PROJECT SUMMARY

RURAL INDUSTRIES
Research & Development Corporation

Project Summary - Early detection of Hendra virus infection by microRNA profiling

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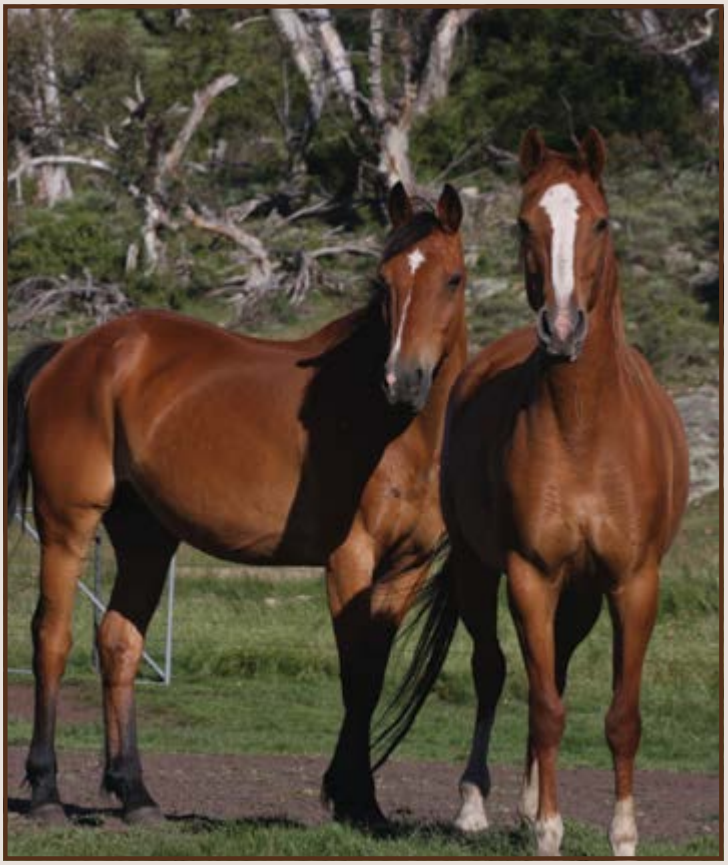
Background

Early and rapid diagnosis of Hendra virus infection in horses is important for the controlling of Hendra virus spread. Early detection of infection in other horses that may have been exposed to flying foxes as well as in-contact horses would allow further separation of such animals from other susceptible horses prior to the onset of high levels of virus shedding and would therefore provide an improved strategy for preventing transmission from horses to people.

Who should be interested in these findings?

This report is targeted at the National Hendra virus taskforce, state and federal authorities responsible for dealing with equine cases of Hendra virus, staff in veterinary diagnostic laboratories and interested individuals.



Background

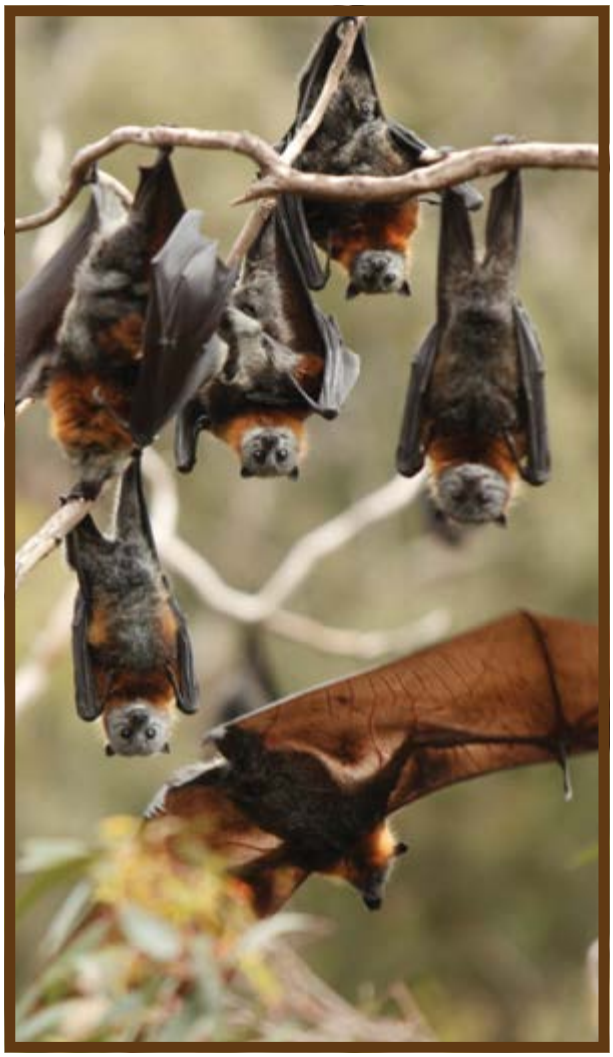
Hendra virus is a serious zoonotic disease for which stringent biosecurity and safety measures apply. Risk management is an important component in the management of Hendra virus outbreaks. The guidelines for veterinarians handling potential Hendra virus infection in horses (Version 4.2, Queensland Government) states that horses assessed as being at risk of infection on an outbreak property are tested and their health status assessed and monitored. Furthermore, testing and monitoring may apply to horses that have moved off the property in the last few weeks, or are present on neighbouring properties.

