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Nipah: The deadly menace

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Abstract:

Nipah Virus (NiV) infection is an emerging zoonosis of public health importance in the South East Asia region. The most recent outbreak occurred in Kozhikode district, Kerala in May 2018.NiV belongs to a new genus Henipavirus under the family Paramyxoviridae. Fruit bats of the family Pteropodidae are the natural hosts of Nipah Virus. Pigs are the secondary and amplifier hosts. Infection is transmitted either directly from infected bats or pigs or indirectly through contaminated food (fruits, fruit juices). Human to human transmission has also been documented. Clinical manifestations in human beings range from asymptomatic infection to acute respiratory distress syndrome and fatal encephalitis. Case fatality rate has varied in different outbreaks from 40 –75%.In the recent Kerala outbreak a high case fatality of 91%was documented. Nipah virus is one of the most deadly viruses known to infect humans. Diagnosis is mainly by RT-PCR. There is no specific treatment or preventive vaccine for Nipah. Stringent infection control measures including standard precautions, contact and droplet precautions in health care facilities help in containing nosocomial outbreaks.

Keywords:

Encephalitis, Henipa, infection control, Nipah, outbreaks, *Pteropodidae*

Introduction

Human race is under the threat of many emerging and reemerging viral infections. Microbial genetic variations and environmental influences contribute to disease emergence. Bats play an important role in transmission of many of these viral zoonotic diseases. Nipah virus (NiV) infection is a bat-borne emerging disease endemic in Southeast Asia. The disease was first recognised as a large outbreak in 1998–1999, among pigs and human beings in Malaysia.[1,2] The disease was initially diagnosed as Japanese encephalitis (JE) as all humans acquired the infection from sick pigs. Pigs had a highly contagious respiratory disease with low mortality, while in humans, it occurred as severe febrile encephalitis. The virus was introduced into pig farms by fruit bats and they spread fast among the pigs. The outbreak caused widespread panic and fear in Malaysia with social disruption and tremendous economic loss as about 1.1 million pigs had to be culled.[1,3] The virus was carried to Singapore by infected pigs from Malaysia causing a small outbreak there. Two outbreaks were reported from West Bengal in India, Siliguri in 2001[4] and Nadia district in 2007.[5] From 2001 to 2012, 17 outbreaks have occurred in the Southeast Asia, of which 15 were confined to various districts in Bangladesh and two in India.[4,6] Last outbreak occurred in Kerala in May 2018. The best method for diagnosing NiV infection is by reverse transcription polymerase chain reaction (RT-PCR). Immunoglobulin M (IgM) and IgG antibodies can be detected by enzyme-linked immunosorbent assay (ELISA). NiV is a biosafety level-4 (BSL-4) pathogen, which requires stringent precautionary measures while handling the patients and their samples. Diagnosis is possible only in centres where BSL-3 facilities are available. Prompt diagnosis is essential for controlling the spread of infection in the community and among healthcare workers.

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Aetiology

NiV belongs to the paramyxoviridae family, genus *Henipavirus*. Henipa viruses are characterised by a wide host range and large genome. Other viruses of the family paramyxoviridae generally show a narrow host range and are genetically stable with somewhat uniform genome size.[7] The genus has two other members, Hendra virus which is the prototype virus and Cedar virus – an apparently non-pathogenic virus found in Australian bats.[8] NiV is named after the Malaysian village 'Sungai Nipah' where the disease first occurred and the virus was first isolated.[1,9] Centers for Disease Control and Prevention (CDC) and National Institute of Allergy and Infectious Diseases have classified NiV as a Category C priority pathogen with potential for bioterrorism.[10,11]

NiV is an enveloped, negative sense, single-stranded RNA virus 40–100-nm diameter. Electron microscopy shows the virions as tangled collections of filamentous, helical nucleocapsids surrounded by viral envelope. They are pleomorphic, varying greatly in size. Average size is about 500 nm in diameter. Herringbone appearance is typically seen following negative-stain electron microscopy.[7]

