Nipah/Hendra virus outbreak in Siliguri, West Bengal, India in 2001


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Background & objectives: The viral encephalitides caused by animal or human viruses are characterized by sudden outbreaks of neurological disease in both tropical and temperate regions. An outbreak of acute encephalitis occurred in Siliguri (West Bengal) town of India between January 31 and February 23, 2001. This outbreak was investigated by a team of scientists from four major institutions, and the findings are presented here.

Methods: Detailed information about the outbreak was collected with the help of local health authorities. Limited entomological investigations were also done. Samples collected from cases and contacts were sent for analysis.

Results: A total of 66 probable cases and 45 deaths were reported. Epidemiological linkages between cases point towards person-to-person transmission and incubation period of around 10 days. There was neither any concurrent illness in animals nor was there any exposure of cases to animals. Centres for Disease Control and Prevention, Atlanta, USA concluded on the basis of tests carried out on serum specimen from four cases and two contacts that the causative pathogen appears to be Nipah/Hendra or closely related virus.

Interpretation & conclusion: This outbreak highlights the importance and urgency of establishing a strong surveillance system supported by a network of state-of-the-art laboratories equipped to handle and diagnose new pathogens and including patient isolation techniques, use of personal protective equipment, barrier nursing and safe disposal of potentially infected material in the prevention and control measures for Nipah/Hendra virus infection.

Key words Acute encephalitis - Nipah/Hendra virus - Siliguri

Globally in the last three decades, more than 30 new pathogens have been detected. Some of these are responsible for entirely novel and life-threatening disorders, such as AIDS, Ebola fever, Hanta virus pulmonary syndrome and Nipah virus encephalitis\(^1\).
The emerging viral encephalitides are caused by animal or human viruses and are characterized by sudden and unexpected outbreaks of neurological disease, not only in tropical and sub-tropical regions but also in temperate areas. During 2002, North America experienced the largest ever outbreak of West Nile encephalitis. Globally, Japanese encephalitis (JE) is the most important emerging viral encephalitis. The 1998-1999 outbreak of severe febrile encephalitis among pig farmers in Malaysia, caused by the newly emergent Nipah virus showed that emerging infectious diseases involving zoonoses have become important global health problem. This outbreak caused widespread panic and fear in Malaysia leading to considerable social disruptions and tremendous economic loss because of mass culling of over one million pigs. Absence of drug therapies effective in treating Nipah infection further increased case fatality and fear among general public.

An outbreak of encephalitis occurred in Siliguri town of West Bengal in India in February 2001 which caused widespread panic among the residents. Occurrence of cases and deaths among treating medical, nursing and paramedical personnel further compounded the matter and led to the closure of private health facilities. The outbreak was investigated by a team drawn from All India Institute of Medical Sciences (AIIMS), New Delhi; National Institute of Communicable Diseases (NICD), Delhi; National Institute of Virology (NIV), Indian Council of Medical Research, Pune and WHO Country Office. A retired health officer from Gujarat also participated in the investigations. Results of investigation of this outbreak are presented in this communication.

Material & Methods

Siliguri is a strategically located town near international borders with China, Bangladesh, Nepal and Bhutan, and has a tropical climate. It has a population of around 5,00,000 with many migrant labourers and a few slums inhabited by socio-economically poor people. Major health facilities are a 200 bedded Sub Divisional Hospital (Hospital ‘A’) located in Siliguri town and North Bengal Medical College Hospital (NBMCH) situated about 20 km away besides a network of private practitioners and hospitals/nursing homes.

Any person with an acute onset of fever with altered sensorium of unknown aetiology reporting to health facilities of Siliguri during the period of outbreak was considered as a case. Detailed information about the outbreak was collected through discussion with local health authorities, clinical examination of cases, study of case records, visits to affected nursing homes/hospitals and residences of the cases, interview of persons who had recovered, community leaders, medical and paramedical staff and verbal autopsy in fatal cases. Entomological investigations were limited to survey and mosquito collections in and around the areas from where the cases/deaths were reported in wards 9, 31, 33, 43 and 44 of Siliguri town to find out the density of vector mosquitoes. Biological samples from cases as well as contacts [blood, cerebro spinal fluid (CSF), throat swab, urine, rectal swab] and necropsy materials (brain, liver, lung tissue/aspirate and blood clot from five fatal cases) were collected and processed at NICD, NIV, AIIMS and Defence Research and Development Establishment (DRDE), Gwalior; and Centres for Disease Control and Prevention (CDC), Atlanta, USA as summarized below:

