PHLN
Public Health Laboratory Network

Hendra virus

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *hendra virus*.

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1 PHLN Summary Laboratory Definition

1.1 Condition:

Hendra virus

1.1.1 Definitive Criteria

- Isolation of *Hendra virus* from clinical material (requires PC4 laboratory)
- Detection of *Hendra virus* RNA in clinical material by nucleic acid testing (NAT)
- Detection of seroconversion or four-fold or greater rise in *Hendra virus*-specific IgM or IgG titres

1.1.2 Suggestive Criteria

- Detection of Hendra virus-specific IgM

1.1.3 Special Considerations

- Isolation of *Hendra virus* should only be performed in a PC4 laboratory
- Hendra virus antibody detection should be confirmed by at least one alternative method e.g. ELISA, microsphere immunoassay (MIA), IFA techniques or serum neutralisation (SNT).

2 Introduction

*Hendra virus (*HeV) is the prototype virus, along with Nipah virus (NiV), of the genus *Henipavirus* in the *Paramyxoviridae* family. It was originally named equine *morbillivirus*, but subsequently renamed HeV following genetic analysis which determined it to be distinct from other paramyxoviruses. It is named after the Brisbane suburb of Hendra where the initial outbreak was recognised in 1994 (1,2).

As with other paramyxoviruses, HeV is a nonsegmented, negative-stranded RNA virus composed of helical nucleocapsids enclosed within an envelope to form roughly spherical, pleomorphic virus particles. HeV has one of the larger genomes among the paramyxoviruses, with 18,324 nucleotides. Six transcriptional units encode six structural proteins, (from the 3' end) nucleocapsid protein (N) phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G), and polymerase (L). The F and G glycoproteins are important for viral attachment and entry (3). The host cell receptor for the G glycoprotein is ephrin B2/B3, which is ubiquitously distributed throughout the arteriolar endothelial cells, neurones, and certain other cells of most animal species. Such a ubiquitous distribution explains both the very wide species susceptibility to HeV infection and the pathophysiology of HeV infection-induced disease (4).

The epidemiology of HeV involves periodic "spillover" events from its natural host (fruit bats – Pteropus spp., among which the seroprevalence is between 20-50%) to horses and then from horses to humans. In total (to October 2009), there have been 13 recognised "spillover" events, involving 43 equine and seven human infections. All "spillover" events have occurred in Queensland and northern New South Wales. Transmission from fruit bats to horses likely involves contamination of pastures or feed with infected fruit bat fluids or tissues, such as gestational products, urine, or saliva. Horse-to-horse transmission appears relatively inefficient but has occurred within stabled environments. Horse-to-human transmission also appears inefficient and has occurred in seven instances – on each occasion involving heavy exposure to infected horse secretions, tissues or body fluids. There has been no evidence of bat-to-human transmission despite extensive surveillance of people with occupational or recreational exposure to flying foxes (1,5,6). There have been no cases of human-to-human transmission of HeV infection, although this is likely to have occurred with NiV.

HeV infection in humans has caused a variety of manifestations: two patients have experienced self-limited influenza-like illnesses, one fatal acute pneumonitis and multiorgan failure, three acute encephalitis (two fatal), and one relapsed encephalitis following aseptic meningitis 13months previously (1,5,6). HeV infection in horses causes an acute rapidly progressive illness characterised by respiratory and neurological manifestations.

There are no effective antiviral therapies available for HeV infection. Although ribavirin has been reported to improve outcomes in NiV infection, there is no clinical evidence of efficacy in HeV infection. Candidate Henipavirus vaccines (for animals) and passive immunotherapeutic therapies for postexposure prophylaxis have been developed and show promising efficacy in animal models. Preventing human HeV infections currently relies upon the prompt recognition of infection in horses so that appropriate infection control precautions to prevent horse-to-human transmission can be implemented (6).

3 Tests

In most circumstances, the diagnosis of HeV infection should be made by detection of HeV RNA (by real time (RT-PCR) or conventional reverse transcriptase PCR confirmed by nucleotide sequencing or detection of HeV-specific antibodies (by EIA, MIA, IFA, or SNT). Culture of HeV is definitive for diagnosis but should only be attempted in PC4 facilities.

Suitable specimens: serum, plasma or whole blood, saliva, nasopharyngeal aspirate/swab, CSF), urine or tissue (especially kidney, lung or neural, although isolation is possible from most tissues).

Swabs should be rayon or dacron-tipped with plastic or aluminium shafts. They may either contain their own viral transport media (VTM), Universal Transport Medium or can be placed into a vial of VTM/UTM immediately after collection. Other specimens should be placed in a sterile container. All

specimens should be stored at 4 °C (according to the appropriate IATA standards), or if they cannot be processed within 72 hours, they should be frozen at -70 °C. Freezing at -20 °C is not acceptable.

3.1 Viral isolation

3.1.1 Suitable specimens

See section 3 overview.

