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Department of Agriculture,
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Biosecurity Innovation Program



The Research Discovery of a variant genotype of Hendra virus (HeVg2) affording prospective spillover detection and revision of geographical spillover risk.

Dr Edward J Annand

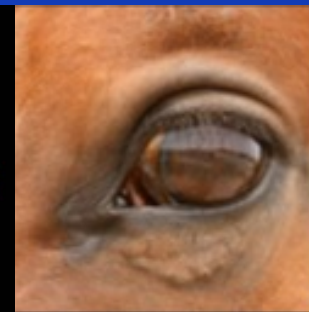
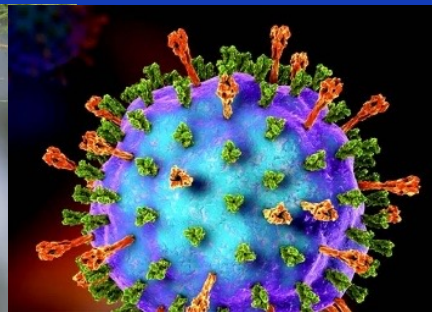
University of Sydney | Sydney School of Veterinary Science and Sydney Institute of Infectious Diseases, Sydney, NSW.

CSIRO | Health and Biosecurity, Black Mountain, ACT.

EquiEpiVet | Equine Veterinary and One Health Epidemiology, Aireys Inlet, Vic.



EQUINE
VETERINARIANS
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Horses as Sentinels for
Emerging Infectious Disease



with **BAT** ONE HEALTH

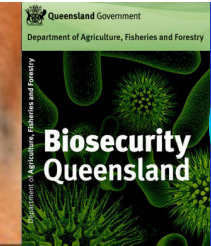
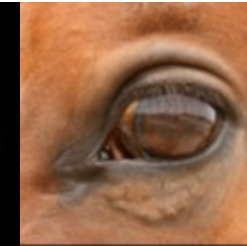
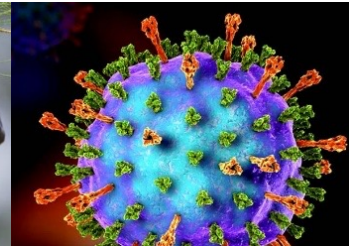


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HeV spillover to horses and humans as a low sporadic incidence but HIGH CONSEQUENCE disease

Any single missed detection of an infected case (false negatives) place humans and animals at risk of fatal illness

And yet **missed detections are inevitable** due to LOW and SPORADIC INCIDENCE of disease

YEAR/S	QLD	NSW	TOTAL	HORSE FATALITIES	Human	
					Exposures / illnesses requiring intensive treatment	Fatalities
1994-2010	13	1	14	48	10 (7+3)	4 (3+1)
2011	10	8	18	24	0	0
2012	8	0	8	10	1	0
2013	4	4	8	8	(1*)	0
2014	3	1	4	4	6	0
2015	2	2	4	4	(1*)	0
2016	0	1	1	1	0	0
2017	1	3	4	4	3	0
2018	0	1	1	1	0	0
2019	0	1	1	1	0	0
2020	0	1	1	1	0	0
2021	0	1	1	1	0	0
TOTALS	41	24	65	107	22 (13)+2*	4

Horse cases listed in blue include recently identified HeVg2 disease events. Human cases listed in blue indicate received monoclonal antibody m102.4. Cases marked with an * represent human laboratory exposures (2013 US NiV 2015 CSIRO HeV) and thus are not included in the spillover tally.

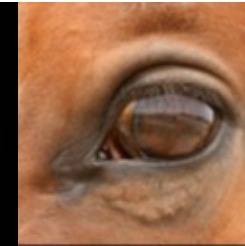
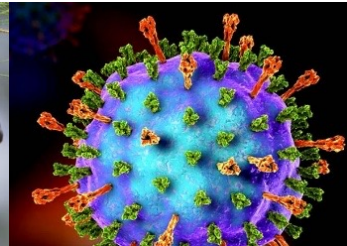
HeV Outbreaks in Horses 1994 - 2021





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Veterinary Testing for unvaccinated horses

- Samples tested for Hendra virus exclusion
- Negative samples are not thoroughly investigated for other pathogens
- Significance realised with detection of two fatal cases of ABLV in 2013



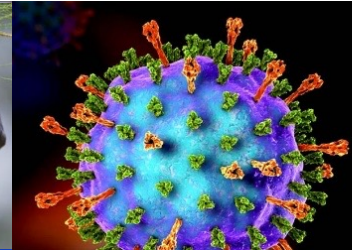


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Horses as Sentinels of Emerging Infectious Disease

Which pathogens are causing neurological and respiratory symptoms in Hendra negative horses?

Testing Hendra negative horses to Discover Emerging Pathogens causing Hendra like illnesses.

