Emerging paramyxoviruses in Australia
Hendra and Menangle

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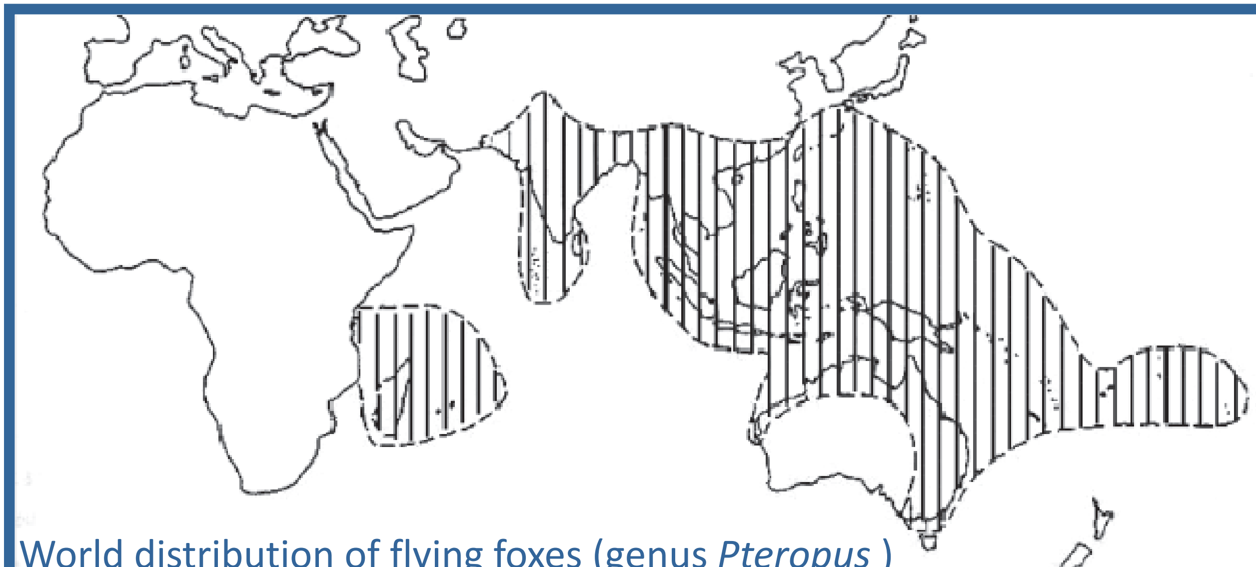
Emerging paramyxoviruses in Australia
Overview

- 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*)

Pteropus poliocephalus

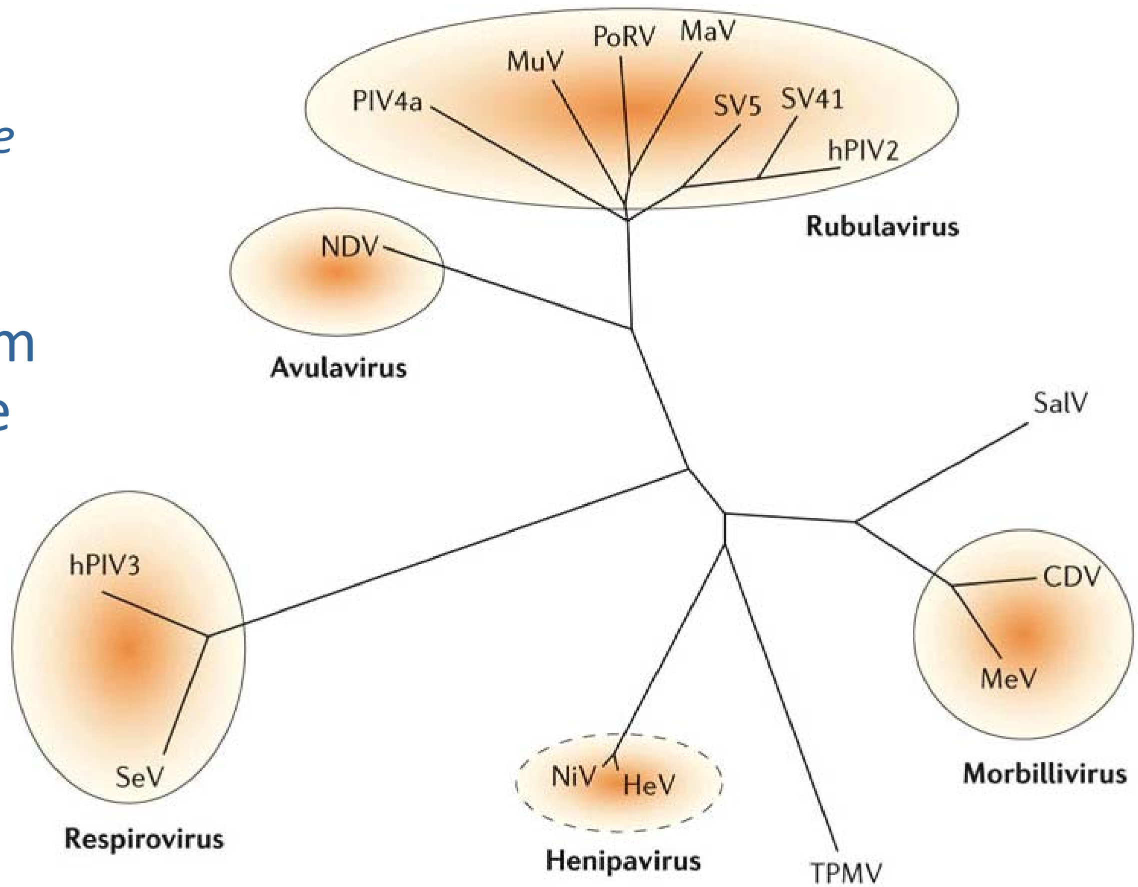
From: Field et al., CTMI 2007;315:133-59

Background
Hendra virus

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

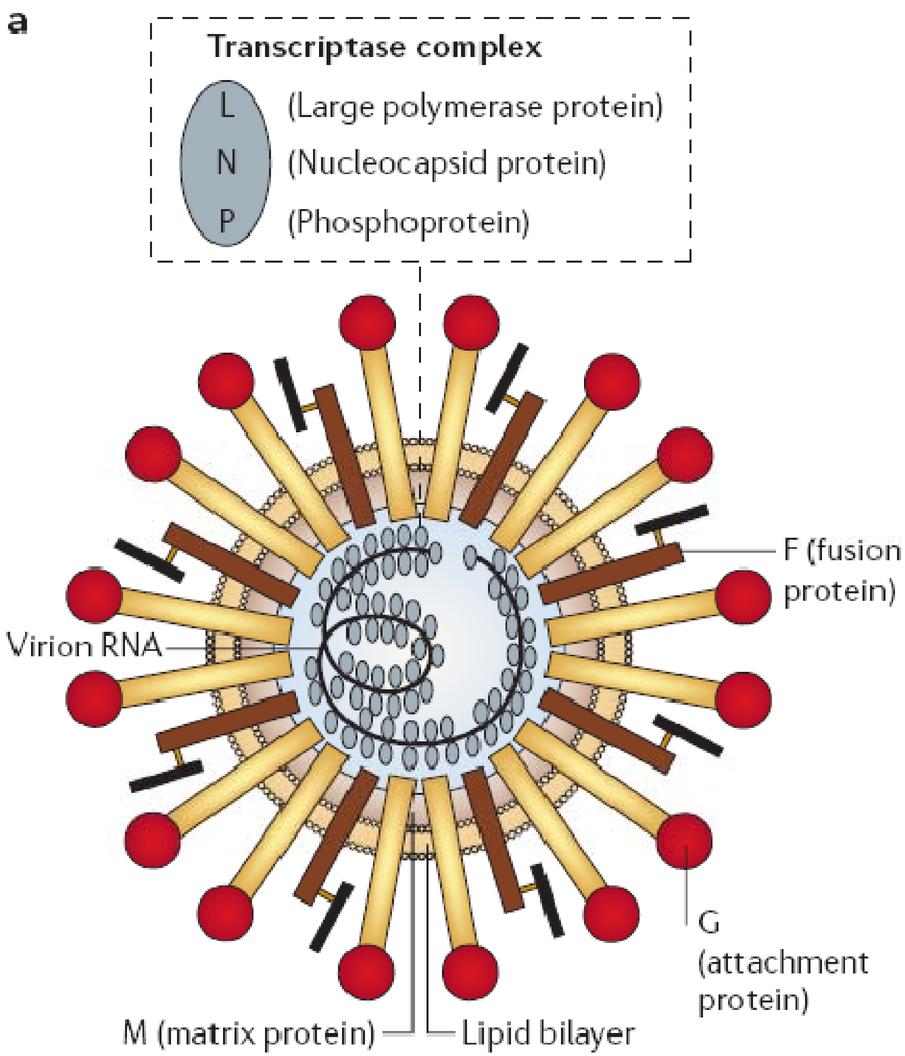
- First recognised 1994 from outbreak at Hendra horse stables in Brisbane

