Compendium of findings from the National Hendra Virus Research Program
Foreword

Hendra virus is a zoonotic disease that spills over from flying foxes to horses, and then can be transmitted to other horses, humans and dogs. It has a high fatality rate (>79 per cent in horses and 57 per cent in humans) giving it both veterinary and public health significance.

Hendra virus was first identified in Australia in 1994 and occurred intermittently, with a total of 14 incidents in the period 1994 to 2010. However, there was an increase in the number of reported incidents between June and August 2011 (18 incidents). These caused the deaths of 23 horses and the euthanasia of the first dog infected with Hendra virus.

This sparked a great deal of concern across the horse industry, as well as in the veterinary and public health sectors and with the public at large. The unprecedented year of Hendra virus incidents prompted the establishment of the National Hendra Virus Research Program, to fund research leading to strategies to minimise the impact of Hendra virus.

In July 2011, a Joint Government Hendra Virus Taskforce was established and the Australian Government and the Governments of New South Wales and Queensland each announced funding commitments of $3 million for Hendra virus research, development and extension activities.

A multi-jurisdictional Steering Committee of the National Hendra Virus Research Program was established to recommend the allocation of these funds to high-impact research projects and to monitor and review project deliverables. The National Health and Medical Research Council (NHMRC) also allocated $3 million to better understand Hendra virus, and CSIRO devoted funds to Hendra virus-related research through its Australian Animal Health Laboratories.

A total of 20 projects were funded under the National Hendra Virus Research Program. Eight of these were managed by the Rural Industries Research and Development Corporation, eight were managed by the NHMRC, and the remaining four by the Queensland and the New South Wales State Governments.

This compendium outlines the findings of these 20 projects under the National Hendra Virus Research Program; the collaborative effort leading to a better understanding of Hendra virus. This understanding will lead to improved management of flying foxes and horses when selecting strategies to mitigate the risk of Hendra virus infection in Australia.

The researchers are to be congratulated on the delivery of high quality research. The cooperative multi-jurisdictional commitment of the Australian Government and the Governments of New South Wales and Queensland has been critical to ensure the funds delivered research that helped minimise the impact of Hendra virus.

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Photos on this page by Justin Welbergen.
Contents

P3
Foreword

P6
Research highlights

FLYING FOXES AND HENDRA VIRUS
P8
Implementing a national flying fox monitoring program
P10
Models to predict Hendra virus prevalence in flying fox populations
P12
Hendra virus infection and transmission dynamics
P14
Spikes in Hendra virus spillover: early warning through the bat urinary metabolome
P16
Models that predict risk for Hendra virus transmission from flying foxes to horses

HORSES, OTHER ANIMALS AND HENDRA VIRUS
P18
Early detection of Hendra virus infection by microRNA profiling
P20
Assessing the risks posed by Hendra virus antibody positive animals
P22
Development of improved diagnostics and therapeutics for Hendra virus infections
P24
Hendra virus in dogs

REDUCING THE RISK OF HENDRA VIRUS IN HORSES
P26
Flying fox dispersal and Hendra virus risk
P28
Additional research required in relation to the development of the horse Hendra virus vaccine
P30
Longitudinal cohort study of horse owners

HENDRA VIRUS IN HUMANS
P32
Structural studies of Hendra virus replication
P34
Defining the role of microRNAs in human Hendra virus infections
P36
Identification of viral-cellular interacting factors for an in-depth understanding of the Hendra virus life cycle and pathogenesis
P38
Understanding pathogenicity and immunity in an encephalitic mouse model of Hendra virus infection

REDUCING THE RISK OF HENDRA VIRUS IN HUMANS
P40
Can the bat immune response to Hendra virus inform drug and vaccine development in other species?
P42
Hendra virus vaccine
P44
New Hendra virus treatments
P46
Development and testing of a monoclonal antibody
Research highlights

The key objective of the National Hendra Virus Research Program was to better understand Hendra virus by learning more about factors that lead to its transmission, and modelling and predicting the nature and impacts of the virus.

The research funded through the Program has been carried out with the aim of better managing and mitigating the risk of Hendra virus infection in humans, horses and other animals.

The projects outlined in this compendium are the result of a remarkable and borderless collaboration between policy makers, research scientists here and overseas, and Commonwealth, state and local authorities, to enhance research, expertise and capacity in relation to Hendra virus.

Most prominent in the highlights of this research has been the development of a post-exposure prophylaxis for Hendra virus in humans, trialled in 40 volunteers to date without issue, and a pre-exposure vaccination for the virus in horses.

At the same time there have been promising results from preliminary research that may lead to a self-administered vaccine, that people working with horses or who have been exposed to a Hendra virus outbreak can apply via the mouth or nose.