THE LAND

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NEWS EVENTS PROPERTY PHOTOS LIFE & STYLE OPINION MORE

Researchers target new wave of emerging infectious diseases

Penelope Arthur
16 Jul 2018, 5:43 AM

The work on emerging infectious zoonotic diseases associated with horses is funded by the Dalara Foundation and is a collaboration between the University of Sydney, School of Veterinary Science, Marie Bashir Institute for Infectious Diseases and Biosecurity and the CSIRO Australian Animal Health Laboratory (AAHL).

The project aims to:

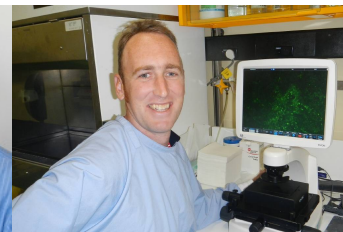
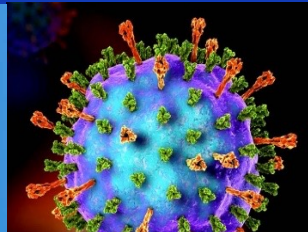
- Identify pathogens in horse cases where the Hendra Virus was confirmed negative
- Confirm and characterise unknown pathogens
- Develop additional diagnostic testing tools for the diagnosis of equine respiratory and neurological diseases and see them incorporated into state diagnostic laboratories.

Driven by the hypothesis that:

Some cases of severe Hendra virus-like disease in Australian horses is due to spillover infection by related but divergent bat borne paramyxoviruses such as those in the Rubulavirus and Henipavirus families of which our awareness is emerging.

One Health

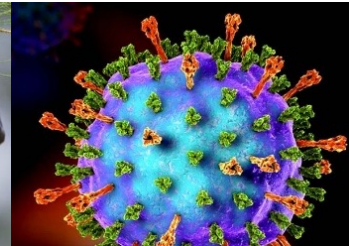
Combating the threat of infectious diseases to protect
Equine and Human Health



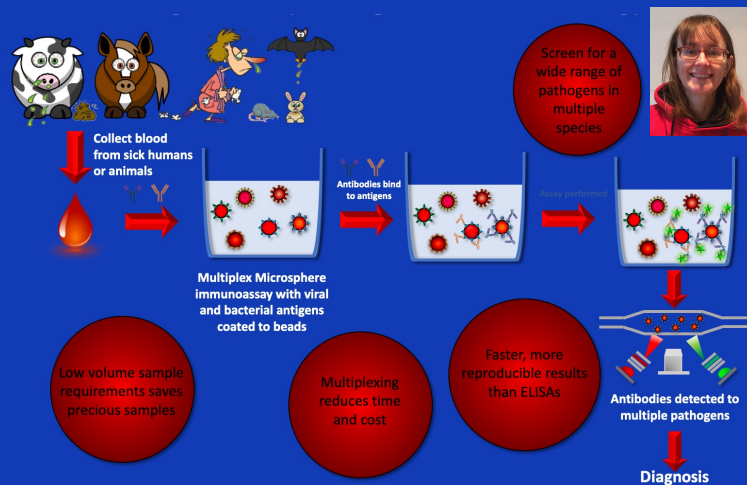


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33 plex Microbead Fluorescent Immunoassay Targeting EIDs of Putative EID Relevance to Australian Geography and Horses

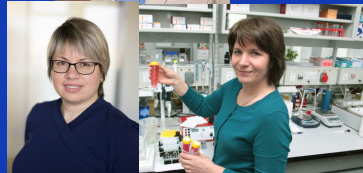


Antigens

Brucella suis repA	Paramyxoviruses	Filovirus	Coronavirus
Brucella OMP	Hendra	EBOV (G)	SARS1
Leptospira	Nipah	Bundibugyo	SARS2 (S and N)
Chikungunya virus E1 protein	Cedar	Reston	MERS
Sindbis virus E1 protein	Mojang	Marburg	Rs4874
Hepatitis E virus Capsid protein	Ghana	Ravn	
Hervey pteropid gammaretrovirus	Menangle (N and HN)	Llovlu	
La Crosse virus N protein	Grove	Mengla	
West Nile virus E protein	Yeppoon	Zaire N	
	Timor N		

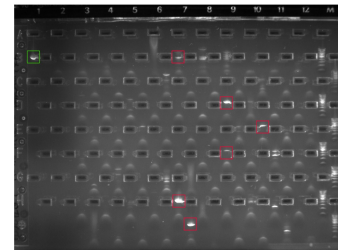


Sources: Commercial, collaborators-CSIRO, Burnett Institute, Vilnius University, Lithuania, Uniformed Services University