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

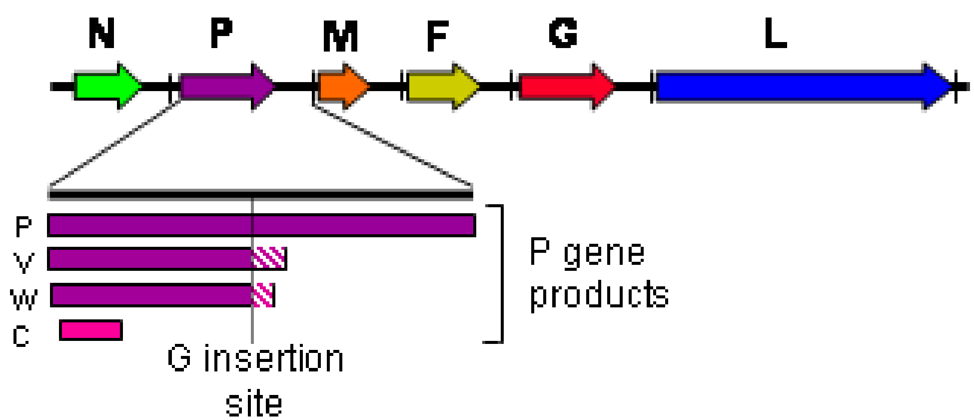


From: Eaton et al., Nature Rev Microbiol 2006;4:23-35

Hendra virus structure and genome

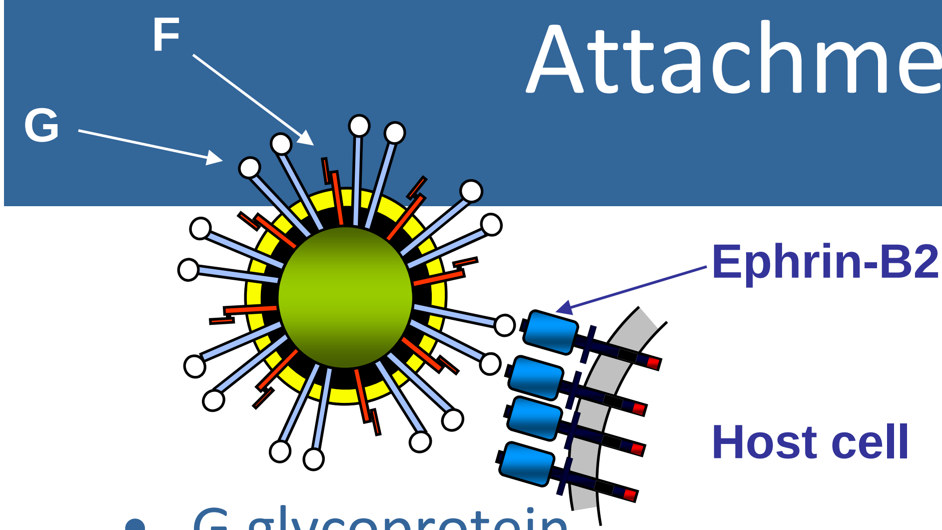


- Large genome: 18,234 nucleotides (~15% longer than most other paramyxoviruses)
- Typical paramyxovirus genome structure



From: Eaton et al., Nature Rev Microbiol 2006;4:23-35

Attachment, fusion, cell entry

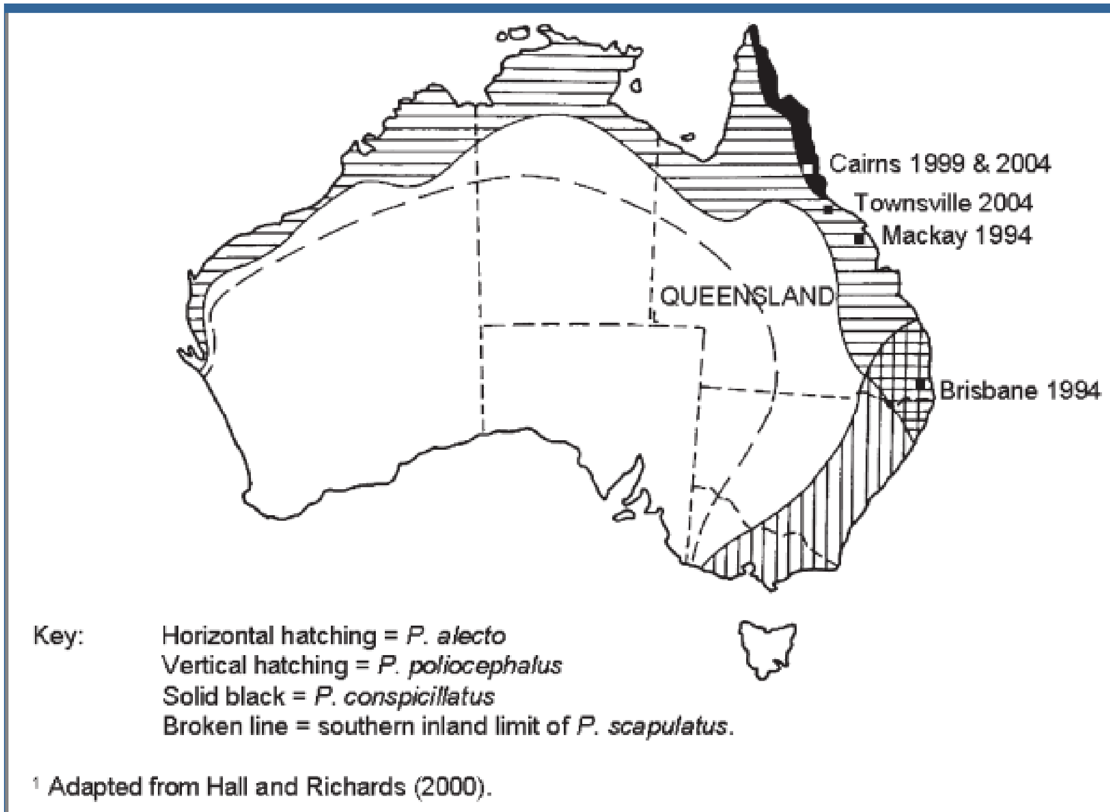


- Explains:
  - Broad host range
  - Systemic nature of infection

- 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 (F_0) cleaved into biologically active F_1 & F_2 by lysosomal cysteine protease **Cathepsin L** after endocytosis
  - Conformational change into **trimer-of-hairpins** structure

Bonaparte MI, et al. (2005). PNAS 102: 10652-57.
Negrete OA, et al. (2005) Nature 436: 401-405.
Negrete OA, et al. (2006) PLoS Pathog 2: e7.
Bishop KA, et al. (2007) J Virol 81: 5893-5901.
Bossart K et al. (2005). Virol J 2:57.
Pager CT et al. (2005). J Virol 79:12714-20.

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

Field et al. CTMI 2007;315:133-59; Halpin et al. J Gen Virol 2000;81:1927-32

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?

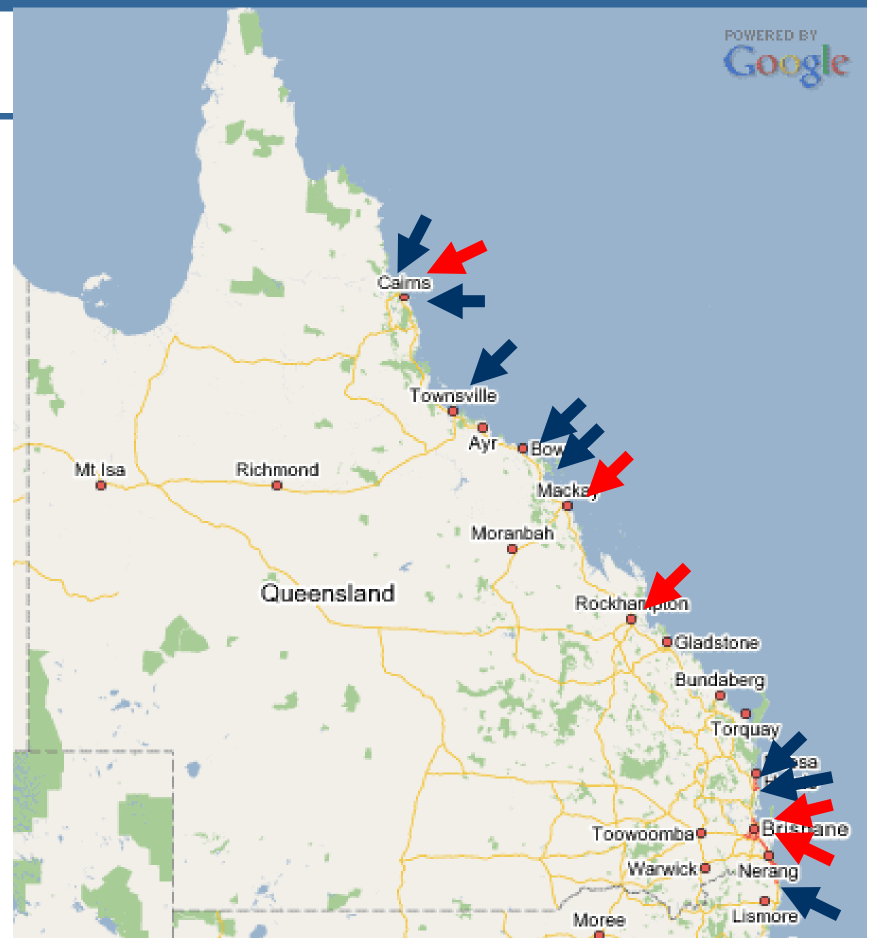
Observed epidemiology of NiV suggests bat-to-human & human-to-human potential of HeV

