Henipavirus: Research and Diagnosis of Hendra and Nipah virus at AAHL

C. J. Morrissy
CSIRO Livestock Industries, Australian Animal Health Laboratory (AAHL), Geelong, Victoria, Australia.
Outline

1. Henipaviruses
   - Nipah & Hendra

2. AAHL (BSL4 laboratory)

3. Diagnosis

4. Summary
Nipah and Hendra Viruses

1. Different viruses
   • Molecularly & Serologically

2. New genus within paramyxoviruses
   • Henipavirus

3. Share antigens

4. Biosecurity Level 4 (BSL 4)
   • Must handle virus at BSL4
   • Expensive: facility & maintenance
Paramyxoviruses

Rubulavirus
- PoRV
- MaV
- MuV
- SV5
- SV41
- PIV2
- MenV
- TiV
- SalV

Respirovirus
- PIV3
- SeV

Henipavirus
- NiV
- HeV

Morbillivirus
- PDV
- CDV
- RPV
- MeV
- PPRV
- CMV
- TPMV
Emerging Viral Diseases: The Hendra Story

Late September, 1994, an outbreak of severe respiratory disease in horses. A single pregnant mare (Drama Series) was moved from a spelling paddock in Cannon Hill, to a stable complex in the Brisbane suburb of Hendra. The disease was characterised by high fever and severe respiratory difficulty.
Grossly apparent pulmonary edema with frothy hemorrhage in the major airways, with severe focal necrotising alveolitis and intraalveolar hemorrhage.
Inteststitial Pneumonia.

Human EMV Fatality Brisbane

Pre-admission Sept 19, 1994

Sept 24, 1994
An Overview of the Outbreak

Date in September 1994

Number of Horses

0 2 4 6 8 10 12

Vic Rail dies

SICK
DEAD

07 09 11 13 15 17 19 21 23 25 27
Hendra virus disease

- 38 horses
  - 27 died
- 6 people
  - 4 died
- 47% of 1043 pteropid bats were seropositive
- Hendra Virus (HeV) isolated from free living bats

After an incubation period of 9–16 days, influenza-like illnesses developed in the 2 persons before progressing to encephalitis; 1 died. Both patients were given ribavirin. Both patients were exposed to infected horses, 1 during the late incubation period in a horse.
The wildlife reservoir....

- 47% of 1043 *pteropid* bats were seropositive

- HeV isolated from free living bats (2/465)
Queensland Horse Density
As at: 06 October 2009

Legend
- Flying Fox Roost Sites
- Flying Fox Additional Jan2009

APS Horse Counts
Estimated number
- 0 - 5
- 6 - 20
- 21 - 60
- 61 - 150
- 151 - 450
- 451 - 3000

Cairns (3)
Townsville (1)
Bowen (1)
Proserpine (1)
Mackay (1)
Cawarral (1)
Peachester (2)
Brisbane (2)
Murwillumbah (1)
Critical control points for preventing transmission
Nipah Virus (NiV)

- Outbreak of viral encephalitis in Malaysia
  - Late 1998 and early 1999
  - 265 human cases, of whom 105 died
  - mainly pig farm workers affected
- Singapore, 11 cases with 1 death
- Bangladesh (NiVB), Variant strains of NiV have caused repeated disease outbreaks in with an increased mortality rate (approximately 75%)
  - bat-to-human and human-to-human transmission.
- Cases in pigs which required development of diagnostic tests
  - Rapid test for surveillance (Indirect ELISA)
The wildlife reservoir......

• Serology in Malaysian bats
  • *Pt. vampyrus* 5/29 +ve
  • *Pt. hypomelanus* 11/35 +ve

• NiV isolated from bat urine on Tioman Is. Malaysia

• NiV reisolated from urine of experimental bats
Eradication of NiV in pigs

1. Serological surveillance
2. Culling seropositive farms

- From 28 February 1999 to 26 April 1999, more than 900 000 pigs from affected areas were culled.
- Ongoing surveillance demonstrates the success of the control program, with no new cases detected since May 1999.
Henipaviruses

• Since the identification of fruit bats as the reservoir hosts, it has been shown henipaviruses have extensive geographical range, with seropositive bats in Indonesia, Thailand, Cambodia and Madagascar. More recently, antibodies have also been found in bats in Northern India, West Africa and China. Isolates of henipaviruses have been obtained from bat populations in Australia, Malaysia and Cambodia.
Australian Animal Health Laboratory (AAHL)
Biosafety level 4 Large Animal Facility
Biosafety Level 4 Animal Facility
Experimental design: Horse

- n=3
- $2 \times 10^6$TCID$_{50}$ Redland Bay 2008
  - spleen
  - 10 ml intranasal/ 10 ml per os
- Daily swabs
  - nasal
  - oral
  - rectal
- Daily urine/feces
- Daily blood
  - VI/PCR
  - Serology
- Post-mortem
Diagnosis