Nested Conventional RT-PCR and Total RNA NGS

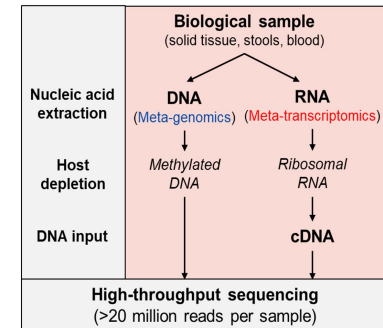
Viral family pan-RT-PCR



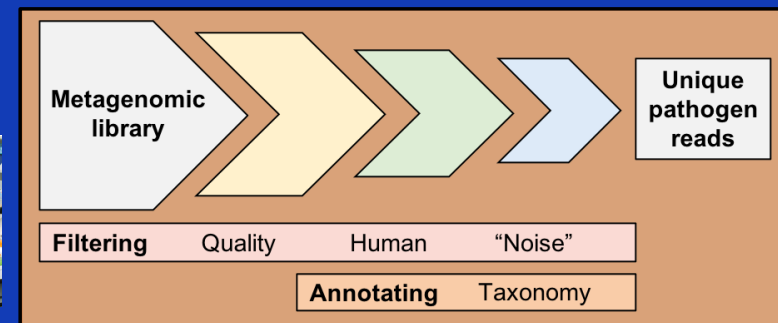
Paramyxo, Corona, Filo,
Alpha & Flaviviruses



Deep RNA sequencing



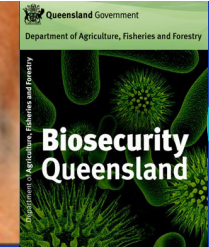
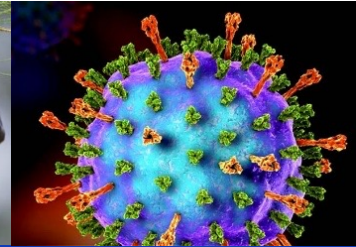
Sensitive Bioinformatical Pipeline





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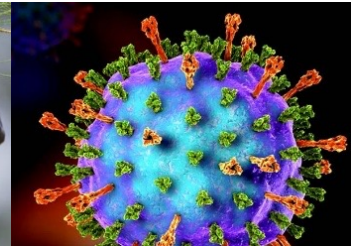
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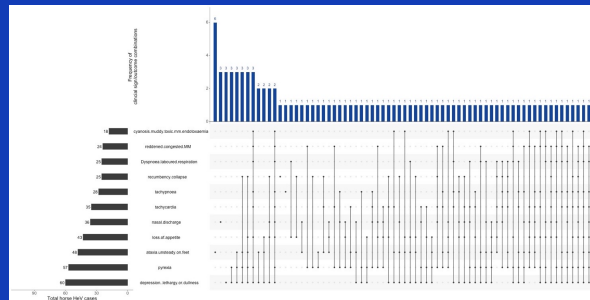
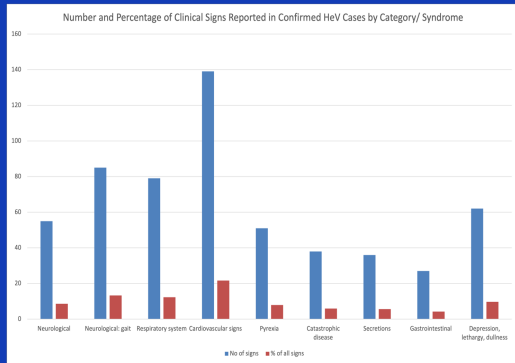
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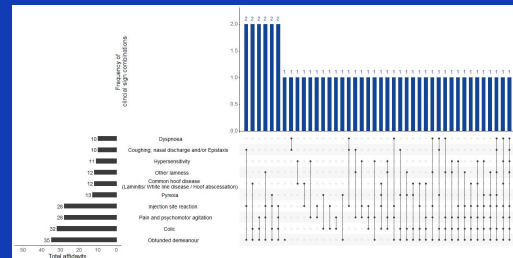
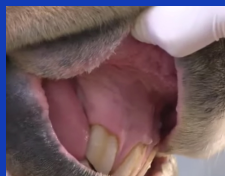


Horses as Sentinels – Targeted Disease Surveillance via research partnering with and extending routine Biosecurity

Understanding HeV disease in horses in its pathogenic basis and the syndromic presentation



Upset plot reported HeV cases



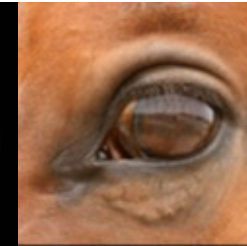
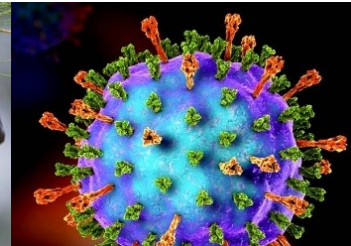
Upset plot AEFI



Table 1. Infectious disease prioritization categories (with examples) used in this study to identify Hendra-negative equine disease cases with highest likelihood of similar undiagnosed viral cause from larger cohort for further investigation