In DRDE, Gwalior, 6 clotted blood samples were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) for flaviviruses, Hanta virus, Nipah virus and measles virus. An attempt was also made to isolate the virus from one clotted blood sample and three blind passages were carried out in Vero cell line. At AIIMS, New Delhi, agglutination test by gelatin particle for IgM antibodies and ELISA for IgG antibodies to Mycoplasma pneumoniae were done on three serum samples. Three CSF samples, one Vero cell culture lysate of inoculated samples and one Vero cell culture lysate of un-inoculated samples were tested by PI gene specific PCR for Mycoplasma pneumoniae.

Serum, CSF, throat and rectal swabs, brain biopsies, lung aspirates and urine samples were processed at NICD, Delhi, for Legionella pneumophila, measles, plague, malaria, leptospirosis, dengue, Hanta virus, Herpes simplex virus and enterovirus infections. The
techniques included culture as well as direct antigen detection in samples by fluorescent antibody test for *Legionella pneumophillia*, ELISA for IgG antibodies for dengue and JE using kits from NIV, Pune; Measles using kits from Serion, Germany, Herpes simplex using kits from Meridian Diagnostics, UK and HAI test for plague etc., virus culture by inoculations in cell lines and RT-PCR. NIV, Pune carried out various investigations including ELISA for IgM and IgG antibodies and virus culture to rule out JE, dengue, leptospirosis, West Nile, measles and enterovirus infection.

Four serum specimens from cases that tested positive for measles IgM at the NIV, Pune, four companion infected Vero cell culture lysates derived from the serum specimens, two case contact serum specimens (one reported as IgM positive, and one as IgM negative for measles at the NIV, Pune), four urine specimen, and one brain aspirate were sent to Centers for Disease Control and Prevention (CDC), Atlanta. The tests performed included CDC capture IgM and IgG assay on all six serum samples following gamma irradiation for measles and Nipah/ Hendra viruses. An IgM capture assay was performed within the BSL-3 laboratory using the non-irradiated specimens and appropriate controls and a commercial IgM capture ELISA. Virus culture was attempted from serum specimen, infected Vero cell lysates, urine specimen and the brain aspirate by inoculation into cell culture using vero cells and B95a cells (marmoset lymphocyte line).

### Results

A total of 66 probable cases and 45 deaths were reported between January 31 and February 23, 2001. Of the 66 cases, 5 left against medical advice, 45 expired and 16 survived. The case fatality was 68 per cent (45/66). A total of 10 cases developed illness between January 31 and February 4, 2001. No cases occurred between 5th and 9th February. From February 10 to 23 cases occurred daily except on February 11. Maximum of 10 cases occurred on February 15 followed by 8 on February 18. Date of onset of illness of last case was February 23 (Fig. 1).

Study of the information collected during the course of investigations revealed possible epidemiological linkages in 43 cases (the same could not be ascertained for rest of the cases due to lack of proper information) and following hypothesis emerged (Fig. 2):

1. A case admitted in the Male Medical Ward (MMW) of Hospital ‘A’, during January 20-24, 2001 probably acted as a source and started the outbreak. However, this source could not be identified due to poor quality and incompleteness of case records.

2. This case probably transmitted infection to other patients admitted concurrently in MMW or visitors to this ward resulting in occurrence of four sets of cases as described: (i) Case 1, admitted in MMW of Hospital ‘A’ for hypertension on January 14, 2001, developed fever on February 1. Outer limit

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**Fig. 1.** Epidemic curve by date of onset, Siliguri outbreak, 2001.
of incubation period comes to 18 days. It is not possible to calculate shortest possible incubation period as he developed illness while still admitted in this hospital; (ii) cases 2 and 3 visited MMW of Hospital ‘A’ between 22 and 23 January to see their elder brother admitted for one day for diabetes related complication. Both of them developed fever on January 31 and died of encephalitis later. There was no other history of exposure. These cases clearly suggested incubation period of about 10 days; (iii) one attendant of case 1 developed fever on January 31, two attendants and one staff got fever on February 1 while one staff had onset on February 2. Both the affected staff of Hospital ‘A’ were performing duty at the MMW while attendants of case 1 visited this ward during January 20-24. All these cases probably got infection from the same source. The incubation period was in the range of 7-11 days and (iv) case 9, admitted in male ward of Hospital ‘A’ between January 20-24 for chronic respiratory problem, developed fever on February 1. The incubation period in this case ranges between 8-12 days.

