Emerging paramyxoviruses in Australia
Hendra and Menangle

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Emerging paramyxoviruses in Australia
Overview

- Virology, pathogenesis
- Epidemiology
- Clinical disease in animals & humans
- Recent developments in therapeutics & prevention
- Implications for veterinary infection control
Recently described paramyxoviruses associated with bats

- Hendra virus (Australia, 1994)
- Menangle virus (Australia, 1997)
- Nipah virus (Malaysia, 1998)
- Tioman virus (Malaysia, 1999)

World distribution of flying foxes (genus Pteropus)

From: Field et al., CTMI 2007;315:133-59
Background
Hendra virus

- Family *Paramyxoviridae*
  - Subfamily *Paramyxovirinae*
    - Genus *Henipavirus*

- First recognised 1994 from outbreak at Hendra horse stables in Brisbane

- Initially designated:
  - Acute equine respiratory syndrome
  - Equine morbilliform virus

Hendra virus structure and genome

- Large genome: 18,234 nucleotides (~15% longer than most other paramyxoviruses)
- Typical paramyxovirus genome structure

Attachment, fusion, cell entry

**G glycoprotein**
- Binds to Ephrin-B2
- Highly conserved and ubiquitously-distributed surface glycoprotein
- Present in small arterial endothelial cells & neurones
- Ligand for Eph class of receptor tyrosine kinases

**F glycoprotein**
- Precursor (F₀) cleaved into biologically active F₁ & F₂ by lysosomal cysteine protease Cathepsin L after endocytosis
- Conformational change into trimer-of-hairpins structure

- Explains:
  - Broad host range
  - Systemic nature of infection

Epidemiology

Flying foxes

- Flying foxes of genus *Pteropus*

- Seroprevalence ~50%
- HeV isolated from birthing fluids, placental material, and aborted pups
- Experimentally isolated from urine
- No apparent clinical disease
- Duration of infection, immunity & dynamics of infection within/between colonies uncertain
- Overlapping distribution of HeV & NiV between Australian & SE Asia

Epidemiology
Transmission

- Virions susceptible to desiccation\(^1\)
- Bat-to-horse:
  - Contamination of pastures & feed with bat gestational products, urine, &/or spats
- Horse-to-horse:
  - More likely in stabled (rather than paddocked) situations
  - Contamination of stable environment/common equipment?
- Horse-to-human:
  - Droplet spread to mucosal surfaces with infected respiratory secretions
  - Direct contact to non-intact skin
  - Attack rate among highest risk exposures \(\sim 10-20\%\)
- Bat-to-human:
  - Not documented in bat handlers despite extensive contact with saliva, urine, faeces etc
- Human-to-human:
  - Not documented in close domestic or HCW contacts

Observed epidemiology of NiV suggests bat-to-human & human-to-human potential of HeV

\(^1\text{Fogarty et al. Virus Res 2008;132:140-4}\)
HeV epidemiology

“Spill over” events: 1994-Sept 2009

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Equine cases</th>
<th>Human cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug 1994</td>
<td>Mackay, Qld</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Sept 1994</td>
<td>Hendra, Qld</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>Jan 1999</td>
<td>Cairns, Qld</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Oct 2004</td>
<td>Cairns, Qld</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dec 2004</td>
<td>Townsville, Qld</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>June 2005</td>
<td>Peachester, Qld</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Oct 2006</td>
<td>Murwillambah, NSW</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>June 2007</td>
<td>Peachester, Qld</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>July 2007</td>
<td>Clifton Beach, Qld</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>July 2008</td>
<td>Thornlands, Qld</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>July 2008</td>
<td>Proserpine, Qld</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Aug 2009</td>
<td>Cawarral, Qld</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Sept 2009</td>
<td>Bowen, Qld</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

- All events temporally associated with flying fox activity in region
- Equine index cases typically paddocked in areas attractive to flying foxes (fruiting trees etc)
- Apparent increase in “spill over” events
Clinical manifestations
Equine infection