Projects were developed under five key themes:
- flying foxes and Hendra virus
- horses and other animals and Hendra virus
- reducing the risk of Hendra virus in horses
- Hendra virus in humans
- reducing the risk of Hendra virus in humans.

A National Flying Fox Monitoring Program documented the distribution and abundance of four species across more than 700 camps along the eastern seaboard, and developed a spatial model of flying fox colony dynamics, both of which will assist in developing risk maps for the transmission of the virus.

Research monitoring Hendra virus excretion and infection in flying fox roosts over a 2300km stretch from Cairns to Batemans Bay has highlighted the need for a management strategy at a regional or larger scale, and independent of state borders.

For the first time scientists have identified biomarkers that could indicate periods of increased Hendra virus risk, by analysing the urinary metabolic profiles for flying foxes when they experience conditions, yet to be identified, that cause an increase in the replication of Hendra virus.

Another project revealed that the length of time the virus survived did not influence the pattern of ‘spillover’ events from flying foxes to horses, but rather that transmission of Hendra virus was likely to involve relatively direct contact of horses with flying fox excreta shortly after excretion.

Research into the possibility that Hendra virus could persist in animals that had clinically recovered from the disease, posing a risk of re-infection or later transmission to other humans and animals, ruled out the likelihood of recurrence.
There is the potential for transmission of Hendra virus to people from acutely infected dogs.

Researchers found that most infected dogs in the trial did not show any signs of illness at a time when they were shedding virus.

A series of projects examined the way in which Hendra virus and its family group of henipaviruses replicate and interact with hosts; information with important ramifications for finding antidotes to other global viruses such as Nipah and the distantly related Ebola virus. One therapy was found to reduce Hendra virus by up to 98 per cent and may prove its potential in future studies.

The wide-ranging social, regulatory and policy impacts of Hendra virus were revealed in a longitudinal cohort study of 1149 horse owners. The study used surveys and interviews to assess how horse owners perceived the risk of Hendra virus, their uptake of risk mitigation practices such as the vaccine, and their engagement with government and industry stakeholders such as veterinarians.

After initial increases in vaccination, it revealed increasing intentions to withdraw from the booster program and delay boosters, poor uptake of recommended property management practices, and a decreasing interest in Hendra virus generally. The findings, including reasons for horse owner attitudes, identify challenges for future engagement between horse owners and authorities, and for the valuable relationship between horse owners and veterinarians.

The body of work in this compendium has obvious public health benefits. While significant advances have been made, continuing research effort and funding support are needed to address the ongoing risks posed by the Hendra virus, its family group of henipaviruses and other potential rapid and unpredictable transmissible viruses.
Implementing a national flying fox monitoring program

This project established a National Flying Fox Monitoring Program to document the distribution and abundance of four species across more than 700 camps along the eastern seaboard. This information is vital for conservation planning and to better manage the risk of Hendra virus.

It found the distribution of flying foxes to be highly variable, with the animals moving in and out of camps seasonally, apparently in response to varying food resources. The grey headed and spectacled flying foxes - whose entire distributions were covered by the monitoring - have shown a dramatic change in their distribution over the last decades with a shift to smaller camps located in urban and peri urban areas. This is a similar distribution to that of horses.

The data showed a severe decline in the abundance of the spectacled flying fox, sufficient to warrant a change in its status to endangered, while the number of grey headed flying foxes was found to be stable to declining.

Hendra virus in horses mostly correlated with incursions of the spectacled flying fox and black flying fox.

Background

Determining trends in both the population and distribution of flying foxes is essential to predict and manage their associated disease risk to humans and livestock, and for their conservation.

The relationship between humans and flying foxes is fraught. On the one hand, flying foxes come into conflict with humans because of the disease risk they pose to industry and the community, and because of their impacts on amenity and agriculture. On the other hand, impacts such as deforestation, landscape modification and persecution are thought to have led to a decline in flying fox populations, to such an extent that two of the four most common species in Australia are currently listed as threatened.

This project contributed objective information to inform public discussion of Hendra virus and flying fox management, and provided baseline data to support management decisions.
IMPLEMENTING A NATIONAL FLYING FOX MONITORING PROGRAM

Methods used

Researchers used a combination of field, analytical and modelling methods. Monitoring of flying fox camps was conducted by professional and volunteer staff distributed from Adelaide in South Australia to Cooktown in far north Queensland. Field ecological work was required to trap animals, attach transmitters and establish download base stations. A range of statistical and modelling methods were used for analysing population numbers and movement data.

It was necessary to establish intergovernmental arrangements for a monitoring program across four states – South Australia, Queensland, New South Wales and Victoria – and the Australian Capital Territory. Unfortunately the project coincided with reorganisations of Queensland and NSW departments and was challenged by changing staff and priority settings. These challenges were successfully resolved through collaboration and communication.

Outcomes

A monitoring method was designed and implemented on a quarterly basis (monthly for spectacled flying foxes), with 518 camps active at some point during the project.