3. Two staff nurses of Nursing Home A (NH ‘A’) exposed to case 1 during his one day stay (3-4 February) developed fever on February 14 and 15 respectively indicating the incubation period to be 10-12 days.

4. Case 9 was admitted in Nursing Home B (NH ‘B’) on 3rd and died on 7th February. A total of 32 people among those who visited or performed duty at NH ‘B’ during February 3-7 developed the illness. Of them, 24 were staff of NH ‘B’, 5 attendants of case 9 and rest three attendants of other patients. The date of onset in these 32 cases was between 12 and 23 February (12 Feb-1, 14 Feb-4, 15 Feb-8, 16 Feb =5, 17 Feb-3, 18 Feb-1, 19 Feb-6, 20 Feb-2, 23 Feb-1 and unknown-1). The incubation period thus ranged from 5-20 days.

These epidemiological linkages point towards person to person transmission and give a clear indication of the incubation period to be around 10 days with a range of 5-20 days.

Age of the cases ranged from 12 to 70 yr; the median age was 30 yr. Fatality was maximum in older age group (> 50 yr). Fifty nine per cent (39/66) of cases were males (Table). There was clustering in
space around NH ‘B’ as many of its staff who got affected stayed in or around it. Another clustering was around residence of case 9 where his family and friends stayed.

All the cases that could be interviewed had a definite history of exposure to a case. No association with factors like travel outside Siliguri, visit by guests from outside Siliguri, attending funeral, exposure to injections, contact with animals/birds including pigs, exposure to any new or old insecticide or homeopathic remedies during one month prior to date of onset in cases was found.

Community survey in affected areas did not reveal any unusual rise in fever cases.

**Entomological investigations:** Entomological investigations did not reveal the presence of dengue or JE vector. *Culex quinquefasciatus*, a filariasis vector was found to be the predominating species (per man hour density = 9-14) breeding in various drains and sewerage. Regarding malaria vector, only one specimen of *Anopheles culicifacies* could be collected from the periphery of the town and three specimen of *Anopheles annularis* were collected from the same area.

**Zoonotic investigations:** An appraisal of the environment of Siliguri town in September, 2001 to study its potential for possible zoonoses revealed dogs as seen usually in urban areas of India, very few pigs, few flying foxes (bats) *Megachiroptera* species and no horses. Cattle rearing was observed in the outskirts of the town. No definitive ecological evidence was available which might suggest the existence of an ecological support system for the natural harbourage of zoonotic infection, in medium sized mammals, with the possibility of its transmission to man.

**Clinical presentation:** The clinical illness started with fever, generally of mild to moderate degree and usually without chills or rigors. Headache was a prominent associated symptom; others being vomiting, bodyache and generalized weakness. Altered sensorium presenting with confusion and incoherent talks developed on day 3-4 followed by decreasing level of consciousness leading to coma in majority of the cases; 34 per cent of the cases had convulsions while 54 per cent had associated respiratory symptoms particularly in the later stage of illness.

There was no rash, haemorrhagic manifestation, jaundice, lymphadenitis, urinary or gastrointestinal symptoms, neck rigidity, cranial nerve involvement, hepatosplenomegaly, pleural effusion, or ascitis. Papilloedema was found in the terminal phase of illness in a few cases. Deep tendon reflexes were diminished, plantars were extensor and intracranial tension was found to be raised in most cases. Heart was normal with no congestive cardiac failure but ECG suggested myocarditis in late stage of illness in some. In cases with respiratory involvement, tachypnoea, bilateral crepitations and evidence of acute respiratory distress syndrome (ARDS) were present.