Infectious disease priority	Description	Example
<i>Category 1</i> Highest infectious disease suspect	Case features 'pyrexia' or 'abnormal mucous membranes AND one or more other clinical signs related to infectious disease OR the presence of either change AND 'epidemiological observation indicative of infectious cause' based on temporal and/or spatial relationship to similar disease cases	Pyrexia with tachycardia and acute onset respiratory consolidation and/or secretions. Pyrexia and neurological symptoms. Pyrexia and 'injected/congested' mucous membranes. 'Congested/injected mucous membranes' with acute severe respiratory dysfunction. Clustering of similar cases on same or neighboring properties
<i>Category 2</i> High infectious disease suspect	Pyrexia OR other clinical signs associated with infectious disease of interest	Acute onset abnormal respiratory secretions. Fever of unknown origin. Colic with the presence of neurological symptoms
<i>Category 3</i> Moderate infectious disease suspect	Clinical signs may be associated equally with infectious and non-infectious causes	Colic with the presence of dehydration and mucous membrane changes. Ataxia with the absence of pyrexia or known trauma
<i>Category 4</i> Low infectious disease suspect	Non-infectious etiologies more common or most likely on differential diagnosis list, but infectious cause still possible	Ataxia following known traumatic event. Traumatic wounds following unusual behavioral event. Acute lethargy following chronic non-infectious disease condition
<i>Category 5</i> No infectious disease suspect	No clinical signs of illness or no infectious cause considered likely	Traumatic wounds in the absence of underlying disease. Screening in unvaccinated horses to manage biosecurity risk prior to invasive procedures addressing non-infectious disease such as a common requirement for dentistry or admission to equine hospitals in Australia
<i>Category 6</i> Confirmed infectious disease	Other infectious disease confirmed via diagnostic testing	A case submitted for HeV testing, found negative and then testing positive for alternative known infectious disease such as ABLV, WNV, EHV or RRV*

*ABLV, Australian bat lyssavirus; EHV, Equine herpes virus; HeV, Hendra virus; RRV, Ross River Virus; WNV, West Nile virus



Important update for HeV surveillance in humans and animals. *Hendra-virus-variant - Horse South-East Qld 2015*

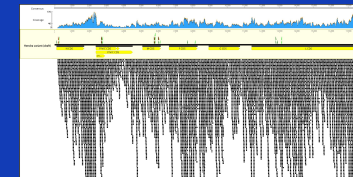
A Hendra virus variant (HeV-var) detected:

- 2015 South-east Queensland case of fatal equine disease;
- Previously found negative by routine state PCR testing for HeV at the time;
- Not detected by the molecular methods routinely relied upon due to sufficient nucleotide mismatches in genomic sequence (approx. 15%).
- Equine HeV disease caused by this variant was clinically indistinguishable from the most severe reported form of acute HeV disease.
- This 2015 horse HeV-variant shares near-identical genomic sequence to that detected in a grey-headed flying fox (*Pteropus poliocephalus*) from Adelaide in 2013.

2013 viral sequence was shared with us in good-will prior to publication will by relevant CSIRO scientists.

Initial action:

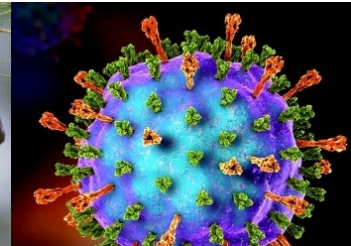
- Timely communication with state biosecurity research partners and CVO allowing for immediate actions relevant to local stakeholders.
- Redesigned PCR assays suitable for routine biosecurity screening and shared these with state and national human and animal health laboratory network.
- Undertook genomic and phenotypic analysis of the HeV-variant to support the understanding that immune-protection should be afforded as for the prototypic HeV by both the Equivac HeV® vaccine in horses and the mono-clonal antibody m102.4 post-exposure therapy in humans.



1. Hendra_ref_prototype_genome	CTTCGACAAAGACGGAACCAA	TGGCATCTTTCATGCTCCATCTCGG	AATTTTGTCCGACGAGCTGG
FND 2. M_fnd	CTTCGACAAAGACGGAACCAA	TGGCATCTTTCATGCTCCATCTCGG	AATTTTGTCCGACGAGCTGG
FND 3. M_prb	CTTCGACAAAGACGGAACCAA	TGGCATCTTTCATGCTCCATCTCGG	AATTTTGTCCGACGAGCTGG
REV 4. M_rev	CTTCGACAAAGACGGAACCAA	TGGCATCTTTCATGCTCCATCTCGG	AATTTTGTCCGACGAGCTGG
FND 5. Hendra_var_QLD_horse_genome	CTTCGACAAAGACGGAACCAA	TGGCATCTTTCATGCTCCATCTCGG	AATTTTGTCCGACGAGCTGG
FND 6. Hendra_var_SA_bat_genome	CTTCGACAAAGACGGAACCAA	TGGCATCTTTCATGCTCCATCTCGG	AATTTTGTCCGACGAGCTGG
FND 7. Mv_fnd_1	CTTCGACAAAGACGGAACCAA	TGGCATCTTTCATGCTCCATCTCGG	AATTTTGTCCGACGAGCTGG
FND 8. Mv_prb_1	CTTCGACAAAGACGGAACCAA	TGGCATCTTTCATGCTCCATCTCGG	AATTTTGTCCGACGAGCTGG
REV 9. Mv_rev_1	CTTCGACAAAGACGGAACCAA	TGGCATCTTTCATGCTCCATCTCGG	AATTTTGTCCGACGAGCTGG