- Horse-to-human:
  - Droplet spread to mucosal surfaces with infected respiratory secretions
  - Direct contact to non-intact skin
  - Attack rate among highest risk exposures ~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

[1]Fogarty et al. Virus Res 2008;132:140-4

HeV epidemiology
"Spill over" events: 1994-Sept 2009

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



- 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

- 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

- Fever, tachycardia
- Respiratory manifestations:
  - Tachypnoea, respiratory distress, nasal discharge,

  and/or
- Neurological manifestations:
  - Ataxia, head tilt, facial nerve paralysis

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

Field et al. CTMI 2007;315:133-59

Hendra, Brisbane


075 GCH


SCHMIDT
RACING
STABLES

Hendra, September 1994

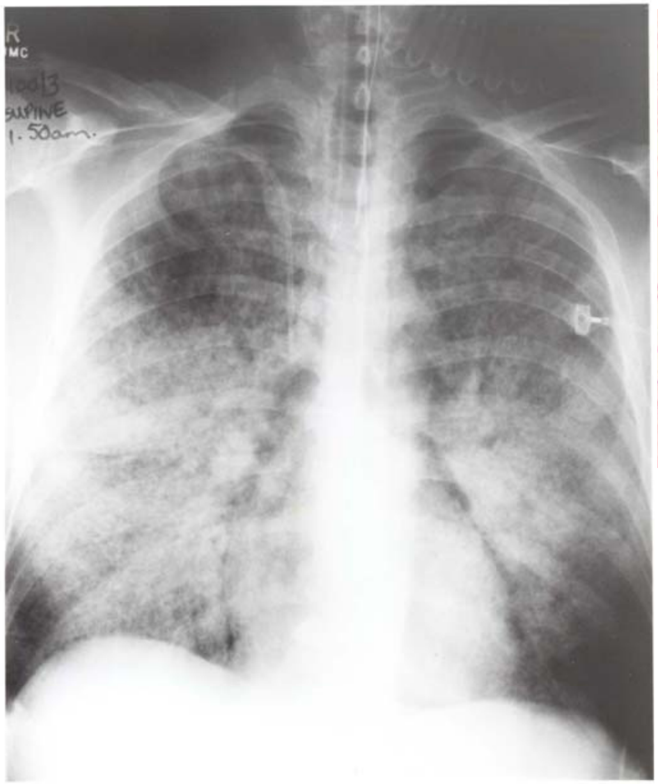
- Explosive outbreak of unknown infectious agent involving 22 horses and 2 humans (1 fatal)

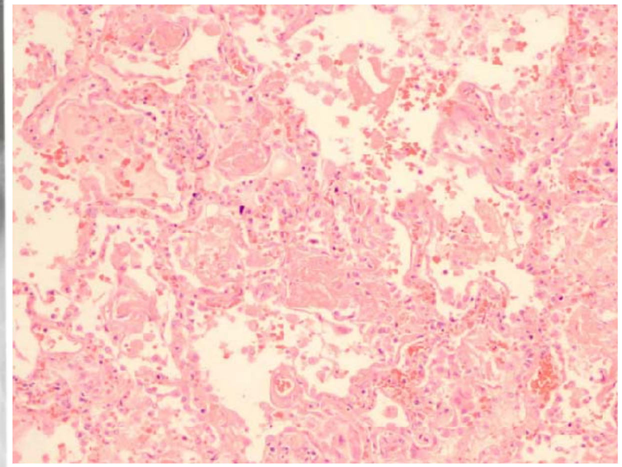
Clinical manifestations
1994 Hendra outbreak

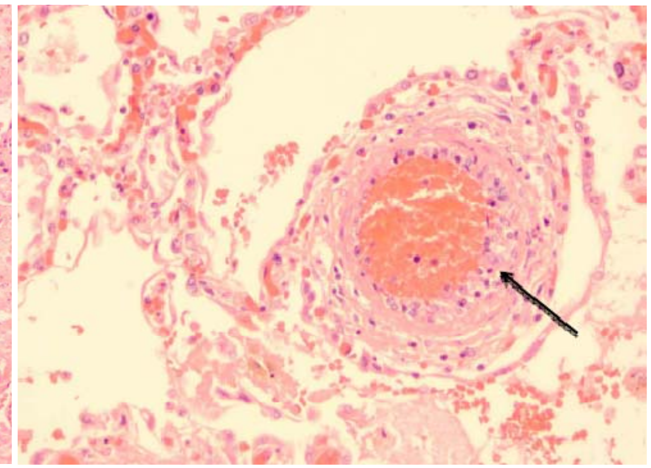
Case	Date	Exposure to infected horses	Clinical syndrome	Outcome Post mortem findings
1: 40-yo stable hand	Sept 1994	Respiratory secretions	'Influenza-like' illness	Recovery, no relapse
2: 49-yo trainer	Sept 1994	Respiratory secretions	Pneumonitis, multi-organ failure, fatal	Died (day 13 illness) PM: widespread vasculitis (incl. brain), severe pulmonary necrotising alveolitis, mild meningeal/brain parenchymal inflammation

Selvey et al. Med J Aust 1995;162:642-5

HeV clinical manifestations
Case 2: Pneumonitis



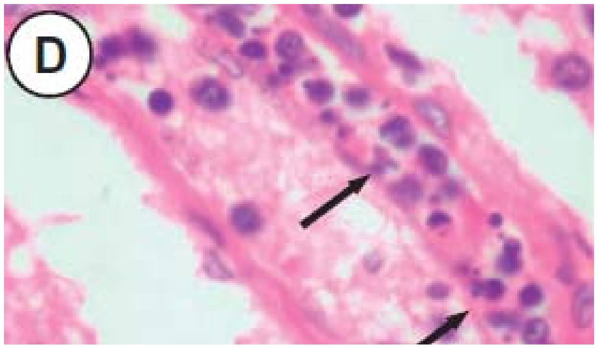


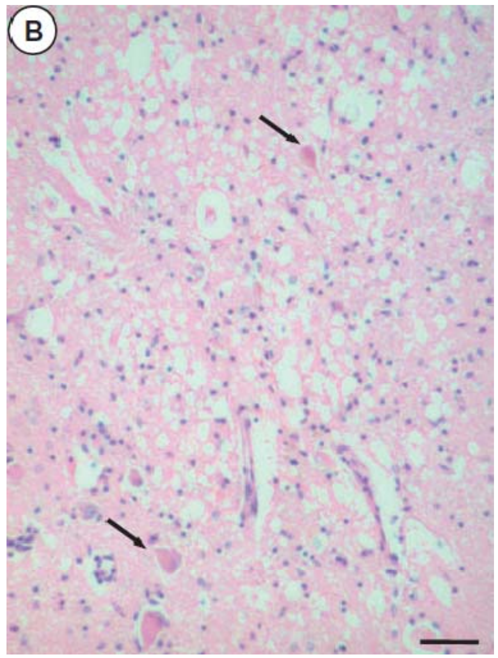


- Diffuse alveolar damage
  - Widespread hyaline membranes
  - Numerous intra-alveolar macrophages
- Pulmonary thromboemboli
- Viral antigens on IHX

