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Review Article

Nipah: A killer virus

Gurjeet Singh[1], Raksha[2], Anant D. Urhekar[3]

Assistant Professor[1&2], Professor and Head[3].

[1&2]Department of Microbiology, N.C. Medical College and Hospital, Israna, Panipat-132107, Haryana, India.
[3]Department of Microbiology, MGM Medical College and Hospital, Kamothe, Navi Mumbai-410209, Maharashtra, India.

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ABSTRACT

Nipah virus (NiV) is member of the genus Henipavirus in the family Paramyxoviridae. NiV disease is a zoonotic disease characterized by fever, constitutional symptoms, and encephalitis, sometimes accompanied by respiratory illness. The name 'Nipah virus' originated from Sungai Nipah (Nipah River Village). A Nipah virus disease outbreak was reported from Kozhikode district of Kerala, India on 19 May 2018. This is the first NiV outbreak in South India. As of 28 May, there are 14 deaths, 16 confirmed cases and 12 suspected cases. Transmission of the NiV disease occurred from direct contact with sick pigs or their contaminated tissues also via respiratory droplets, contact with throat or nasal secretions from the pigs, or contact with the tissue of a sick animal. Consumption of fruits or fruit products (e.g. raw date palm juice) contaminated with urine or saliva from infected fruit bats was the most likely source of infection. Limited human to human transmission of NiV has also been reported among family and care givers of infected NiV patients. In Siliguri, India, transmission of the virus was also reported within a health-care setting (nosocomial), where 75% of cases occurred among hospital staff or visitors. This infection can occur in humans without showing any symptoms. However, it is essential for people to look out for influenza-like symptoms. Fever, sore throat, headaches, vomiting and muscle pain (myalgia) are some of the common signs. The infection progresses to acute respiratory infection (mild to severe) causing interference in breathing. During this phase, people experience atypical pneumonia and acute respiratory distress, which further leads to severe problems (fatal encephalitis). Initially develop influenza-like symptoms of fever, headaches, myalgia (muscle pain), vomiting and sore throat, followed by dizziness, drowsiness, altered consciousness, and neurological signs that indicate acute encephalitis. Some people can also experience atypical pneumonia and severe respiratory problems, including acute respiratory distress. Encephalitis and seizures occur in severe cases, progressing to coma within 24 to 48 hours. The incubation period (interval from infection to the onset of symptoms) is believed to range from 4 to 18 days. However, an incubation period as long as 45 days has been reported. BSL 2 facilities are sufficient if the virus can be first inactivated during specimen collection. Laboratory diagnosis of NiV includes serology, histopathology, Polymerase Chain Reaction (PCR) and virus isolation. Serum Neutralization Test, Enzyme Linked Immunosorbent Assay (ELISA), Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) are used for laboratory confirmation. The 2018 review of the WHO list of Blueprint priority diseases indicates that there is an urgent need for accelerated research and development for the Nipah virus.

INTRODUCTION

Nipah virus (NiV) is member of the genus Henipavirus in the family Paramyxoviridae. It is classified as a Biosafety Level-4 (BSL-4) agent due to its highly pathogenicity and relative new findings. The Centers for Disease Control and Prevention (CDC) and the National Institute of Allergy and Infectious Diseases (NIAID) have classified NiV as a Category C priority pathogen. [1] NiV disease is a zoonotic disease characterized by fever, constitutional symptoms, and encephalitis, sometimes accompanied by respiratory illness. NiV has an envelope with filamentous nucleocapsids, the genome consists of a single-stranded negative-sense RNA of approximately 18.2 kb. The genome encodes for six major structural proteins: nucleocapsid (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G), and large protein or RNA polymerase (L). [2]

The name 'Nipah virus' originated from Sungai Nipah (Nipah River Village), where the first isolates were obtained [3–5]. Bats of the genus Pteropus appear to be the natural reservoir of the virus. Nipah virus swept through numerous piggeries in Malaysia and killed 1100 people during the period from 1998 through 1999. [6-7] NiV was identified as the etiological agent responsible of an outbreak, in pigs and humans, in Malaysia and Singapore. Transmission may be from consumption of contaminated food by bats secretion, or contact with infected pigs. Another way can be human-to-human spread. Since 1998 there have been several cases of infections in Bangladesh and India, collectively causing hundreds of cases with lethality rates sometimes in excess of 70%. [8–22]

The natural reservoir for NiV is pteropid fruit bats [2], and direct bat to human transmission can occur, frequently as a result of consumption of date palm sap contaminated with saliva or urine from infected bats [22]. Alternatively, transmission to the human population can proceed via an amplifying host, as during the initial NiV outbreak in Malaysia, in which transmission was from close contact with infected domesticated swine [14]. Transmission is primarily via the oronasopharyngeal route, with initial infection in the respiratory mucosa, followed by viral dissemination and high levels of viral replication in the endothelial cells of the central nervous system vasculature, causing the often-fatal encephalitis [23,24]. No intermediate host was implicated in the Nipah virus outbreaks in India and Bangladesh. Rather, epidemiological data suggest transmission of Nipah virus from bats to humans through the consumption of fruit or date palm sap contaminated by infected fruit bats. [17,25] In most outbreaks, limited chains of direct human-to-human transmission have been documented, usually from an infected patient with respiratory symptoms to a direct care-giver, both in community and hospital settings [22,26–28].

