Important update regarding Hendra virus (HeV) to the Australian Veterinary Association (AVA) and Equine Veterinarians Australia (EVA) members

"Horses as Sentinels for Emerging Infectious Viral Disease Research"

Representing the Research Group: Dr Ed Annand (University of Sydney - School of Veterinary Science, Marie Bashir Institute for Infectious Diseases and Biosecurity , CSIRO), Dr John-Sebastian Eden (University of Sydney - Medical School, The Westmead Institute for Medical Research) , Dr Ina Smith (Risk Evaluation and Preparedness Program - Health and Biosecurity - CSIRO), Dr Andrew Breed (Animal Health Policy Branch, Biosecurity Animal Division, Australian Government Department of Agriculture, Water and the Environment), Dr Peter Reid (Private Equine Veterinarian representing AVA/EVA).

New findings

Using innovative molecular testing combined with world-leading next-generation-sequencing and bioinformatics approaches our research has recently identified and characterised **a novel Hendra virus (HeV) variant** from a previous 2015 case of fatal equine disease in south-east Queensland which was found negative for HeV by routine PCR testing at the state laboratory.

Notably, this variant is not detected by the molecular methods routinely relied upon due to sufficient mismatches in genomic sequence (approx. 15% at a nucleotide level).

We have also determined that the 2015 horse HeV variant virus shares near identical (~99%) genomic sequence with virus identified in pooled grey-headed flying fox (GHFF - *Pteropus poliocephalus*) extracted samples from Adelaide in 2013.

Significance of the findings

These findings serve to inform the re-assessment of HeV risk to horses. Most notably, a previously perceived view by some of a negligible risk of HeV disease associated with GHFF's, and a perceived limitation of HeV risk to regions frequented by black flying foxes is no longer supported by our updated understanding. It confirms our view that HeV disease in horses could and likely does result from virus spillover from all Australian flying fox species.

The variant's confirmed association with GHFF's supports consideration of HeV disease as a differential diagnosis in unvaccinated horses anywhere in Australia that flying foxes are present. It is our interpretation that this variant has not just recently emerged but more likely has been circulating for some time with its detection going largely unrecognised except for one instance in flying foxes in Adelaide in January 2013.

Veterinarians in all regions should become familiar with correct biosecurity and workplace health and safety practices and obligations relevant to HeV investigations, including implementation of infection risk mitigation and prevention strategies in practice protocols, and protocols for engaging in notifiable disease investigations.

Chief Veterinary Officers, BSL, CSIRO ACDP (formerly AAHL) and the 2015 horse case attending veterinarian, have been notified of the new findings. Chief Health Officers are also being informed.

Implications of the new findings

1. Redesign of qRT-PCR assays available at State Government Labs and at ACDP

Members of our research group have redesigned the PCR assays suitable for routine biosecurity screening and have shared these assays with ACDP DSR (Diagnosis, Surveillance and Response) and QDAF BSL and will share these updated assays with all relevant state and national animal and human health laboratories as soon as possible. In the meantime, all samples sent to state animal health laboratories can be forwarded to ACDP for confirmatory testing.

2. Horse vaccine and human monoclonal antibody

Following our group's urgent sharing of sequence data and related information with, and largely because of, established close collaborative ties with leading USA scientists, our analysis of this HeV variant supports the understanding that immune protection should be afforded as for the prototypic HeV by both the Equivac HeV® vaccine and the already developed and trialled monoclonal antibody m102.4 for early post-exposure therapy.

3. Serology

Serological assays that use the reference HeV soluble G glycoprotein antigen (sG) are also expected to readily detect target immunoglobulins in horses with exposure to this HeV variant. This has implications for human, flying fox and horse testing and importantly offers an understanding for the previously observed mismatch between relatively high sero-positivity in GHFF and relatively lower viral detections and attributed spillover events to this species. Our research team hopes to support the validation of the DIVA assay that has been in development for some years at CSIRO with our most appropriate serology samples and test results from our "Horses as Sentinels" research horse sample cohort.

4. Urgent scientific publication

Our group will soon be finalising and submitting a scientific research paper to the *Emerging Infectious Diseases Journal* to update the local and international scientific community. Further investigations into the variant's tropism are also ongoing.

The September 2015 horse case

A 12-year-old male Arabian of local origin ('homebred') presented with acute severe disease featuring severely 'injected' mucous membranes, tachycardia (75), tachypnoea (60), normal rectal temperature, muscle fasciculations, head pressing and recumbency with rapid deterioration over 24 hours resulting in euthanasia on humane grounds. Flying fox roosts proximal to the spillover event are known to host both grey-headed flying foxes (GHFF's) and black flying foxes (BFF's). GHFF numbers in the area have greatly increased in the last few years.

Notably, the equine HeV disease attributed to this variant was clinically indistinguishable from the most severe reported form of acute HeV disease caused by the previously recognised prototypic strain.

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Approach and Methodology

The initial detection resulted from our batched molecular research testing pathway that combines next generation sequencing with the use of nested-conventional-PAN Paramyxovirus PCR screening to samples from disease cases prioritised utilising our SQL database which aims to facilitate this research support of routine biosecurity while leaving sensitive information with the state laboratory. Suspect cases were prioritised based on their likelihood of infectious cause following our pathogenic-basis-of-disease syndromic analysis.

Co-Hosted by: The University of Sydney (USYD) Sydney School of Veterinary Science, Faculty of Medicine and Health; Marie Bashir Institute for Biosecurity and Infectious Diseases and CSIRO (Health and Biosecurity Business unit).

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