

NIPAH VIRUS



The mission of the Swine Health Information Center is to protect and enhance the health of the United States swine herd through coordinated global disease monitoring, targeted research investments that minimize the impact of future disease threats, and analysis of swine health data.

September 2015 | Updated May 2021

SUMMARY

IMPORTANCE

- Nipah virus (NiV) is a zoonotic paramyxovirus that causes serious disease in people. It is carried subclinically by pteropid bats. Pigs can become sick and act as amplifying hosts.
- Entry of NiV into the United States is a public health concern and could lead to swine industry losses.

PUBLIC HEALTH

- NiV causes febrile encephalitis and respiratory disease in humans, with high case-fatality rates.
- In an outbreak in Malaysia (1998-99), NiV was associated with swine contact. Continuing outbreaks in Bangladesh and India are due to ingestion of raw date palm sap. A 2015 occurrence in the Philippines was associated with slaughter and consumption of horse meat.

INFECTION IN SWINE

- Many NiV infections are subclinical in swine. Disease manifestations vary by age.
- Suckling piglets develop neurological disease with high mortality. A characteristic “barking” cough can occur in growing pigs, along with CNS signs. Mortality is low.
- Acute death is sometimes seen in adult pigs, with bloody nasal discharge postmortem. Neurological signs and abortion have been reported.

TREATMENT

- There is no specific treatment for NiV infection.

CLEANING AND DISINFECTION

- NiV survives for days in fruit juices and date palm sap. It is relatively stable in the environment.
- Paramyxoviruses are susceptible to acids, alcohols, aldehydes, alkalis, halogens, and oxidizing agents. Sodium hypochlorite (household bleach) has been used to disinfect pig farms.

PREVENTION AND CONTROL

- NiV is reportable to the World Organization for Animal Health (OIE).
- Pteropid bats must be kept out of swine farms. Fruit trees should not be planted near farms, and swine barns should be screened if possible. Roof run-off should be diverted away from pig areas, and potentially contaminated fruit should not be fed to pigs.
- Stop movement and stamping out may be instituted in an NiV outbreak. The health of swine workers and responders must be protected since NiV is zoonotic.

TRANSMISSION

- Among pigs, NiV spreads via direct contact and possibly inhalation.
- Pigs acquire NiV from bats through ingestion of contaminated fruit (urine or saliva), water, aborted bat fetuses, or birth products.

PATHOGENESIS

- NiV targets the vascular, nervous, and lymphoreticular systems.

DIAGNOSIS

- NiV is a BSL-4 agent. Tests that do not amplify virus and minimize sample handling are preferred. People working with suspect cases must wear appropriate personal protective equipment.
- Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assays are described for the N, P, L, and M genes. Use of recombinant proteins makes testing safe for BSL-2 facilities.
- Enzyme linked immunosorbent assays (ELISAs) have been developed for NiV IgG and IgM, as well as the viral proteins G, N, M, and P. Inactivated serum can be tested in BSL-2 labs. Positive test results must be confirmed by virus neutralization in a BSL-4 facility.

EPIDEMIOLOGY

- Antibodies to NiV have been found in most fruit bat species that have been tested. In addition to pigs, NiV infection has occurred in dogs, cats, goats, and possibly cattle.
- Geographic distribution of NiV mirrors the range of the fruit bat, its reservoir. It includes Oceania, South and Southeast Asia, and sub-Saharan Africa. Annual outbreaks occur in Bangladesh and India.

ETIOLOGY

- NiV is a single-stranded RNA virus belonging to the genus *Henipavirus*, family *Paramyxoviridae*. It is closely related to Hendra virus and Cedar virus.
- There are two distinct NiV strains: mNiV, found mostly in Malaysia and Cambodia, and bNiV, found mostly in Bangladesh and India.

HISTORY IN SWINE

- Widespread NiV outbreaks occurred in people and pigs in Malaysia and Singapore from 1998-99. Intensified pig farming and growing commercial fruit production led to increased contact between pigs and bats in affected areas.
- Retrospective analysis showed that NiV was present in pigs several years prior to the outbreak.

