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Genomic Surveillance of Nipah Virus: Tracking Evolution and Transmission Pathways"

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ABSTRACT

The Nipah virus has had many devastating reappearances after its first 1998–1999 appearance in Singapore and Malaysia, infecting humans severely and resulting in a high mortality rate. This disease is classified as a Biosafety Level-4 (BSL-4) due to its high level of pathogenicity and the limited availability of vaccines and treatments. The fruit bat, or Pteropus, is known to be a natural host and reservoir for the NiV virus. After infecting pigs, the virus made its way to people. Direct contact or the transfer of body fluids from infected animals are the main vectors for the spread of NiV. A newly discovered zoonotic paramyxovirus, Nipah Virus (NiV) causes respiratory and neurological problems in people. The incubation period for the virus in humans may range from two weeks to two months. Severe NiV encephalitis is characterized by a high temperature, headache, nausea, and vomiting. Other symptoms include anomalies in the brainstem, seizures, aberrant eye reflexes, and vasomotor abnormalities. A wide variety of methods, such as ELISA, virology, molecular biology, and serology, may be used to diagnose NiV infection. In order to better manage and prevent future outbreaks, research is underway on vaccinations and antivirals.

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

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INTRODUCTION

First reported cases of Nipah occurred in Malaysia and Singapore between September 1998 and May 1999. A patient in a Malaysian hamlet was the first to have the virus, thus the name "Nipah" for short. Over 276 cases of acute encephalitis and 105 deaths were reported as a consequence of this epidemic. 1 The fact that adult males, rather than youngsters, constituted the bulk of the patients in this epidemic of JE is rather unusual. 2 The Nipah Virus (NiV), which is carried by bats and may cause deadly encephalitis in humans, has been detected in four countries: India, Bangladesh, Singapore, and Malaysia. 3-5 It is a member of the Mononegavirales order, which includes Marburg, Hendra, and Ebola, among other terrifying diseases. There are many dangerous zoonotic viruses, and this one is among them. The virus is believed to naturally reside in fruit bats belonging to the genus Pteropus. 6,7 The encapsulated, pleomorphic Nipah virus (NiV) has a genome that ranges in size from 40 to 1900 nanometers and is composed of a single-stranded negative-sense RNA. 7,8, 9 When examining NiV and other members of the Paramyxoviridae family under an electron microscope, one may see comparable morphological structural patterns. NiV and HeV are both members of the genus Henipavirus, which belongs in the family Paramyxoviridaea. Compared to HeV, NiV was 68% to 92% identical in the protein-coded parts and 40% to 67% similar in the non-translated areas. 9-11 The viral genome is composed of six structural proteins: the nucleocapsid, phosphoprotein, matrix, fusion, glycoprotein, and RNA polymerase. A crucial complex that controls transcription and viral RNA production, the

ribonucleoprotein complex is formed by the N, P, and L amino acids in addition to the viral RNA. F and G proteins regulate adhesion and host cell entrance; they transcend the cell membrane. 12, 13

Epidemiology

Natural reservoirs for NiV include fruit bats of the genus Pteropus, which is also called flying foxes. Because of their diet of fruits and nectar, which is abundant in areas near farms and orchards, they help to transmit viruses more easily. The worldwide occurrence of NiV outbreaks has been linked to bats, which are endemic to tropical and subtropical areas of East Africa, Australia, Asia, and some maritime islands. 6,7 Bats may not show any symptoms of NiV infection, yet they may still spread the virus via their bodily fluids. 7-14 A small number of fruit bat populations in Cambodia,15 Thailand,16 Madagascar,17, and Ghana were positive for NiV-neutralizing antibodies during the serological surveillance investigation. 18 Infected individuals may transmit the virus by direct touch with their body fluids, infected animals can transmit the virus through their excretions or secretions, and contaminated fruit can be consumed. 1,19 times