**Clinical laboratory investigations:** Cases showed mild leucocytosis, normal haemoglobin and packed cell volume (PCV) and adequate platelets. Few cases showed albuminuria and microscopic haematuria. Peripheral smear was negative for malaria parasite and blood negative for falciparum malaria antigen. Renal and liver function tests were normal. CSF was clear with total cell count of 0-4 cells (all lymphocytes)/cu mm and normal proteins and sugar levels.

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**Table. Age distribution of cases, deaths and case fatality rate (CFR), Siliguri outbreak, 2001**

<table>
<thead>
<tr>
<th>Age group (yr)</th>
<th>Cases</th>
<th>Total</th>
<th>Deaths</th>
<th>Total</th>
<th>CFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 - 20</td>
<td>4</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>67</td>
</tr>
<tr>
<td>21 - 30</td>
<td>17</td>
<td>28</td>
<td>11</td>
<td>19</td>
<td>68</td>
</tr>
<tr>
<td>31 - 40</td>
<td>7</td>
<td>15</td>
<td>5</td>
<td>8</td>
<td>53</td>
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<tr>
<td>41 - 50</td>
<td>9</td>
<td>10</td>
<td>7</td>
<td>8</td>
<td>80</td>
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<tr>
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<td>2</td>
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<td>2</td>
<td>100</td>
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<td>66</td>
<td>28</td>
<td>45</td>
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Though initial laboratory investigations indicated the outbreak to be due to non-exanthematous measles, subsequent epidemiological and laboratory investigations including those conducted at CDC, Atlanta, where viral culture in Vero and B95a cell lines were negative for measles virus cytopathic effects as conveyed in its first report, did not support this diagnosis.

In its second report, CDC reported that the outbreak may have been the result of Nipah/Hendra virus infection, or a closely related virus. All the four serum specimen from patients were IgM positive while three were IgG positive in the ELISA assays. In contrast, the contact serum specimens were negative for Nipah virus. The results were, however, stated to be preliminary as it cannot be said as to whether this agent is Nipah virus, Hendra virus, or some new member that might belong to this genus (International Committee on Taxonomy of viruses designated as Henipah virus) of paramyxoviruses. CDC further stated that apparent nosocomial and possible person-to-person transmission of infections in Siliguri, was different from those described earlier for Nipah/Hendra infections. However, CDC findings concluded that on the basis of tests on 4 serum specimens from cases and 2 from contact, it appeared to be Nipah/Hendra or closely related virus.

Post-mortem investigations: Examination of post-mortem liver tissue from three cases revealed focal necrosis with fatty changes. Brain tissue showed generalized oedema, neuronal degeneration, discrete mononuclear cell infiltration and mild gliosis. Lung tissue showed alveoli filled with amorphous eosinophilic material and interstitial space showed fair number of inflammatory cells.

Treatment & follow up: The treatment included broad spectrum antibiotics, anti-malarials, drugs to reduce cerebral oedema besides antipyretics and other supportive measures (intravenous fluids, vitamins, etc.). Antiviral drug, acyclovir was given to seven cases but did not seem to affect the progress of illness.

Of the 8 recovered cases examined in a follow up visit about 10 wk after the outbreak subsided, 7 did not show any neuromotor deficit while one case that had developed paraplegia, had improved a lot and was able to walk with support. However, all the cases continued to have varying degree of dizziness and weakness.

Discussion

The first reported outbreak of acute encephalitis among pig handlers in Malaysia in 1998-1999 led to the discovery of a novel paramyxovirus named Nipah virus. Afterwards, outbreaks of Nipah virus encephalitis have been reported from Singapore (1999) and Bangladesh (2001, 2003 and 2004). Nipah virus is closely related to another new zoonotic virus named Hendra virus discovered in 1994 after outbreak of respiratory illness among horses and humans in Australia. There have been three outbreaks of Hendra virus in Australia in the years 1994, 1995 and 1999.

Hendra and Nipah viruses have neurological and pneumonic tropisms. With Nipah virus, the predominant clinical syndrome in humans is encephalitic whereas in pigs, the infection was characterized by acute fever and respiratory involvement with or without neurological signs. The pathogenesis of Nipah infection is associated with its ability to infect blood vessels and extravascular parenchyma in many organs, particularly in the central nervous system. The incubation period in Nipah virus encephalitis ranges between 4 and 18 days. The onset is usually with influenza-like symptoms and the disease may progress to encephalitis with disorientation, convulsions and coma. About 50 per cent of clinically apparent cases die.