IMMUNITY

- Multiple vaccines are in development, but none are commercially available for people or pigs.
- A sub-unit vaccine based on G glycoprotein from Hendra virus is licensed for use in horses in Australia and provided good cross-protection against NiV in an experimental ferret model.

GAPS IN PREPAREDNESS

- NiV is highly infectious in pigs and causes serious disease in humans. There are no licensed vaccines in the United States.
- Diagnosis is challenging because clinical signs are non-specific. Additionally, most U.S. laboratories are not equipped to detect NiV.
- Once NiV enters the human population, it can spread person-to-person. NiV has the potential to become a pandemic agent.

LITERATURE REVIEW: NIPAH VIRUS

IMPORTANCE

Nipah virus (NiV) is a zoonotic paramyxovirus that causes serious disease in people. It is carried subclinically by pteropid bats, and pigs can act as amplifying hosts. A human outbreak in Malaysia and Singapore was associated with swine contact. However, in Bangladesh and India, disease has been linked to ingestion of raw date palm sap. NiV is highly contagious in swine and causes high morbidity. Entry of NiV into the United States could be devastating for public health and for the swine industry.

PUBLIC HEALTH

The first known outbreak of NiV occurred from 1998-99 in Malaysia and Singapore. Febrile encephalitis, with a high case fatality rate, was diagnosed in men with close swine contact including swine farm and abattoir workers. A few cases of respiratory disease also occurred.¹ Experimental evidence has since shown that Malaysian NiV (mNiV, see *Etiology*) produces higher viral titers and spreads more rapidly in human airway epithelial cells compared to porcine airway epithelial cells.²

During the Malaysian outbreak, encephalitis and respiratory disease also occurred in pigs. Approximately one million pigs in affected areas were culled by military personnel. Serological monitoring showed that 0.4% (6/1412) people had detectable antibody against NiV.³ Of those six individuals, two were later hospitalized with encephalitis.³ No additional outbreaks have been detected in Malaysia or Singapore.⁴ However, seroprevalence in humans in peninsular Malaysia is nearly 11%.⁵

In Bangladesh, NiV appeared in 2001⁶ and has since caused nearly annual outbreaks.^{4,7} Symptoms include febrile encephalitis and respiratory illness, with frequent cough and difficulty breathing.⁶ Human-to-human transmission was suspected, but the source of NiV was undetermined initially.⁶ Later, NiV was linked to ingestion of raw date palm sap contaminated by fruit bats,⁸ and pteropid bats were confirmed as the reservoir host for NiV⁹ (see *Epidemiology*).

Human NiV infection has also been reported in other Southeast Asian countries. As described by Hauser and colleagues, in India, most transmission has been nosocomial.⁴ In the Philippines, a 2014 NiV outbreak was associated with horse slaughtering and consumption of horse meat.¹⁰ Rapidly progressing neurological disease was concurrently seen in horses, and several cats that consumed the horse meat died. Human-to-human transmission also occurred.¹⁰

INFECTION IN SWINE

NiV infection may be subclinical or acute in swine, and signs can vary by age.¹¹ Disease caused by mNiV is described below.

- Suckling pigs exhibit leg weakness, muscle tremors, and neurological twitches.¹¹ Mortality can be high.¹¹
- In growing pigs, febrile illness develops with respiratory or neurological signs.¹¹ A characteristic “barking” cough can be seen, along with open-mouth breathing.¹¹ Additionally, CNS signs include muscle fasciculation, leg weakness, ataxia, and spastic paresis progressing to lateral recumbency.¹¹ Mortality is low. Dead pigs may present with a bloody nasal discharge.^{11,12}
- In adult pigs, acute death has been noted occasionally.^{11,12} Bloody nasal discharge is frequently seen postmortem. Neurological signs are common and include head pressing, agitation, tetanic spasms and seizures, and pharyngeal muscle paralysis which leads to salivation and tongue drooping.^{11,12} Abortion has been reported in sows.^{11,12}

Gross lesions caused by NiV include pulmonary consolidation and distended interlobular septa on cut surfaces.¹¹ Frothy exudate, sometimes laced with blood, can fill the trachea and bronchi in affected pigs.¹¹ Enlarged bronchial, submandibular, and mesenteric lymph nodes are commonly found.¹¹ Neurological lesions include meningeal congestion and edema.¹¹ Meningitis or meningoencephalitis is more frequently seen in neurological cases than encephalitis.¹¹