NiV genome comprises six major genes: nucleocapsid, phosphoprotein, RNA polymerase genes, (N, P and L); envelope membrane protein genes (F and G) and the matrix protein (M). Envelope glycoproteins are responsible for host cell infection, the 'G' glycoprotein binds the viral receptor and the fusion (F) glycoprotein helps in virus–host cell membrane fusion[7,11] [Figure 1]. Two major genetic lineages are identified among NiVs known to cause disease in humans: NiV Malaysia (NiV-MY) and NiV Bangladesh (NiV-BD). The genome of Malaysian NiV is 18,246 nucleotides long, while that of Bangladesh has 18,252 nucleotides.[6] A third lineage of NiV isolated from Lyle's flying fox (*Pteropus lylei*) in Cambodia is more closely related to NiV-MY than to NiV-BD.[12]

Studies carried out in African green monkeys showed that NiV-BD is more pathogenic. Experimental NiV-BD infection in ferrets was associated with increased oral shedding than NiV-MY and higher levels of virus replication in the respiratory tract. These differences have been suggested as the reasons why the cases from Bangladesh and India had shorter incubation periods, more respiratory symptoms, greater human-to-human transmission and higher case fatality rates.[6]

Resistance of Virus

The viability of NiV in the environment is uncertain. NiV can survive for days in the urine and other secretions of fruit bats and in contaminated fruit juices. The virus can survive up to three days in fruit juices and for seven days in date palm sap held at 22°C. Sodium hypochlorite was used effectively in outbreaks for cleaning and disinfection purposes including the pig farms.[2] They are stable between pH 4 and 10. They are readily inactivated by soaps, detergents, lipid solvents such as alcohol and ether and many of the disinfectants. Effect of heat varies depending on the substrate. Usually, it is inactivated at 60°C for 60 min.[13]

Transmission

Natural hosts of NiVs are fruit bats of the family *Pteropodidae*. There is no apparent disease in fruit bats. They are simple reservoirs and shed the virus in urine, other secretions and excretions such as faeces, saliva and birthing fluids. Fruit bats commonly drop partially eaten saliva-laden fruit which are eaten by other animals

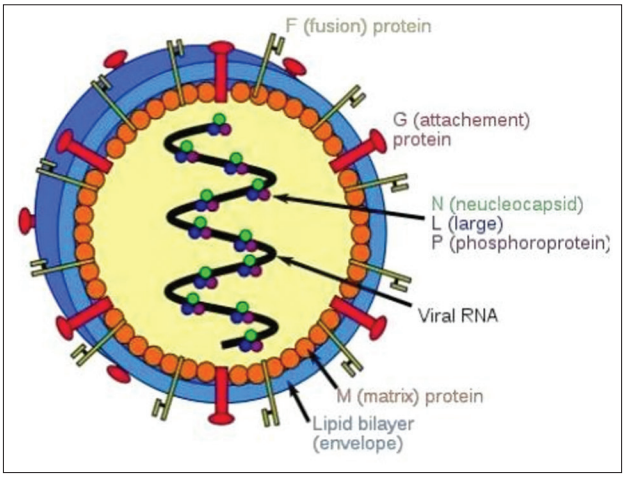

Figure 1: Schematic representation of structure -Nipah virus (https://commons.wikimedia.org/wiki/File: Henipavirus_structure.sv)