- Fever, tachycardia
- Respiratory manifestations:
  - Tachypnoea, respiratory distress, nasal discharge, and/or
- Neurological manifestations:
  - Ataxia, head tilt, facial nerve paralysis

- 42 recognised equine cases
- 75% case fatality rate
- Course of illness for fatally infected horses: 2 days from initial signs of infection to death
- HeV excreted in respiratory samples at least 2 days prior to symptoms

- Post mortem findings:
  - Widespread systemic vasculitis, endothelial syncytial cells

Field et al. CTMI 2007;315:133-59
Hendra, Brisbane
Hendra, September 1994

- Explosive outbreak of unknown infectious agent involving 22 horses and 2 humans (1 fatal)
## Clinical manifestations
### 1994 Hendra outbreak

<table>
<thead>
<tr>
<th>Case</th>
<th>Date</th>
<th>Exposure to infected horses</th>
<th>Clinical syndrome</th>
<th>Outcome</th>
<th>Post mortem findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:</td>
<td>Sept 1994</td>
<td>Respiratory secretions</td>
<td>‘Influenza-like’ illness</td>
<td>Recovery, no relapse</td>
<td>PM: widespread vasculitis (incl. brain), severe pulmonary necrotising alveolitis, mild meningeal/brain parenchymal inflammation</td>
</tr>
<tr>
<td>2:</td>
<td>Sept 1994</td>
<td>Respiratory secretions</td>
<td>Pneumonitis, multi-organ failure, fatal</td>
<td>Died (day 13 illness)</td>
<td></td>
</tr>
</tbody>
</table>

1: 40-yo stable hand
2: 49-yo trainer

HeV clinical manifestations
Case 2: Pneumonitis

- Diffuse alveolar damage
  - Widespread hyaline membranes
  - Numerous intra-alveolar macrophages
- Pulmonary thromboemboli
- Viral antigens on IHX

From: J McCormack & Kathryn Uranker
HeV neuropathological findings:  
Case 2

Vasculitis in meningeal & brain parenchymal blood vessels with HeV antigens

Necrotic/vacuolar plaque in the dentate nucleus

HeV antigens in neuronal cytoplasm & nucleus

Summary:
- Focal vasculitis & endotheliitis in meninges & parenchyma
- Viral antigens in endothelial cells
- Small necrotic/vacuolar plaques
- Mild lymphocytic leptomenigitis

Clinical manifestations
1994 Mackay outbreak*

<table>
<thead>
<tr>
<th>Case</th>
<th>Date</th>
<th>Exposure to infected horses</th>
<th>Clinical syndrome</th>
<th>Outcome Post mortem findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>36-yo farmer</td>
<td></td>
<td></td>
<td>Relapsed with fatal encephalitis 13 months later (Sept 1995)</td>
<td>PM: confluent areas neuronal loss, brain parenchymal &amp; perivascular inflammation; no significant non-CNS pathology</td>
</tr>
</tbody>
</table>

*Outbreak recognised retrospectively in 1995

HeV neuropathological findings:

Case 3

Summary:
• Severe widespread multifocal necrotising encephalitis
• Severe neuronal loss
• Viral antigens in surviving neurons
• Widespread lymphocytic cuffing
• No true vasculitis


HeV antigens in neurones within & surrounding inflammatory lesions
### Clinical manifestations

#### 2004 Cairns outbreak

<table>
<thead>
<tr>
<th>Case</th>
<th>Date</th>
<th>Exposure to infected horses</th>
<th>Clinical syndrome</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>4: Veterinarian</td>
<td>Nov 2004</td>
<td>Performed necropsy</td>
<td>‘Influenza-like’ illness</td>
<td>Recovery, no relapse</td>
</tr>
</tbody>
</table>

Hanna et al. Med J Aust 2006;562-4
Horses
Horse A
Horse B
Horse C
Horse D
Horse E