Microscopic lesions seen in affected pigs include giant-cell pneumonia with multinucleated syncytia in the respiratory epithelium.¹³ Syncytia can also be found in endothelial cells of small blood and lymph vessels.² Some affected pigs presented with meningeal inflammatory infiltrate.¹³ Lymphangitis may also be seen coupled with lymphocyte necrosis and depletion.¹¹

Experimentally, pigs are susceptible to bNiV, the NiV strain associated with human illness in Bangladesh and India. Following oronasal inoculation, viral shedding (oral, nasal, rectal) and tissue invasion were confirmed; however, pigs did not develop clinical signs.¹⁴

TREATMENT

There is no specific treatment for NiV infection. However, as described by Hauser *et al.*, drugs with potential antiviral activity against NiV include chloroquine, ribavirin, acyclovir, favipiravir, remdesivir, balapiravir, poly(I)-poly(C₁₂U), ephrinB2, human mAb m102.4, and human mAb h5B3.1.⁴

CLEANING AND DISINFECTION

SURVIVAL

NiV reportedly survives for up to three days in fruit juices, and for seven or more days in artificial date palm sap.⁶⁶ In fruit bat urine, NiV remains infectious for days,¹⁶ (half-life 18 hours). In the environment, the virus is relatively stable. It is inactivated by heating to 100°C for more than 15 minutes.⁶⁶

NiV survives in blood in closed tubes for more than one week at room temperature.¹⁵ NiV survival in animal feed has not been directly assessed; however, a surrogate (canine distemper virus) did not survive well under conditions simulating transport between continents.¹⁷ NiV has been detected on environmental surfaces in a hospital setting.¹⁸ In Southeast Asia, NiV outbreaks occur during the cool, dry portion of the year.¹¹

DISINFECTION

Paramyxoviruses are susceptible to acids, alcohols, aldehydes, alkalis, halogens, and oxidizing agents. Sodium hypochlorite was used to disinfect pig farms in Malaysia.¹⁹ NiV has limited susceptibility to biguanides, phenolic compounds, and quaternary ammonium compounds.¹⁹

PREVENTION AND CONTROL

DISEASE REPORTING

NiV is an OIE-listed disease and must be reported internationally according to the *Terrestrial Animal Health Code*. Any suspicious clinical or necropsy findings should always be reported to the USDA and your State Animal Health Official.

DISEASE PREVENTION

In areas with pteropid bats, farms should be managed to prevent contact between pigs, fruit bats, and their secretions. Fruit trees should be located away from swine farms.¹¹ Fruits that may be contaminated by bat urine or saliva should not be fed to pigs.¹⁹ Barns should be screened if possible and roof run-off should be diverted away from pig pens.¹⁹ New animals should be quarantined before they are introduced to the herd.¹¹ Multiple vaccines are being developed for NiV, though none are commercially available in the United States (see *Immunity*).

DISEASE CONTROL

In the United States, there is no known reservoir host for NiV. However, in affected areas, swine movements must be restricted and infected herds rapidly depopulated.¹¹ Strict biosecurity measures should also be in place. People working in an NiV outbreak need personal protective equipment, including respiratory protection, to prevent zoonotic transmission.

TRANSMISSION

NiV is highly contagious in swine; transmission is via contact with infected secretions and possibly inhalation.¹¹ Experimentally, infected 6-week-old pigs excrete NiV as early as 4 days post-infection (dpi) from the oropharynx and in nasal secretions.²⁰ Pigs may acquire NiV from bats through ingestion of contaminated fruit (urine or saliva), water, aborted bat fetuses, or birth products.^{19, 21}

Spatial risk factors for transmission of NiV from bats to pigs include:

- Bat presence in the area and distance to the nearest bat colony
- Distance to the nearest forest, fruit orchard, and water body
- Human population density
- Swine population density²²

