

Recent Advancement and Novel Approaches of Nipah Virus: An Overview

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ABSTRACT

Since the Nipah Virus (NiV) first appeared in Malaysia and Singapore in 1998-99, the NiV virus has reappeared several times, infecting people critically and having a high fatality rate. It is categorized as a Biosafety Level-4 (BSL-4) pathogen because of its extreme pathogenicity and the scarcity of vaccinations and medicines. It is well recognized that fruit bats (Genus Pteropus) constitute a natural host and NiV reservoir. The virus spread from pigs to humans after infecting pigs. The primary means of NiV transmission are direct interactions or animal bodily fluids. Nipah Virus (NiV) is a recently identified zoonotic paramyxovirus that causes neurological and respiratory disorders in humans. In humans, the virus can incubate for two weeks to two months. High fever, headache, nausea, and vomiting are common signs of severe NiV encephalitis, as are aberrant eye reflexes, vasomotor abnormalities, seizures, and myoclonic jerks, which are signs of brainstem dysfunction. Many diagnosis techniques for NiV infection include serological, molecular, virological, and ELISA. Research is going on antivirals and vaccines that will aid in preventing and controlling outbreak situations in the future.

Keywords: Nipah virus, Paramyxovirus, Virology, Phosphoprotein, Encephalitis.

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INTRODUCTION

From September 1998 to May 1999, Malaysia and Singapore were the locations of the first Nipah outbreak. The virus was known as "Nipah" after the Malaysian village where it was discovered for the first time in a patient. This outbreak resulted in 105 fatalities and more than 276 cases of acute encephalitis.¹ It is rare for JE that adult male made up the majority of the cases in this outbreak rather than children.² Malaysia, Bangladesh, Singapore, and India have all recorded cases of the bat-borne Nipah Virus (NiV), which can cause fatal encephalitis in people.³⁻⁵ It belongs to the Mononegavirales order, which is home to other feared pathogens including Hendra, Ebola, and Marburg. It is one of the deadly zoonotic viruses that have been found. Fruit bats from the genus Pteropus are thought to be the virus's natural reservoir.^{6,7} Nipah Virus (NiV) is a single-stranded negative-sense RNA genome of the encapsulated, pleomorphic Nipah virus measures between 40 and 1900 nanometers.^{8,9} Similar morphological structure patterns can be seen in NiV and other Paramyxoviridae members under an electron microscope. NiV is included in the family Paramyxoviridae's genus Henipavirus along with HeV. In the protein-coded sections and non-translated regions, respectively,

NiV demonstrated 68% to 92% and 40% to 67% similarity with HeV.⁹⁻¹¹ The Nucleocapsid (N), Phosphoprotein (P), Matrix protein (M), Fusion protein (F), Glycoprotein (G), and RNA polymerase (L) are the six structural proteins that comprise the genome of the virus. Together with the viral RNA, the N, P, and L form the ribonucleoprotein complex, an important complex that regulates transcription and the synthesis of viral RNA. The envelope-spanning F and G proteins control attachment and entry into the host cell.^{12,13}

Epidemiology

Fruit bats of the species Pteropus, sometimes known as flying foxes, are the natural reservoirs for NiV. They mainly live in places near farms and orchards and consume fruits and nectar, which lowers the barrier to viral spread. The native to tropical and subtropical regions of East Africa, Australia, Asia, and some oceanic islands, bats have been found to be associated with NiV epidemics that are reported globally.^{6,7} Although bats are asymptomatic carriers of NiV, they do transmit the viruses in their saliva, urine, semen, and excreta.⁷⁻¹⁴ During the serological monitoring research, a few fruit bat populations were discovered to be positive for NiV-neutralizing antibodies in Cambodia,¹⁵ Thailand,¹⁶ Madagascar,¹⁷ and Ghana.¹⁸ The three main ways that the virus spreads are through the consumption of fruit contaminated with NiV, direct contact with bodily fluids from infected humans, or contact with the excretions or secretions of infected animals.^{1,19}