Hendra virus variant primers shared with Human and Animal Health laboratory and biosecurity network 24th February 2021 courtesy of 'Horses as Sentinels Research' group in recommendation for urgent prepublication use on compassionate grounds. Primer design by JS Eden WIMR and I Smith CSIRO.



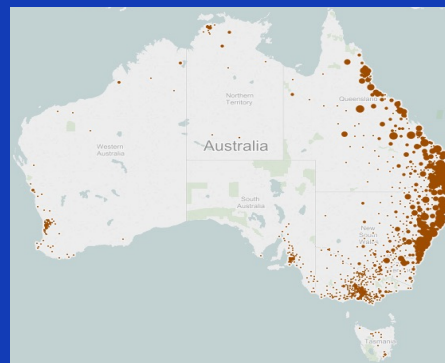


It should be emphasized, that the HeV RBP shares only 79% amino acid identity to NiV RBP, yet the HeV-sG subunit vaccine provides 100% protection against lethal challenge with both HeV and NiV in animal models (11).

Both the higher similarity between the HeV-var and HeV RBP (92.5% amino acid identity) and structural consistency of critical epitopes mentioned above, suggest that current vaccination utilizing the HEV RBP will elicit similarly protective antibodies against this HeV-var. Current serological assays based on the HeV RBP are not expected to distinguish between exposure to the variants.

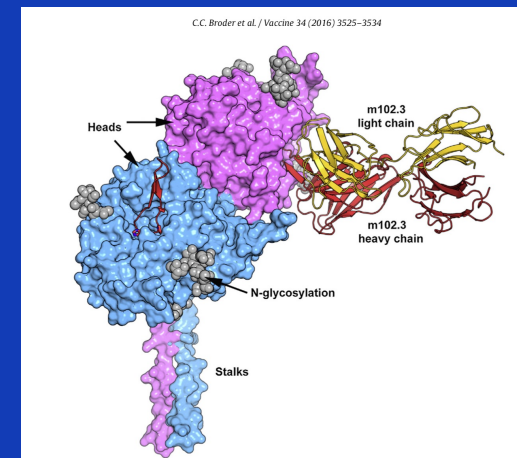
Neutralisation Titres on Vaccinated Horses

Serum	HeV	HeV-var/g2
1	64	64
2	256	256
3	128	128
4	128	128
5	64	64
6	2048	1024
7	16	16
8	1024	1024
9	2048	2048
10	64	64
11	512	1024
12	2048	2048
13	512	512
14	512	512



>150,000 horses vaccinated from 2012 to 2019

>650,000 doses administered.



Vaccine 34 (2016) 3525–3534

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Hendra virus and Nipah virus animal vaccines

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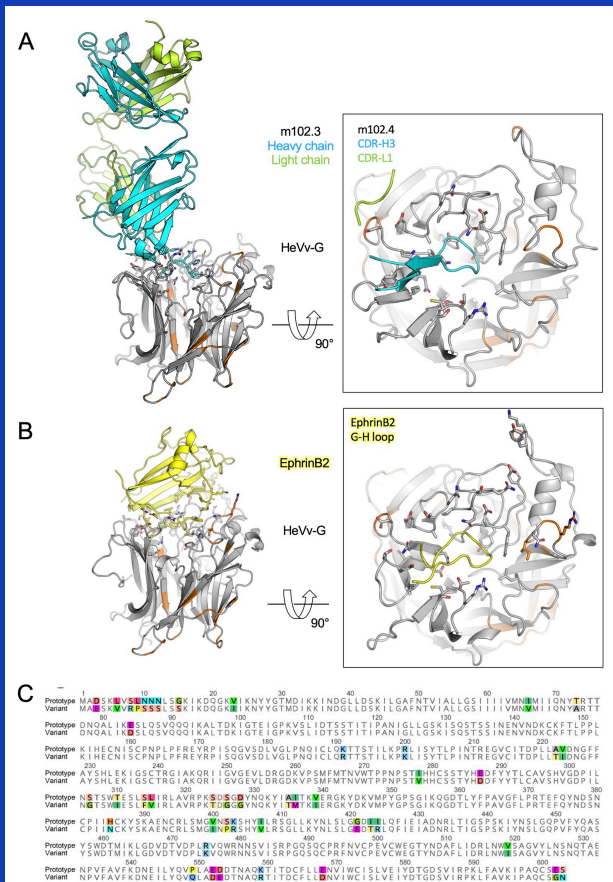
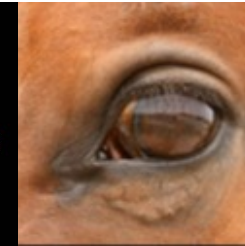
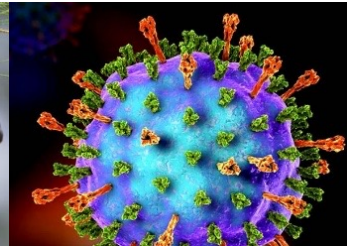


