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Review Article

Nipah Virus: A Hidden and Continuous Threat to Humankind

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Abstract
Nipah virus, a member of family Paramyxoviridae, genus Henipavirus causes acute and severe respiratory illness and encephalitis in humans. The primary source of infection is through infected pigs and bats. Virus was first isolated in 1999 post 1998 outbreak in Malaysia, where pigs were the primary source of infection. Unlike Malaysia, fruit bats of family Pteropodidae were the main reservoir in Bangladesh and India. Several outbreaks have been reported from Bangladesh and India in past 20 years. Most of the infections are associated with ingestion of date palm sap contaminated by bats and even human to human transmission is also known. Viral isolation, Nucleic acid amplification tests and serology are the main diagnostic methods. Several ELISA based tests are available for serological diagnosis. As so far no approved vaccine or effective antiviral drugs are available, the mainstays of management relies on preventive and supportive management.

Keywords: *Henipavirus, Pteropus bats, Encephalitis, Outbreak, ELISA, Vaccine.*

Introduction

Nipah virus a member of family *Paramyxoviridae* closely resembles Hendra virus, the two recognized spices of genus *Henipavirus.* The first reported outbreak occurred during September 1998 – April 1999 in Malaysia[(1)]. India has also witnessed few outbreaks during 2001 and 2007 in West Bengal and recently in Kozhikode district of Kerala (2018). The first viral isolation was from the Kampung Sungai Nipah (Nipah River Village) and therefore named as Nipah Virus (NiV)[(2)]. Nipah virus is mostly zoonotic and the main sources of infection are pigs and bats, though human to human transmission is also known. The incubation period is highly variable ranging from few days to months, with 90% within two weeks[3]. Initially the people develops influenza like symptoms like high grade fever, sore throat, headache, myalgia and weakness followed by impaired consciousness and spatial perception accompanied by nausea and vomiting suggestive of acute encephalitis[(3)]. The mortality rate during Malaysian outbreak was around 40% while during Bangladesh outbreak approached to more 70% which was due to more respiratory involvement [(4)].

Epidemiology

The first outbreak of Nipah virus occurred during 1998-1999 among the pig farm workers in the north west part of Malaysia[1,2]. Pig farming and agriculture have been directly implicated in the transmission of Nipah virus. Pigs by consuming the bat fed fruits became infected with Nipah virus, which eventually spread to the pig farm workers. During outbreak in Malaysia, out of 283 cases of viral encephalitis, 265 cases were identified to be acute Nipah encephalitis on the basis of laboratory investigations. Out of these 265 cases, 105 people lost their life accounting for 40% mortality. More than 80% of cases occurred in males and majority of them were directly involved in pig-farming[5]. Singapore witnessed Nipah virus outbreak in 1999 accounting for 11 cases with one death, probably because of importation of infected pigs from Malaysia. Various outbreaks because ofNipah Virus is summarized in **Table 1**[6,7].

Bangladesh is endemic for Nipah virus outbreaks, particularly in districts where date palm sap is produced. Transmission occurs by the consumption of raw date palm sap. *Pteropus* fruit bats, the reservoir of Nipah virus, visit the date palm and contaminates the sap by lickingand urinating into the collection pots[8]. In Bangladesh Nipah virus outbreak occurs almost every year with more 75% mortality rate. In India the disease was reported in humans without involvement of pigs. The two outbreaks that occurred in West Bengal during 2001 and 2007 in Siliguri and Nadia districts respectively accounted for more than 75% mortality. *Pteropus* species fruit bats crossing the border were the probable source of Nipah virus in these districts. Recently three deaths due to Nipah virus infection were reported on 19 May 2018 from Kozhikode District of Kerala and a fourth death of a health care worker who was involved in providing medical care to the deceased. Laboratory testing at National Institute of Virology, Pune confirmed positive for Nipah virus in three out of four deaths by RT-PCR and IgM ELISA for Nipah virus. Until 28 May 2018, 15 people have been tested positive for Nipah virus from Kozhikode and Malappuram districts of Kerala. Out of these 15 cases, thirteen already lost their life. This is the first reported outbreak from Kerala and third from all over the country[9].

Table 1: Nipah virus Outbreaks

Year	Country	Cases	Deaths	Case Fatality
1998	Malaysia	265	105	40%
1999	Singapore	11	1	9%
2001	Bangladesh	13	9	69%
2001	India	66	49	74%
2003	Bangladesh	12	8	67%
2004	Bangladesh	67	50	75%
2005	Bangladesh	12	11	92%
2007	Bangladesh	18	9	50%
2007	India	5	5	100%
2008	Bangladesh	11	9	82%
2009	Bangladesh	4	1	25%
2010	Bangladesh	16	14	88%
2011	Bangladesh	44	40	91%
2012	Bangladesh	12	10	83%
2013	Bangladesh	24	21	87%

The Virus

The Nipah virus is classified as:

Subfamily: Paramyxovirinae
Family: Paramyxoviridae
Genus: Henipavirus

The genus Henipavirus contains two most pathogenic viruses to humans namely Hendravirus and Nipah virus which were identified in 1994 and 1998 respectively[2]. Nipah virus is highly pathogenic and thus Biosafety Level-4 containment is required for dealing with live Nipah virus[10]. Nipah virus is closely related to Hendra virus, which caused lethal disease in horses and humans during 1994 in Australia. Morphologically, Nipah virus is similar to other paramyxoviruses with slightly larger genome. They are pleomorphic, spherical to filamentous with size ranging from 40 to 1900nm. Unlike other paramyxoviruses, Nipah virus lacks hemagglutinin and neuraminidase properties and contain a single layer of surface projections with an average length of 17nm[11].

The RNA genome consists of six genes N, P, M, F, G and L encoding for nucleoprotein,

phosphoprotein, matrix, fusion protein, glycoprotein and large RNA polymerase respectively[12]. Unlike the HN protein of other paramyxoviruses, G and F proteins of Nipah virus mediated viral entry into the host cells and antibodies produced against these protein neutralizes viral particles[13]. In addition to P protein, P gene also encodes for three other non-structural protein C, V and W, usually not required for viral replication but often serves as virulence factors[14].

There are two major genetic lineage, Nipah virus-Malaysia (NiV-MY) with 18,246 nucleotides and Nipah virus- Bangladesh (NiV-BD) with 18,252 nucleotides genome length[11]. Functionally, the two strains are indistinguishable, but animal model studies have suggested certain differences in these strains. African green monkey model indicated that NiV-BD is more pathogenic with narrower window for passive antibody therapy than NiV-MY[15]. Similar study using ferret model shown increased oral shedding with more rapid onset and higher levels of viral replication in the respiratory tract of NiV-BD than NiV-MY[16,17]. These properties of NiV-BD explained the shorter incubation, more respiratory symptoms, human to human transmission and higher case fatality in cases from Bangladesh and India.

Transmission

Members of paramyxoviruses are known to have a limited number of host range with rare interspecies transmission. Unlike other paramyxoviruses, Nipah virus uses highly conserved mammalian ephrinB2/B3 molecules as their entry receptors, thus displaying a wide species tropism along with interspecies transmission[18,19]. Fruit bats commonly known as flying foxes member of family *Pteropodidae* have been identified as the main reservoir for Nipahvirus. Bats primarily shed NiV via urinary route and capable of infecting humans directly or through pigs and horses as intermediate amplifier host[20].

During Malaysia outbreak, bats were assumed to introduce virus into the swine population by shedding viruses in their urine and saliva. Pigs while consuming fruits contaminated by bats acquired the viruses and then transmitted to the pig-farm workers. This was confirmed by the serological survey during 1998-99 outbreak of *Pteropus* bats, demonstrating positive antibodies for Nipah virus [21].

Two outbreaks in India and several outbreaks in Bangladesh between 2001 to 2013 did not show any involvement of pigs. Outbreak investigations in Bangladesh identified another routes of viral transmission which included climbing tree, consumption of raw date palm sap and contact with sick person or animals[22]

Pteropidusgiganteus (fruit bats) drinks the sap from the collecting pots at night and even contaminates the pot through their urine. Consumption of contaminated raw date palm sap transmits the virus to humans[23]. In India, the presence of Nipah virus RNA was detected from the liver homogenate of *P. giganteus* captured from Myanaguri, West Bengal[24]. In Siliguri, India, 75% of cases occurred among hospital staffs and visitors, strongly suggestive of human to human transmission within a health care setting[25]. Similarly approximately half of the patients in Bangladesh between 2001-2007 developed their disease following human to human transmission [20].

Pathogenesis

The incubation period of Nipah virus is highly variable from days to months, with more than 90% at 2 weeks or less[3]. Patients commonly presents with highgrade fever, dizziness, headache, vomiting with gradual development of severe encephalitis. Majority of them develops reduced level of consciousness and signs of brain stem dysfunctions in form of abnormal pupillary reflex, vasomotor changes, seizures and myoclonic jerks[3]. Respiratory involvement was rare during Malaysian outbreak, while two thirds cases from Bangladesh and India had respiratory

involvement and few of them even developed acute respiratory distress syndrome. These differences was may be because of two different strains of Nipah virus as discussed earlier.

Respiratory Infection: In humans, Nipah virus can be detected in the bronchial epithelium and are shed mainly in nasopharyngeal and tracheal secretions during the early phase of disease[26]. This accounts for the human to human transmission during the early phase of illness. Nipah virus leads to recruitment of immune cells by induction of inflammatory cytokines that can progress to an Acute Respiratory Distress Syndrome like disease[27].

Viremia: Viremia usually develops late in disease when virus replicating in respiratory epithelium gain access to circulation and disseminate throughout the body leading to multi organ failure [28].