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Mode of Transmission NiV

Human infection was caused by NiV-infected pigs, the virus's intermediate hosts in Malaysia. Pigs contracting NiV from bats is primarily caused by fruits that the bats have partially eaten or contaminated. Direct contact with the sick pigs was necessary for the virus to spread from pig to human, and fomites, aerosols, or direct touch with humans might also cause the virus to spread.^{20,21} Butcher staff frequently come into contact with the excretions and secretions of infected pigs, such as their urine, saliva, pharyngeal secretions, and respiratory secretions, as well as raw pig meat and other products contaminated with the NiV.²¹ Aerosol transmission of NiV to humans is also thought to be a crucial respiratory route of infection as pigs had considerable respiratory discomfort.²² The importation of pigs from Malaysia to Singapore resulted in infection among pig farmers living close to the pig sites.²³ A small number of affected people swallowed raw palm sap approximately 30 days before the illness appeared, according to a Bangladeshi investigation, suggesting that the sap was contaminated with bat secretions infected with NiV.²⁴ The epidemiological similarities between the outbreaks in West Bengal and Kerala, India, and Bangladesh pertain to the non-transmissible transmission of the NiV virus between individuals.²⁵

The structure of Nipah virus is shown in Figure 1.²⁶

Etiology

Members of the Henipavirus genus, Paramyxovirinae subfamily, Paramyxoviridae family, and Mononegavirales order include the paramyxovirus known as the Nipah virus. NiV is the name of an enclosed, single-stranded, negative sense RNA virus. The reservoir host for NiV is the *Pteropus* fruit bat, and the virus has an 18-hr half-life in the urine of these bats.¹⁹ The three major ways that NiV spreads are through the consumption of contaminated food, contact with bodily fluids from infected humans or animals, and exposure to droplets or aerosols. One of the risk factors for catching the virus is being in close proximity to an infected person. Polluted food includes fruits and date palm sap that has been polluted by the bat's bodily secretions.²⁷ In Bangladesh, there were a total of 8 documented cases of NiV infection in 2023, of which five patients passed away. Recently, a 35-year-old woman from Bangladesh passed away as a result of the infection. According to the Institute of Epidemiology and Disease Control, someone died within three days of consuming date juice. He grew ill and passed out, and it was determined that he had NiV after he was transported to a hospital. A centre for NiV has always been Bangladesh. It has experienced NiV case outbreaks virtually every year since its first case in 2001, and as of this writing, there have been 331 cases with 236 fatalities (71.3% mortality rate). Nearly 40% of all NiV cases worldwide are also shared by it.²⁸ In reaction to a recurrence of the potentially fatal Nipah virus, the Indian state of Kerala stopped schools, offices, and transportation in the Kozhikode area. The decision

on September 13 was made as a preventative measure against the virus's spread, which has already resulted in two fatalities and six confirmed cases. Testing at the National Institute of Virology in Pune established that the Nipah virus was to blame for the death of a 49-year-old man in Kerala on August 30. On September 11, a 40-year-old man who was the state's second victim passed away.²⁹ West Bengal outbreaks caused 66 cases, 45 fatalities, and five cases of 100% mortality in India in 2001 and 2007, respectively. Kerala in the South has seen an increase in cases from India in recent years; Kerala saw the first epidemic in 2018, which resulted in 18 cases and 17 fatalities.^{6,30}

Pathophysiology

The main cause of encephalitis in those who are affected is the Nipah virus. Additionally, this virus has been associated with respiratory diseases.³³ During the Nipah virus, patients frequently reported with fever and altered mental status or decreased consciousness.³ With time, the disease's neurological characteristics worsened and eventually transformed into a coma, which in fatal situations causes death. The majority of instances indicate a need for mechanical ventilation. When suffering from both acute and chronic illnesses, the brain develops localised lesions, primarily in the subcortical and deep white matter of the cerebral hemisphere.^{3,34,35} Patients' lungs, spleen, and central nervous system all showed some histological changes.² The results of autopsies revealed vasculitis in capillaries and tiny blood vessels. Segmental endothelial extermination, karyorrhexis, and mural necrosis were used to define the vascularity's that were found. The vascular system of the grey and white matter showed signs of damage. In addition to vasculitis, the pulmonary system frequently displayed aspiration pneumonia, pulmonary oedema, and alveolar haemorrhage. Spleen vasculitis were not seen, however a reduction in white pulp and acute necrotizing inflammation were seen.³⁶

Clinical Presentation

In humans, the virus can incubate for two weeks to two months. Seizures, aberrant eye reflexes, vasomotor abnormalities, high fever, headache, nausea, and vomiting are all common indicators of severe NiV encephalitis. Myoclonic jerks are a sign of brainstem dysfunction.³ The most common neurological symptoms in affected people are aseptic meningitis, severe encephalitis, and localised brainstem involvement, which are frequently accompanied by cerebellar symptoms. Psychological symptoms like sadness, personality disorders, and trouble speaking and attention might also appear in certain people. Relapses and late-onset encephalitis that might last months or years after the first acute sickness are possible for NiV patients in some circumstances.^{37,38} Compared to outbreaks that occurred in Bangladesh and India, segmental myoclonus, which is characterised by rhythmic involuntary contractions of muscle groups supplied by adjacent segments of the brainstem or spine,