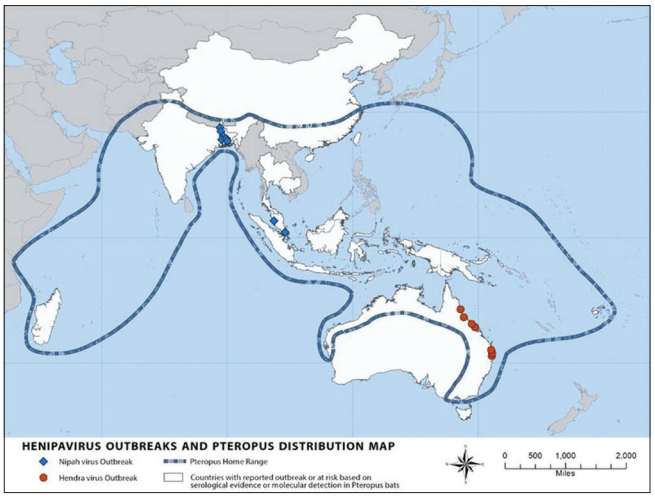

Figure 2: Geographical distribution of Nipah virus outbreaks and fruit bats (Ang BSP, Lim TCC, Wang L. Nipah virus Infection. J Clin Microbiol 2018;56[6])

including pigs. NiV is highly contagious among pigs and pigs can act as both intermediate and amplifier host. From pigs, transmission can be direct or indirect. Direct contact with infected pigs was the major mode of transmission in the 1999 Malaysian outbreak.[14] Pigs were not the transmitting hosts in both Siliguri and Bangladesh. Strong evidence of human-to-human transmission was found in Siliguri in 2001,[15] Bangladesh in 2004[16] and Nadia, West Bengal in 2007[4] outbreaks. In Siliguri outbreak in India, 33 health workers and hospital visitors contracted the disease from NiV-infected patients suggesting nosocomial infection.[14] Respiratory secretions are more important in person-to-person transmission.

There are about 60 species of bats in the genus *Pteropus*, commonly known as flying foxes. All species eat plant products (fruits, flowers and pollen). They can travel long distances, certain species even up to 600 km. Migratory *Pteropus* bats are distributed from the West Indian Ocean islands of Mauritius, Madagascar and Comoros, Pakistan, Southeast Asia, Philippines, Indonesia, Pacific Islands and Australia. The distribution of Indian flying fox (*P. giganteus*) extends from Thailand and Burma to India and Pakistan. The geographic distribution of *Henipa viruses* overlaps with that of *Pteropus* bats[6,17-19] [Figure 2]. The reservoir hosts of NiV have been identified as *Pteropus vampyrus* and *Pteropus hypomenalus* in Malaysia and *Pteropus giganteus* in Bangladesh and India.[20]

All the outbreaks of NiV infection in Southeast Asia have a seasonal pattern, occurring during December to May. This is the breeding season of bats when there is increased shedding of the virus. This also coincides with the date palm sap harvesting season.[13] Drinking of raw date palm sap contaminated with fruit bat urine or saliva-containing NiV is the only known cause of outbreak of disease in Bangladesh.[21] Peak time when viral RNA becomes detectable in bat urine is April to June.[14] It is, during this time, the young bats leave the nest to fly. The seasonal preference of transmission in *Pteropus* bats is documented in various studies. Strong evidence exists between the loss of natural habitat of bats and bat-related viral infections in humans and animals.[13] As bats are long distant flying birds living in large colonies, novel viruses can easily be transferred from one species to another and the seroprevalence of *Henipa viruses* among bat colonies are relatively high.[22]

Species Susceptible to Nipah Virus Infection

Humans, pigs, bats, dogs, cats, goats and horses are susceptible to NiV infection. NiV infection has been reported in sheep but is not confirmed.[2,23] Experimental infection can be produced in ferrets, guinea pigs, squirrel monkeys, African green monkeys,[24] hamster[25] and suckling mouse.[26] NiV antibodies have been demonstrated in all the animals exposed to NiV infection in pigs in Malaysia.[1,2]

Geographic Distribution of Nipah Outbreaks [Table 1]