June
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

July
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

Humans
Case 1
Case 2

Incubation period
Sick
Body at RVC

Case 1 performs necropsy
Last day Case 1 & 2 perform nasal lavage
Case 1 tests negative for HeV
Case 2 tests negative for HeV
Case 1 PCR HeV positive
Case 2 PCR positive
Case 1 seroconverts; develops encephalitis
Case 2 seroconverts; develops encephalitis
ILI
Encephalitis
ILI
Encephalitis

From: Brad McCall & Field et al. Emerg Infect Dis 2010;16
Clinical manifestations
Thornlands, July 2008: Patient 1

- 33-yo veterinarian: severe progressive encephalitis:
  - Initially developed ILI (onset 16 days after performing necropsy/9 days after performing nasal lavage)
  - Cognitive impairment, ataxia, dysarthria (day 5), seizures (day 10), non-responsiveness, and death (day 31)
  - CSF: RT-PCR positive on CSF
  - EEG: absent stable rhythm, periodic sharp waves
  - MRI: progressive widespread hyperintense foci and restricted diffusion c/w severe ischaemic damage
  - Received high-dose iv Ribavirin (days 5-16)
Day 5 (T2 FLAIR):
- Multifocal cortical & pontine lesions

Day 18 (T2 FLAIR):
- Innumerable cortical and subcortical hyperintense foci
- New areas involving thalami and midbrain

Day 25 (T2 FLAIR):
- Progressive generalised involvement

Clinical manifestations
Thornlands, July 2008: Patient 2

- 21-yo veterinary nurse: milder encephalitic features:
  - Initial ILI (onset 11 days after performing nasal lavage on infected horse)
  - Cognitive impairment, ataxia, dysarthria
  - CSF: pleocytosis but negative RT-PCR
  - EEG: slow wave activity
  - MRI: scattered hyperintense cortical, deep white matter foci and leptomeningeal enhancement
  - Recovery (day 29) with residual high-level deficits
  - Received iv Ribavirin (days 4-29), then oral (to 6 months)
  - No relapse (at 18 months) but residual high-level deficits

Four infected horses, manifesting febrile illnesses with neurological & respiratory signs:
- Horse 1: died 28/7/09, initially attributed to snake bite
- Horses 2 & 3: died 7/8/09 & 8/8/09; suspected as HeV infection
- Horse 4: illness 11/8/09; confirmed positive/euthanized 24/8/09

Four persons with “high risk” exposures:
- One exposed to index horse 14 days previously
- Three exposed 5 days previously
- All received 5-day course iv Ribavirin (30 mg/kg load; 15 mg/kg q6h) & oral hydroxychloroquine (400mg q12h)

Multiple others with lower risk exposures
Clinical manifestations
Cawarral, August 2009

- 51-yo veterinarian:
  - Previously well
  - Performed endoscopy without PPE on index equine case day prior to death
  - Had received 5-day course iv Ribavirin/ po Hydroxychloroquine at 14 days post-exposure
  - Next day (i.e. day 21 post-exposure):
    - Presented with rapidly progressive encephalitis:
    - Seizures, obtundation → intubated/ventilated/sedated
    - Multifocal MRI involvement
  - Administered human iv anti-HeV/NiV G glycoprotein MAb (day 3)
  - Progressive encephalitic manifestations → death (day 19)
Day 2 (T2 FLAIR):
- Multifocal cerebral & deep white matter hyperintensities

Day 9 (T2 FLAIR):
- Extensive multifocal cerebral & brainstem hyperintensities

Day 9 (DWI):
- Extensive areas of cerebral diffusion restriction (c/w infarction)
### Human HeV infections Summary

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. human cases</th>
<th>No. deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-limited ‘influenza-like’ illness</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Multi-organ failure with predominant pulmonary involvement</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Aseptic meningitis with late relapsing encephalitis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acute encephalitis</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7</strong></td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>

- **Incubation period:**
  - Range, 5-21 days

- **Exposures:**
  - All experienced close unprotected mucosal &/or cutaneous exposures to respiratory secretions &/or blood from horses with unrecognised infection
### Public Health investigations
Follow-up of humans exposed to equine HeV