CNS Infection: Nipah virus in human induces expression of pro-inflammatory cytokines (TNF-α and IL-1β) which have been shown to increase blood brain barrier (BBB) permeability in addition to neural injury and death in animal models[27]. Disruption of BBB is by direct cytopathic effect of viral replication or indirect effect of TNF-α and IL-1β expression is still doubtful. Several animal model experiments have shown direct entry of Nipah virus into CNS through the olfactory nerve. Nipah virus infects neurons through the cribriform plate extends into olfactory bulb and from there directly into CNS[29].

Autopsy Findings: Pathological lesions were seen mainly in brain with disseminated microinfarction due to vasculitis induced thrombosis and direct neuronal involvement amongst victims from Malaysian outbreak. Similar vasculitis lesions were also seen in other organs like respiratory tract, heart and kidneys. Vasculitis in Nipah virus infection commonly involved small and medium sized vessels resulting into endothelial multinucleated syncytia formation and fibrinoid necrosis[30].

Diagnosis

Nipah virus is highly pathogenic and thus for isolation and propagation, Biosafety Level-4 containment is needed[10]. It is a potential agent for bioterrorism and is listed as a category C agent by the Centers for Disease Control and Prevention [31]. Nipah virus infection can be diagnosed by various methods:

i. Viral Isolation
ii. Serology
iii. Molecular

Viral Isolation: Viral isolation can be performed using African green monkey kidney (Vero) and Rabbit kidney (RK-13) cell lines (32). Viral growth is indicated by the appearance of cytopathic effects (CPE) within 3 days in form of large multinucleated syncytia formation containing viral antigen. Additional two 5-days passages are recommended if no CPE develops to confirm negative for Nipah virus. To characterize viral isolation and to look for cross reactivity within Henipaviruses, immunostaining and virus neutralization tests like plaque reduction, microtitre neutralization and immunoplaque assay are applied[32].

Serology

Antigen Detection: Monoclonal antibody based antigen capture ELISA.

Polyclonal antibodies derived from rabbit by injecting NiV-G protein was used for development of antigen capture sandwich ELISA.

Antibody Detection: ELISAs are the most common serological assay. Infected cell lysate antigen coated ELISAs are used to demonstrate circulating IgM/IgG Antibodies.

Molecular

RT-PCR: Reverse Transcriptase Polymerase chain reaction

Real Time RT-PCR

Duplex Nested RT-PCR

Confirmed by the sequencing of the amplified products.

In Fatal cases, post autopsy immunohisto-chemistry is performed to confirm a diagnosis.

In India, NIV, Pune has got the preparedness for the diagnosis of Nipah virus whenever a suspected event occurs in the country.

Treatment and Prevention

The treatment options in form of antiviral drugs are limited. Though Ribavirin has been shown to be effective in vitro but their trials in humantill date is inconclusive and clinical usefulness remains uncertain[33]. In ferret model passive immunization, using Human monoclonal antibody against Nipah-G glycoprotein has been found to be effective[(33)].

Thus preventive strategies are the mainstay of controlling Nipah virus infection. Important preventive measures includes:

a) Preventing farm animals from acquiring Nipah virus by eating fruits contaminated by bats.
b) Avoid overcrowding of farm animals to prevent rapid spread of disease and animals should not be kept near fruit trees that attracts fruit bats.
c) Avoid unnecessary contact with sick animals.
d) Avoid consumption of raw date palm sap.
e) Use of physical barriers to prevent bats from accessing and contaminating sap.
f) Use of proper physical barrier protection while handling a suspected case of Nipah virus.

Vaccines

In several pre-clinical studies, number of vaccine candidates have been found to be capable of providing complete protection against Nipah virus in small animal and non-human primate models. Protection was demonstrated in hamster, ferret and African green monkey using a Vesicular stomatitis virus candidate vaccines[(34)].

Hendra G protein subunit vaccine producing cross-protection against Hendra virus and Nipah virus has been used recently in Australia to protect horses against Hendra virus and offers great potential for protection in humans against other Henipaviruses[(33)].

Vaccination should also be extended to cover farm animals especially pigs in areas where Nipah virus is endemic.

WHO has declared Nipah virus to be a priority pathogen, and pharmaceutical companies may be funded to carry out trials in underdeveloped countries where affording medication and vaccination is a troublesome task. Coalition for Epidemic Preparedness Innovations (CEPI) an International coalition of governments and pharmaceutical companies was formed in January 2017 to develop safe, effective and affordable vaccines for diseases with pandemic potential including Nipah virus[(30)].

Conclusion

The emergence of new virus called Nipah virus twenty years ago with potential to cause severe fatal neurological and respiratory complications leading to death both in humans and animals, and it continues to be like a hidden threat to re-emerge. Several outbreaks in past twenty years especially in Bangladesh and India had led to severe fatal outcome. *Pteropus* bats, which is widespread beyond these endemic regions constitutes a potential threat for outbreaks to occur in new regions.

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