For the Nipah virus outbreaks in Bangladesh between 2001 and 2007, it was estimated that,50% of Nipah virus cases were the result of human-to-human transmission events. Nipah virus has been isolated from human urine, saliva, nasal and oropharyngeal secretions and epidemiological data suggest that direct contact with these secretions of Nipah virus spreaders resulted in greater risk of Nipah virus infection. Three potential modes of human-to-human transmission of Nipah virus could be transmission via fomites, direct contact or aerosols. [29] Currently there are no vaccines or therapeutic medicine specifically recorded to either prevent or treat patients infected with Nipah virus. [30] The World Health Organization has included Nipah in its priority list of emerging diseases that could cause a global pandemic, along with Crimean Congo fever, Ebola, Middle East respiratory syndrome coronavirus and Zika. [31]

OUTBREAK IN KERALA (INDIA)

According to the world health organization (WHO), a Nipah virus disease (NiV) outbreak was reported from Kozhikode district of Kerala, India on 19 May 2018. This is the first NiV outbreak in South India. As of 28 May, there are 14 deaths, 16 confirmed cases and 12 suspected cases. The two affected districts are Kozhikode and Mallapuram. A multi-disciplinary team led by the Indian Government's National Centre for Disease Control (NCDC) is in Kerala in response to the outbreak. WHO is providing technical support to the Government of India as needed. WHO does not recommend the application of any travel or trade restrictions or entry screening related to the Nipah virus outbreak. Nipah virus disease is an emerging infectious disease spread by secretions of infected bats. It can spread to humans through contaminated fruit, infected animals, or through close contact with infected humans. [32]

EPIDEMIOLOGY

Paramyxoviruses are characterized by broad host range and for this reason they show an important zoonotic potential, like Nipah virus originating from bats. Bats represent the most successful mammals on earth including about 1200 chiropteran species distributed worldwide. In the last decades Hendra virus, Nipah virus and other zoonotic viruses like Ebola, Marburg, and SARS virus, have been identified in various Pteropus species fruit bats [33-36, 56].

Fruit-eating bats (Pteropus species) are the natural reservoir for the henipaviruses. Humans are usually infected via the intermediate hosts. In case of Nipah virus, pigs are the usual intermediate hosts. But exposures to infected fruit bats or materials contaminated by infected bats or direct human-to-human transmission is also possible. Bats are classified in the order Chiroptera (from the Greek 'cheiros,' meaning hand; and 'pteros,' meaning wing) and it is within the genus Pteropus in the family Pteropodidae or old world fruit bats, that we find the natural hosts of HV and NV. Pteropid bats are commonly referred to as 'flying foxes.' Sixty-five Pteropus species are distributed from Madagascar through the Indian subcontinent to southeastern Asia and Australia and as Far East as the Cook Islands. Some Pteropus species are among the largest of all bats, weighing as much as 1.2 kg and displaying a wingspan of up to 1.7 m.

Pteropus species are unique because they lack the complex neural and behavioral mechanisms required for echolocation that characterize the vast majority of bat species. Instead, they have large eyes and they navigate visually, feeding mainly on fruits and flowers, which they locate by smell.[37-39]

Although brought into much attention by the epidemic of NV encephalitis in Malaysia in 1998-99 (vide infra), isolated cases of Hendra virus causing encephalitic illness amongst animal handlers were being reported since as early as 1994. NV and HV having close genomic similarity were difficult to differentiate serologically earlier. In fact, even during the Malaysian epidemic of 1998-99, initial results indicated the causative organism to be Hendra virus. Later of course, with viral isolation and development of a specific serological marker, the identity of the NV was made known. The word 'Nipah' originated from the name of a village 'Sungai Nipah' in the Malaysian peninsula, one of the first villages where pig farmers developed an encephalitic disease. Studies have been reported Nipah encephalitic illnesses among human and animal [40-62].

The first known human infection with NV was detected during an outbreak of severe febrile encephalitis in peninsular Malaysia and Singapore [61-62] in 1998-1999. Direct contact with pigs was the primary source of human infection. A total of 276 patients with viral encephalitis were reported in that epidemic. Most of the victims were adult males involved in pig farming or pork production. The spread of virus within the pig farms and between states of Malaysia was due to movement of pigs. NiV disease transmission among pigs in the same farm was attributed to direct contact with excretions and secretions i.e. urine, saliva, laryngeal and pharyngeal secretions. Iatrogenic transmission by use of same needles was also implicated. [39,62]