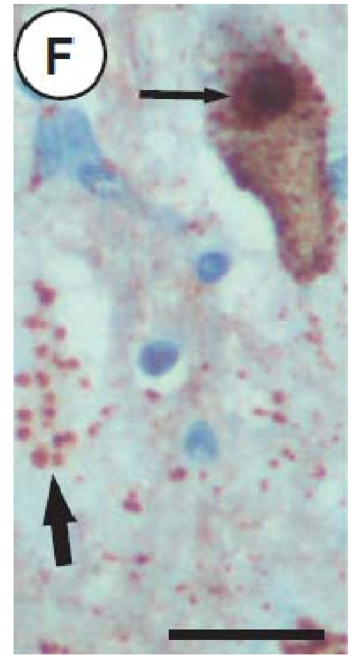
From: J McCormack & Kathryn Uranker

HeV neuropathological findings:
Case 2



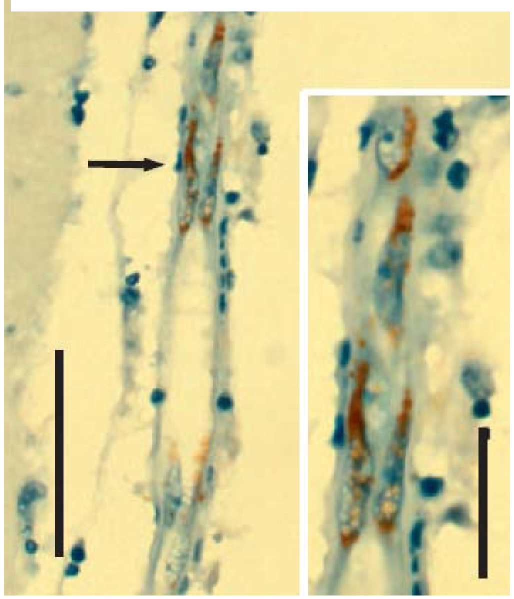


Necrotic/ vacuolar plaque in the dentate nucleus



HeV antigens in neuronal cytoplasm & nucleus

Vasculitis in meningeal & brain parenchymal blood vessels with HeV antigens



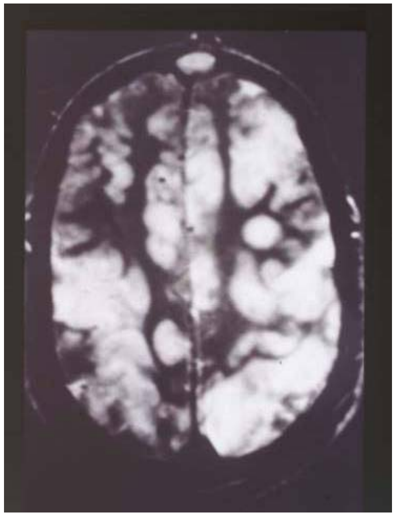
Summary:

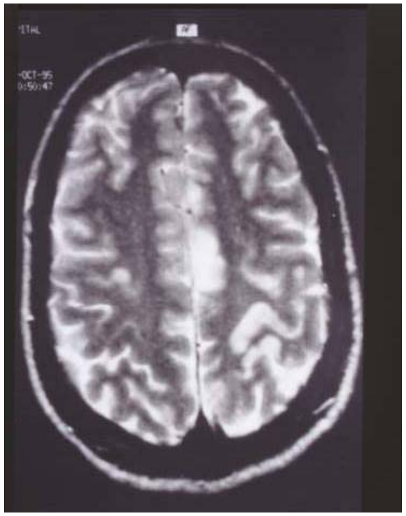
- Focal vasculitis & endotheliitis in meninges & parenchyma
- Viral antigens in endothelial cells
- Small necrotic/vacuolar plaques
- Mild lymphocytic leptomeningitis

Wong et al. Neuropathol Appl Neurobiol. 2009;35:296-305

Clinical manifestations
1994 Mackay outbreak*

Case	Date	Exposure to infected horses	Clinical syndrome	Outcome Post mortem findings
3: 36-yo farmer	Aug 1994	Assisted with necropsy	Aseptic meningitis with recovery (Aug 1994) Relapsed with fatal encephalitis 13 months later (Sept 1995)	Died (day 25 illness) PM: confluent areas neuronal loss, brain parenchymal & perivascular inflammation; no significant non-CNS pathology

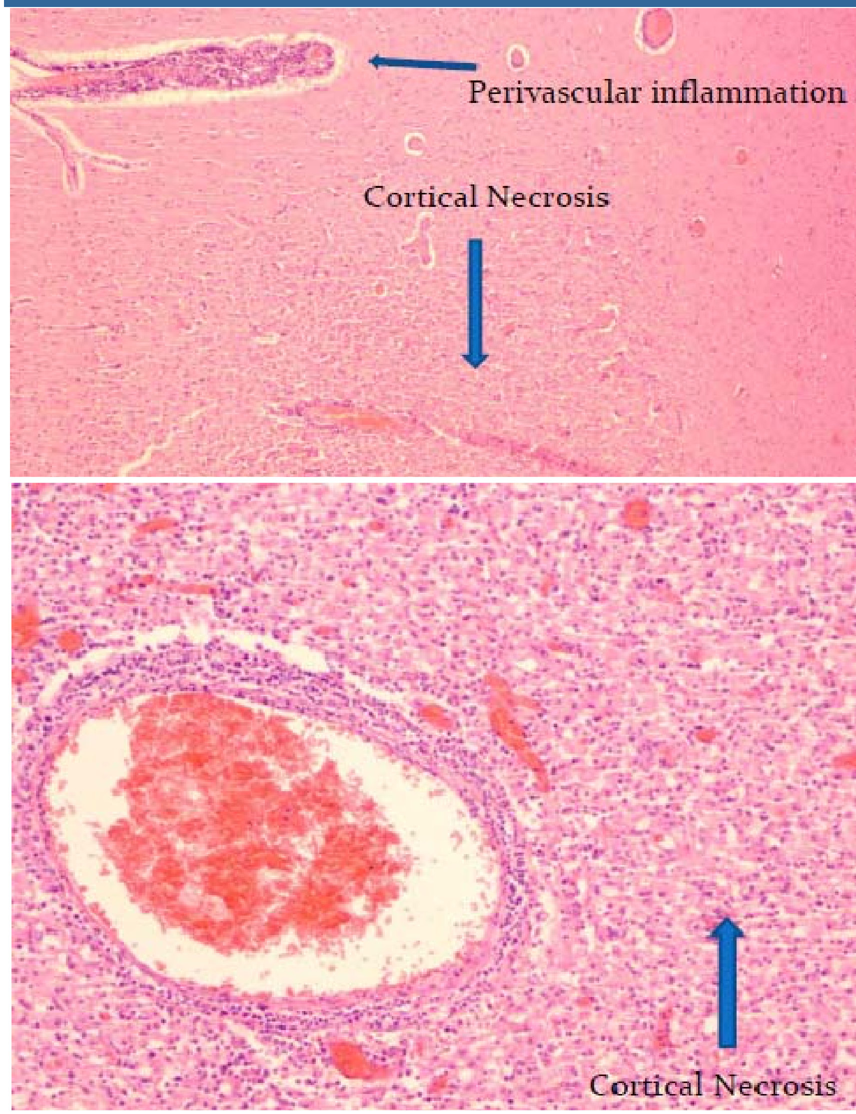




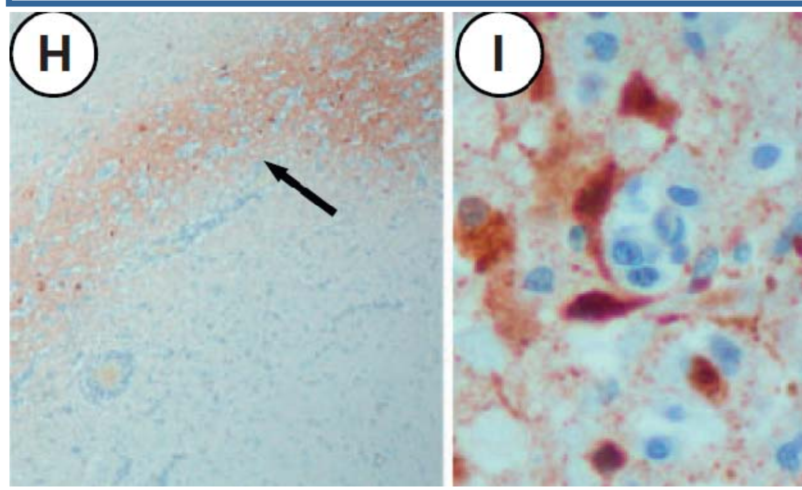
*Outbreak recognised retrospectively in 1995