Mode of Transmission NiV

In Malaysia, the virus's intermediate hosts—pigs infected with NiV—were the ones who infected humans. The most common way for bats to transmit NiV to pigs is via infected or partly chewed fruits. The virus could only pass from infected pigs to people via close physical contact; other potential vectors include fomites, aerosols, or even just coming into close proximity to infected humans. 20,21 Raw pig flesh and other items infected with the NiV are common sources of contamination for butcher workers, who often come into touch with the fluids and excretions of infected pigs. These include saliva, pharyngeal secretions, respiratory secretions, and urine. 21 In addition to the respiratory distress that pigs experienced, the aerosol transmission of NiV to humans is considered a critical infection pathway. 22 Pig farmers in close proximity to pig sites in Singapore were infected after pigs were imported from Malaysia. 23 A Bangladeshi inquiry found that some of the afflicted individuals had consumed raw palm sap about 30 days before to the onset of the sickness, which may have been tainted with bat secretions infected with the NiV virus. 24 Similarities in the epidemiology of the NiV virus's non-transmissive transmission between people have been observed in the outbreaks in West Bengal, Kerala, India, and Bangladesh.

Etiology

The paramyxovirus genus Henipa, subfamily Paramyxovirinae, family Paramyxoviridae, and order Mononegavirales includes the Nipah virus. RNA viruses with a single strand and a negative sense are known as NiV. With a half-life of 18 hours in bat urine, the Pteropus fruit bat is NiV's reservoir host. 19 Food contamination, direct contact with infected blood or other body fluids, and breathing in infected droplets or aerosols are the three main vectors for the transmission of NiV. Being in close quarters with an infected individual is one way to get the virus. The bat's biological fluids contaminate fruits and date palm sap, among other foods. 27 There were eight confirmed instances of NiV infection in Bangladesh in 2023, with five people succumbing to the virus. The virus recently claimed the life of a 35-year-old lady from Bangladesh. The Institute of Epidemiology and Disease Control reports that date juice may cause death in as little as three days. He became sick and blacked out; upon arrival at the hospital, it was found that he had NiV. In terms of NiV, Bangladesh has always been a hub. Since the first case in 2001, there have been outbreaks of NiV almost annually; there have been 331 cases so far, with 236 deaths (a mortality rate of 71.3%). Additionally, it is shared by about 40% of all NiV patients globally. 28 Schools, workplaces, and transportation in the Kozhikode region were closed by the Indian state of Kerala in response to a resurgence of the potentially lethal Nipah virus. The choice The decision was taken on September 13 to stop the spread of the virus, which has already killed two people and caused six cases to be verified. A 49-year-old man from Kerala passed away on August 30th, and tests conducted at Pune's National Institute of Virology proved that the Nipah virus was the culprit. The state lost its second casualty, a man in his 40s, on September 11. In 2001, 29 outbreaks in West Bengal resulted in 66 illnesses and 45 deaths; in 2007, five cases were deadly. There has been a rise in the number of cases reported from India in the southern state of Kerala; the first pandemic in Kerala occurred in 2018, with 18 confirmed cases and 17 deaths. 6,30

Pathophysiology

The Nipah virus is the primary aggravating factor in cases of encephalitis. There is evidence that this virus may cause respiratory illnesses. 33 Fever, altered mental state, and reduced awareness were common symptoms described by Nipah virus patients. 3 Over time, the neurological symptoms of the condition progressed to the point that they became life-threatening comas, which ultimately led to death. In most cases, mechanical ventilation is necessary. Acute and chronic brain

diseases cause localized lesions to form, most often in the cerebral hemisphere's subcortical and deep white matter. 3,34,35 dollars Histological alterations were seen in the spleen, central nervous system, lungs, and spleen. 2 The findings of the autopsies showed that there was vasculitis in the small blood vessels and capillaries. Vascularities were identified by karyorrhexis, mural necrosis, and segmental endothelial elimination. There were indications of injury to the vascular system of both the white and gray matter. The pulmonary system often showed aspiration pneumonia, pulmonary edema, alveolar hemorrhage, and vasculitis. Although there was no evidence of spleen vasculitis, there was a decrease in white pulp and acute necrotizing inflammation. 36

Clinical Presentation

The incubation period for the virus in humans may range from two weeks to two months. It is usual for severe NiV encephalitis to be accompanied with seizures, altered eye reflexes, vasomotor abnormalities, high temperature, headache, nausea, and vomiting. A dysfunctional brainstem is indicated by myoclonic jerks. 3 Aseptic meningitis, severe encephalitis, and localized brainstem involvement are the most prevalent neurological symptoms in afflicted individuals. Cerebellar symptoms are often present as well. Depression, personality problems, and difficulties with speech and concentration are among psychological symptoms that may manifest in certain individuals. In rare cases, NiV patients may have relapses and late-onset encephalitis, which may manifest months or even years after the first acute illness. 37; 38 Segmental myoclonus, which is different from the epidemics in India and Bangladesh, causes the muscles supplied by neighboring segments of the brainstem or spine to contract involuntarily and rhythmically.