NiV outbreak occurred in Malaysia with 265 suspected cases and 105 deaths with 40% case fatality. The virus was transmitted from bats to pigs and from pigs to pig farmers. Imported live pigs from Malaysia to Singapore during that time resulted in an outbreak in Singapore involving 11 abattoir workers with 1 reported death (case fatality 9%). Malaysian and Singapore outbreak ended in 1999. Mass culling of pigs and banning of pig farming in certain high-risk places was necessary for preventing the spread.[1,27] Successive outbreaks of Nipah encephalitis have occurred in Bangladesh since 2001 and NiV infection has become endemic in Bangladesh causing regular outbreaks.[4] From 2001 to 2013, about 238 NiV cases occurred in Bangladesh with 182 (76%) deaths. In 2014, 18 cases were reported from 11 different districts with nine deaths. India reported two outbreaks of NiV encephalitis in the Eastern state of West Bengal, Siliguri in 2001 and Nadia district in 2007. The case fatality was 70% with a reported 50 deaths from 71 cases.[4,28] Kozhikode district of Kerala, India, suffered from the last and recent outbreak during May–June 2018. Twenty three people were infected and 18 were laboratory confirmed with 16 deaths with a case fatality of 89%.

Clinical Presentation

Human infections range from asymptomatic to acute respiratory infection and fatal encephalitis. Incubation period ranges from four days to two weeks and even up to 45–60 days.[2,29] Initial symptoms are fever, headache, myalgia, vomiting and sore throat followed by dizziness, drowsiness, altered sensorium and neurological signs of acute encephalitis. Atypical pneumonia, acute respiratory distress, septicaemia, gastrointestinal bleeding and renal impairment are seen in seriously affected patients.[23,30] Encephalitis and seizures occur in severe cases, progressing to coma in 24–48 h.[29] Most patients who survive acute encephalitis make a full recovery, but reports show that about 20% of patients tend to have residual neurological consequences such as persistent convulsions and personality changes. Relapse or delayed-onset encephalitis is seen in a small number of patients who recover.[31-33]

Blood parameters are usually normal. Abnormalities are thrombocytopenia (30%) and leucopoenia (11%). Electrolytes are normal except for hyponatremia. Liver enzymes are elevated in 40% of patients. Haemoglobin

Table 1: Reported Nipah Virus outbreaks

Year/Month	Location	No. of cases	No. of deaths	Case Fatality Rate
Sep 1998-April 1999	Malaysia (Perak, Selangor, and Negeri Sembilan states)	265	105	40%
Mar 1999	Singapore	11	1	9%
Jan-Feb 2001	Siliguri (India)	66	45	68%
April-May 2001	Meherpur (Bangladesh)	13	9	69%
January 2003	Naogaon (Bangladesh)	12	8	67%
January 2004	Rajbari (Bangladesh)	31	23	74%
April 2004	Faridpur (Bangladesh)	36	27	75%
Jan-Mar2005	Tangail (Bangladesh)	12	11	92%
Jan-Feb 2007	Thakurgaon (Bangladesh)	7	3	43%
Mar 2007	Kushtia, Pabna, and Natore (Bangladesh)	8	5	63%
April 2007	Naogaon (Bangladesh)	3	1	33%
April 2007	Nadia (India)	5	5	100%
Feb 2008	Manikgonj (Bangladesh)	4	4	100%
April 2008	Rajbari and Faridpur (Bangladesh)	7	5	71%
Jan 2009	Gaibandha, Rangpur, and Nilphamari (Bangladesh)	3	0	0%
	Rajbari (Bangladesh)	1	1	100%
Feb-Mar 2010	Faridpur, Rajbari, Gopalganj, and Madaripur (Bangladesh)	16	14	87.5%
Jan-Feb 2011	Lalmohirhat, Dinajpur, Comilla, Nilphamari, and Rangpur (Bangladesh)	44	40	91%
Feb 2012	Joypurhat, Rajshahi, Natore, Rajbari, and Gopalganj (Bangladesh)	12	10	83%
Mar-May 2014	Philippines	17	9	53%
Kozhikode outbreak, Kerala May-June 2018	Kozhikode, Kerala (India)	18	16	89%(confirmed cases)

and renal indices are also normal. Seventy-five percentage cases have shown elevated cerebrospinal fluid (CSF) proteins and white cell counts. CSF glucose levels are often within normal limits.[13]