O'Sullivan et al. Lancet 1997;349:93-5

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

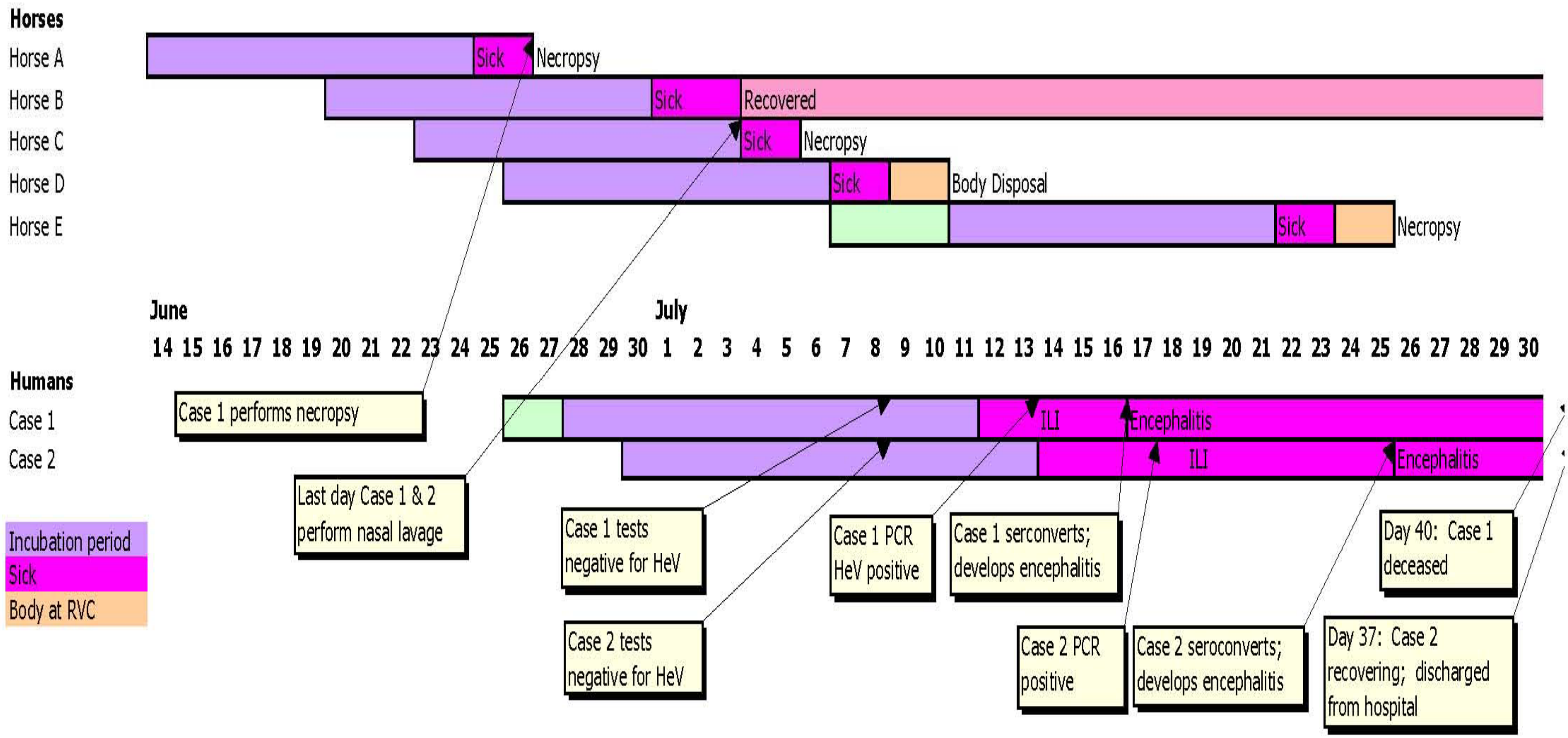
From: K Uranker and Wong et al. Neuropathol Appl Neurobiol. 2009;35:296-305

Clinical manifestations
2004 Cairns outbreak

Case	**Date**	**Exposure to infected horses**	**Clinical syndrome**	**Outcome**
4: Veterinarian	Nov 2004	Performed necropsy	'Influenza-like' illness	Recovery, no relapse

Hanna et al. Med J Aust 2006;562-4

Redlands Veterinary Hospital
Thornlands, July 2008

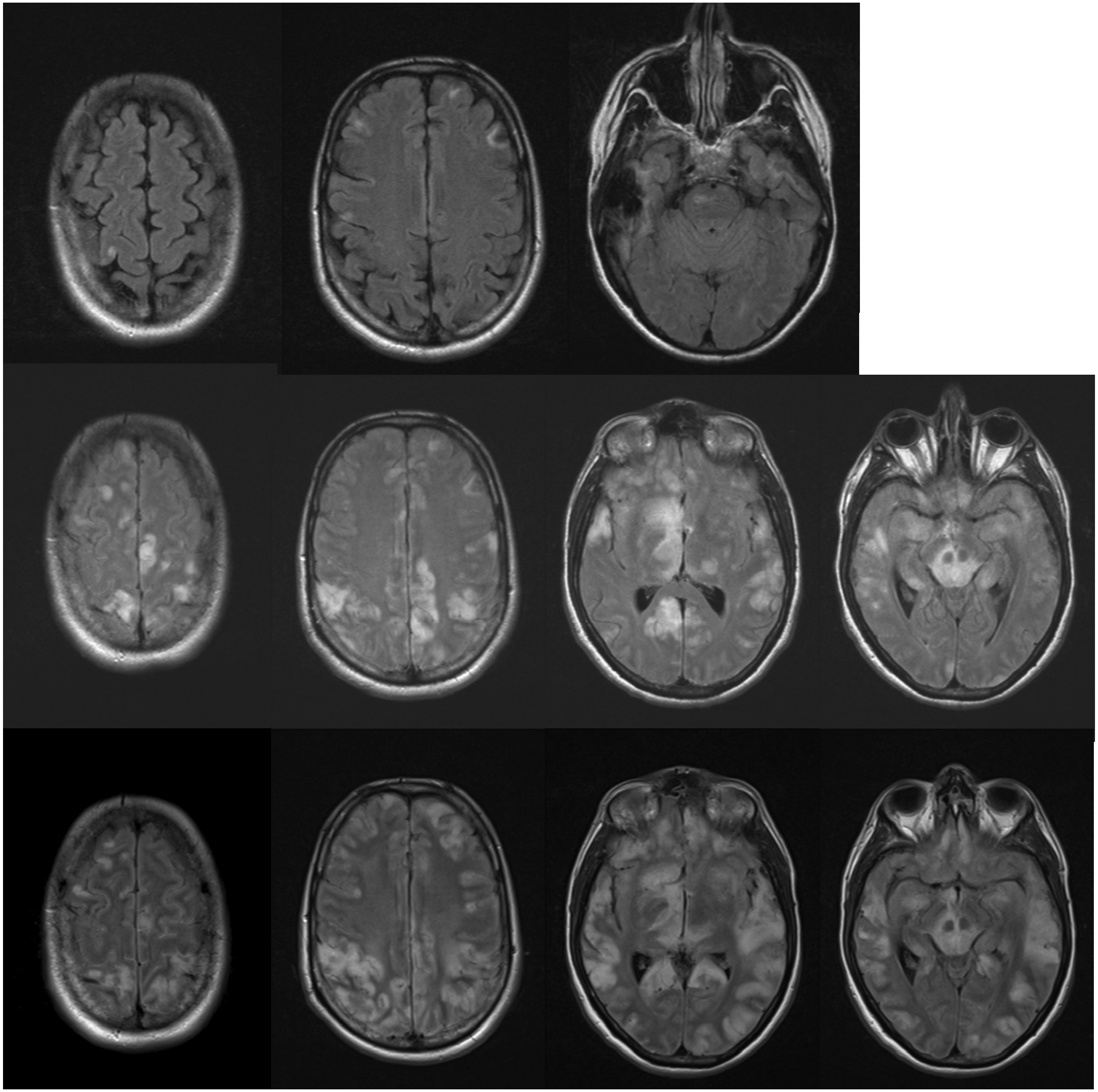


From: Brad McCall & Field et al. Emerg Infect DIs 2010;16

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)

Playford et al. Emerg Infect Dis 2010;16:219-23



Day 5 (T2 FLAIR):

- Multifocal cortical & pontine lesions

Day 18 (T2 FLAIR):

- Innumerable cortical and subcortical hyperintense foci
- New areas involving thalami and midbrain