NiV outbreaks in chronological order. Mortality and morbidity caused by the Nipah virus in various parts of the world.^{31,32}

Years	Country	No of Cases	Deaths	Mortality Rate (%)
1998	Malaysia	265	105	40
1999	Singapore	11	01	09
2001	Bangladesh	13	09	69
2001	India	66	45	68
2003	Bangladesh	12	08	67
2004	Bangladesh	67	50	75
2005	Bangladesh	12	11	92
2007	Bangladesh	18	09	50
2007	India	05	05	100
2008	Bangladesh	11	07	64
2009	Bangladesh	04	01	25
2010	Bangladesh	18	16	89
2012	Bangladesh	43	37	86
2013	Bangladesh	31	25	81
2014	Bangladesh	37	16	43
2014	Philippines	17	09	53
2015	Bangladesh	15	11	73
2017	Bangladesh	03	02	67
2018	Bangladesh	04	03	75
2018	India	18	17	94
2019	Bangladesh	08	07	88
2019	India	01	00	00
2020	Bangladesh	07	05	71
2021	Bangladesh	02	00	00
2021	India	01	01	100
2022	Bangladesh	03	02	67
2023	Bangladesh	11	08	73

was more prevalent in Malaysia. About one-third of NiV survivors have demonstrated neurological and cognitive problems. They almost all suffered from chronic fatigue syndrome and more than half of them showed behavioural and neuropsychiatric changes similar to those seen in Malaysia and Singapore.³⁹

Diagnosis

Numerous etiological diagnosis methods for NiV infection include Immunohistochemistry (IHC) testing, molecular, virological, and serological methods.

Enzyme-Linked Immunosorbent Assay (ELISA)

There are several techniques for determining the aetiology of NiV infection, including serological, molecular, virological, and immune Enzyme-Linked Immunosorbent Assay (ELISA), which is used to both find the NiV antigen and evaluate the antibody response. It is an easy and cheap way to check samples that seem

suspicious.¹⁹ This serological test makes use of several methods, including: In ELISA-capture, monoclonal antibodies are used to detect NiV and differentiate it from HeV⁴⁰ variations or even from the recombinant N protein-using NiV variant.⁴¹ Also discussed is the creation of indirect ELISAs for IgG and IgM to screen porcine and human serum and detect seroconversion in bats.^{22,42,43} Another variation of the technique uses rabbit polyclonal antibodies against the NiV G protein in a sandwich ELISA.⁴⁴ In India (High Security Animal Disease Laboratory [HSADL], Bhopal, India), one of the screening procedures for suids was created using a recombinant protein N.¹⁹

Virus Neutralization Test (VNT)

Which was created immediately after the outbreak in Malaysia, was used as the standard serological test. The traditional NiV VN test frequently employs Vero cells, and it is thought that the suppression of the cytopathic impact by the serum being tested

represents a positive neutralization. Additionally, plate VN tests have been developed.⁴⁵ In order to establish the neutralization test, a pseudo-type vesicular stomatitis virus with NiV envelope proteins was also used. This allowed it to be neutralized by a serum containing specific antibodies.⁴⁶

Molecular biology methods

The most sensitive and specific approach is PCR, and this is how the neutralisation test was created using a pseudo-type vesicular stomatitis virus that contains NiV. A common target of RT-PCR and nested-PCR is the viral N, M, and P sequences.⁴⁷ Phylogenetic investigations greatly benefit from the use of NiV-targeted PCR, as well.^{43,48} The gold standard for NiV detection from various biological samples is RT-PCR (and its derivatives). In 2004, the N gene sequence was used to construct RT-PCR for the NiV. NiV RNA was detectable in blood samples from infected hamsters, whereas HeV was not, suggesting that the test had high specificity.⁴⁹

Viral isolation

When the NiV is suspected in early instances and fresh outbreaks, it is highly helpful. Brain, lung, kidney, and/or spleen are examples. Vero cells are a perfect substrate for NiV growth, and the cytopathic effect typically manifests after three days of culture in the form of characteristic syncytia and plaques in the cell monolayer.⁴⁷ The culture supernatant is subjected to immunostaining, Seroneutralization (SN), and PCR as the following step in the virus identification process. The NiV's

structure can be determined using electron microscopy, and virus-antibody interactions can be found using immunological electro-microscopy.^{42,48}