The number of animals to be tested during a Hendra virus outbreak and the time required to release animals from quarantine are significant issues in the context of outbreak management. Research conducted at CSIRO AAHL demonstrates that horses infected with Hendra virus do not display signs of disease (for example, elevated heart rate, temperature) or detectable Hendra virus genome until around the time of florid disease, which can be up to 16 days post-exposure.

A diagnostic test for the early detection of Hendra virus infection in horses would aid the decision-making process surrounding Hendra virus outbreaks. Host molecules known as microRNAs, are produced during early stages of virus infection, and can report the identity of a virus responsible for infection. MicroRNAs are currently employed in diagnostic testing for several reasons: they can be detected from easily-collected bodily fluids such as blood and urine, are stable once secreted, and can be detected using common laboratory techniques such as PCR.

We have previously reported that a single microRNA is up-regulated in horses infected with Hendra virus (Stewart et al., 2013).

Acknowledging that a single molecule cannot itself act as a diagnostic tool, due to a lack of specificity, the purpose of this work was to assess a transcriptome-wide microRNA profile associated with Hendra virus infection in horses, and to assess whether this profile manifests before other measures of infection.

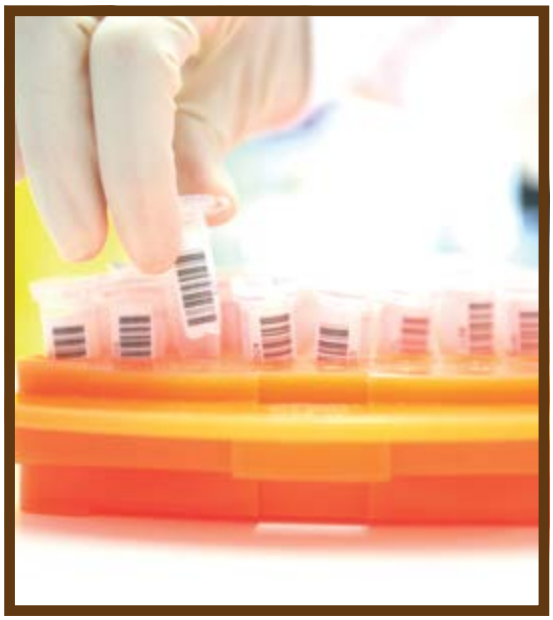


Aims/objectives

The specific objective was to explore the feasibility of microRNA profiling as an early marker of Hendra virus infections in horses, with the potential to detect infection prior to antigen or genome detected. The sensitivity and specificity of this approach was assessed both *in vitro* and *in vivo*.

The project had three main areas:

1. Analysis of RNA samples from Hendra virus infected and non-infected horses by deep sequencing technologies to identify microRNAs associated with Hendra virus infection.
2. To assess how microRNA profiles associated with Hendra virus infection in horses compare to other measures of infection, such as the detection of virus itself, or the onset of signs of disease.
3. Assess new technologies, namely surface enhanced Raman scattering (SERS), as a method to rapidly detect microRNA levels in biological samples using hand-held devices with field applications.



Methods used

To identify microRNAs associated with Hendra virus infection, RNA was purified from infected and non-infected horses as part of an experimental infection of horses with Hendra virus that was conducted at bio-safety level 4 conditions at the CSIRO Australian Animal Health Laboratory (described in (Marsh et al., 2012). Samples were also taken from horses that tested positive to Hendra virus in the field. RNA was sequenced using Illumina deep sequencing technology and microRNAs were identified using bioinformatics software called mirDeep (Friedlander et al., 2008). PCR and surface-enhanced Raman spectroscopy (SERS) were trialled to detect microRNAs in biological samples, in a view to expedite diagnosis.

Key findings

Hendra virus infection in horses induced rapid changes in select host microRNAs, supporting the notion that this approach can aid diagnostic testing. When measured by next generation RNA sequencing and bioinformatics, several molecules were differentially-regulated in Hendra virus-infected horses, compared to healthy horses. These candidates were associated with infection in both field cases and a controlled Hendra virus infection trial, suggesting that measuring these molecules would be relevant for field testing, and are reflective of both the early and latter stages of infection. Efforts to develop a PCR or SERS test to rapidly streamline the detection process proved unsuccessful and require further work.

Recommendations

This research shows clear differences in host microRNA responses between healthy horses and horses infected with Hendra virus. These differences can be detected by RNA next generation sequencing, followed by bioinformatic analysis. A rapid diagnostic test, most likely based around Taqman PCR-based detection of select microRNAs, will require further work to develop.

Current recommendation is that microRNA profiling, as measured by RNA sequencing and bioinformatics, could be used to assess infection status in horses. There is also considerable scope for future work:

i. The development of a PCR-based test for the detection of select microRNAs.

ii. Testing of the specificity of host microRNA responses by analysing microRNAs profiles of horses infected with other viruses .

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More information

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