Henipavirus infection can be diagnosed by a number of different tests:

- virus neutralization (VNT): BSL4
- enzyme-linked immunosorbent assay (ELISA)
  - critical for surveillance & eradication programs
  - animal movement (cats, dogs, horses)
- polymerase chain reaction (PCR) assay
- Electron Microscopy (EM)
- Immunofluorescence & immunoperoxidase assays
  - Immunohistochemistry & EM
- virus isolation by cell culture: BSL4
### HeV ELISA

<table>
<thead>
<tr>
<th></th>
<th>Bats</th>
<th>Horses</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Tested</td>
<td>247</td>
<td>939</td>
<td>347</td>
</tr>
<tr>
<td>Positive</td>
<td>42</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>205</td>
<td>907</td>
<td>338</td>
</tr>
</tbody>
</table>

#### Sensitivity and Specificity Relative to VNT

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bats</td>
<td>100%</td>
<td>88.8%</td>
</tr>
<tr>
<td>Horses</td>
<td>96.9%*</td>
<td>99.6%</td>
</tr>
<tr>
<td>Humans</td>
<td>78.8%*</td>
<td>100%</td>
</tr>
</tbody>
</table>

*Based on small numbers of sera.

*Several sera from early infection.*
## Comparison of Nipah ELISAs/VNT

450 sera (Malaysia)

<table>
<thead>
<tr>
<th></th>
<th>Nipah ELISA</th>
<th>VNT</th>
<th>New Nipah ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>150</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Eq</td>
<td>150</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Neg</td>
<td>150</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>
### New Nipah Indirect ELISA

<table>
<thead>
<tr>
<th>VNT</th>
<th>POS</th>
<th>NEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA POS</td>
<td>98</td>
<td>5</td>
</tr>
<tr>
<td>NEG</td>
<td>1</td>
<td>5333</td>
</tr>
</tbody>
</table>

Specificity: 99.91%
Sensitivity: 98.99%

#### Expressed Antigens
- Reduce need to work at BSL4
- Specific antigens or reagents for Hendra and Nipah
- Need for a specific ELISA for serosurveillance of bats
Summary Research

- **Lab-based research**
  - Diagnostics
  - Therapeutics
  - Vaccine
  - Animal model
  - Pathogenesis
  - Virus-bat interaction

- **Field-based study**
  - Longitudinal study in Geelong
  - Henipavirus infection dynamics in Queensland
  - Surveillance study overseas
• Improved real-time PCR compatible with all known henipaviruses (*J. Virol. Meth.* 161: 52-57, 2009)
• Virus neutralization based on pseudovirus with reporter genes: more sensitive, more quantative and more reproducible (*J. Virol. Meth.* 160: 7-13, 2009)
• Virus sensor for “pen-side” test (on going collaborative research)
Therapeutics & Vaccine

**Therapeutics**

- Recombinant human monoclonal antibody effective in an in vivo ferret infection model (*PLoS Path. 5: 1-11, 2009*)
- Although showing some promise in vitro, chloroquine is not effective in vivo (*J. Vriol. 83: 11979-11982, 2009*)

**Vaccine**

- Recombinant soluble G proteins of henipavirus is a promising vaccine candidate in small animal models (*Vaccine 26: 3842-3852, 2008*)
- The Hendra virus soluble G protein is current being tested as a potential vaccine in horses to protect horses from disease/infection.
Animal model

- Ferrets are becoming the animal of choice for henipavirus infection studies at AAHL (*PLoS Path. 5: 1-11, 2009*)

- In collaboration with US scientists, African green monkey is shown to be a suitable non-human primate model for henipavirus infection and disease (*PLoS One 5(5): e10690, 2009*).

- Also animal models in Horse, Cats, Pigs & Bats
Pathogenesis

- Establishment of a reverse genetics system for Hendra virus to examine the molecular determinant for pathogenesis
- Comparison of transmission and pathogenesis of Nipah virus-Malaysia versus Nipah virus-Bangladesh
- Study of henipavirus infection in mice using knock-out mice to determine factors important for innate immunity and pathogenesis
- Screening of human genome-wide siRNA library to identify genes important for henipavirus infection
Virus-bat interaction

- Bat genomics and transcriptomics

- Bats important reservoir for diseases transmitted to Humans: Henipaviruses, SARS & Ebola
Field studies

• Longitudinal study of a bat colony in Geelong (next to AAHL): weekly sampling of bat urine for 12 months to study virus infection dynamics and to identify potential triggers for “viral spikes” in the bat population

• Survey of Queensland bat population to study Hendra virus genetic diversity and factors important for spill over events

CSIRO Livestock Industries, Australian Animal Health Laboratory (AAHL)

Name: Chris Morrissy
Title: Scientific Coordinator, AAHL Regional Program, Diagnostic Virologist

Phone: +61 3 5227 5425
Email: chris.morrissy@csiro.au

Thank you