<table>
<thead>
<tr>
<th>Outbreak</th>
<th>Number exposed</th>
<th>PPE use</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hendra, 1994</td>
<td>8 close contact with infected horses</td>
<td>0%</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Thornlands, 2008</td>
<td>14 staff with “high risk” exposures</td>
<td>7%</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Cawarral, 2009</td>
<td>4 stud workers with “high risk” exposures</td>
<td>0%</td>
<td>1 (25%)</td>
</tr>
</tbody>
</table>

Candidate therapies

- Attachment, fusion, entry:
  - Passive Ab therapy (targeting F,G)
  - Soluble receptor-based strategies (targeting G, Ephrin B2)
  - Fusion inhibitors

- Viral replication
  - Ribavirin

Ribavirin

- **In vitro:**
  - 50-fold reduction in HeV yield & 9-fold reduction in RNA synthesis at 12 mg/L\(^1\)

- **In vivo (Hamster model):**
  - RBV (25-50 mg/L) delayed but did not prevent death\(^2\)

- **Observational study from two Malaysian centres\(^3\):**
  - 140 patients with NiV encephalitis treated with (mostly oral) RBV vs 54 controls
  - Mortality: 32% vs 54% (p=0.006)

- **Clinical experience with high dose iv RBV for human HeV infection:**
  - Achievable serum/CSF concentrations =10-13 mg/L
  - IC\(_{90}\) (in Vero E6 cells) =64 mg/L

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Chloroquine

- **In vitro:**
  - 50% inhibition of HeV & NiV infection with Chloroquine (2 µM)\(^1\)
  - ?inhibition of Cathepsin L

- **In vivo:**
  - No protective effect as prophylactic or post-exposure therapeutic for NiV infection in ferret model\(^2\)

Passive antibody approaches

- Target NiV &/or HeV F & G glycoproteins
- Polyclonal\textsuperscript{1}, murine MAb\textsuperscript{2}:
  - Protective in animal models (hamster, ferret) with NiV challenge
  - Greater efficacy with pre- (rather than post-) challenge administration
- Human MAb:
  - Several candidate anti-G MAbs identified\textsuperscript{3} (most potent cross-reactive MAb designated 102.4)\textsuperscript{4}
  - Protective in ferret model administered 10h post-NiV challenge\textsuperscript{5}
  - 102.4 administered to Case 7 with encephalitis

Soluble receptors & fusion inhibitors

- **In vitro data:**
  - **Soluble receptors:**
    - Soluble Ephrin-B2\(^1\)
    - Soluble EphB4,B1\(^2\)
  - **Several potential fusion inhibitors:**
    - Heptad-derived peptides\(^3\)
    - F protein binders\(^4\)
    - Cathepsin L inhibitors (chloroquine)

Vaccine progress

- All based upon G &/or F glycoproteins
- Using either viral vectors or recombinant soluble antigens
- All induce neutralising antibodies

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Animal model</th>
<th>Adjuvant</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinia virus encoding NiV F or G</td>
<td>Hamsters</td>
<td>None</td>
<td>Up to 5 months’ immunity demonstrated</td>
</tr>
<tr>
<td></td>
<td>NiV challenge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canarypox virus encoding NiV F or G</td>
<td>Pigs</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NiV challenge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recombinant HeV or NiV soluble G</td>
<td>Cats</td>
<td>CSIRO triple</td>
<td>Cross protection from HeV sG</td>
</tr>
<tr>
<td></td>
<td>NiV challenge</td>
<td>adjuvant</td>
<td></td>
</tr>
<tr>
<td>Recombinant HeV soluble G</td>
<td>Cats</td>
<td>CpG</td>
<td>Cross protection from HeV sG Mucosal IgA detected</td>
</tr>
<tr>
<td></td>
<td>NiV challenge</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other Henipaviruses

Geographic distribution of Henipavirus outbreaks and fruit bats of Pteropodidae Family
Nipah virus infections

