

NIPAH VIRUS



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SUMMARY

Etiology

- Nipah virus (NiV) is a single-stranded RNA virus belonging to the genus *Henipavirus*, family *Paramyxoviridae*, which was discovered in Malaysia in 1998–1999. It is closely related to Hendra virus.
- There are two lineages: a Malaysian strain (mNiV) and a Bangladesh strain (bNiV).

Cleaning and Disinfection

- NiV survival in the environment is unclear; however, infections seem most common in cool, dry weather in India and Bangladesh.
- NiV can be inactivated by 0.1% formalin and 0.5% household bleach. In general, paramyxoviruses are susceptible to acids, alcohols, aldehydes, alkalis, halogens, and oxidizing agents.

Epidemiology

- The known geographic distribution of NiV correlates with the range of reservoir species—flying foxes—including Australia and Southeast Asia to India and the Eastern African islands. Annual outbreaks occur in India and Bangladesh.
- Pigs are an amplifying host for NiV. Dogs and cats are susceptible but are not thought to be involved in transmission.
- NiV is zoonotic and causes high fatality rates in humans.

Transmission

- Among pigs, NiV spreads via direct contact and possibly aerosolization.
- Human outbreaks in Bangladesh are caused by consumption of date palm juice contaminated with saliva and urine from infected flying foxes.

Infection in Swine/Pathogenesis

- NiV in swine has respiratory and neurological manifestations. Young piglets are most affected. Infections in older swine may be asymptomatic. A characteristic “barking” cough can occur.

Diagnosis

- Virus isolation is required for definitive diagnosis, but handling NiV requires a BSL4 laboratory.

- Quantitative real-time polymerase chain reaction (qRT-PCR) is available, as is immunohistochemistry. Immunofluorescence may be complicated by cross-reactivity with Hendra virus, although several monoclonal antibodies are now available for henipavirus differentiation.
- An indirect enzyme linked immunosorbent assay (ELISA), virus neutralization tests using pseudotype particles, and multiplexed microsphere assays have all been developed to detect antibodies to NiV at the BSL2 level.

Immunity

- Multiple vaccines are in development but none are commercially available. Vector-based vaccines include those utilizing canarypox virus, Newcastle disease virus, and vesicular stomatitis virus.
- The Hendra virus vaccine used in horses has shown some cross-protection against NiV in animal models.

Prevention and Control

- Stamping out would likely be implemented to limit the spread of NiV should an outbreak occur in the United States. Stop movements would also keep the virus from being widely transmitted. Emergency vaccination may be possible if a commercial vaccine becomes available.
- Standard biosecurity measures should be in place in swine operations.
- Use of diagnostics available at BSL2 laboratories would improve timeliness of the response.
- Because NiV is zoonotic and causes high fatality rates in humans, the health of swine workers and responders must be protected and assessed during an outbreak.

Gaps in Preparedness

- No NiV vaccines are commercially available.
- The difficulty of using BSL4 laboratories for diagnosis increases the time and cost to identify NiV.

OVERVIEW

Nipah virus (NiV) is a paramyxovirus in the genus *Henipavirus*. Two lineages are currently circulating in Southeast Asia: a Malaysian strain (mNiV) and a Bangladesh strain (bNiV). Experimentally, mNiV appears to be the more virulent of the lineages. NiV is closely related to Hendra virus (HeV), which causes high mortality in both horses and humans and is endemic in parts of Australia.

Following its discovery in swine and humans in Malaysia (1998–1999), outbreaks have occurred in Bangladesh and India nearly every year. The NiV reservoir host is the flying fox, a fruit bat. In Malaysia the virus jumped from flying foxes to pigs; human outbreaks in Bangladesh are caused by consumption of date palm juice contaminated with saliva and urine from infected flying foxes. Outbreaks in humans seem to be most common during the cool, dry season from September to May.