3.1.2 Cell culture techniques

A variety of cell lines may be used to isolate HeV, including Vero, Vero E6, RK13, MDBK, LLC-MK2, BHK, Hep-2, HeLa, and embryonated chicken eggs. Several passages may be required to determine if isolation is successful. Cytopathic effect (CPE) typically manifests in the form of large syncytia containing multiple nuclei.

3.1.3 Suitable Test Acceptance Criteria

Isolation can be confirmed by IFA, immuno-electron microscopy, RT-PCR or genomic sequencing. Verificaion at a second reference lab should be undertaken.

3.1.4 Suitable External QC Programme

None available.

3.1.5 Special Considerations

HeV is a PC4 rated organism. Any work involving live virus should only be conducted in a PC4 laboratory after appropriate training.

3.2 Nucleic acid testing (NAT)

3.2.1 Suitable specimens

see section 3 Note: Fixed tissues and environmental samples have limited diagnostic value due to contaminating and inhibitory substances which may interfere with the tests.

3.2.2 Suitable tests

Real time or conventional gel-based RT-PCR, gene sequencing.

3.2.3 Test Sensitivity

There is currently insufficient data available to determine test sensitivity but real-time RT-PCR has been observed to have significantly greater sensitivity than conventional gel-based PCR (7).

3.2.4 Test Specificity

At least one real time RT-PCR targeting the G gene has been reported to have ~100% specificity for all Hendra virus isolates recovered to date (7) Significant nucleotide heterogeneity has been observed between different Hendra virus isolates and care should be taken in selecting conventional or RT-PCR primers (6). Care should be taken in using RT primer sets targeting the nucelocapsid gene as some published primer sets do not detect all Hendra virus isolates.

3.2.5 Predictive Values and relevant population

Given the low incidence of infection there is insufficient data to enable meaningful calculation of predictive values.

3.2.6 Test Acceptance Criteria

Real time PCR – detection to 40 amplification cycles can be considered definitive of infection. Sequencing should be undertaken of conventional PCR products to confirm definitive diagnosis.

3.2.7 Suitable Internal Controls

e.g. positive controls and negative controls must be run to ensure validity of test run. Inhibition controls may be run dependent upon specimen type. The use of synthetic or oligo-based positive controls may be useful to limit possible false positives or for laboratories that do not have access to HeV RNA.

3.2.8 Suitable External QC Programme

None available.

3.2.9 Special Considerations

Failure to detect HeV nucleic acid does not necessarily exclude infection. Serology should be attempted on sera collected from patients providing samples for RT-PCR or virus isolation. Where possible, samples should be tested in duplicate or positive samples should be confirmed by repeat extraction and RT-PCR confirmation. Use of two or more RT-PCR tests targeting different areas of the Hendra virus genome should be considered. Sequencing should be undertaken to confirm amplicons from conventional PCR.tests.

3.3 Serology

3.3.1 Suitable specimens

Serum (minimum volume 0.5 mL). Specimens should be repeated during the course of the illness to detect an IgM or IgM to IgG seroconversion or a fourfold or greater rise in IgM or IgG titres.

3.3.2 Serological techniques

A variety of serological techniques can be used to detect HeV-specific antibodies, including EIA(8), MIA (9), IFA using acetone fixed Hendra virus infected Vero E-6 cells, and SNT(8).

3.3.3 Test Sensitivity

Insufficient data to determine although the IFA test and MIA test have demonstrated a higher level of specificity and sensitivity in the limited testing conducted to date.

3.3.4 Test Specificity

Insufficient data to determine although the IFA test and MIA test have demonstrated a higher level of specificity and sensitivity in the limited testing conducted to date.

3.3.5 Predictive Values and relevant population

Given the low incidence of infection there is insufficient data to enable meaningful calculation of predictive values.

3.3.6 Suitable Test Acceptance Criteria

A single positive result (with cut offs determined by the laboratory performing the test) should be accepted as suggestive of infection. A seroconversion or four fold rise in HeV-specific antibody titre is indicative of infection.

3.3.7 Suitable Internal Controls

Positive (high, low) and negative controls should be included to validate each test run.

3.3.8 Suitable External QC Programme

None available.

3.3.9 Special Considerations

Serology in the viraemic phase of illness may not demonstrate the presence of anti-HeV antibodies. Virus isolation and/or RT-PCR should also be attempted in this instance. Result variations may exist between assays depending on whether or not recombinant (partial genome represented) or whole viral antigens are employed.

4 Agreed Typing & Subtyping Methods

HeV is a member of the Family Paramyxoviridae: Sub Family Paramyxovirinae: Genus Henipavirus: Species Hendra virus. There are no recognised subtypes.

4.1 Laboratory Nomenclature for National Database Dictionary

SNOMED CT concept	SNOMED CT Code
Hendra virus (organism)	115510008

5 References

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