- **Malaysia/Singapore 1998-9:**
  - 283 infected (40% CFR)
  - Natural host=bats (*Pteropus* spp.), intermediate host=pigs
  - Single strain
  - Primarily neurological presentation
  - Late encephalitic manifestation: 10%
  - **Transmission:**
    - Pig-to-human
    - No convincing evidence human-to-human transmission\(^1,\)\(^2\)
  - Outbreak terminated with culling >1,000,000 pigs

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\(^1\)Chan et al., Infect Epidemiol 2002; \(^2\)Mounts et al., J Infect Dis 2001
Nipah virus infections

- Bangladesh/West Bengal 2001-9
  - 8 smaller outbreaks
  - Multiple strains
  - Clinical features:
    - 70% CFR
    - Prominent pulmonary disease
    - No relapsing/late encephalitis
  - Transmission:
    - Bat-to-human (via raw date palm sap); Human-to-human
    - Other mammals-to-human

Menangle virus

- **Family** *Paramyxoviridae*
  - Subfamily *Paramyxovirinae*
    - Genus *Rubulavirus*

- First recognised 1997 from outbreak at piggery in New South Wales

- Closely related to Tioman virus
Menangle virus
Piggery outbreak, 1997

- Large commercial piggery (2,600 sows), Apr-Sept 1997:
  - Reduction in conception rates & litter size
  - Large numbers of mummified and stillborn foetuses
  - Severe skeletal & craniofacial defects, pulmonary hypoplasia
- No evidence of clinical disease in pigs of any age after birth
- Novel paramyxovirus isolated
- High rate (>95%) of seropositivity among pigs at affected piggery and two contact growing farms
- Outbreak terminated with control measures (disinfection, depopulation, restocking with immune sows etc)

Menangle virus
Human infection

- Serological assessment of ~250 persons with potential exposure to infected pigs
- Two workers with history of severe ILI/rash and heavy occupational exposure to infected pigs tested seropositive (NTs 128 & 512)
  - Close contact with birthing pigs
  - Necropsies on pigs
  - Both recovered with no subsequent relapse

Menangle virus
Epidemiology

- Variety of mammalian species (rodents, cattle, sheep, cats, dog) and birds in vicinity: seronegative
- Colony of *Pteropus poliocephalus* & *P. scapulatus* within 200m of piggery
- Seropositivity from fruit bats in northern & eastern Australia:
  - *P. alecto* (46%)
  - *P. poliocephalus* (41%)
  - *P. conspicillatus* (25%)
  - *P. scapulatus* (1%)

- No disease described in fruit bats

• Hendra virus:
  – Closely related to Nipah virus within the Henipavirus genus
  – Associated with severe – often fatal – equine & human infections
  – Reservoir within flying foxes (*Pteropus* spp.)
  – Emerging epidemiology: apparent increasing frequency of ‘spill over’ events
  – Biosafety class 4 pathogen: limits study to appropriate facilities
  – No established therapeutic agents or vaccines (although promising candidates)

• Menangle virus:
  – Single outbreak affecting piggery, including two humans with ILIs
  – Reservoir within flying foxes (*Pteropus* spp.)

• Emerging zoonoses: demands an urgent paradigm shift in veterinary infection control procedures
Infection Control: a veterinary priority

Stop Putting Yourself at Risk
Start Making Changes for Life

AVA Qld Division presents
An Infection Control Workshop for Veterinary Practices
Events Centre Maroochy, Sunshine Coast
Sunday, 16 May 2010, 8.50am to 4.45pm
Acknowledgments

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  - Katharine Bossart
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  - Chris Broder
Discussion points

- **Prevention:**
  - Veterinary/Medical Infection Control precautions
  - Early recognition of equine infections
  - Implications of infectiousness prior to clinical disease in horses
  - Risk assessment of exposed humans
  - Development of equine HeV vaccines

- **Treatment:**
  - Poor apparent efficacy of currently available agents: Ribavirin, Chloroquine
  - Development & availability of passive immunotherapy/prophylaxis

- **Natural history:**
  - Risk of relapse in infected humans