Pathogenesis

During the initial phase of illness, NiV is found abundantly in the respiratory secretions and can be detected in bronchoalveolar epithelial cells. The virus spreads from respiratory epithelium to endothelium in the lungs. Once NiV gains entry into the bloodstream, it gets disseminated throughout the body. The virus enters the CNS either through olfactory nerves or through bloodstream.[34] Receptors for the virus is ephrin-B2 which is a highly conserved protein in mammalian species. It is expressed on lymphocytes, neurons, smooth muscle cells and endothelial cells surrounding small arteries. Ephrin-B3 can function as alternate receptor in the brain regions where EB2 is not expressed. Cell entry and cell-to-cell transfer of NiV occurs by initial binding of G protein to ephrin receptors and cell fusion is mediated by F protein.[35,36]

Pathology

Infection of endothelial cells is a hallmark of NiV infection in animals and humans and the key histopathological finding in Nipah is the presence of syncytial multinucleated endothelial cells.[35,36] The syncytia consist of overlapping or sharply molded nuclei with moderate-to-abundant cytoplasm.[37] Severe damage of the microvasculature of the CNS is thought to be the basis for the development of NiV encephalitis which often leads to coma and death.[36] Marked vasculitis with endothelial damage, cellular lysis in the arterioles, venules and capillaries of various organs are the major pathological changes observed in confirmed NiV patients. Extensive involvement of blood vessels is observed in the CNS, lung, heart and kidney.

Differential Diagnosis

Differential diagnosis includes measles, JE, cerebral malaria, bacterial meningitis, herpes simplex encephalitis and other viral encephalitis[38] [Table 2]. First outbreak of NiV infection in Malaysia was initially considered as JE, as the disease occurred in pigs and also because JE was endemic in Southeast Asia. When it occurred in Siliguri where person-to-person transmission was the predominant mode, it was confused with measles. Often in outbreaks, Nipah cases occur in clusters.[13]

Laboratory Diagnosis

NiV is a highly pathogenic organism and is classified as a BSL-4 agent.[11] Sample collection and handling should be done according to the biosafety regulations. For isolation and propagation of NiV, BSL-4 facilities are needed. BSL-2 facilities may be used for routine diagnostic tests after inactivating the virus during specimen

collection.[14] Various methods are used for inactivation of virus to reduce the risk.[23] Sera may be gamma-irradiated (6 kilogreys) or diluted 1/5 in phosphate buffered saline (PBS) containing 0.5% Tween-20 and 0.5% Triton X-100 and heat inactivated at 56° for 30 min.[13,39,40]

Samples for Nipah Diagnosis

Throat swabs collected in viral transport medium, 10-ml urine in universal sterile container, 5-ml blood in plain vial and 1–2-ml CSF in sterile container and tissue specimens in viral transport medium (VTM). As most laboratories do not have the facility for diagnosing Nipah infections, samples should be sent to authorised laboratories. NIV Pune and Manipal Centre for Virus Research, Karnataka, are the authorised laboratories in India.

During the early stage of illness virus isolation and RT-PCR from freshly collected throat swabs, CSF, urine and blood and during convalescent-phase antibody detection by ELISA from serum or CSF are recommended. In fatal cases, immunohistochemistry of tissues collected during autopsy may be helpful in diagnosis. From clotted blood samples, serum should be separated within 24 h to avoid haemolysis and stored at –70°C.[39,41]