But what exactly led to the spillage of the virus from its natural reservoir into the pigs remains a subject of speculation. Species jumping of viruses can be due to evolutionary or ecological reasons. But NV is an old virus and has not undergone any evolutionary change. Most authorities believe that ecological factors led to their emergence. [39] This can be due to a change in the number density and management of pigs. But more importantly, the curse of unplanned deforestation of pulpwood has taken its toll on the natural habitat of the fruit bats in the last two decades. This coupled with the EL Nino Southern Oscillation - related drought prompted migration of bats from their natural habitat in the costal forest on to the villages where the pigsties (piggeries) were located. [39]

The southern oscillation refers to an oscillation in the surface pressure (atmospheric mass) between the southeastern tropical Pacific and the Australian-Indonesian regions. When the waters of the eastern Pacific are abnormally warm (an EI Nino event), sea level pressure drops in the eastern Pacific and rises in the west. The reduction in the pressure gradient is accompanied by a weakening of the low-latitude easterly trades. This condition results in redistribution of rains with flooding and droughts. The drought played a major role in animal migration from forestlands towards villages

in several ways. With destruction of trees, there had been shortage of food in the forest, especially for the fruit-eating bats. Second, the dry weather resulted in forest fires, causing further loss of trees and lack of food supply for the fruit-eating bats. Thirdly, the forest fire caused a severe haze, resulting in poor visibility for the bats, which preferred to migrate to the cleaner 'air' of the villages closer to human and pig habitation. These EL Nino related factors coupled with 'intentional' deforestation played a major role in upsetting the ecological balance, perpetuating transmission of NV from bats to pigs and pigs to humans in Malaysia.[39]

The route of introduction of virus into the pigs was also facilitated by the practice of growing fruit trees adjacent to the piggeries. In April and May 2001, a cluster of febrile neurological illnesses with nine deaths was reported in a village in Meherpur district, Bangladesh. Preliminary investigations by the Bangladesh Ministry of Health and the World Health Organization (WHO) excluded a diagnosis of Japanese encephalitis, dengue fever or malaria, but 2 of 42 serum specimens obtained from village residents in May 2001 showed reactive antibodies to Nipah virus antigen in tests performed at the US Center for Disease Control and Prevention (CDC). However, a comprehensive investigation of this outbreak was not conducted. [57]

In January 2003, a further cluster of febrile illnesses with neurological features and eight reported deaths occurred in adjoining villages in Naogaon district, 150 km from the village in Meherpur district. Similarities in the clinical manifestations observed among patients in Naogaon and Meherpur raised the question of whether the outbreaks were caused by the same agent. But unlike the Malaysia epidemic, no intermediate amplifying host could be identified. This led to the conjecture that the virus was transmitted directly or indirectly from bats to the humans. [58] Two outbreaks consisting of 48 cases of NV were detected in 2004 in two adjacent districts (30 km apart) of central Bangladesh (Rajbari and Faridpur) with a case-fatality rate of nearly 75%. Because of heightened surveillance, other small clusters and isolated cases (n=19) were identified during the same period in seven other districts in central and northwest Bangladesh. Although antibodies to NV were detected in fruit bats from the affected areas in 2004, an intermediate animal host was not identified, which suggests that the virus was transmitted from bats to humans. In fact, human-to-human transmission of NV was documented during the Faridpur outbreak of 2004. [59]

A study showed four NV isolates from Bangladesh share 99.1% homology but exhibit more inter strain nucleotide heterogeneity than the sequences of the human isolates in Malaysia, which were nearly identical. These varying amounts of genetic variability may reflect differences in the mode of transmission of NV in the two countries. A further outbreak in Tangail district of Bangladesh occurred in end 2004 early 2005 and has also been reported.[60]

Unlike the Malaysia outbreak, 'bat to pig to human' transmission was unlikely to have occurred in Bangladesh. For religious reasons, pig farming is not practiced in Bangladesh and pig population is low. Hence direct bat-to-human transmission and then human-to-human transmission seemed most likely. Tan, in a talk delivered at the World Congress of Neurology, November 2005, at Sydney,[63] proposed that this route might be through contaminated date palm juice, which humans consume. Collecting date palm juice in earthenware pots hung atop date palm trees (after making an incision in the bark of the trees) is a common practice in rural areas of eastern India and Bangladesh during winter months. The bats feed on the juice, thus contaminating the juice with their saliva, which is subsequently drunk by humans.

The Siliguri epidemic clearly resembles the Bangladesh epidemic. Most of the victims had nothing to do with the pigs. They were medical and paramedical staff of the nursing homes where the affected cases were brought.[64-65] This, together with the demonstration of the viral genome in the urine of the affected patients, clearly supports the case for a human-to-human transmission,[64] perhaps with a similar bat-to-human transmission through contaminated date palm juice, to start with. Unconfirmed reports suggest that the index case in the Siliguri outbreak was brought to a private nursing home from a neighboring village by an ambulance (personal communication). The patient, his accompanying person and the ambulance driver -all succumbed to the illness. [66]