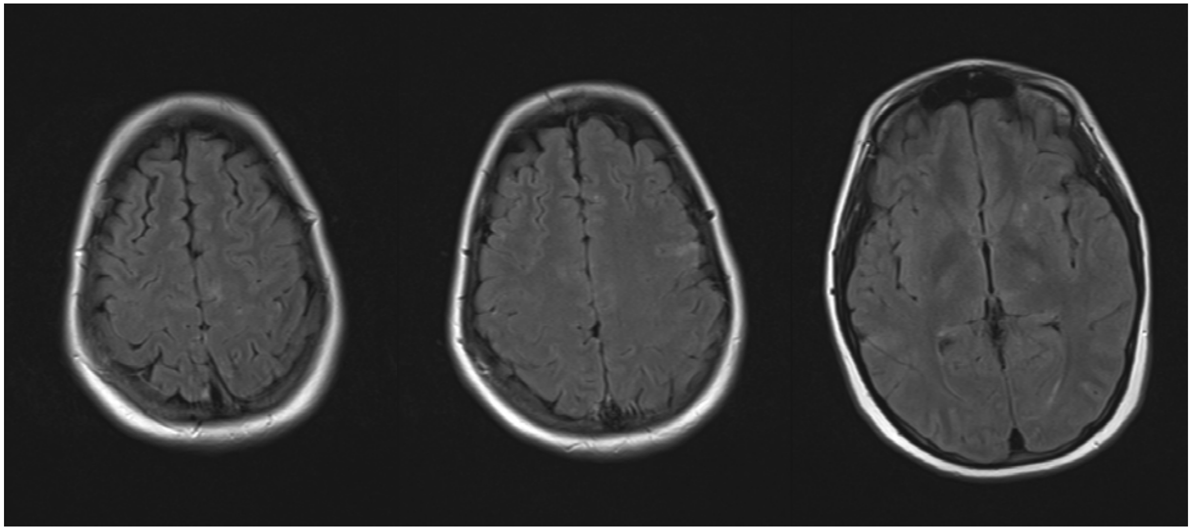
Day 25 (T2 FLAIR):

- Progressive generalised involvement

Playford et al. Emerg Infect Dis 2010;16:219-23

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

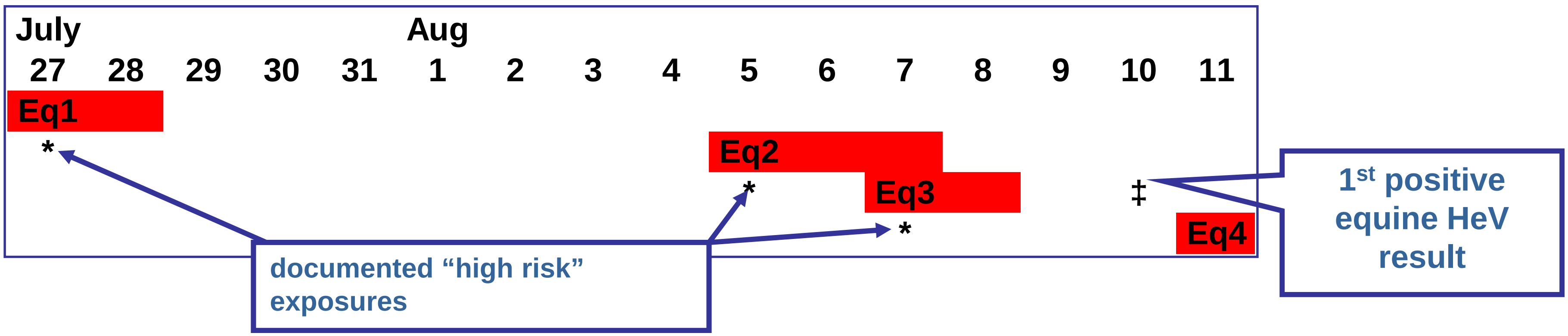


  - Recovery (day 29) with residual high-level deficits
  - Received iv Ribavirin (days 4-29), then oral (to 6 months)
  - No relapse (at 18 months) but residual high-level deficits

Playford et al. Emerg Infect Dis 2010;16:219-23

Thoroughbred stud, Cawarral (Rockhampton)
Early August 2009

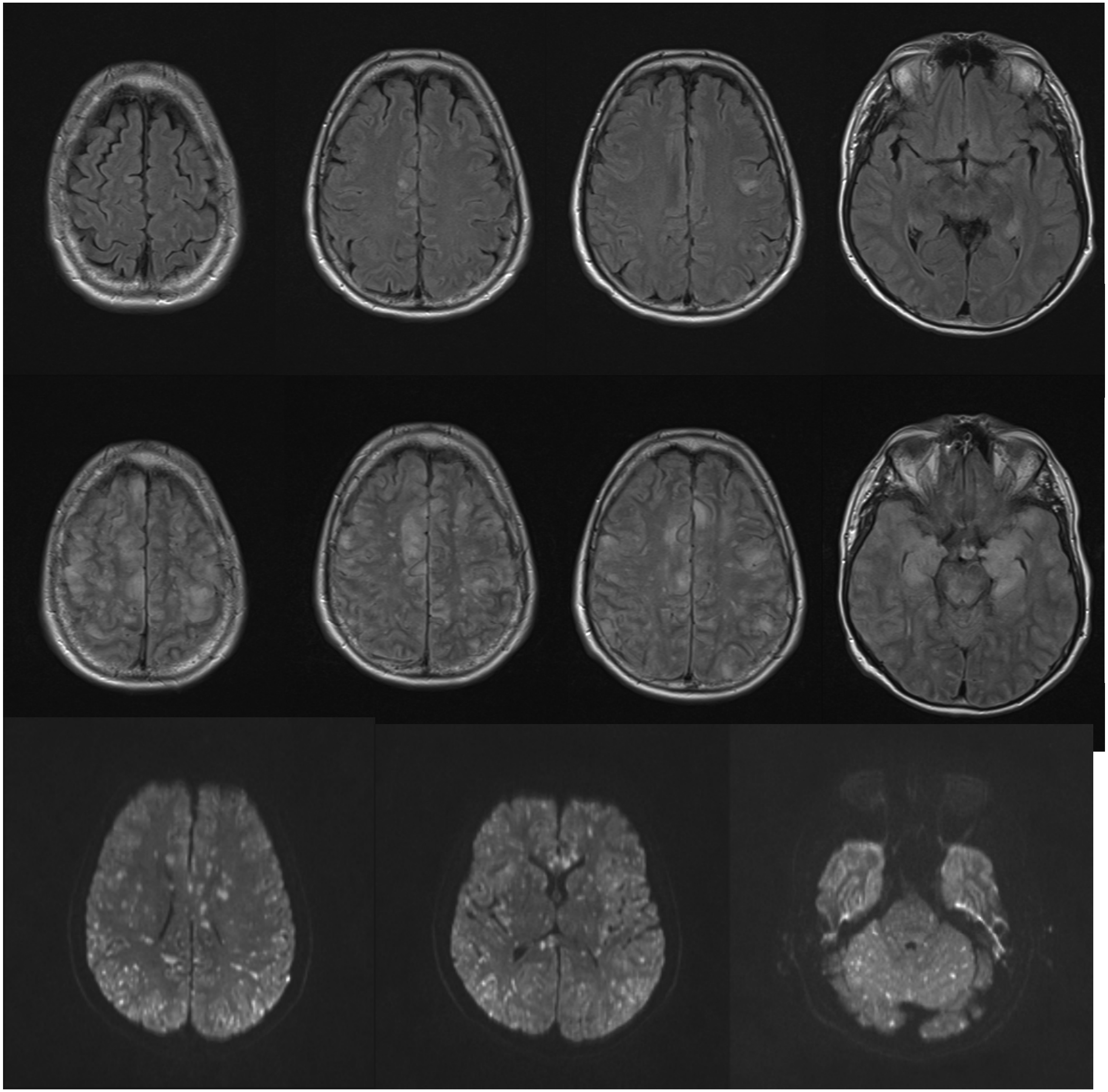
- Four infected horses, manifesting febrile illnesses with neurological & respiratory signs:
  - Horse 1: died 28/7/09, initially attributed to snake bite
  - Horses 2 & 3: died 7/8/09 & 8/8/09; suspected as HeV infection
  - Horse 4: illness 11/8/09; confirmed positive/euthanized 24/8/09



- 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 hydoxychloroquine (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

	No. human cases	No. deaths
Self-limited 'influenza-like' illness	2	0
Multi-organ failure with predominant pulmonary involvement	1	1
Aseptic meningitis with late relapsing encephalitis	1	1
Acute encephalitis	3	2
Total	**7**	**4**