Immunohistochemistry (IHC)

Formalin-fixed tissues were used to stain the heart, kidney, spleen, lung, lymph nodes, and central nervous system in order to search for viral antigens. Flogosis, necrosis, and vasculitis, NiV-associated lesions, can be seen in tissue sections.⁴³

Prevention Approaches

Fruit bats (flying foxes), which are particularly susceptible to the NiV infection, can transmit it to people.⁵⁰ Periodically, it is important to make sure that the general people, medical experts, and government authorities are aware of neglected and related illnesses and are ready to contain any future breakout in areas with a history of outbreaks. Research aimed at better understanding the ecology of bats, their susceptibility to becoming NiV carriers, and the precise prevalence of these infections could help mitigate the risk posed by human intervention in their habitat. By collecting samples and conducting sero-surveillance for the NiV antibody and NiV in humans and bats using ELISA and PCR techniques, it will be feasible to stop an epidemic in the areas where it is most prevalent.⁵¹ By reducing the risk of intimate contact and bodily fluid exposure between patients and attendants, outbreaks like the one in Kerala could be avoided. Only people wearing masks and gloves should handle food products, mats and sheets, clothes, and any type of patient aid. The caretaker's/attendant's gloves, mask,

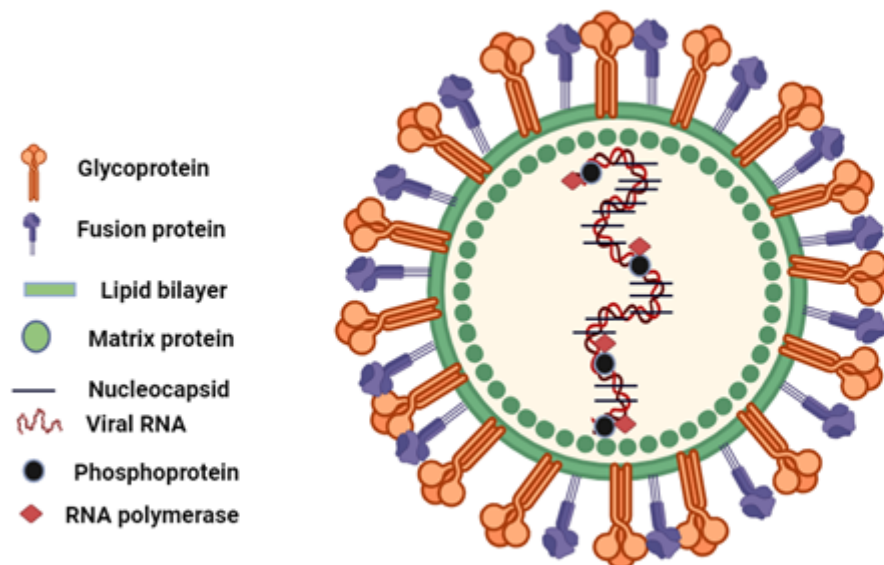
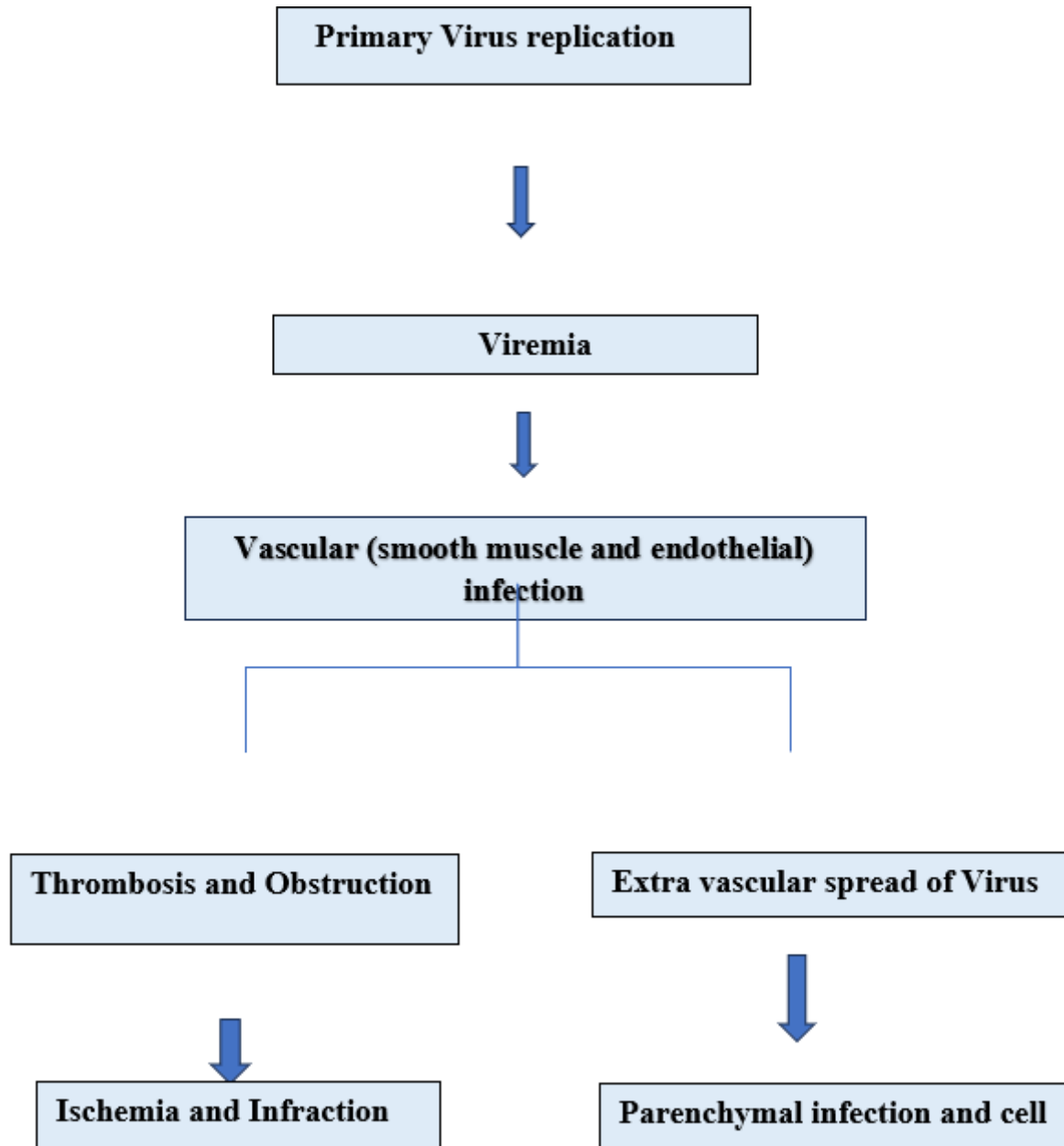