PATHOGENESIS

NiV targets the vascular, nervous, and lymphoreticular systems.¹¹ After entering the oronasal cavity, NiV follows nerves into the brain, causing neurological symptoms, and enters endothelial cells of small vessels and immune cells, leading to widespread viral dissemination.¹¹ NiV infects monocytes and T lymphocytes, and NiV antigen has been found in macrophages and dendritic cells.¹¹ Immune cell depletion, including T lymphocytes, may play a role in increased risk of secondary infections.²³

DIAGNOSIS

NiV is a BSL-4 agent. People collecting samples must wear personal protective equipment including respiratory protection. Diagnostic tests that do not amplify virus and minimize handling of samples are preferred.¹¹

TESTS TO DETECT NUCLEIC ACIDS, VIRUS, OR ANTIGENS

Virus isolation should be performed for definitive diagnosis in an area with a newly suspected outbreak. Vero cells are preferred,¹¹ but NiV can also be isolated in RK-13, BHK, and porcine spleen cells.¹⁹ Formation of large syncytia is usually seen within 2–3 days.¹¹ However, an isolation attempt should not be declared unsuccessful until several passages of least five days are conducted.¹¹

Reverse transcriptase polymerase chain reaction (RT-PCR) assays are more rapid, sensitive, and safe than virus isolation.¹¹ A number of highly sensitive quantitative RT-PCR (qRT-PCR) assays have been developed. As described by Mazzola and colleagues,²⁴ these include RT-PCR (P, N genes),¹³ qRT-PCR (N gene, P gene, M gene),^{25, 26} SYBR qRT-PCR (N gene),^{26, 27} duplex nested RT-PCR (N gene),²⁸ nested RT-PCR (P gene²⁹ or L gene²⁶), multiplex bead-based qRT-PCR (N and P genes, NiV and Hendra virus),³⁰ and multiplex array card qRT-PCR (N gene).^{31, 32}

Additionally, qRT-PCR primers and probes have been developed for the N gene of the two different NiV strains, mNiV and bNiV.³³ High-throughput sequencing for a virus panel including NiV has recently been described.³⁴ Oropharyngeal or nasal swabs are preferred for RT-PCR. Virus is detectable prior to the onset of clinical signs and for three weeks post-infection.¹¹

Detection of viral antigen in fixed tissues, via immunohistochemistry, is also rapid and safe.⁹ An antigen capture enzyme linked immunosorbent assay (ELISA) has been described for NiV and Hendra virus.³⁵

TESTS TO DETECT ANTIBODY

Virus neutralization is the OIE reference standard. A high-throughput micro-fusion inhibition test (mFIT) has recently been described that can identify and quantify neutralizing antibodies to NiV.³⁶ However, ELISA is the most used serological test for NiV.¹¹ Testing is rapid, and use of recombinant proteins makes it safe to perform in BSL-2 facilities.³⁷

As described by Mazzola and colleagues,²⁴ ELISAs have been developed targeting NiV IgG and IgM^{13, 38} and the viral proteins G,³⁹⁻⁴¹ N,^{35, 37, 42-44} M,^{42, 45} and P.^{35, 46} A multiplex bead-based antibody capture ELISA that targets the G glycoprotein from both NiV and Hendra virus has been described.⁴⁷ A positive ELISA result should be confirmed by virus neutralization in a BSL-4 facility.¹¹

Conditions for inactivation of NiV in serum have been described. To be safe for handling in a BSL-2 facility, samples can be UV irradiated, with a cover of aluminum foil, for 30 minutes and heated at 56°C for 30 minutes.

SAMPLES

Oronasal swabs, urine, and serum can be used for isolation from live animals, while brain, lung, kidney, and spleen samples can be used postmortem.⁹ If possible, urine should also be collected for analysis.⁹ Experimentally, oral fluids have been successfully used for NiV detection via qRT-PCR¹⁴ Strict biosecurity protocols, including stringent use of personal protective equipment, should be followed when sampling suspect cases.¹⁹

EPIDEMIOLOGY

SPECIES AFFECTED

Fruit bats in the genus *Pteropus*, also known as flying foxes, are thought to be the main reservoir host for NiV.⁹ Fruit bats roost in forests and swamps, flying to fruit trees at night to feed. Viral shedding can occur throughout the year, and multiyear epizootics in pteropid bats have been associated with increased roost size, decreased immunity over time, and viral recrudescence.⁴⁸