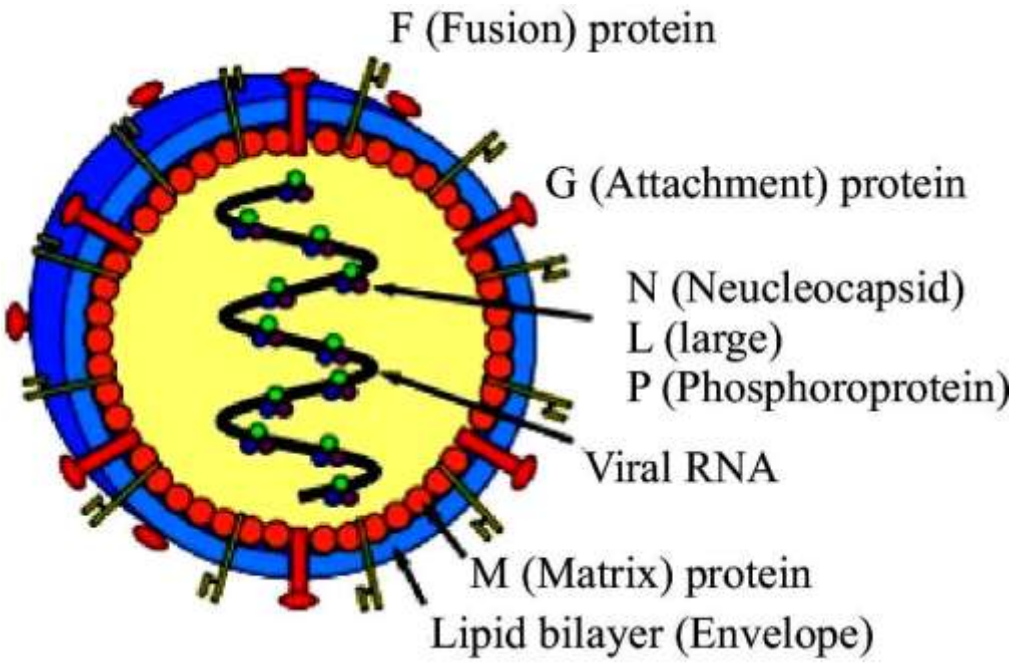
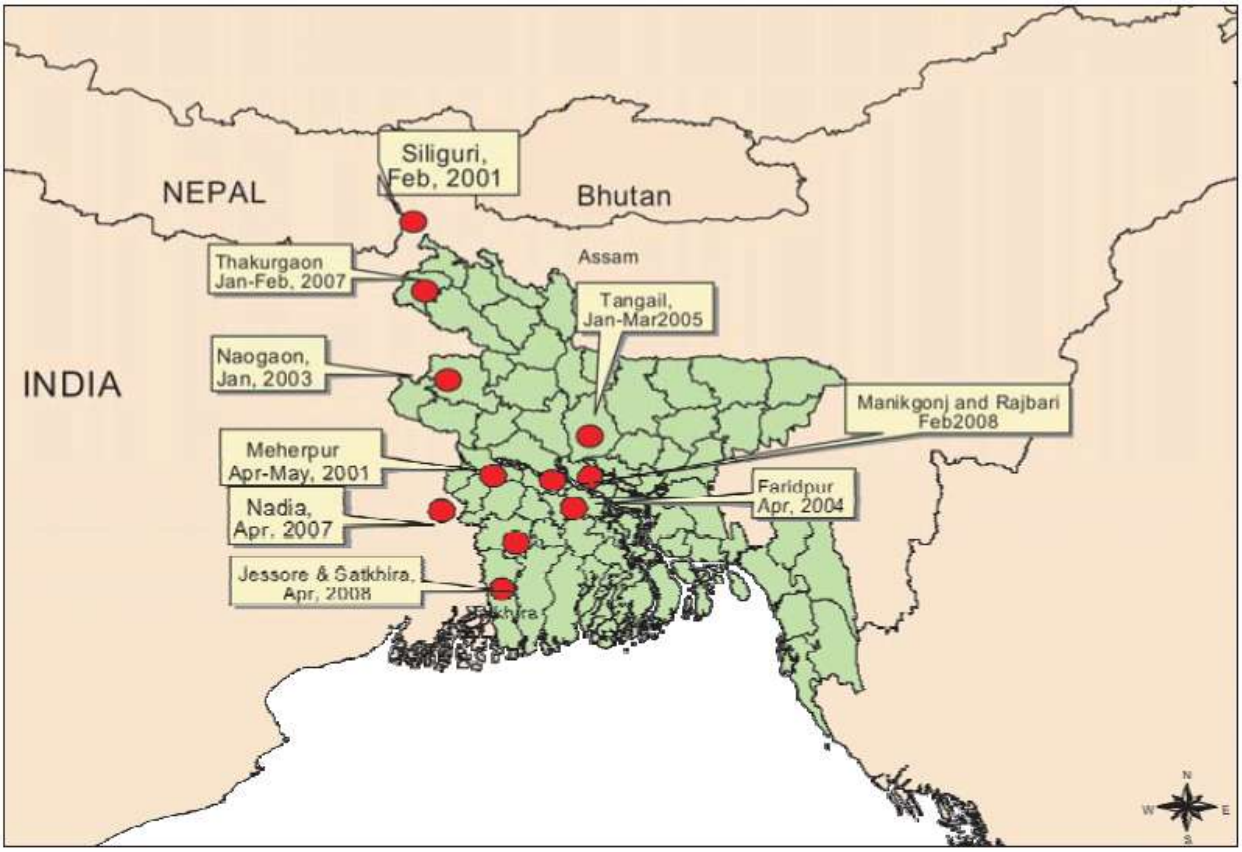