NiV can infect many different species, including bats, humans, cats, dogs, pigs, and other livestock. Pigs are an amplifying host for NiV and person-to-person transmission can also occur. NiV-infected humans present with encephalitis, and case fatality rates can reach 75%. Workers with direct contact with infected swine are most at risk for contracting NiV during an outbreak. Pigs transmit NiV to people through sputum, splashing urine, and large respiratory droplets, facilitated by a characteristic barking cough in infected pigs. Transmission between pigs occurs through direct contact, exposure to infected secretions, and possibly via aerosol transmission. The NiV outbreak in pigs in Malaysia resulted in stamping out of more than one million pigs. NiV has not been reported in pigs in Malaysia since 1999.

Clinical signs seen in swine are mostly respiratory and neurological. Suckling piglets may have loss of neuromuscular function including leg weakness, tremors, and twitches. Mortality is highest in young piglets. Weaned and growing pigs may be asymptomatic or may exhibit acute, febrile disease including respiratory and neurologic signs. A barking cough may be observed along with other respiratory signs including labored or open-mouth breathing. Neuromuscular signs include leg weakness, ataxia, spastic paresis, and muscle twitching. Mortality in growing pigs is generally low (<5%). Boars and sows affected with NiV typically survive infection, although sudden death of animals exhibiting no prior symptoms or less than 24 hours after the onset of symptoms has been seen.

NiV is a biosafety level (BSL) 4 pathogen and select agent, making research on and diagnostic testing for NiV difficult. However, diagnostic tests utilizing recombinant technology have allowed diagnosis of NiV in BSL2 laboratories. An indirect enzyme linked immunosorbent assay, virus neutralization tests using pseudotype particles, and multiplexed microsphere assays have all been developed to detect antibodies to NiV at the BSL2 level. This allows more rapid, cost efficient diagnosis of NiV.

Quantitative polymerase chain reaction (PCR) primers and probes allow detection of the nucleocapsid or matrix gene of NiV. Immunofluorescence or immunohistochemistry can be used to detect henipavirus antigen in infected tissue, and monoclonal antibodies are available for henipavirus differentiation (HeV vs. NiV). Virus isolation may be used as a definitive diagnosis in the case of a newly suspected outbreak. NiV isolation requires using strict biosecurity protocols in a BSL4 laboratory. Oronasal swabs, urine, and serum can be used for isolation from live animals, while brain, lung, kidney, and spleen can be used for post-mortem virus isolation.

Multiple NiV vaccines have been developed and tested experimentally. The HeV vaccine used in horses in Australia has shown cross-protection against NiVs in other animal models. Other vaccines are composed of vector viruses (canarypox virus, Newcastle disease virus, and vesicular stomatitis virus) expressing NiV glycoprotein and/or fusion protein. The recombinant vesicular stomatitis virus vaccine also includes a Zaire ebolavirus glycoprotein, allowing the vaccine to adequately elicit neutralizing antibodies to NiV after one dose, giving it an advantage during possible emergency vaccination scenarios.

The best known reservoir for NiV, the flying fox, is confined to Southeast Asia, Australia, and eastern Africa. This is an advantage for countries who might import NiV through trade, because the lack of a reservoir theoretically allows an outbreak to be stamped out. The ability of pigs to act as amplifying hosts and to transmit NiV to humans means that any infected herds of swine would need to be depopulated immediately. Emergency ring vaccination could be done using a one dose of recombinant VSV vaccine in order to help prevent the spread of NiV to nearby producers, although the vaccine is still experimental and not commercially available. People working around infected swine would need maximum personal protective equipment to prevent infection with NiV. Limiting movement of pigs would also need to be a priority in the outbreak containment to keep NiV from spreading outside an infected location. Diagnostic tests safe to use at a BSL2 level, on samples treated to inactivate virus, could be utilized during a widespread outbreak in order to quickly and safely identify infected herds while other samples could concomitantly be utilized for virus isolation for definitive NiV diagnosis.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Nipah virus (NiV) is in the genus *Henipavirus*, family *Paramyxoviridae*, which also includes Hendra virus (HeV) and Cedar virus (CedV).¹ NiV has morphology similar to other paramyxoviruses with pleomorphic structure, herringbone nucleocapsids, and 10 nm long surface protein spikes on the envelope.²