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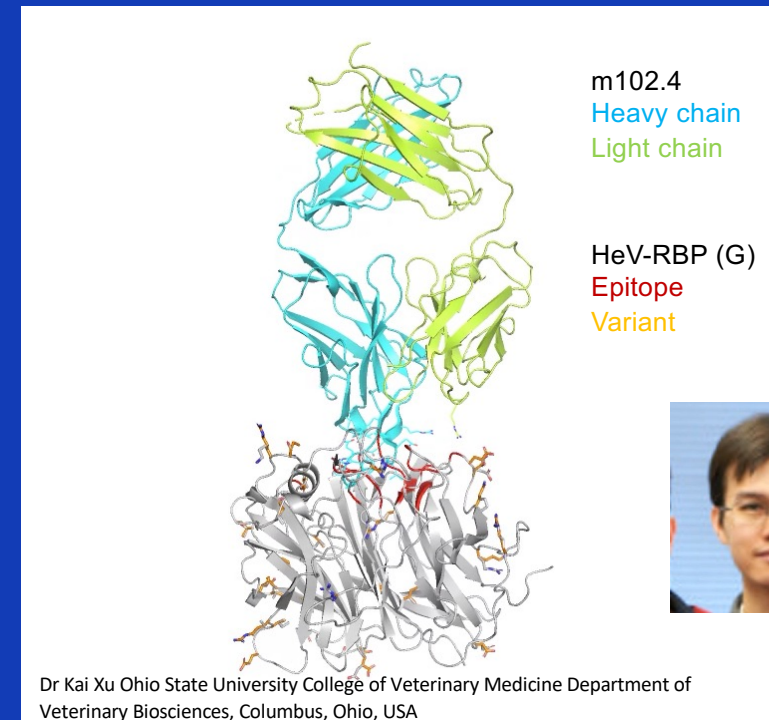
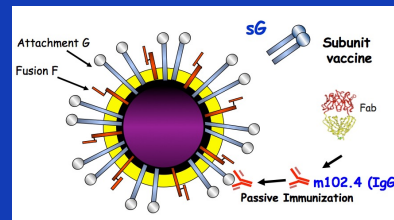


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Comparison of the translated amino-acid sequence of this HeV-var and prototypic HeV RBP *in silico* revealed the ephrin-B2 entry receptor binding site and that of mAb m102.4 to be unchanged. Similarly, m102.4 neutralization was confirmed *in vitro* with HeV-var as for HeV. As such, it is expected that current PEP utilizing mAb m102.4 will remain effective against this HeV-var.

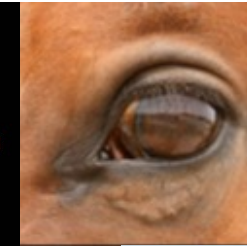
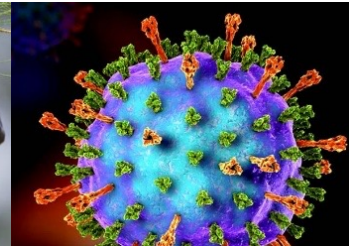


Anticipated efficacy of sG vaccine for horses and post-exposure immunotherapy with m102.4 via *In silico* analysis of HeV variant receptor-binding protein structure based on established structure for HeV via x-ray crystallography.



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Timely communication of equivalent pathogenicity and immunogenicity with updated qPCR testing capacity shared for routine priority disease investigation.



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Special Features

Hendra virus - Two Viruses, Same Fatal Disease

Edward J. Annand

University of Sydney | Sydney School of Veterinary Science |

Marie Bashir Institute for Infectious Diseases and Biosecurity, Sydney, NSW, AUS.

CSIRO | Health and Biosecurity, Black Mountain Laboratories, Canberra, ACT, AUS.

EquiEpiVet | Equine Veterinary and One Health Epidemiology, Airey's Inlet, Surf Coast, Vic., AUS.