Fig. 1: Structure of Nipah Virus Infection (NiV)
Source: https://en.wikipedia.org/wiki/Henipavirus#Nipah_virus



The boundaries and name shown on this map do not imply any expression or any opinion what so ever on the part of World Health Organization concerning the legal status of any country, territory, city or area of its authorities or concerning the delimitation of its frontiers or boundaries.

Fig. 2: Chronological distribution of outbreak of Nipah virus infection in South Asia, 2001-2008.
Source: http://www.searo.who.int/entity/emerging_diseases/links/CDS_Nipah_Virus.pdf?ua=1

Table 1: Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia Region, 2001-2018

Country: Bangladesh

Month/Year	Location	No. of cases	No. of deaths	Fatality Rate
April, May 2001	Meherpur	13	9	69%
January 2003	Naogaon	12	8	67%
Jan 2004 Apr 2004	Rajbari Faridpur	31 36	23 27	74% 75%
Jan- Mar 2005	Tangail	12	11	92%
Jan-Feb 2007 Mar 2007 Apr 2007	Thakurgaon Kushtia Pabna, Natore and Naogaon	7 8 3	3 5 1	43% 63% 33%
Feb 2008 Apr 2008	Manikgonj Rajbari	4 7	4 5	100% 71%
Jan 2009	Gaibandha, Rangpur and Nilphamari Rajbari	3 1	0 1	0% 100%
Feb-Mar 2010	Faridpur Faridpur, Rajbari, Gopalgonj, Kurigram,	8 8 1	7 7 1	87.50% 87.50% 100%
Jan-Feb 2011	Lalmohirhat, Dinajpur, Comilla Nilphamari, Faridpur, Rajbari	44	40	91%
Jan 2012	Joypurhat	12	10	83%
Jan- Apr 2013	Pabna, Natore, Naogaon, Gaibandha, Manikganj	24	21	88%
Jan-Feb 2014	13 districts	18	9	50%
Jan-Feb 2015	Nilphamari, Ponchoghor, Faridpur, Magura, Naugaon, Rajbari	9	6	67%

Country: India

Month/Year	Location	No. of cases	No. of death	Case Fatality Rate
Feb 2001	Siliguri	66	45	68%
Apr 2007	Nadia	5	5	100%
May* 2018	Kerala	14	12	86%

*As of 24 May 2018

Source: http://www.searo.who.int/entity/emerging_diseases/links/morbidity-and-mortality-nipah-sear-2001-2018.pdf?ua=1. [67]

MODE OF TRANSMISSION

During the initial outbreaks in Malaysia and Singapore, most human infections resulted from direct contact with sick pigs or their contaminated tissues. Transmission is thought to have occurred via respiratory droplets, contact with throat or nasal secretions from the pigs, or contact with the tissue of a sick animal.

In the Bangladesh and India outbreaks, consumption of fruits or fruit products (e.g. raw date palm juice) contaminated with urine or saliva from infected fruit bats was the most likely source of infection.

Limited human to human transmission of NiV has also been reported among family and care givers of infected NiV patients. During the later outbreaks in Bangladesh and India, Nipah virus spread directly from human-to-human through close contact with people's secretions and excretions.

In Siliguri, India, transmission of the virus was also reported within a health-care setting (nosocomial), where 75% of cases occurred among hospital staff or visitors. From 2001 to 2008, around half of reported cases in Bangladesh were due to human-to-human transmission through providing care to infected patients. [68]

Transmission also occurs from direct exposure to infected bats. A common example is consumption of raw date palm sap contaminated with infectious bat excretions. [69]

The possible mechanical transmission by repetitive use of same needles or equipment without further sterilization after each use for health intervention and artificial insemination and sharing of boar semen within a farm were also implicated. The possible role of transmission by infected dogs and cats found in the affected farm could not be excluded [39].

SIGNS AND SYMPTOMS

This infection can occur in humans without showing any symptoms. However, it is essential for people to look out for influenza-like symptoms. Fever, sore throat, headaches, vomiting and muscle pain (myalgia) are some of the common signs.

The infection progresses to acute respiratory infection (mild to severe) causing interference in breathing. During this phase, people experience atypical pneumonia and acute respiratory distress, which further leads to severe problems (fatal encephalitis).

Infected people initially develop influenza-like symptoms of fever, headaches, myalgia (muscle pain), vomiting and sore throat. This can be followed by dizziness, drowsiness, altered consciousness, and neurological signs that indicate acute encephalitis. Some people can also experience atypical pneumonia and severe respiratory problems, including acute respiratory distress. Encephalitis and seizures occur in severe cases, progressing to coma within 24 to 48 hours.

