

NIPAH

Aetiology Epidemiology Diagnosis Prevention and Control References

AETIOLOGY

Classification of the causative agent

Nipah virus is an enveloped, negative-sense, single-stranded RNA virus in the family Paramyxoviridae, genus *Henipavirus*. The name of the virus and disease is from the village of "Sungai Nipah" in Malaysia where the first human cases lived.

Resistance to physical and chemical action

Temperature:	Other animal <i>Paramyxoviruses</i> are inactivated by 60°C/60 minutes.
pH	Stable between pH 4.0 and 10.0.
Chemicals/Disinfectants:	<i>Paramyxoviruses</i> are susceptible to common soaps and disinfectants; lipid solvents (alcohol and ether) and sodium hypochlorite solutions were used effectively in outbreaks for cleaning and disinfection.
Survival:	Survives for long periods in favourable conditions; survives for days in fruit bat urine and contaminated fruit juice.

EPIDEMIOLOGY

Hosts

- Primary reservoir for Nipah virus are fruit bats of the genus *Pteropus*
- Domestic swine are extremely susceptible to infection; act as amplifying host
- Infections have also been reported in dogs, cats, horses and goats

Transmission

- Transmission of virus from bats to swine has not been conclusively elucidated; various biologically plausible means for infected secretions of primary hosts to enter installations of pigs
 - swine enclosures in proximity of fruit trees where bats reside; direct contact with infected secretions, contaminated fruit or dead bats
 - scavenging animals may also play a role in transport of virus into proximity of pigs
- Nipah-infected swine may aerosolise virus or transmit disease by direct contact of their respiratory secretions to other swine and humans
- Movement of animals incubating disease or not demonstrating clinical signs is principle means of extending Nipah virus geographically
- Vertical transmission across the placenta, by iatrogenic means and in semen has been suggested but not confirmed in swine
- Intranasal and oral inoculation of cats with virus experimentally produced disease

Sources of virus

- Nipah virus has been found in urine and uterine fluids of wild pteropid bats; experimentally isolated from urine, kidney and uterus of infected bats
- Virus may be found in fruit contaminated with bat saliva or urine
- Other logical sources for infection are contaminated drinking water and access to aborted bat foetuses or other fluids/tissues of parturition

- Once infected, pigs shed Nipah virus in respiratory secretions and saliva; urine of infected swine should also be considered a risk
- Role of other animals as a source of virus in outbreaks is less clear though virus has been isolated from feline respiratory secretions, urine, the placenta and embryonic fluids
- Sources of infective material for humans include swine respiratory secretions or via fruit or juice contaminated by bat secretions (e.g. unpasteurised date palms or juice)

Occurrence

To date, Nipah has only been reported from Malaysia, Bangladesh and India. Human cases among abattoir workers in Singapore were associated with first reports of disease in 1998. Virus isolation and seropositivity has been confirmed from various countries in Southeast Asia and Madagascar. Areas of Southeast Asia where fruit bats of the genus *Pteropus* are present should be considered endemic.

For more recent, detailed information on the occurrence of this disease worldwide, see the *OIE World Animal Health Information Database (WAHID)* interface [<http://www.oie.int/wahis/public.php?page=home>] or refer to the latest issues of the *World Animal Health* and the *OIE Bulletin*.

DIAGNOSIS

Incubation period in pigs is approximately 7–14 days, but may be as short as four days. Experimentally in cats, incubation periods of 6–8 days have been documented. Average incubation period is 4 to 20 days in humans; can be as short as two days or as long as a month.