1.2 Strain Variability

There are two lineages of NiV circulating in Southeast Asia: Malaysian NiV (mNiV) and Bangladesh NiV (bNiV).³ In the 1998 Malaysian NiV outbreak, only a single mNiV isolate was identified; however, an outbreak of bNiV may be caused by multiple strains.⁴ *In vitro* and *in vivo* hamster models show that mNiV causes increased cytopathology, increased disease progression, and higher mortality rates than bNiV.⁵ However, bNiV appears to cause more respiratory disease in people, leading to non-productive cough in 62% of patients vs. only 14% of patients infected with mNiV.⁴

2. Cleaning and Disinfection

2.1 Survival

The initial Malaysian NiV outbreak occurred from September to May of 1998–1999. Subsequent yearly outbreaks, beginning in 2001 in Bangladesh and India, have occurred between December and May. These months include the cool, dry portion of the seasonal climate of these countries.⁴ In date palm sap, NiV remains infectious for days.⁴

2.2 Disinfection

Paramyxoviruses are susceptible to acids, alcohols, aldehydes, alkalis, halogens, and oxidizing agents. NiV has limited susceptibility to biguanides, phenolic compounds, and quaternary ammonium compounds.⁶

3. Epidemiology

3.1 Species Affected

Flying foxes, also known as fruit bats, are the main reservoir host for NiV.³ Flying foxes roost in forests and swamps, flying to fruit trees at night to feed.¹⁰ Neutralizing antibodies to NiV have been found in many of the flying foxes in Southeast Asia, including 75% of sampled flying foxes in Malaysia.⁹ Neutralizing antibodies to NiV found in Ghana from *Eidolon helvum*, the West African flying fox, demonstrate a more widespread distribution of henipaviruses than previously believed.¹¹ There has also been documentation of neutralizing NiV antibodies in a Microchiroptera (microbat) species, *Scotophilus kuhlii*, in Malaysia.¹²

Infected pigs can be amplifying hosts.³ Dogs and cats are also susceptible to NiV infection, but are not believed to be capable of transmitting NiV to humans or other animals.⁷ Rodents and birds near the Malaysia outbreak tested seronegative.³

3.2 Zoonotic Potential

NiV is a zoonotic paramyxovirus posing a significant public health threat.² Mortality in humans can be up to 100%.⁴ In the 1998–1999 Malaysian epidemic, NiV proved fatal in 105 of the 258 human cases of encephalitis.³ Pig-to-human transmission is believed to be facilitated via sputum or large respiratory droplets formed by coughing.²

In Bangladesh and India, NiV outbreaks have resulted in case fatality rates greater than 74%.³ The viral source is believed to be contaminated date palm juice.⁸ Contact with livestock in Bangladesh has also been associated with contracting NiV.⁴

Spread among humans is believed to be through direct contact with infected patients' saliva.³ Caretakers of patients sick with NiV are at an increased risk of infection.⁴

3.3 Geographic Distribution

The first outbreak of NiV started in northern Malaysia in September, 1998 and spread to central Malaysia and Singapore, by February and March 1999, respectively.³ Spread of NiV was facilitated by movement of subclinically infected pigs to new locations.³ Beginning in 2001, outbreaks have occurred nearly every year in Bangladesh and India.⁴

The known geographic distribution of NiV correlates with the range of flying foxes, mainly of the genus *Pteropus*.⁹ Flying foxes are found from Australia and Southeast Asia to India and Eastern African islands.¹⁰

3.4 Morbidity and mortality

During the Malaysian outbreak, there was 10–15% mortality in piglets.³ Mortality occurs in less than 5% of growing pigs.²