Samples should be packed in triple container, transported by triple packaging system under cold chain (2°C–8°C), to the testing laboratory with prior intimation. The samples should reach the designated laboratory within 48 h. Before dispatching the sample, outer surface of the container should be disinfected with 5% lysol or 1:100 diluted bleach. Sample containing vials should be kept in good quality plastic bags. If delay is expected, they have to be sent frozen on dry ice or liquid nitrogen. Samples should not be stored at –20°C for long.[41] NiV grows well on African green monkey kidney (Vero) and rabbit kidney (RK-13) cells where syncytia formation with large multinucleated cells containing viral antigen can be demonstrated. CPE could be visible in five–seven days after inoculation of clinical samples incubated at 37°C. Identification of virus can be done by immune staining or virus neutralisation tests, RT-PCR of culture supernatants and by electron microscopy.[19,39]

ELISA for detecting IgG and IgM antibodies can be performed with inactivated serum in BSL-2 facility.[14] IgG is used for serosurveillance in humans, bats, pigs and other domestic animals.[13] Since Nipah and Hendra are closely related viruses, ELISA using any of the antigen detects antibodies to both viruses. Serological tests can also be carried out in BSL-2 facility after inactivation of samples. RT-PCR is the preferred method for diagnosing NiV infection.[13,42,43] Course of appearance of NiV IgM and IgG antibodies in CSF and serum is shown in Figure 3.[37] Serum neutralisation test is the reference standard for antibody detection.[40]

Treatment

There are no drugs or vaccines specific for NiV infection. Intensive supportive care is the mainstay in treating severe respiratory and neurological complications. *In vitro* studies have shown that ribavirin is effective for both Hendra and NiV.[44] An open-label ribavirin treatment trial carried out during Nipah in Malaysia in 1998 showed a reduction in mortality by 36%.[45] There are studies showing that ribavirin did not have protective effect in NiV infections in animal models.[46] Favipiravir (T-705) a purine analogue antiviral approved for use in Japan against emerging influenza strains is found to have potent antiviral activity against *Henipa viruses*.[47] A neutralising human monoclonal antibody, m102.4, was found to be effective in a ferret animal model. This binds to the NiV G glycoproteins. The trial was also found successful in non-human primate models with related Hendra virus.[48] Many researches are ongoing on the development of vaccines against NiV. Some of them have been found to be effective in animals.[49,50] All these studies are in the experimental stage. A vaccine for prevention of Hendra virus in horses has been licensed in Australia by Pfizer Animal Health under the name EquivacHeV. The horse vaccine is expected to provide substantial benefit to humans.[51]

Prevention

Nipah outbreak is a crisis both for the community and healthcare system. With no vaccines against Nipah, the only way to prevent infection in people is by educating

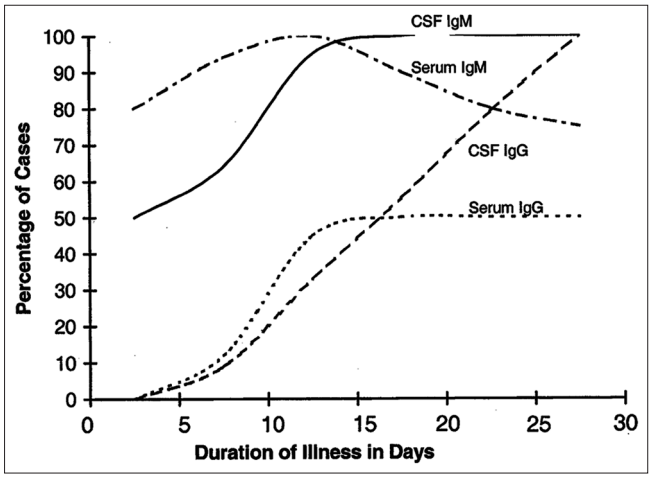

Figure 3: Course of appearance of Nipah virus IgM and IgG antibodies in CSF and serum (Wong KT, Shieh WJ, Kumar S, Norain K, Abdullah W, Guarner J, *et al*. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. Am J Pathol 2002;161[6]:2153-6)