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Title: **Novel Hendra virus variant detected by sentinel surveillance of Australian horses.**

Authors: Edward J. Annand^{1#}, Bethany A. Horsburgh¹, Kai Xu, Peter A. Reid, Ben Poole, Maximilian

C. de Kantzow, Nicole Brown, Alison Tweedie, Michelle Michie, John D. Grewar, Anne E. Jackson,

Nagendrakumar B. Singanallur, Karren M. Plain, Mary Tachedjian, Brenda van der Heide, David T. Williams,

Cristy Secombe, Eric D. Laing, Spencer Sterling, Lianying Yan, Louise Jackson, Cheryl Jones, Raina K.

Plowright, Alison J. Peel, Ibrahim Diallo, Andrew C. Breed, Christopher C. Broder, Philip N. Britton*, Navneet

K. Dhand*, Ina Smith*, John-Sebastian Eden*

¹These authors contributed equally. *Shared senior authorship. *Corresponding author.

Manuscript Number: EID-21-1245 Submitted Mon, May 31, 2021, *In Review*.

Article Summary Line: A novel variant of Hendra virus was discovered and isolated from an Australian horse with fatal disease.

Running Title: HeV variant associated with fatal disease in a horse

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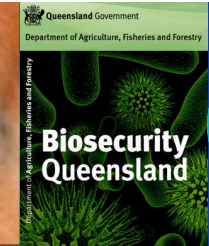
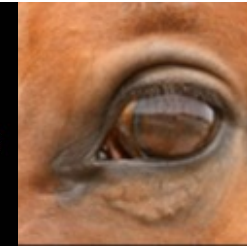
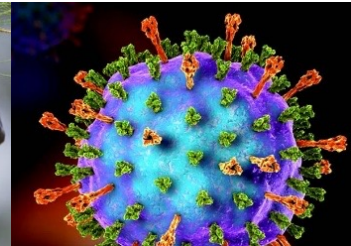


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Timely Prepublication Sharing of Duplex qPCR for Variant (HeVg2) and Original HeV Genotypes Results in Prospective Detection (Southern-most HeV Spillover)



Australian Government

The Hon David Littleproud MP
Minister for Agriculture and Northern Australia



Joint media release: Researchers develop test for new Hendra variant

9 October 2021

Minister for Agriculture and Northern Australia, the Hon David Littleproud MP
Minister for Science and Technology, the Hon Melissa Price MP

- New Hendra virus variant (HeV) confirmed in routine surveillance in New South Wales
- Researchers recently discovered this new Hendra virus variant in historical samples
- New test available nationally to identify Hendra virus variant



Department of
Primary Industries

Hendra variant case confirmed near Newcastle

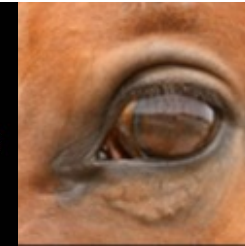
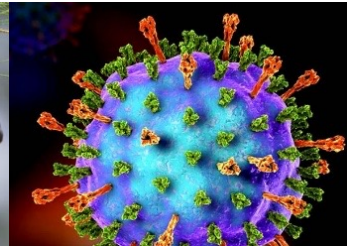
8 Oct 2021

A variant Hendra virus strain has been confirmed in a 7-year-old unvaccinated Clydesdale from West Wallsend, near Newcastle.



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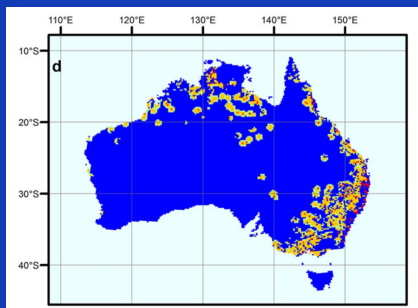
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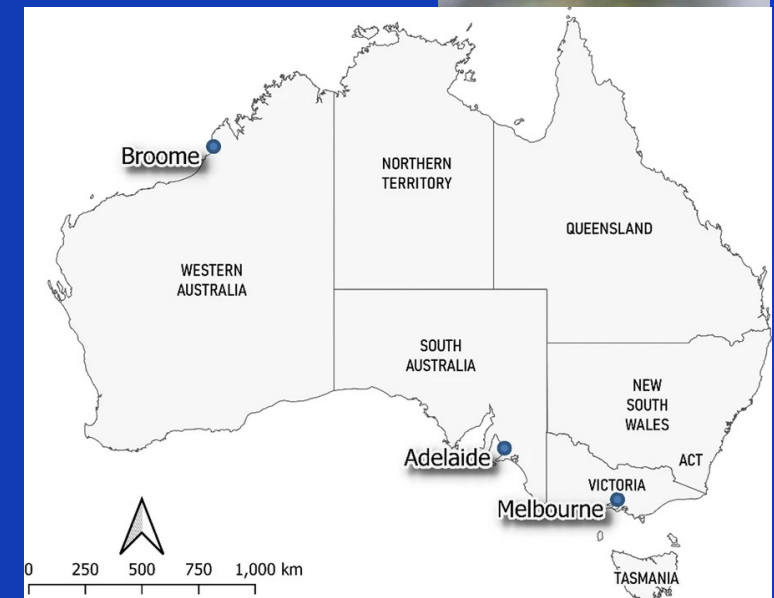
Numerous molecular detections of the same variant genotype of Hendra virus (HeVg2), in grey-headed flying foxes in South Australia and Victoria and a little red flying fox in Western Australia:

Wang, J., Anderson, D.E., Halpin, K. et al. A new Hendra virus genotype found in Australian flying foxes. *Virology* 18, 197 (2021). <https://doi.org/10.1186/s12985-021-01652-7>.