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 HeV[40] 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)

The standard serological test was developed soon after the epidemic in Malaysia. It is believed that the serum under investigation suppresses the cytopathic effect, and the conventional NiV VN test often uses Vero cells for this purpose. stands for a neutralization that is positive. On top of that, VN tests for plates have been created. 45 Additionally, a pseudo-type vesicular stomatitis virus including NiV envelope proteins was used to set up the neutralization test. A serum containing particular antibodies was able to neutralize it because of this. 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

The NiV is very useful for early cases and new epidemics when suspicions of the virus are strong. Samples include the brain, lungs, kidneys, and spleen. The cytopathic effect, which usually appears after three days of culture as distinctive syncytia and plaques in the cell monolayer, is the result of NiV growth on Vero cells. 47 Immunostaining, Seroneutralization (SN), and polymerase chain reaction (PCR) are the subsequent steps in identifying the virus in the culture supernatant. These NiV's

Immunological electro-microscopy may reveal interactions between viruses and antibodies, whereas electron

microscopy can ascertain their structures. 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

The NiV infection may be transmitted to humans by fruit bats, often known as flying foxes. 50 It is crucial to regularly update the public, medical professionals, and government officials about neglected and associated diseases so that they may prepare to limit any future epidemic in places where they have occurred. Bat ecology, vulnerability to NiV infection, and infection prevalence may all be better understood via research, which might reduce the threat that humans provide to bat habitats. It will be possible to halt an epidemic in its most prolific locations by collecting samples and doing sero-surveillance for the NiV antibody and NiV in people and bats using ELISA and PCR methods. 51 Outbreaks like the one in Kerala may be prevented if patients and attendants were to avoid close contact and the danger of exposure to body fluids. All patient aids, food items, clothing, and mats and sheets should only be handled by someone wearing protective gear. The caretaker's or attendant's protective gear, including gloves, a mask,