NiV antibodies have been found in most fruit bat species that have been tested. There are likely unknown reservoir species, and surveillance should be expanded to detect these.⁴⁹ Fruit bats known to harbor NiV include:

- *Cynopterus brachyotis*, *Eonycteris spelaea*, *Pteropus hypomelanus*, *Pteropus vampyrus* (Malaysia)⁵⁰
- *Pteropus medius*, *Pteropus giganteus* (Bangladesh⁶ and northeast India)^{51, 52}
- *Scotophilus kuhlii* (Malaysia)⁵⁰
- *Pteropus lylei* (Cambodia^{53, 54} and Thailand)⁵⁵
- *Eidolon dupreanum*, *Pteropus rufus* (Madagascar)⁵⁶
- *Eidolon helvum* (Ghana)⁵⁷

NiV does not infect the Egyptian fruit bat, *Rousettus aegyptiacus*.⁵⁸

Domestic pigs can be amplifying hosts for NiV.⁵⁹ There is no information on NiV in wild swine.⁶⁰ Horses were associated with the 2014 outbreak in the Philippines.¹⁰ Other livestock species may act as spillover hosts including goats and possibly cattle.⁶¹ Dogs and cats are susceptible to NiV, but are not thought to transmit NiV to humans or other animals.⁷ Experimentally, species that have been infected with NiV include pigs, cats, ferrets, nonhuman primates, guinea pigs, golden hamsters, and mice.¹⁹ Rodents and birds near the Malaysian outbreak tested seronegative.⁵⁹

GEOGRAPHIC DISTRIBUTION

The first known outbreak of NiV in humans and pigs occurred in northern Malaysia in September 1998. The virus spread to central Malaysia and Singapore by February and March 1999, respectively, through movement of subclinically infected pigs.⁵⁹ Since 2001, non-swine-related outbreaks have occurred nearly every year in Bangladesh and parts of India.⁶² The geographic distribution of NiV correlates with the range fruit bats in the genus *Pteropus*⁹ which includes Oceania, South and Southeast Asia, and sub-Saharan Africa.⁴

NiV has not been detected in China, to date. Risk factors associated with possibly NiV introduction include live pig trade and pig smuggling, human movements from South and Southeast Asia to China, seasonal migration of fruit bats, and fruit trade (contaminated fruit, juice, or pulp).⁶³

MORBIDITY AND MORTALITY

During the Malaysian outbreak, there was 10–15% mortality in piglets.⁵⁹ Death occurs in less than 5% of growing pigs.¹¹ Many NiV infections are subclinical in pigs.

ETIOLOGY

CHARACTERISTICS OF PARAMYXOVIRUSES

NiV belongs to the family *Paramyxoviridae*. Paramyxoviruses are large (100–350nm), enveloped, single-stranded RNA viruses.¹¹ Virions are pleomorphic, and can take on a spherical or elongated form. They have a layer of surface spikes and a herringbone-shaped nucleocapsid.¹¹ Paramyxoviruses have six open reading frames (ORFs) encoding the structural genes N (nucleocapsid), L (polymerase), P (phosphoprotein), M (matrix) F, (fusion), and G/H/HN (attachment glycoproteins).⁶⁴

There are seven genera in the family, four of which affect mammals: *Rubulavirus*, *Henipavirus*, *Respirovirus*, and *Morbilivirus*. Paramyxoviruses of swine¹¹ include:

- Menangle virus and porcine rubulavirus (blue eye), genus *Rubulavirus*
- Nipah virus and Hendra virus, genus *Henipavirus*
- Porcine respirovirus 1 (porcine parainfluenza virus 1), genus *Respirovirus*

CHARACTERISTICS OF NIPAH VIRUS

There are two distinct NiV strains that circulate in South and Southeast Asia: mNiV, found mostly in Malaysia and Cambodia, and bNiV, found mostly in Bangladesh and India.⁵⁹ mNiV and bNiV likely diverged around 1995.⁶⁵ Considering both the N and G proteins, inter-clade similarity ranges from 98–100%, while intra-clade similarity is approximately 92–95%.⁶⁶ Isolates that contain mixed N gene sequences have been found in Thailand.⁶⁶