The manuscript describes detections in bat samples (2013-2021) of the same consistent phenotypic second genotypic lineage detected retrospectively (2021) by this research to have caused fatal equine disease in QLD (2015).



East IJ, Wicks RM, Martin PAJ, Sergeant ESG, Randall LA, Garner MG. Use of a multi-criteria analysis framework to inform the design of risk based general surveillance systems for animal disease in Australia. *Preventive Veterinary Medicine*. 2013 Nov 1;112(3):230–47.



Map of Australia showing the locations of the HeV-g2 positive flying foxes collected between 2013 and 2021: one LRFF was from Broome, three GHFF were from Adelaide and 7 GHFF were from Melbourne.

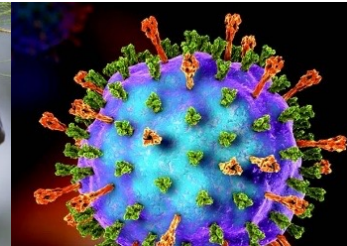


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Revised Geographic HeV Spillover Risk

Horses as Sentinels



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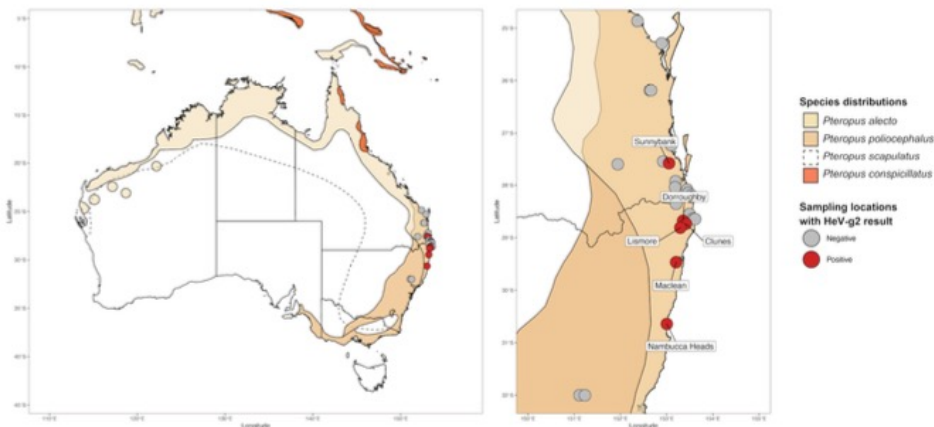


Figure 1: Map of Australia (left) and study area (right) showing the distribution of Australian flying foxes, the sampling locations and positive HeV-g2 detections.

A novel variant of Hendra virus circulates in black flying-foxes (*Pteropus alecto*) and grey-headed flying-foxes (*Pteropus poliocephalus*)

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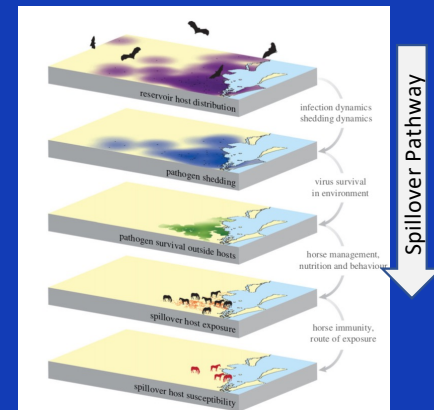
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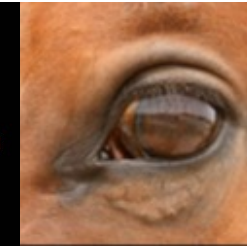
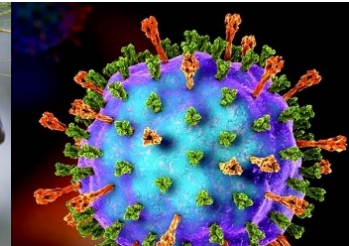


Plowright RK et al. 2015



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HeV and ABLV are both severe zoonotic diseases that circulate amongst Australian bats and are lethal to both horses and humans.

While they are likely ancient viruses, our awareness of them is relatively recent and emerging.

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Horses as Sentinels of Emerging Infectious Diseases Research

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