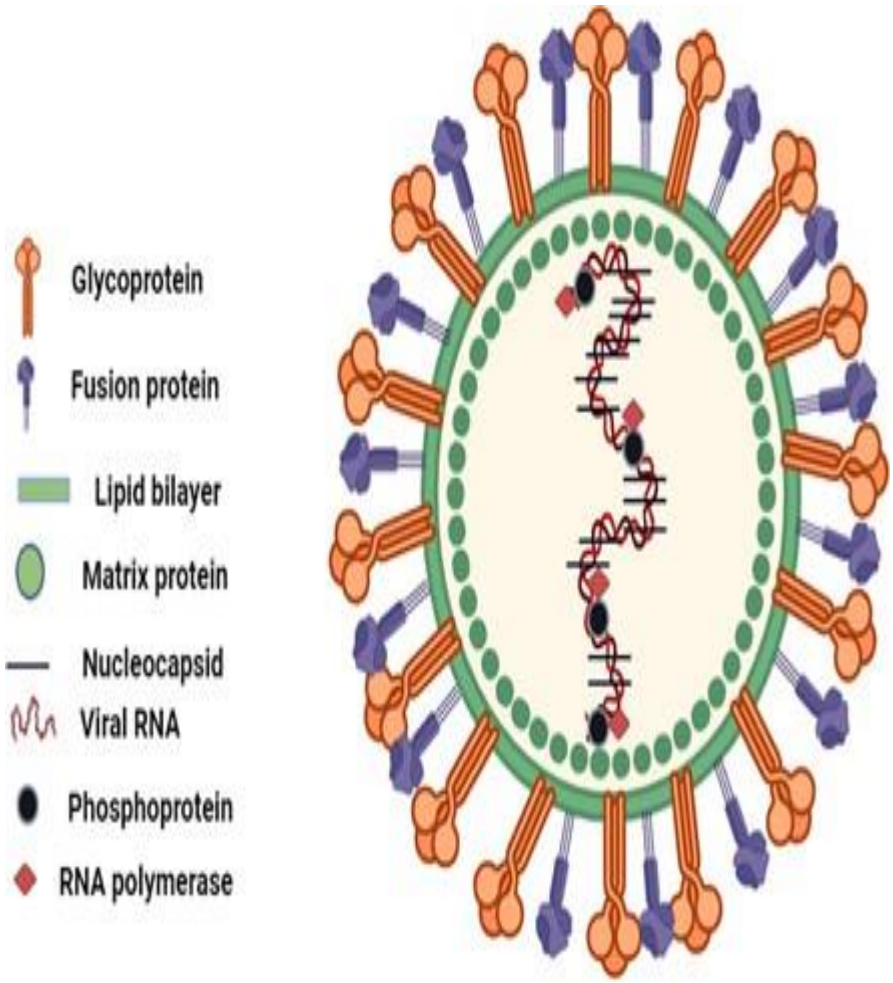
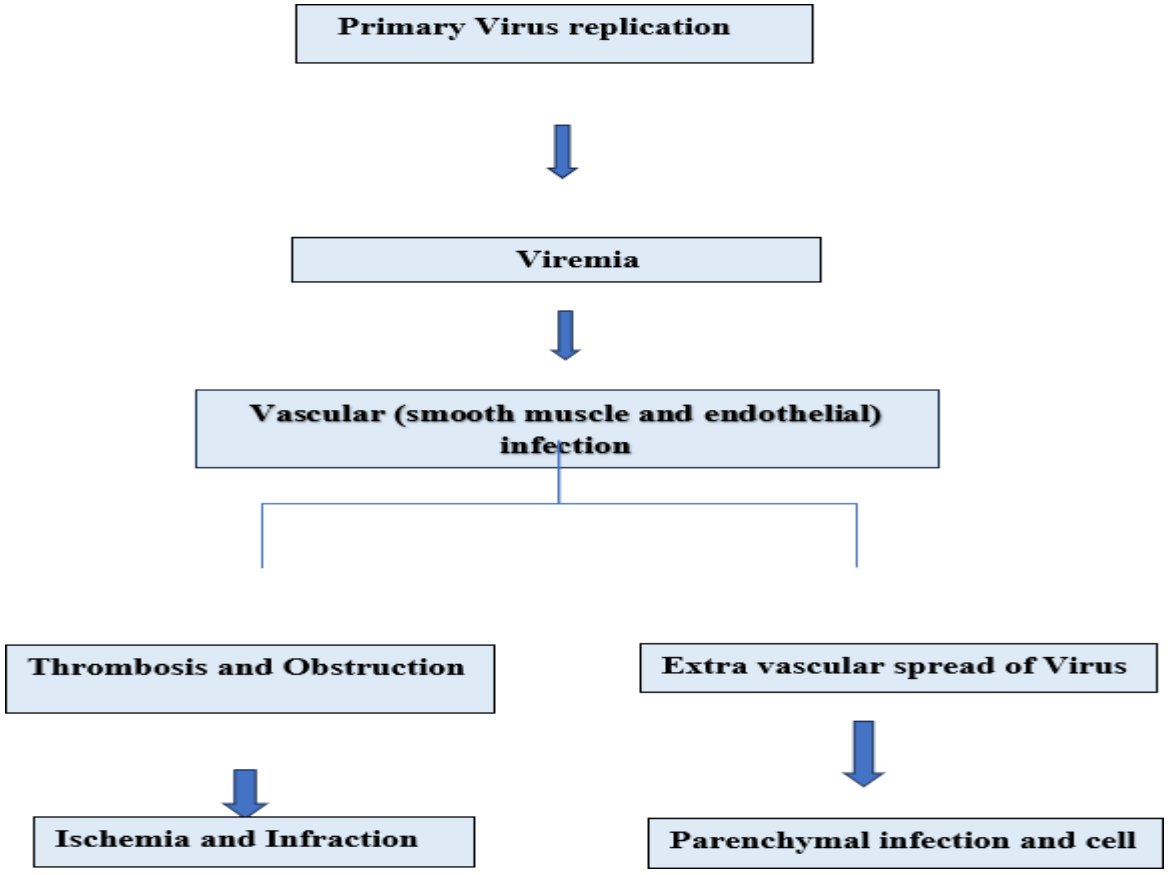


Figure 1: Structure of Nipah virus.