Figure 1: Structure of Nipah virus.



Pathophysiology of Nipah Virus.

and patient aids should all be properly incinerated. To prevent the disease and its nosocomial spread, medical personnel treating encephalitic patients exhibiting NiV symptoms should wear the appropriate Personal Protective Equipment (PPE) and be placed in isolation from the public as soon as possible. Using an incinerator to burn the body is the best course of action.⁵² Purchasing and consuming unwashed raw fruits and products in areas where the NiV virus is widespread should be strictly prohibited. The consumption of raw fruits bitten by bats is considered to be one of the numerous causes of the recent outbreak in Kerala. This is similar to the ingestion of raw date palm sap that caused recurrent outbreaks in Bangladesh and India.²⁴

Treatment

Infection prevention strategies should be used while isolating patients who have NiV infections. The focus of treatment is mostly on supportive. Antiviral ribavirin is effective against paramyxoviruses such as respiratory syncytial virus. Its effectiveness has been disputed, with contradicting studies claiming there has been no change in mortality rates. But in cases of Nipah virus infections, the Indian National Centre for Disease Control advises using ribavirin.^{3,53,54} As prospective treatments, acyclovir, chloroquine, and ephrin-B2 are being considered.^{23,55,56} Favipiravir, which is approved for the treatment of influenza in Japan, was proven successful in hamsters.⁵⁷ It has been proved that ferrets

Medication, vaccines under development for NiV.