The incubation period (interval from infection to the onset of symptoms) is believed to range from 4 to 18 days. However, an incubation period as long as 45 days has been reported. Most people who survive acute encephalitis make a full recovery, but long term neurologic conditions have been reported in survivors. Approximately 20% of patients are left with residual neurological consequences such as seizure disorder and personality changes.

A small number of people who recover subsequently relapse or develop delayed onset encephalitis. The case fatality rate is estimated at 40% to 75%. This rate can vary by outbreak depending on local capabilities for epidemiological surveillance and clinical management. [70-71]

DIFFERENTIAL DIAGNOSIS

- It is important to note that this disease has human health implications and all field investigations should take necessary precautions to prevent infection
- Any respiratory or neurological conditions of swine in an area known to have *Pteropid* bats, should consider Nipah as a rule out
- Also among swine; deaths of suckling pigs and piglets; sudden death in boars and sows o abortions and other reproductive dysfunction respiratory diseases with harsh, non-productive coughing and cases with encephalitic manifestations of trembling, muscular in coordination and myoclonus leading to lateral recumbency.

LABORATORY DIAGNOSIS

Procedures for the laboratory diagnosis of NiV include serology, histopathology, PCR and virus isolation. Serum Neutralization Test, ELISA, RT-PCR are used for laboratory confirmation.

Most countries in the South-East Asia Region do not have adequate facilities for diagnosing the virus or on ways of controlling it. Bangladesh, India and Thailand have developed laboratory capacity for diagnostic and research purposes.

Nipah virus is classified internationally as a biosecurity level (BSL) 4 agent. BSL 2 facilities are sufficient if the virus can be first inactivated during specimen collection. There are a few laboratories in which the virus can be studied safely without a risk of it "escaping" and infecting more people. [72]

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Virus Isolation

- Virus isolation is an important primary diagnostic approach for NiV infections.
- NiV grow well in Vero cells, and the range of specimens yielding isolates in either natural or experimental cases. Brain, lung, kidney and spleen should always be submitted.
- Tissues are handled under sterile conditions for preparation of 10% suspensions in cell culture media.
- These are clarified by centrifugation and the supernatant used for inoculation of cell cultures.
- A CPE usually develops within 3 days, but two 5-day passages are recommended before judging the attempt unsuccessful.
- Initially after low multiplicity infection of cell monolayers, the CPE is manifested by the formation of syncytia that may contain up to 20 or more nuclei.
- Subsequently syncytia lift from the substrate, leaving punctate holes in the cell monolayer.
- The syncytia formed by NiV in Vero cell monolayers are significantly larger than those created by HeV in the same time period.
- Interestingly, the distribution of nuclei differs between NiV-induced syncytia and can be used to differentiate the two viruses (see Hyatt et al. in this Current focus for more details).
- Identification methodologies for virus isolates include immunostaining of fixed, infected cells, neutralization with specific antisera, PCR of culture supernatants, electron microscopy and immunoelectron microscopy.
- The later techniques are useful for preliminary characterization of the isolate since HeV and NiV have distinct ultrastructural characteristics. [74]

Immunohistochemistry

- Immunohistochemistry has proven one of the most useful tests in NiV detection.
- Performed on formalin-fixed tissues, it is safe and has allowed retrospective investigations on archival material.
- In NiV infections there is a wide range of tissues in which viral antigen can be detected, since the primary pathology occurs in the vascular endothelium.
- It has been suspected that viral antigens may be cleared from lung tissue somewhat early in the course of infection and so the diagnostic submission should include a range of tissues, not just lung.
- Ideally a submission for immunohistochemistry would include samples of the brain at various levels, lung, mediastinal lymph nodes, spleen and kidney.
- In pregnant animals the uterus, placenta and foetal tissues should be included.
- Antiserum was used in the initial NiV investigations, but was subsequently replaced by convalescent pig and cat anti-NiV antisera.
- The preferred reagent now is a rabbit antiserum raised to plaque purified NiV.
- Although a biotin-streptavidin peroxidase linked detection system has been used successfully in the past, the preferred detection system at AAHL now is the newer anti-rabbit/anti-mouse dextran polymer linked reagent conjugated with alkaline phosphatase (EnVision+, Daco Corporation). [74]

Electron Microscopy

- Nipah virus grows in cultured cells to titres as high as 108 TCID50 or PFU/mL.
- Visualization of viruses in the medium of infected cells by negative contrast electron microscopy and detection of virus-antibody interactions by immunoelectron microscopy rapidly provide valuable information on virus structure and antigenic reactivity, even during primary isolation of the virus.
- Other ultrastructural techniques such as grid cell culture, in which cells are grown, infected and visualized on electron microscope grids, and identification of replicating viruses and inclusion bodies in thin sections of fixed, embedded cell cultures and infected tissues complement the diagnostic effort. [74]