4. Transmission

NiV is highly contagious among swine populations and spreads via direct contact, exposure to infected secretions, and possibly aerosolization.² Experimentally infected pigs have shown ability to excrete NiV as early as 4 days post-infection from the oropharynx, and NiV can also be shed in nasal secretions.¹³

5. Infection in Swine/Pathogenesis

NiV targets the vascular, nervous, and lymphoreticular systems.² Once NiV enters the oronasal cavity, the virions infect epithelial cells, immune cells, and peripheral nerve endings of cranial nerves.² NiV can then directly follow nerves into the brain in some pigs and cause neurological symptoms.² NiV also targets endothelial cells of small vessels as well as immune cells, leading to widespread viral dissemination.² Viremia results in increased viral load in tissue parenchyma, including the central nervous system and the lower respiratory tract.² NiV infects monocytes and T lymphocytes, and NiV antigen has been found in macrophages and dendritic cells.² Immune cell depletion, including T lymphocytes, may play a role in increased risk of secondary infections.¹⁴

5.1 Clinical Signs

Young piglets are most likely to die from NiV.² Sucking piglets may exhibit leg weakness, muscle tremors, and neurological twitches.²

In growing pigs, NiV infection may be asymptomatic or cause acute, febrile disease with respiratory and CNS signs.² A characteristic respiratory sign in affected pigs is a “barking” cough, although there may only be labored to open-mouth breathing.² CNS signs associated with disease include muscle fasciculation, leg weakness, ataxia, and spastic paresis.² Dead pigs may present with a bloody nasal discharge.¹⁵

Mortality is not common in sows or boars affected by NiV, although adults can die suddenly with no symptoms or within 24 hours after the onset of symptoms.¹⁵ Neurological signs seen in adults include tongue drooping, frothy salivation, head pressing, agitation, tetanic spasms and seizures, and pharyngeal

muscle paralysis.¹⁶ Bloody nasal discharge can occur post mortem, and abortions have also been observed.¹⁵

5.2 Postmortem Lesions

Gross lesions include pulmonary consolidation and distended interlobular septa on cut surfaces.² Frothy exudate, sometimes laced with blood, can fill the bronchi and trachea of affected pigs.² Enlarged bronchial, submandibular, and mesenteric lymph nodes were commonly found.² Lesions associated with neurological symptoms include meningeal congestion and edema.² Meningitis or meningoencephalitis was more frequently seen in neurological cases than was encephalitis.²

Microscopic lesions seen in affected pigs include giant-cell pneumonia with multinucleated syncytia in the respiratory epithelium.¹⁷ Syncytia can also be found in endothelial cells of small blood and lymph vessels.² Some affected pigs presented with meningeal inflammatory infiltrate.¹⁷ Lymphangitis may also be seen coupled with lymphocyte necrosis and depletion.²

6. Diagnosis

6.1 Clinical History

Disease and fatalities in people may be one of the first signs of a NiV outbreak.² Symptoms associated with NiV-induced encephalitis in humans include fever, headache, dizziness, vomiting, and progression to impaired consciousness.² Swine history will include respiratory and neurological symptoms with relatively low mortality in affected populations.²

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

Virus isolation should be performed for definitive diagnosis in an area with a newly suspected outbreak. Experimentally, NiV is detectable in oropharyngeal and nasal swabs as early as two days post-infection; experimentally infected animals continued to shed virus until three weeks post-infection.² African green monkey kidney or rabbit kidney cell lines are commonly used for virus isolation.⁹ A cytopathic effect (CPE) is usually seen within two to three days, but multiple passages of five days each are recommended before concluding that a sample is NiV negative.⁹

Quantitative real-time PCR (qRT-PCR) primers and probes have been developed for the nucleocapsid (N) gene of NiV.⁹ Primers for conventional PCR and sequencing of the matrix (M) gene have also been described.⁹ Specific qRT-PCR primers and probes for the N gene of bNiV and mNiV have been described.⁵