- 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

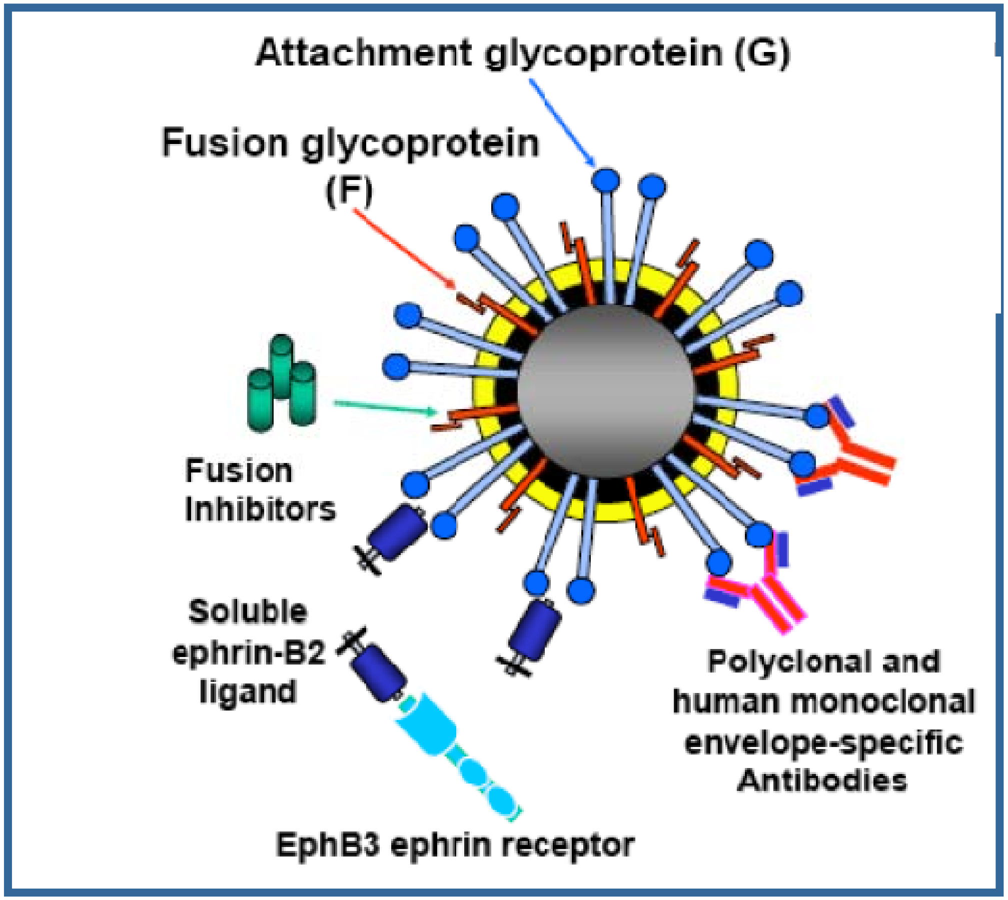
Public Health investigations
Follow-up of humans exposed to equine HeV

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

Selvey et al. Med J Aust 1995;162:642-5 ; Playford et al. Emerg Infect DIs 2010;16:219-23

Candidate therapies

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



From: Bossart et al. Open Virol J 2007;1:14-25

Ribavirin

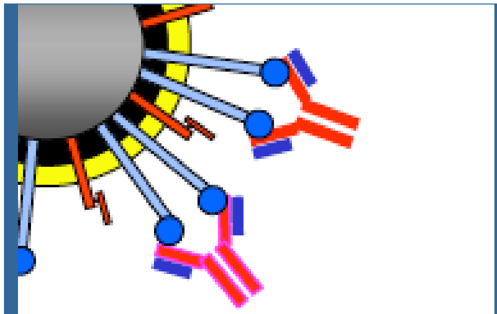
- In vitro:
  - 50-fold reduction in HeV yield & 9-fold reduction in RNA synthesis at 12 mg/L[1]
- In vivo (Hamster model):
  - RBV (25-50 mg/L) delayed but did not prevent death[2]
- Observational study from two Malaysian centres[3]:
  - 140 patients with NiV encephalitis treated with (mostly oral) 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

[1]Wright et al. Arch Virol 2005;150:521-32; [2]Geogres-Coubot et al. Antimicrob Agents Chemother 2006;50:1768-72 [3]Chong et al. Ann Neurol 2001;49:810-3;

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]

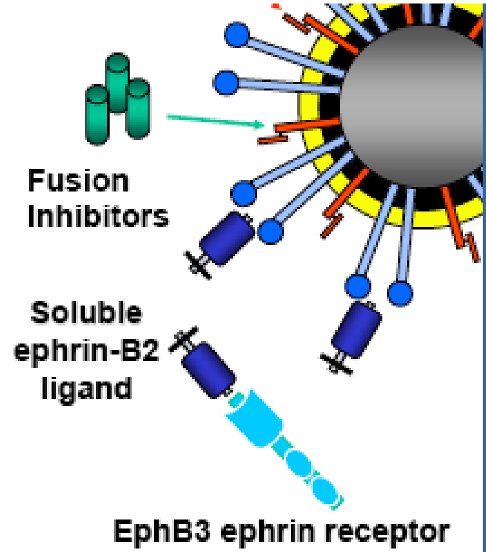
[1]Porotto et al. J Virol 2009;83:5148-55; [2]Pallister et al. J Virol 2009;83:11979-82



Passive antibody approaches

- Target NiV &/or HeV F & G glycoproteins
- Polyclonal[1], murine MAb[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[3] (most potent cross-reactive MAb designated 102.4)[4]
  - Protective in ferret model administered 10h post-NiV challenge[5]
  - 102.4 administered to Case 7 with encephalitis

[1]Guillame et al. J Virol 2004;78:834-40; [2]Guillame et al. J Virol 2006;80:1972-8; [3]Zhu et al. J Virol 2006;80:891-9; [4]Zhu et al. J Infect Dis 2008;197:846-53; [5]Bossart et al. PLoS Pathogens 2009;5:e1000642



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)

[1]Negrete et al. Nature 2005;436:401-5; [2]Bonaparte et al. PNAS 2005;102:10652-7; [3]Bossart et al. J Virol 2002;76:11186-98; [4]Niedermeier et al. J Med Chem 2009;52:4257-65

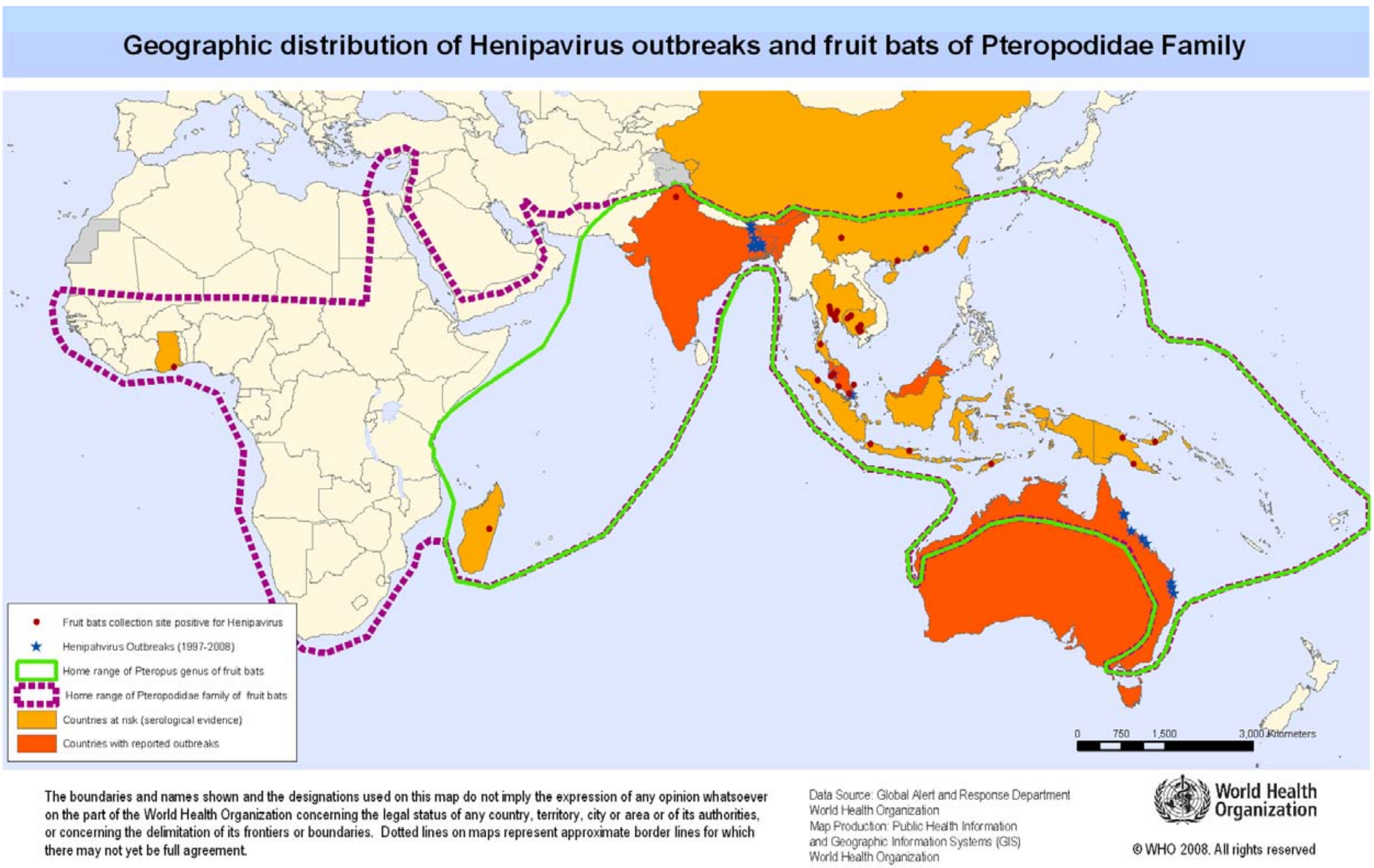
Vaccine progress

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

Vaccine	Animal model	Adjuvant	Notes
Vaccinia virus encoding NiV F or G	Hamsters NiV challenge	None	Up to 5 months' immunity demonstrated
Canarypox virus encoding NiV F or G	Pigs NiV challenge	None	
Recombinant HeV or NiV soluble G	Cats NiV challenge	CSIRO triple adjuvant	Cross protection from HeV sG
Recombinant HeV soluble G	Cats NiV challenge	CpG	Cross protection from HeV sG Mucosal IgA detected