Serum Neutralization Test

- For serology the SNT is accepted as the reference standard.
- In the test, performed under PC4 conditions, sera are incubated with virus in the wells of 96-cell microtitre plates prior to the addition of Vero cells.
- Sera are screened at a 1:2 dilution, although this occasionally leads to problems with serum-induced cytotoxicity.
- Where sample quality is poor or sample volumes small, as may be the case with flying fox (Pteropus species) or microbat sera, an initial dilution of 1:5 may be used.
- Cultures are read at 3 days, and those sera that completely block development of CPE are designated as positive.
- NiV have been quantified using a plaque assay procedure and the procedure modified to create a second neutralization procedure.
- The viruses are titrated on Vero cell monolayers in 96-well plates and after 18–24 h, foci of infection are detected immunologically in methanol-fixed cells using an antiserum to a bacterial expressed portion of the HeV P protein. Rabbit anti-P antibodies are detected using immuno-peroxidaseconjugated secondary antibody.
- This procedure has been modified in the traditional manner by incubating a specific number of plaque-forming units with dilutions of test serum prior to adsorption to the cell monolayers. Unadsorbed virus is removed and virus-induced syncytia detected 24 hours later.
- Such a plaque-reduction neutralization test has merit if cytotoxicity is a problem, because virus-serum mixtures are removed after an adsorption period, and if the volumes of sera available are low. [74]

ELISA

- In initial investigations of outbreaks caused by NiV, in subsequent epidemiological studies and for ongoing surveillance there is a need for serological tests that can be conducted safely and quickly without access to PC4 facilities.
- ELISA meets these requirements.
- The first development was an indirect ELISA for detection of IgG antibodies to HeV in horses.
- Lysates of NiV-infected cells have been prepared using non-ionic detergents and virus-specific material removed from the cytoskeleton by shearing in a Dounce homogenizer.
- Nuclei are removed by centrifugation and for safety purposes the antigens are irradiated with 6 kilo-Grays prior to use.
- This treatment has negligible effect on antigen titre.
- At CDC the approach has been to not only have an indirect ELISA for detection of IgG but to also employ a capture ELISA for detection of IgM.
- The CDC ELISAs for detection of both anti-HeV IgG and IgM antibodies were the initial tests transferred to Malaysia in response to the NiV outbreak.
- They were used to confirm the diagnosis in both human and porcine populations, the identification of cases and to establish the serological profile of infected pig herds as a prelude to designing a national surveillance program.
- A panel of sera from farms involved in the outbreak was tested in this ELISA, and the neutralization results on the panel were used to calculate a relative sensitivity and specificity of > 70 and > 95%, respectively. [74]

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

- Diagnostic PCR assays for NiV are in routine use in CDC.

- Sets of nested primers are employed for amplification of segments of the M genes, coding for the relatively conserved matrix protein.
- RT-PCRs can be used for detection of viral sequences in fixed or fresh tissue or cerebrospinal fluid diagnostic specimens or as an adjunct to the rapid characterization of viral isolates from cell culture.
- An alternative approach at CDC has been to develop a diagnostic PCR based on the N gene coding for the nucleoprotein, although the initial work was done with a PCR for the P gene.
- In the NiV outbreak in Malaysia isolates from human cases and from pigs were shown to be identical, as was an isolate from a human case in Singapore.
- A phylogenetic analysis of members of the subfamily Paramyxovirinae has been conducted on the basis of N gene sequences. NiV was shown to group and to be more closely related to viruses in the genus Morbillivirus than to viruses in the genus Rubulavirus. [74]

RISK OF EXPOSURE

The risk of exposure of Nipah virus is high for hospital workers and caretakers of those infected with the virus. In Malaysia and Singapore, Nipah virus infection occurred in those with close contact to infected pigs. In Bangladesh and India, the disease has been linked to consumption of raw date palm sap (toddy) and contact with bats. [18]

PREVENTION

Human-to-human transmission of NiV has been reported in recent outbreaks demonstrating a risk of transmission of the virus from infected patients to healthcare workers through contact with infected secretions, excretions, blood or tissues. The Healthcare workers who working for patients with suspected or confirmed NiV should implement Standard Precautions when caring for patients and handling specimens from them.

The following general measures can be effective from prevention of Nipah virus infection:

- ✓ Avoid close (unprotected) physical contact with infected people
- ✓ Wear NH95-grade and higher masks
- ✓ Wash hands regularly with soap
- ✓ Avoid consuming partly eaten fruits or unpasteurised fruit juices
- ✓ Avoid being around anima pens
- ✓ Boil freshly collected date palm juice before consuming
- ✓ Thoroughly wash and peel fruits before consuming
- ✓ Maintain your and children's personal hygiene
- ✓ Cover your household properly.