Immunohistochemistry (IHC) can be performed to detect NiV.⁹ The N protein antigen is commonly targeted. Detection of phosphoprotein (P) antigen can also be used with IHC, although N protein antigen is expressed in greater quantities than P protein antigen and is therefore of better diagnostic value.⁹

Immunofluorescence can rapidly detect NiV but cannot differentiate between henipaviruses, since mono-specific antisera to individual proteins of NiV will cross react with HeV.⁹ Negative contrast electron microscopy can be used to identify viral particles.⁹ Two monoclonal antibodies (MAb) with affinity for the N protein or P, V, and W protein of henipaviruses have been developed.¹⁸ MAb 1A11 C1 can detect the N protein of both NiV and HeV, while MAb 2B10 p4 can detect HeV antigen better than NiV antigen, making it possible to use 2B10 p4 for differentiation between NiV and HeV.¹⁸ To differentiate the two henipaviruses using immunofluorescence assays, reactivity to NiV or HeV specific antisera must be compared to positive controls of NiV or HeV.⁹ Anti-HeV antiserum neutralizes HeV at a four-fold greater dilution than it neutralizes NiV, and anti-NiV antiserum neutralizes NiV four times more efficiently than it neutralizes HeV.⁹

6.3 Tests to Detect Antibody

An indirect enzyme linked immunosorbent assay (ELISA) using recombinant NiV N protein as an antigen has been described for use as a diagnostic test.¹⁹ Recombinant proteins allow use of the ELISA to test samples that have been treated to inactivate the virus in biosafety level (BSL) 2 diagnostic labs.¹⁹ When compared to CDC inactivated-virus ELISA assays, the indirect ELISA for IgG detection had accordance of 98.6% sensitivity and 98.4% specificity using human serum samples, and 100% accordance (albeit only 16 samples) using swine serum samples.¹⁹ In surveillance programs, a positive test would need to be followed with a positive virus neutralization result to confirm the diagnosis⁹ and prevent a costly and unnecessary response to a false positive result.

Virus neutralization tests (VNT) have been developed for high-throughput screening in BSL2 diagnostic laboratories using recombinant vesicular stomatitis virus (rVSV) expressing NiV fusion (F) protein and glycoprotein (G).²⁰ A VNT utilizing secreted alkaline phosphatase (SEAP) has been developed to detect antibodies to NiV.²⁰ Using 75% reduction as the cut-off threshold, this VNT gave the same diagnostic results as a live NiV VNT performed in a BSL4 lab.²⁰ Another VNT with similar efficacy to a live NiV VNT test has been described using a luciferase reporter gene to detect neutralization of NiV proteins.²¹

Multiplexed microsphere immunoassays have been developed to detect either antibody binding to recombinant soluble NiV or HeV G protein, or antibody inhibition of ephrin-B2 receptor binding.²² Spectrally distinct microspheres allow specific and sensitive quantification and differentiation between HeV and NiV antibodies in a sample.²² Because of the recombinant subunit proteins used, this assay does not have to be conducted in a BSL4 lab if the samples are treated to inactivate virus.²²

6.4 Samples

Oro-pharyngeal/nasal swabs, urine, and serum can be used for isolation from live animals, while brain, lung, kidney, and spleen samples can be used post mortem.⁹ If possible, urine should also be collected for analysis.⁹ Strict biosecurity protocols, including stringent use of personal protective equipment, should be followed when sampling pigs with suspected NiV infection.