Pathophysiology of Nipah Virus.

together with patient assistance devices, must be appropriately burned. In order to stop the illness from spreading, medical staff treating patients with encephalitis who are showing signs of NiV should immediately isolate them from the public and wear the proper protective gear. The most humane thing to do would be to burn the corpse in an incinerator. 52 It should be absolutely forbidden to buy and eat raw, unwashed fruits and goods in regions where the NiV virus is prevalent. One of the many possible reasons of the latest epidemic in Kerala is the eating of raw fruits that have been bitten by bats. A same situation occurred in India and Bangladesh when people ingested raw date palm sap, which led to repeated outbreaks. 24

Treatment

When isolating patients with NiV infections, it is important to follow infection prevention techniques. Supportive care is the primary emphasis of therapy. Respiratory syncytial virus and other paramyxoviruses are susceptible to the antiviral ribavirin. Studies showing no change in death rates contradict one another, casting doubt on its usefulness. The Indian National Centre for Disease Control, however, recommends ribavirin for Nipah virus infections. 3,53,54 Ephrin-B2, acyclovir, and chloroquine are being examined as potential therapies. 22,35,56 days The hamster study confirmed the efficacy of favipiravir, a medicine that has received Japanese approval for the treatment of influenza. 57 Evidence suggests 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 1.6×104 PFU NiVM OR IP 3.3×104 NiVM	Hamster	60
Venezuelan equine encephalitis virus replicon particles.	NiV F or G	Footpad inoculation 3.1×105 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 5×103 TCID50 NIVB SC 5×102/5×103 TCID50 NiVM	Ferret	62
Recombinant measles virus.	NiV G	IP 2×104 TCID50 NiV G for hamster. Boost after 21 days. SC 1×105 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 1×107 PFU NiVB F, G or F and G IM 1×106 infectious particles NiVMF IM 108 PFU NiV F.	ON 5×103 PFU NiVM IP 105 TCID50 NiVM ON 2.5×105 PFU NiVM	Ferret Hamster Pig	62 64
Recombinant vaccinia virus.	NiV F and/or G	SC 107 NiV F.	IP 1×103 PFU NiVM	Hamster	65
Polyclonal serum.	NiV F and/or G	IV 0.2 mL of antiserum.	IP 1×103 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 5×103 TCID50 NiVM ON 5×103 TCID50 NiVM	Ferret African Green Monkey	40,67

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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.

In 2021, a ground-breaking method known as the "Drug-target-drug network-based approach" was put out. 68 utilizing the currently licensed pharmaceuticals in the US by the FDA, the Nipah virus and thirteen others were investigated utilizing drug-target-drug network analysis. An evaluation of US FDA-approved drugs was conducted utilizing a confidence score as a repurposing hypothesis in the computer research. A total of sixteen drugs have been found to have new uses in the fight against NiV and Hepatitis E Virus (HEV). To further verify this approach and find the most promising options for the individual viruses, molecular docking is used. In 2019, the "anti-Nipah" online source was supplied by Rajput et al. The inhibitory impact was predicted using the QSAR model with the help of 313 chemicals from the database. 69

CONCLUSION

NiV is classified as BSL-4.70 because of its low transmission rate and great lethality. In the last 20 years, many countries have reported cases of NiV, including Singapore, Malaysia, and Bangladesh. Recent reports indicate the emergence of NiV in Kerala, India. These epidemics have been devastating to the economics and public health of the nations hit by the high death tolls and quick spread of NiV infection. The prospect of a NiV breakout is currently quite unlikely. Some drugs have shown effective viral suppression in vitro but not in animals, and the US FDA has not yet authorized any medicines for human use. The Syrian hamster model recently revealed that mice challenged with a fatal amount of NiV had 100% protection when administered subcutaneously twice daily or once daily for 14 days with favipiravir. More study is needed to fully understand the antiviral activities of the small molecule medicine favipiravir, which has been utilized to treat henipavirus infections in vivo for the first time.

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