Guillame et al. J Virol 2004;78:834-40; Weingartl et al. J Virol 2006;80:7929-38; Mungall et al. J Virol 2006;80:12293-302; McEachern et al. Vaccine 2008;26:3842-52

Other Henipaviruses



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

[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

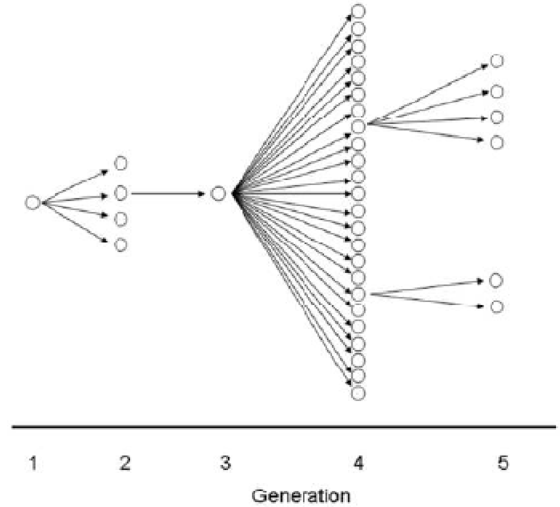


Figure 2. Chain of person-to-person transmission in Nipah outbreak in Faridpur, Bangladesh, 2004.

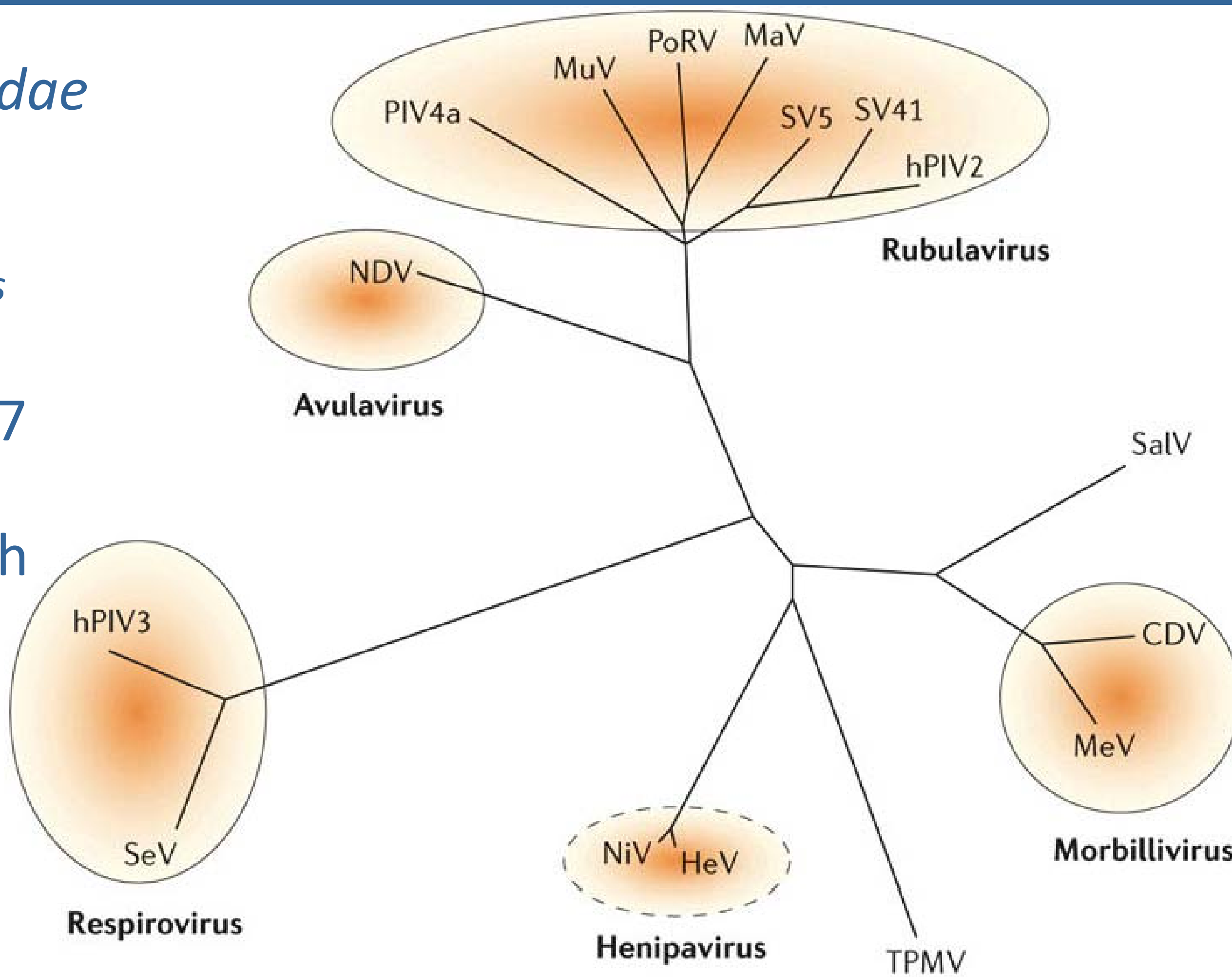
Hsu et al., Emerg Infect Dis 2004; Luby et al., Emerg Infect Dis 2006; Gurley et al., Emerg Infect Dis 2007; Luby et al. Clin Infect Dis 2009;49:1743-8

Menangle virus

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

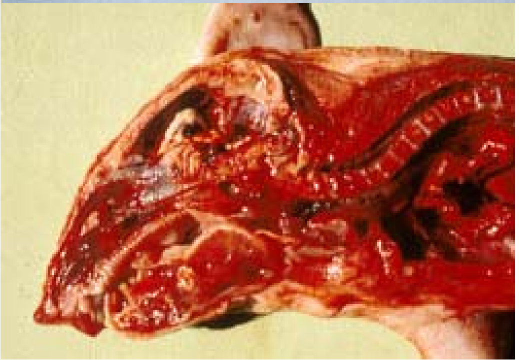
- First recognised 1997 from outbreak at piggery in New South Wales

- 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)



Philbey et al. Emerg Infect Dis 1998;4:269-71; Kirkland et al. Aust Vet J 2001;79:199-206

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

Chant et al. Emerg Infect Dis 1998;4:273-5

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%)

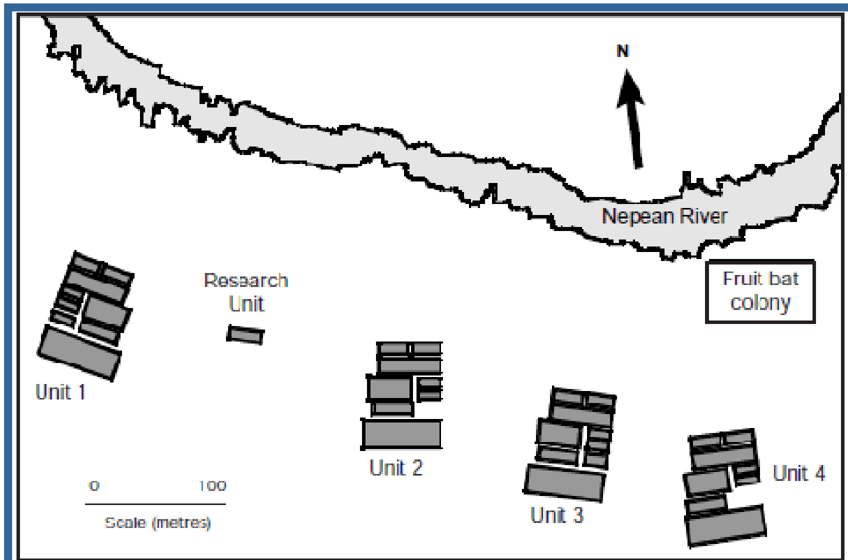


Figure 1. Plan of the affected piggery showing the location of the four production units and the fruit bat colony (rectangle) in relation to the Nepean River.

- No disease described in fruit bats

Philbey et al. Aus Vet J 2008;86:449-54; Kirkland et al. Aust Vet J 2001;79:199-206

Overview

- 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

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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