 99.95%, & 97.11% homology with Bangladesh and Malaysian Sequences respectively (Figure 2). Amplification of M gene was possible for 3 urine samples. The Indian sequences (320 nt) showed 98.91% and 94.37% identity with the Bangladesh & Malaysian strains respectively (Figure 3).

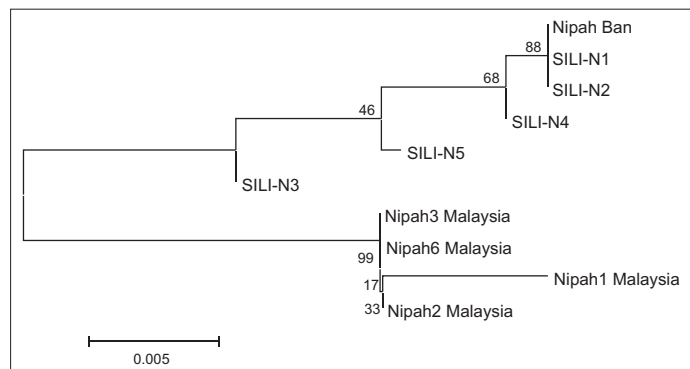


Figure 2: Phylogenetic tree of Nipah viruses isolated from encephalitis cases in Siliguri based on the N gene (159 nucleotides) sequences

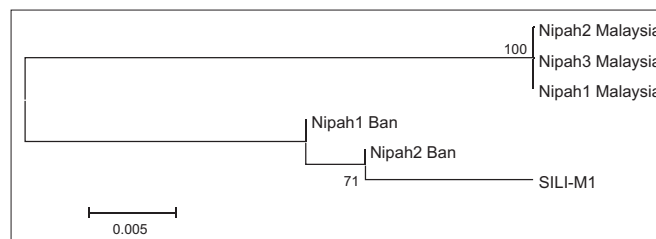


Figure 3: Phylogenetic analysis of M gene (320 nt) of Nipah virus in an urine sample from Siliguri outbreak

## **Karnal, Haryana**

An outbreak of encephalitis among children was reported from villages around Karnal, Haryana during October 2004. Children in the age group of 1-5 years were affected. The important clinical symptoms were fever, vomiting, convulsions and coma. Acute phase serum, blood clot, throat swab were collected from four cases. Urine sample was obtained from two of the four cases. One convalescent serum sample from a recovered case, eleven sera from contacts of cases and three sera from unaffected children were also collected. No virus could be isolated from acute serum samples processed in infant mice/cell culture. No viral aetiology could be established in these encephalitis cases investigated.

## **Bijnore, UP**

A total of 25 sera, 18 CSF, 7 TS, 4 urine and 5 liver specimens were collected from encephalopathy cases occurring in Bijnore, UP. All these cases were from pediatric age group below 10 years. Of the 11 cases, 10 died. These specimens were processed in Vero cells for virus isolations. No virus was isolated. Serology for JEV, WNV IgM antibodies were negative. Measles serology indicated measles IgM positive in one case. Serology for Hepatitis A and E was done. Two cases and one contact of encephalitis case were found to be positive for Hepatitis A IgM antibodies. One contact was HAV RNA positive.

## **Bagpath, Haryana**

Thirteen deaths were reported from one village in Bagpath district in October 2nd and 3rd week, 2004. There were four deaths in a family. All were children below 10 years of age. On the visit, there were no active cases, only two fever cases were found and specimens taken. Etiology remained unknown, as there were no active cases during the visit.

## **Maharashtra**

Cases of encephalitis were reported from 11 districts of Vidarbha region and 4 districts of Marathwada region. Incidentally many of these districts are bordering Andhra Pradesh where a similar outbreak of encephalitis was reported during the same period. Maharashtra Public Health authorities reported total of 393 encephalitis cases and 115 deaths during the period of June to August 2003 mainly from Vidarbha (11 districts) and four districts of Marathwada region of Maharashtra (Table 1 & figure 4).