Channel	Targeted antigen	Schedule of vaccination	Dose	Animal models	References
VLPs	NiV F, G and M	IM 30 g VLP. Boost after 21 and 42 days OR IM 30 g VLP.	IP 1.6×10 ⁴ PFU NiVM OR IP 3.3×10 ⁴ NiVM	Hamster	60
Venezuelan equine encephalitis virus replicon particles.	NiV F or G	Footpad inoculation 3.1×10 ⁵ infectious units. Boost after 5 and 18 weeks.		Mice	61
Recombinant subunit.	NiV/HeVsG	SC 4, 20 or 100 g HeVsG. Boost after 20 days SC 100 g NiVsG or HeVsG. Boost after 2 and 4 weeks.	ON 5×10 ³ TCID50 NIVB SC 5×10 ² /5×10 ³ TCID50 NiVM	Ferret	62
Recombinant measles virus.	NiV G	IP 2×10 ⁴ TCID50 NiV G for hamster. Boost after 21 days. SC 1×10 ⁵ TCID50 for NiV G for AGM.	IP103 TCID50 NiVM IP105 TCID50 NiVM	Hamster African Green Monkey	63
Recombinant adeno associated virus.	NiV G	IM (2.1010 infectious particles) D (1.1010) for mice; IM (6.1011) for hamster.	IP104 PFU NiVM	BALB/c mice Hamster	63
Recombinant VSV. Recombinant canarypox virus.	NiV F and/or G, or N NiV F and/or G	IM 1×10 ⁷ PFU NiVB F, G or F and G IM 1×10 ⁶ infectious particles NiVMF IM 108 PFU NiV F.	ON 5×10 ³ PFU NiVM IP 105 TCID50 NiVM ON 2.5×10 ⁵ PFU NiVM	Ferret Hamster Pig	62 64
Recombinant vaccinia virus.	NiV F and/or G	SC 107 NiV F.	IP 1×10 ³ PFU NiVM	Hamster	65
Polyclonal serum.	NiV F and/or G	IV 0.2 mL of antiserum.	IP 1×10 ³ PFU NiVM	Hamster	41,66
m102.4 Human monoclonal antibody.	HeV G/NiV	IV 15 mg/kg, 1, 3 or 5 days post challenge, then again after 2 days IV 50 mg (24 hr before) Pre challenge dose (10 hr after) post challenge dose.	ON 5×10 ³ TCID50 NiVM ON 5×10 ³ TCID50 NiVM	Ferret African Green Monkey	40,67

and non-human primates benefit from the human monoclonal antibody.^{58,59} Only when a throat swab has undergone a negative RT-PCR test may patients be allowed to leave the hospital. After the infection has been confirmed, individuals who have been discharged are kept in isolation for 21 days.⁵³

Novel approaches for NiV medication discovery.

A brand-new strategy called the "Drug-target-drug network-based approach" was proposed in 2021.⁶⁸ Utilising

drug-target-drug network analysis, the Nipah virus and thirteen other viruses were examined using the current US FDA-approved medications. The computer study was used to evaluate US FDA-approved medications using a confidence score as a repurposing theory. Between the Hepatitis E Virus (HEV) and NiV, sixteen medications have been repurposed. Additionally, molecular docking is used to validate this method and identify the best candidates for the specific viruses. Rajput *et al.* provided the "anti-Nipah" web source in 2019. 313 compounds that were

part of the data bank were used to forecast the inhibitory effect using the QSAR model.⁶⁹

CONCLUSION

NiV is a highly fatal virus with a low transmission rate; because of these traits, it is categorized as BSL-4.⁷⁰ During the past 20 years, outbreaks of NiV have been documented in a number of nations, including Bangladesh, Malaysia, and Singapore. Kerala, India, has reported NiV outbreaks most recently. Because of the high death and rapid spread of NiV infection, these outbreaks have posed a serious threat to the public health and economies of the affected countries. There is now little hope for a potential NiV breakout.

There are currently no human-useable pharmaceuticals approved by the US FDA, and some medications have demonstrated good viral suppression *in vitro* but not in animals. Favipiravir, when given subcutaneously twice a day or once a day for 14 days, gave mice challenged with a lethal dosage of NiV total protection, according to new research using the Syrian hamster model. Small molecule medication favipiravir has been successfully used to treat henipavirus infections *in vivo* for the first time, indicating that this therapy approach warrants more research due to its antiviral properties.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NiV: Nipah virus; **BSL-4:** Biosafety level-4; **HeV:** Hepatitis E virus; **HSADL:** High security animal disease laboratory; **SN:** Seroneutralization; **PPE:** Personal protective equipment.

AUTHORS' CONTRIBUTIONS

Shiv Dhananjay collected and organized the main manuscript text and prepared figures. Romi kumari provided helped to write article. Dr. Ranjeet Kumar given concept, review and revised article. All authors have critically reviewed and approved the article and are responsible for the context and similarity index of the manuscript.

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