7. Immunity

7.1 Post-exposure

Neutralizing antibody titers appear 7–10 days post-experimental infection with maximum titers seen 14–16 days post-infection.² Ferrets administered a HeV soluble G protein vaccine were protected against live NiV challenge 14 months post-vaccination.²³

7.2 Vaccines

Multiple vaccines have been developed for NiV, though none are commercially available. A HeV soluble G subunit-based vaccine has shown promise in preventing infection in animals exposed to lethal doses of NiV or HeV.⁹ A HeV soluble G vaccine has been produced and licensed, and is available for use in horses in Australia.⁹ A vaccination study has shown the HeV soluble G vaccine capable of preventing NiV from causing disease in ferrets.²³

Canarypox virus vectors expressing the NiV G protein (ALVAC-G) or F protein (ALVAC-F) have been generated.²⁴ In one study, pigs were vaccinated with a combination ALVAC-G/F vaccine given as two doses with a booster at day 14. Adequate neutralizing titers to NiV were found and all pigs were protected against NiV upon challenge.²⁴ NiV was not isolated from tissues or oropharyngeal/nasal swabs from vaccinated pigs following the challenge, showing ALVAC-F/G prevents shedding of NiV.²⁴

Recombinant Newcastle disease virus-vectored NiV vaccines expressing the G and F proteins of NiV have shown to be experimentally effective in producing long-lasting neutralizing NiV antibodies in pigs.²⁵ This vaccine requires a booster four weeks after the initial vaccination to produce adequate neutralizing titers.²⁵ Advantages of this vaccine are the ability to easily culture the vaccine virus in chicken eggs and the amenability to lyophilization,²⁵ facilitating both production and storage of the vaccine.

A recombinant vesicular stomatitis virus (rVSV) vector vaccine expressing NiV G or F protein has been shown to induce a strong humoral anti-NiV response in hamsters following a single vaccine dose.²⁶ The rVSV platform is believed to add a cell-mediated immune response not seen in other vaccine platforms due to the addition of Zaire ebolavirus G protein to the virus vector in place of the VSV G protein.²⁶ A vaccine of this nature could be employed during an emergency ring vaccination approach to an outbreak.²⁶ A rVSV vector vaccine expressing NiV N protein was found to provide only partial protection to NiV.²⁶

7.3 Cross-protection

It is likely that novel henipaviruses induce NiV cross-reactive antibodies.²⁷ There is good cross-protection provided by the G protein of HeV. Passive administration of monoclonal antibodies to HeV G protein have prevented disease in ferrets challenged with a lethal dose of NiV.²⁸ Vaccinations using HeV soluble G protein have been found to protect against challenge with live NiV.²³

8. Prevention and Control

With no flying fox species present in North, Central or South America, a reservoir host is lacking for NiV. Assuming unknown, capable reservoirs do not exist, NiV elimination would be possible should an outbreak occur in the United States.

Limiting the spread of NiV is important because of its swine industry impact and zoonotic potential. Restricting movement of pigs in an affected area and rapidly depopulating infected herds, while implementing strict biosecurity protocols, will occur in the case of an outbreak. Workers involved in an outbreak will require personal protective equipment when dealing with potentially infected pigs, including particle respirators. Utilization of an rVSV-vectored vaccine could provide emergency protection to pigs nearby an affected herd if the vaccine becomes commercially available.

There are diagnostics available to detect NiV at a BSL2 level, allowing more rapid cost effective diagnosis of infected herds in the event of an outbreak. ELISAs, VNTs, and bead-based assays have all been developed for potential use at the BSL2 level using recombinant technology.

9. World Organization for Animal Health (OIE) Terrestrial Animal Health Code

NiV is not covered in the 2015 OIE Terrestrial Animal Health Code and there are no recommendations on importation of swine or pork.

10. Gaps in Preparedness

There is no NiV vaccine available for emergency use in pigs or humans, although experimental vaccines have shown promising efficacy. NAHLN laboratories are not equipped to diagnose NiV infection, or to handle BSL4 pathogens safely.

An emergency response plan for dealing with an outbreak of a zoonotic pathogen like NiV in swine should be developed. There is very limited ability to conduct research on BSL 4 pathogens in swine in the United States.

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