Clinical diagnosis

Swine

Available observations of clinical signs in swine would suggest a respiratory and neurologic foundation; clinical manifestations associated with age groups

Suckling pigs and piglets (< 1 month old)

- Laboured breathing and muscle tremors with limb weakness
- Mortality in piglets can be high (40%)

Young swine (1 to 6 months old)

- Begins as an acute fever with respiratory signs; laboured breathing, nasal discharge and loud non-productive cough (“barking pig syndrome” and “one-mile cough”)
- Accompanying neurologic signs: muscular fasciculation, myoclonus, limb weakness, and spastic paresis; in some cases, lateral recumbency with paddling and tetanic spasms
- Disease presentation can be mild to fulminant with high morbidity and low mortality (< 5%)

Older animals (> 6 months old)

- Acute febrile condition with marked neurologic signs
- Central nervous system involvement: nystagmus, bruxism, head pressing, aggressive behaviour, tetanic spasms and seizures
- Respiratory signs may include open-mouthed breathing, nasal discharge and sialorrhoea (possibly due to pharyngeal paralysis)
- Morbidity in confined animals approaches 100%
- Fulminant death in this age group with few signs has been reported; mortality still tends to be low
- First trimester abortions have also been reported

Other species

- Limited clinical information exists for other species
- In dogs, distemper-like syndrome observed with pyrexia, depression, dyspnoea and conjunctivitis with purulent ocular-nasal discharge
- Nipah affected cats were observed on farms during outbreaks in Malaysia and some of these resulted in death; acute febrile disease with respiratory complications was observed experimentally in cats
- Fruit bats show no serious signs of infection

Humans

- Mild or subclinical presentation common; clinical cases manifest acute neurologic condition
- Begins as influenza-like illness: high fever, headache, muscular pain, and weakness.
- If central nervous system involvement progresses: drowsiness, dizziness, convulsions and/ or coma sometimes accompanied by nausea and vomiting; 50% of cases develop impaired consciousness with brain stem dysfunction.
- Respiratory signs less common but could manifest as acute respiratory distress syndrome
- Seriously affected patients can develop septicaemia, gastrointestinal bleeding, and renal impairment; in some instances leading to death
- Recovered patients may experience encephalitic relapse up to years later and subclinically infected individuals may show central nervous signs up to 4 years later

Lesions

- Principal gross and microscopic lesions associated with Nipah in swine found in lungs and/or central nervous system
- Lung lesions may vary from mild to severe pulmonary consolidation with petechial or ecchymotic haemorrhages and distended interlobular septa
- Trachea and bronchi may be filled with frothy exudate which varies in appearance from clear to blood-tinged
- Meningeal oedema with congestion of the cerebral blood vessels has been observed in the brain; some cortical renal congestion may be evident
- Microscopically, epithelia of all the major respiratory pathways are affected with presence of syncytial cells in vascular endothelium
- A mononuclear vasculitis with fibrinoid necrosis is often observed associated with thrombosis
- Principal histologic changes in the brain, if present, are perivascular cuffs and gliosis
- Reports of a generalised vasculitis in cats and non-suppurative meningitis in horses have been recorded

Differential diagnosis

- It is important to note that this disease has human health implications and all field investigations should take necessary precautions to prevent infection
- Any respiratory or neurological conditions of swine in an area known to have pteropid bats, should consider Nipah as a rule out
- Also among swine;
 - deaths of suckling pigs and piglets; sudden death in boars and sows
 - abortions and other reproductive dysfunction
 - respiratory diseases with harsh, non-productive coughing
 - cases with encephalitic manifestations of trembling, muscular incoordination and myoclonus leading to lateral recumbency

Laboratory diagnosis

At the outset, it is important to consider that Nipah is classified as a biosafety level 4 (BSL4) agent and special precautions must be undertaken in the collection, submission and processing of samples. See Chapter 1.1.1 of the OIE *Terrestrial Manual*, Collection and shipment of diagnostic specimens.