It is currently believed that certain species of fruit bats of *Pteropus* species are the natural hosts of both Nipah and Hendra viruses. In the 1998 Malaysia outbreak, evidence suggested spill-over of Nipah virus from its reservoir host into the domestic pigs and ultimately to humans and other animals. Transmission from animal to animal and from animal to human appears to require close contact with contaminated tissue or body fluids from infected animals. Nipah antibodies have been detected in pigs and other domestic and wild animals. The role of species other than pigs in transmitting infection to other animals has not yet been determined.
Of the 265 cases of Nipah virus encephalitis cases in Malaysia during September 1998 to April 1999, 105 died (CFR= 40%). 93 per cent of cases had occupational exposure to pigs. In 32 fatal cases that were studied, fever (100%), decreased level of consciousness (89%), headache (82%), disorientation (76%), giddiness (61%) and respiratory symptoms (40%) were the major symptoms. The mean incubation period was 10 days.

A cross-sectional study by Institute for Medical Research, Malaysia in collaboration with CDC, USA and others after this outbreak revealed that 1.6 per cent of 435 abattoir workers who slaughtered pigs showed antibody to Nipah virus verses nil among 233 workers who slaughtered ruminants. Eleven cases with one death were reported in 1999 among abattoir workers of Singapore handling pigs imported from the affected areas in Malaysia. There was no evidence of man–to- man transmission.

Retrospective investigations of Nipah virus encephalitis outbreaks in Meherpur (2001) and Naogaon (2003) in Bangladesh suggested that transmission may occur through close contact with other patients or from exposure to a common source. Although bats in the region had serologic evidence of infection, person- to-person spread may have been an important mode of transmission.

In outbreak of Nipah virus encephalitis in Bangladesh in January 2004, 23 cases and 17 deaths (CFR 74%) were reported from six districts. No link could be established between these cases and sick pigs or other mammals.

Another cluster of 30 encephalitis cases with 18 deaths (CFR= 60%) was reported during 13 March-14 April, 2004 in Faridpur district of Bangladesh. Laboratory investigations at CDC, Atlanta confirmed Nipah virus infection in 16 cases. Direct contact with ill patents is suspected to have played a role in the transmission of the disease and spread of this outbreak.

Evidence of a small focus of frugivorous bats and the availability of several fruit bearing trees in the residential area of Siliguri town, points to possible link of these bats to the residential area of the town. However, further investigations, including, serological studies in bats could not be done though it would have helped in identifying possible reservoir of infection. In the absence of detailed investigations on bats or other animals, the role of zoonotic transmission of virus in this outbreak cannot be ruled out.

The incubation period and clinical profile of cases in the Siliguri outbreak was very similar to the laboratory confirmed outbreaks of Nipah virus encephalitis in Malaysia, Singapore and Bangladesh. The case fatality was also high and comparable.

In the Siliguri outbreak there were definite linkages between cases indicating man-to- man transmission best illustrated by clustering of 32 cases occurring between February 12-23 among staff of NH ‘B’ or attendant of case 9 or other patients who visited NH ‘B’ during stay of case 9. Also, cases 2 and 3 (brothers) had common history of exposure to Hospital ‘A’ and both developed illness on the same day indicating that they contracted infection from the same source to which they were exposed in Hospital ‘A’.

Though the results have been stated to be preliminary by CDC, the similarity in clinical picture, case fatality and incubation period with laboratory proved Nipah outbreaks in Malaysia, Bangladesh and Singapore support the hypothesis of this outbreak to be due to Nipah virus.

This outbreak highlights the importance as well urgency of establishing of strong surveillance system supported by state-of -the-art laboratories equipped to handle and diagnose new pathogens. Simultaneously, regular orientation and training of doctors towards emerging pathogens should be done so that the outbreaks of new pathogens are suspected and diagnosed early. A network of laboratories needs to established to include laboratories within the country as well as the Region so that laboratory diagnostic back up facilities for emerging pathogens are available.

In view of strong possibility of person-to-person transmission of Nipah virus, there is also a need to
include patient isolation, use of personal protective equipment, barrier nursing and safe disposal of potentially infected material in the prevention and control measures.

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