In the Malaysian outbreak, one NiV variant spread rapidly and caused most cases of disease in pigs and people.⁶⁶ Greater sequence heterogeneity is seen in NiV variants in Bangladesh, possibly because NiV has been introduced from fruit bats to humans multiple times.⁶⁶

Compared to mNiV, bNiV has a shorter incubation period, more commonly induces respiratory disease, and less commonly causes myoclonus. Experimentally, bNiV also seems to be more pathogenic than mNiV.^{33, 67} In addition to Hendra virus, NiV is similar to Cedar virus, a paramyxovirus found in Australian bats.⁶⁸ Other emerging paramyxoviruses have been described by Thibault and colleagues.⁶⁴

A number of NiV isolates have been isolated and sequenced from fruit bats and humans.^{69, 70} Full genome sequencing of an NiV isolate from a fruit bat in Cambodia, 2003, showed high pathogenicity related to the production of nonstructural proteins V and W, which counteract host innate immunity.⁷¹

HISTORY IN SWINE

Widespread NiV outbreaks occurred in people and pigs in Malaysia and Singapore from 1998-99.¹⁹ Exactly how and when NiV jumped from pteropid bats to pigs is unknown. In affected areas, pig farming had recently intensified. Increasing commercial fruit production led to more trees being planted near farms.⁷² Together, these factors facilitated contact between pigs and fruit bats.⁷² Retrospective analysis showed that NiV was present in pigs several years prior to this outbreak but went unnoticed. NiV does not produce pathognomonic signs in pigs and diagnosis may be complicated by co-infection with other pathogens.¹¹ No major swine outbreaks have occurred in subsequent years.

IMMUNITY

POST-EXPOSURE

Experimentally, neutralizing antibodies can be detected 7–10 dpi, with maximum titers seen 14–16 dpi.¹¹ The cellular immune response is also a component of NiV protection in pigs.^{11, 73} Persistent infections do not occur.¹¹

VACCINES

There are no commercially available NiV vaccines for humans or swine. Vaccines being investigated for use in pigs include an adjuvanted NiV sG protein (similar to the sub-unit vaccine used in horses),⁷⁴ a simian adenoviral vector expressing NiV G, and an adjuvanted, molecular clamp stabilized NiV F protein.^{75, 76}

Additional NiV vaccines have been described by Hauser and colleagues⁴ and others.⁷⁷ They include:

- A sub-unit vaccine based on soluble Hendra virus G glycoprotein which protects against both Hendra virus and NiV. This vaccine is licensed for use in horses in Australia.⁷⁴
- Vector-based vaccines that utilize a number of recombinant virus/protein expression combinations including simian adenovirus/bNiV G,⁷⁸ vesicular stomatitis virus/bNiV F or G,^{79, 80} rabies virus/NiV G and F,^{81, 82} vesicular stomatitis virus/Ebola glycoprotein and NiV G,⁸³ measles virus/NiV G,⁸⁴ bovine herpesvirus/NiV G or F,⁸⁵ and vaccinia virus/NiV G.⁸⁶
- A virus-like particle-based vaccine utilizing purified Nipah-like particles (G, F, and M proteins).⁸⁷
- An mRNA-based vaccine encoding a soluble Hendra virus glycoprotein subunit.⁸⁸

CROSS-PROTECTION

Novel henipaviruses likely induce NiV cross-protection.⁸⁹ Experimentally, there is also good cross-protection between NiV and Hendra virus G protein using a ferret model.⁸⁹

GAPS IN PREPAREDNESS

NiV is highly infectious in pigs, and it causes serious disease in humans. There is no vaccine available for pigs or people. Diagnosis is challenging because clinical signs are non-specific. Additionally, U.S. laboratories are not equipped to detect NiV—the virus must be handled only in BSL-4 facilities. If an outbreak occurred, mass culling would likely ensue. This is dangerous for people, putting them in close contact with sick pigs. Once NiV enters the human population, it can spread person-to-person. NiV has potential to become a pandemic agent.⁹⁰

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