Identification of the agent

- Virus isolation and characterisation
 - sampling and submission of samples – brain, lung, kidney and spleen transported at 4°C in 48 hours or frozen if over 48 hours
 - isolation in cultured cells – African green monkey kidney (Vero) and rabbit kidney (RK-13) cells; CPE usually develops within 3 days but two 5-day passages are recommended before judging the attempt unsuccessful
- Methods of identification
 - Immunostaining of fixed cells - cross reactivity within henipaviruses requires differentiation
 - Test procedure:
 - Vero or RK-13 cells grown on glass cover-slips or chamber slides
 - Monolayers are examined for the presence of syncytia after incubation for 24–48 hours at 37°C
 - Henipavirus-induced syncytia characterised by presence of large polygonal structures containing viral antigen
 - Immunoelectron microscopy – visualisation of culture medium by negative-contrast electron microscopy; grid cell culture technique complement diagnostic efforts
- Virus neutralisation
 - Plaque reduction: virus–antiserum mixtures incubated at 37°C for 45 minutes, adsorbed to monolayers of Vero cells at 37°C for 45 minutes; number of plaques determined by traditional plaque assay procedures after incubation at 37°C for 3 days; titre is calculated as plaque forming units (PFU)
 - Microtitre neutralisation: virus–antiserum mixtures used on microtitre plate; after 3 days at 37°C, test is read using inverted microscope and wells scored for degree of CPE observed; titre is calculated as the tissue culture infectious dose capable of causing CPE in 50% of replicate wells (TCID₅₀)
 - Immune plaque assay: viruses titrated on Vero cell monolayers (96-well plates); infection detected immunologically using anti-viral antiserum; titre is expressed as focus-forming units (FFU)/ml
- Nucleic acid based recognition methods
 - complete genomes of both HeV and NiV have been sequenced thus allowing PCR-based methods; real-time PCR provides benefit of not propagating live infectious virus
 - Laboratories wishing to establish molecular detection methods should refer to published protocols or consult the OIE Reference Laboratory
- Henipavirus antigen detection in fixed tissue – immunohistochemistry
 - Possible samples: brain at various levels, lung, mediastinal lymph nodes, spleen and kidney; in pregnant animals the uterus, placenta and foetal tissues should be included
 - Formalin-fixed tissues or formalin-fixed cells of vascular endothelium
 - Antisera to HeV and NiV; rabbit antisera to plaque-purified HeV and NiV have been found to be particularly useful
 - Biotin–streptavidin peroxidase-linked detection system has also been used successfully

Serological tests

Various strategies have been developed to reduce the risk of laboratory sera; gamma-irradiation or sera dilution and heat-inactivation

- Virus neutralisation tests: accepted as the reference standard
 - Henipaviruses can be quantified by plaque, microtitre or immune plaque assays; these assays can be modified to detect anti-virus antibody
 - cultures read at 3 days; those sera that completely block development of CPE are designated as positive
 - Immune plaque assay is an option in the event of cytotoxicity as virus/serum mixtures are removed from the Vero cell monolayers after the adsorption period, thereby limiting their CPE
- Enzyme-linked immunosorbent assay (ELISA)
 - Henipavirus antigens derived from tissue culture for use in ELISA are irradiated with 6 kiloGreys prior to use; negligible effect on antigen titre

- Indirect ELISA for detection of IgG
- Capture ELISA for detection of IgM
- Modified ELISA developed based on relative reactivity of sera with NiV antigen
- due to false-positives related to specificity of ELISA, all suspect-positive ELISA confirmed by VNT

For more detailed information regarding laboratory diagnostic methodologies, please refer to Chapter 2.9.6 Hendra and Nipah virus diseases in the latest edition of the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* under the heading “Diagnostic Techniques”.

PREVENTION AND CONTROL

Sanitary prophylaxis

- Strict biosecurity of swine installations with the aim of avoiding contact with fruit bats and their secretions is essential, including: fruit tree set-back, using screens at open-air access and appropriate disposal of roof run-off
- An active surveillance program with rapid detection and immediate culling of seropositive swine is critical in preventing spread of disease and infection of humans
- Effective quarantines and control of animal movements must also be implemented early in an outbreak
- All materials and equipment from affected farms should be cleaned and disinfected before transport
- Control of any access to swine by wild or domestic animals must be enacted

Medical prophylaxis

- No vaccines yet exist but recent experiments in cats seem promising

For more detailed information regarding safe international trade in terrestrial animals and their products, please refer to the latest edition of the *OIE Terrestrial Animal Health Code*.

REFERENCES AND OTHER INFORMATION

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The OIE will periodically update the OIE Technical Disease Cards. Please send relevant new references and proposed modifications to the OIE Scientific and Technical Department (scientific.dept@oie.int). Last updated October 2009.