All the six districts in Nagpur region were affected. Total 203 encephalitis cases were reported between 15th June to 31st August 2003. Of these 111 were males and 92 were

Table 1: Encephalitis cases in Maharashtra in 2003 (Data provided by Maharashtra Public Health Department)

District	Reported: Deaths/ Attack (CFR %)
Akola	1/1 (100%)
Amravati	3/8 (38%)
Bhandhara	16/30 (53%)
Buldhana	0/14 (0%)
Chandrapur	21/52 (40%)
Gadchiroli	4/9 (44%)
Gondia	6/14 (43%)
Nagpur	29/123 (24%)
Wardha	9/29 (31%)
Washim	0/2 (0%)
Yavatmal	4/12 (33%)
Hingoli	6/12 (50%)
Latur	0/2 (0%)
Nanded	13/43 (30%)
Parbhani	3/42 (7.1%)
Total (15 districts)	115/393 (29%)

Maharashtra State Report, 2003 (Only selected districts)

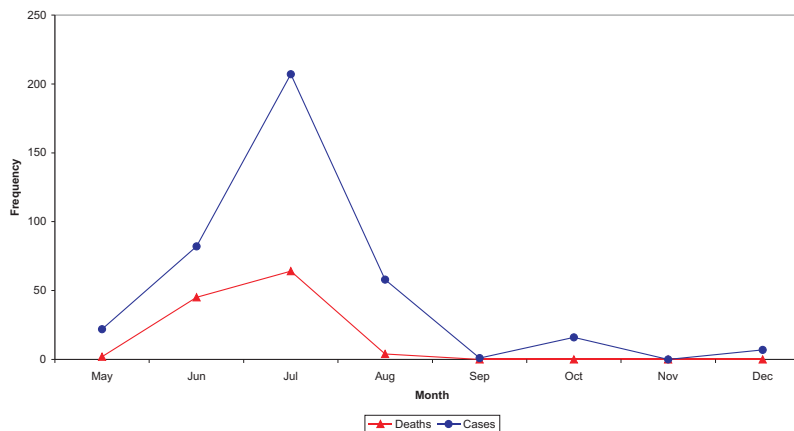


Figure 4: Epidemic curve of encephalitis cases in Maharashtra

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females. Eighty cases (39.4%) belonged to age below 5 years, 86(42.3%) between 5-10 years and 37(18.2%) between 10-15 years. No adult cases were found during the outbreak. Cases started appearing from 15th June 2003. Seventy-one (34.9%) cases were reported in month of June, 109 (53.6%) cases in July and 23 (11.5%) in August. Only one case was reported in month of September 2003. Case fatality in 2003 was 85/203(41.9%), that was more than that from 2002 (23.8%). Mortality was more in age group below 10 years. Clinical features included fever, vomiting, convulsions, altered sensorium, respiratory distress, coma condition and death. No meningeal signs were observed in majority of cases. All the cases were negative for malarial parasites.

Cases of suspected encephalitis were reported from 5<sup>th</sup> June 2003 in Nanded district of Maharashtra. According to the state government authorities a total of 43 cases and 13 deaths were reported from the district (CFR-30%). Clinical features included a short duration of moderate to high grade fever without chills/rigors, headache, intermittent vomiting followed by convulsions within first 6 hours followed by loss / alteration in consciousness. One patient had respiratory paralysis. Fundoscopy showed hemorrhagic papilladema in few patients. Patients were treated with Diazepam, phenobarbitone, Dilantin Sodium, IV fluids, Quinine IV drip, chloroquine injectable., mannitol and Dexamethasone. The patients admitted in district hospital (n=34) were in the range of 1 to 12 years of age. Mortality was higher in below 5 yrs age group of the patients admitted in district hospital, majority (n=12) died within 9 hours of hospitalization (>9 hours = 8: 10-24 hrs = 1: >24 =3). The case distribution appeared to be sporadic. Majority of the cases were clustered in Nanded Taluka. Only two wards reported two cases, otherwise distribution was one in each village.

## **Conjunctivitis**

Outbreaks of conjunctivitis were reported from many parts of Maharashtra and Gujarat states during September to November 2003. Clinical specimens in the form of eye swabs, throat swabs and sera samples from representative cases were collected and investigated for viral etiology. Out of all the seventy eye swabs inoculated, 15 virus isolates could be obtained in HeLa or Hep-2 cell cultures. These virus isolates were all identified as Coxsackie Virus A- 24 by various serological and molecular approaches.