Sanitary prophylaxis

- ✓ Strict biosecurity of swine installations with the aim of avoiding contact with fruit bats and their secretions is essential, including: fruit tree set-back, using screens at open-air access and appropriate disposal of roof run-off
- ✓ An active surveillance program with rapid detection and immediate culling of seropositive swine is critical in preventing spread of disease and infection of humans
- ✓ Effective quarantines and control of animal movements must also be implemented early in an outbreak
- ✓ All materials and equipment from affected farms should be cleaned and disinfected before transport
- ✓ Control of any access to swine by wild or domestic animals must be enacted. [75]

TREATMENT

Currently, there is no vaccine or drug available for humans or animals. The primary treatment is intensive supportive care for people suffering from severe respiratory and neurologic complications.

There is no effective treatment, but ribavarin may alleviate the symptoms of nausea, vomiting, and convulsions. Treatment is mostly focused on managing fever and the neurological symptoms. Severely ill individuals need to be hospitalized and may require the use of a ventilator. From contracting the disease to the onset of the symptoms, the incubation period ranges between 4 and 14 days. In some case, an incubation period of 45 days has also been reported. People are expected to make full recovery after surviving acute encephalitis. However, survivors have shown long-term neurological conditions like seizure disorder and personality changes. After recovery, a small number of people are seen to have relapsed or developed delayed onset encephalitis. [68, 70, 75]

Table 2 Brief summary of lab diagnostic tests developed in various laboratories

Sr. No.	Technique/product developed	Place	Reference
1.	Rapid immune plaque assay for the detection of Nipah viruses and anti-virus antibodies	CSIRO, Australia	[76]
2.	Solid-phase blocking ELISA for detection of antibodies to Nipah virus.	DVS, Malaysia	[77]
3.	Real-time RT-PCR (TaqMan)	Institute Pasteur, France	[78]
4.	MAb against formalin-inactivated NiV	National Institute of Animal Health Japan.	[79]
5.	Recombinant nucleocapsid protein produced in Escherichia coli	University Putra Malaysia	[80]
6.	MAb-based immunohistochemical diagnosis NiV	National Institute of Animal Health, Japan	[81]
7.	Recombinant glycoprotein produced in insect cells	University Putra Malaysia,	[82]
8.	Recombinant nucleocapsid protein produced in insect cells	University Putra Malaysia,	[83]
9.	Recombinant glycoprotein produced in E. coli	University Putra Malaysia	[84]
10.	Monoclonal antibodies against NiV (4 MAbs against ‘‘N’’ protein and 1 against ‘‘M’’ protein	NCFAD, Canada	[85]
11.	Indirect ELISA for the detection of Henipavirus antibodies based on a recombinant nucleocapsid protein expressed in E. coli	Chinese National Diagnostic Center for Exotic Animal Diseases,	[86]
12.	Indirect IgG ELISA for human and swine sera and an IgM capture-ELISA for human sera using the recombinant NiV-N protein as an antigen	Institute of Tropical Medicine, Japan	[87]
13.	Neutralization assays for differential Henipavirus serology using Bio-Plex Protein Array Systems	CSIRO, Australia	[88]
14.	Duplex nested RT-PCR for detection of Nipah virus RNA from urine specimens of bats	Chulalongkorn University Hospital, Thailand	[89]
15.	Monoclonal antibodies against the nucleocapsid proteins	Institute of Veterinary Sciences, China	[90]
16.	Neutralization test for specific detection of NiV antibodies using pseudotyped VSV	National Inst. Inf. Diseases, Japan	[91]
17.	Recombinant matrix protein produced in *E. coli*	University Putra Malaysia Malaysia	[92]
18.	Neutralization assay using VSV pseudotype particles expressing the F and G proteins of NiV (pVSV-NiV-F/G) as target antigens	CDC, Atlanta	[93]
19.	MAb based antigen capture ELISAs for virus detection and differentiation between NiV and HeV	CDC, Atlanta	[94]
20.	Antigen capture ELISA using polyclonal antibodies obtained by DNA immunization	National Institute Inf. Diseases, Japan	[95]
21.	Second generation of pseudotype-based serum neutralization assay for NiV antibodies	National Institute Infectious Diseases, Japan	[96]

CONCLUSION

Nipah virus (NiV) causes a recently discovered zoonotic disease endemic in South Asia, where sporadic outbreaks have been reported in Malaysia, Singapore, India, and Bangladesh. NiV infection in humans causes a range of clinical presentations, from asymptomatic infection (subclinical) to acute respiratory infection and fatal encephalitis. The case fatality rate is estimated at 40% to 75%. This rate can vary by outbreak depending on local capabilities for epidemiological surveillance and clinical management. NiV can be transmitted to humans from animals (i.e. bats or pigs), or contaminated foods and can also be transmitted directly from human-to-human. Fruit bats of the Pteropodidae family are the natural host of NiV. At present there is no antiviral drug available for NiV disease and the treatment is just supportive. There is no vaccine available for either people or animals. The primary treatment for humans is supportive care. The 2018 review of the WHO list of Blueprint priority diseases indicates that there is an urgent need for accelerated research and development for the Nipah virus.

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Corresponding Author: Dr. Gurjeet Singh
Assistant Professor, Department of Microbiology, N.C. Medical College and Hospital, Israna, Panipat-132107, Haryana, Indian
E-mail: gurjeetsingh360@gmail.com

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