about the risk factors and the measures that can be taken to reduce exposures. Bat-to-human transmission can be reduced by minimising the access to date palm sap and fruit juices. Freshly collected date palm juice should be boiled and fruits thoroughly washed and peeled before consuming. Fruits with bite marks should be discarded. To reduce the risk of animal to human transmission, people should avoid contact with infected pigs. Gloves and other protective clothing should be worn while handling sick animals or their tissues. To reduce the risk of human-to-human transmission, healthcare workers caring for patients with suspected or confirmed infection or persons who handle specimens should follow standard infection control measures. Contact and droplet precautions should be used in addition to standard precautions as person-to-person transmission was the major mode of infection in all reported outbreaks from India. Contact and droplet precautions include appropriate patient placement, use of personal protective equipment, limit transport and movement of patients, use of disposable and dedicated patient care equipment, cleaning and disinfection of rooms. Airborne precautions also may be required in certain circumstances and include wearing of mask and observing respiratory/cough etiquette.[30] All levels of precautions include hand hygiene.

Nipah Virus Outbreak in Kerala

In early May 2018, Kozhikode , one of the northern districts in Kerala, witnessed the catastrophic NiV outbreak. There occurred a total of 23 cases with 21 deaths with a case fatality of 91%. This includes the primary case (not labelled as index case as this was not laboratory confirmed) 18 laboratory confirmed cases and 4 probable cases. The case fatality rate among the laboratory-confirmed cases was 89%. The outbreak occurred as three clusters. Nineteen cases were contacts of the primary case and three were secondary contacts.[42] The major clinical presentations of the patients included encephalitis, acute respiratory distress syndrome and/or myocarditis.

Throat swab, urine, blood and CSF in indicated cases were collected following the standard precautions donning full personal protective equipment. Samples were immediately packed appropriately and transported maintaining cold chain to Manipal Centre for Virus Research, Karnataka, were diagnosis was done by RT-PCR.[42]

Clinical details of 18 laboratory-confirmed cases are given in Table 2. Of the 18 laboratory confirmed cases, 10 received full course of ribavirin therapy including the two survivors. Ribavirin was given at a dose of 2 gm stat and 1 g six hourly for four days, followed by 500 mg six hourly for five days (based on the WHO guideline for other haemorrhagic fevers).

Table 2: Clinical and lab findings of patients from Kerala outbreak (Courtesy-Department of Emergency Medicine, Government Medical College, Kozhikode)

Symptoms/Signs/Lab parameter	*n*	Present	%
Fever	19	19	100.0
Vomiting	19	11	57.8
Headache	19	12	63.1
Cough	19	4	21
Breathlessness	19	6	31.5
Altered sensorium	19	15	78.9
Convulsions	19	7	36.8
Tachypnoea	19	12	63.1
Crepitations	19	16	84.2
Infiltrations in chest x-ray	17	14	82.3
Platelet count <140 × 10^3/µl	18	9	50
Platelet count <100 × 10^3/µl	18	4	22.2

The Indian Council of Medical Research has confirmed fruit bats as the source of infection in this outbreak.[52]

The disease could efficiently be controlled by prompt and effective infection control measures. Training on Nipah infection control practices (ICP) was given initially by the experts from CDC and National Institute of Virology which was then taken up by the infection control committee of Government Medical College, Kozhikode. Dedicated space was identified in the hospital payward wing so that each patient/suspect could be admitted in individual rooms. Training on ICP was given not only to healthcare workers but also at community level. Drivers of taxi, auto rickshaw, bus, ambulance; the police department; staff of civil station and many others attended the training sessions. The awareness and ICP classes were held daily for a period of one month in May–June. Daily infection control rounds were carried out and any lapse in ICP were promptly and strictly addressed and corrected. By strict adherence to infection control measures and effective contact tracing Nipah could be contained in a short time in Kozhikode. With the last case reported on 30 May 2018, Kerala Government declared the state free from Nipah infection on 1 July 2018.

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Conflicts of interest
There are no conflicts of interest.

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