## **Viral hepatitis**

An outbreak of hepatitis was reported at Chikhali in Pimpri-Chinchwad Corporation area of Pune during March - April 2004. Forty eight cases, mostly adults were registered in a population of approximately 4000. No deaths were recorded. The area received intermittent piped water supply where leakages could be detected in pipe lines. Blood samples of Nine patients could be collected. Five of these were IgM-anti-HEV positive. All tested negative for IgM anti HAV. This indicated HEV to be the causative agent.

An outbreak of hepatitis was investigated at Alandi, which is a historical temple village with a resident population of approximately 15000. After a house-to-house survey carried by the local health authorities, 64 cases of hepatitis were detected up to 28th of March. The first case was recorded on the 1st of March 2005. The age group affected was 15-45 years and cases were spread throughout the village. The villagers had noticed turbid water in the month of January and February. Water supply was derived from the River Indrayani, purified and distributed through pipes. No leakages could be detected in the pipeline. Sewage was collected into a septic tank through open drains. An underground pipe drained this untreated water into the river downstream to drinking water inlets and bathing ghats

Water samples at the inlet to waterworks, post purification, at the bathing ghat & downstream of sewage outlet were taken for virological analysis. Blood samples of 6 patients and two suspected hepatitis cases were collected. All six patients were IgM anti-HEV positive and the suspect cases were negative. Tests were suggestive of HEV etiology of the outbreak. An outbreak of hepatitis was investigated at Loni Kaalbhor, taluka Haveli, district Pune. In a population of 5222, sixty nine cases were recorded between the 3rd of January and 4th of February 2005, peaking in the 4th week of January. Majority of the patients were adults (58/68, 85.3%). Cases among pregnant women were not recorded. Male to female ratio was 1: 1.1. Mortality was not observed.

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Drinking water was derived from two wells, one of which was fed from a nearby canal. Water from both these wells was pumped to two overhead tanks where it was chlorinated. Outlets from both tanks led to a single pipeline below ground level. At this particular point, a leakage was detected. The water, which had accumulated in this area, was being utilized for washing purposes after defecation around the tank. Further downstream, at a distance of 50 meters, a leaking valve on the main water pipeline was located.

Twenty-eight blood samples of patients were taken for sero-diagnosis. 23 were found to be IgM-anti HEV positive. Of the negatives, two adult patients had onset of disease one day prior to sample collection, and the remaining were children. All the above patients were HBsAg negative. Three stool samples, from patients with five days of onset of symptoms, were collected for virus detection. On the basis of serological results, it could be concluded that HEV was the etiological agent of this outbreak.

### *Emergence of hepatitis A in epidemic form*

An outbreak of suspected viral hepatitis at Medical College Hospital area, Kottayam, Kerala state, was reported in September 2004. Of the 1180 cases of viral hepatitis reported from the district, 540 were from the Medical college area (medical community, 170 and students of school of medical education, 67). Of the 285 patients investigated, 248 (87%) were IgM-anti-HAV positive. Two deaths were reported among doctors. Cases from the community gave previous history of visit to Medical college hospital area. The sewage treatment plant at the campus was non-functional since 1990 and the untreated sewage was constantly overflowing and getting mixed with a canal. At the time of investigations, all the water sources were superchlorinated. HAV RNA was shown to be present in the feces of hepatitis A patient, sewage tank and the canal. Viral load as estimated by real time PCR was highest in the fecal sample ( $1.36 \times 10^7$  copies/ml). In the sewage tank and the overflowing sewage collected about 10 meters away, the viral load was  $2.57 \times 10^3$  and  $2.65 \times 10^3$  copies/ml respectively. The viral load in the canal sample positive in nested PCR was below the detection limit of real time PCR assay (100 copies/ml). None of the 13 water samples concentrated almost 10,000-fold and the soil sample showed presence of HAV RNA. Phylogenetic analysis based on 5'-NCR and P2 regions showed HAV genotype IIIA. This is the first report of hepatitis A epidemic in Indian adults.