The dramatic drop in spectacled flying fox numbers over a 10-year period appears to have been caused in large part by cyclones Larry and Yasi.

While the research identified general patterns of landscape and habitat use, flying foxes appear to make individual assessments of food resources around the camp they are roosting in, and as a consequence the area foraged will alter significantly between seasons.

Individual flying foxes change camps regularly and can forage up to 150km from their current base. Modelling food resources and their usage by flying foxes in the future will enable prediction of the distribution of flying fox activity. Simulation modelling of flying fox use of specific landscapes is now possible, making it possible to predict flying fox distribution and movement, and potentially the risk of Hendra virus.

While urbanisation of flying foxes poses little direct threat to human health, all parts of the landscape are used in foraging activities. No landowner can assume that their location gives them immunity, and horse owners in peri-urban settings should maintain recommended property management to minimise the risk of Hendra spillover.

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FURTHER INFORMATION


Optimal Levy-flight foraging in a finite landscape. Journal of the Royal Society Interface 12. 20141158 http://rsif.royalsocietypublishing.org/content/12/104/20141158

This project is developing models that will enable prediction of flying fox colony dynamics, patterns of high prevalence and intensity of Hendra virus infection in such colonies, and the subsequent risk of transmission of Hendra virus to horses.

Already scientists have developed a spatial model of flying fox colony dynamics, and detected evidence that ‘pulses’ of Hendra virus activity in southeast Queensland are associated with changes in colony size, which in turn can be predicted in advance from remotely sensed satellite data. This will assist in developing risk maps for transmission of the virus and identify where and when to prevent transmission.

Background

The principal objective of this project is to provide ongoing modelling support to the Hendra virus program. A series of models will be developed, with increasing complexity and level of detail as data becomes available. These predictions focus on identifying high levels of Hendra virus infection in flying foxes and the spatial and temporal distribution of transmission risk to horses. Much of this information is already available or being collected in current Queensland Government search programs, and this project will use the collaborative network to access information from other flying fox-henipavirus studies, to help refine models. This will be supplemented by further targeted collection of empirical data, where initial models identify critical information gaps to which qualitative outcomes are sensitive, where such data cannot be supplied from other projects underway. Such gaps are expected to include uncertainty about the nature of host-viral interactions (including the possibility of carrier states, super shedders and waning immunity) and information on flying fox colony fidelity and movements.
Methods used

Models will combine modern methods of statistical analysis, including hierarchical Bayesian approaches and generalised mixed models, together with process-based models using network modelling approaches.

The models will be based on analysis of data on flying fox colony size through time, coupled with information on dynamics of prevalence of infection at a colony level and flying fox movements. A range of scenarios will be modelled to evaluate the possible effects of dispersal of flying fox colonies on Hendra spillover risk, comparing the possible consequences of dispersal under two competing scenarios: excretion driven by pulses of prevalence in Hendra virus in flying foxes, and recurrent pulses of excretion from persistently infected flying foxes.

The initial phases of the project used models to synthesise the existing information and to identify information gaps, in particular demographic and epidemiological parameters to which Hendra virus dynamics in flying fox populations are particularly sensitive.

As the project progresses, models incorporating the critically important biological features will be built using information collected following the initial modelling phase.

Outcomes

The spatial model of colony dynamics, informed by climate and remote sensing vegetation data, has now been extended to southeast Queensland using colony counts from the National Flying Fox Monitoring Program.

Researchers are analysing time series data of Queensland virus prevalence, focusing on an exhaustively-collected data set from Boonah. They have found that Hendra virus is mostly associated with black and spectacled flying foxes, and that an increase in density resulting from influxes of little red flying foxes reduces the capacity to detect Hendra virus.

Statistically significant pulses have been identified in the Boonah data, however the models suggest that these pulses do not recur in a periodic fashion. Results suggest that monthly sampling allows correct detection of all pulses in the time series 85 per cent of the time. Overall, results suggest that future survey designs will benefit from conducting simple simulations of sampling strategies.

As well as the Queensland Hendra virus transmission data, researchers on the project have gained access to the New South Wales transmission data and this will be used to further extend and test models in 2016.
Hendra virus infection
and transmission dynamics

This study sought to identify the key factors associated with infection and transmission of Hendra virus in flying foxes in Queensland and New South Wales, including biological variables associated with an increase in the virus in flying foxes.

It also examined the interactions and behaviours of flying foxes, horses and humans that increase the risk of Hendra virus transmission.

The insights gained from the study allow risk management strategies for Hendra virus to be refined and targeted, and highlight the need for a management strategy at a regional or larger scale and independent of state borders.

Background

Prior to 2011, 13 of the 14 known Hendra virus spillovers had occurred in Queensland.

The cluster of equine cases in NSW in 2011 demonstrated that Hendra virus was not a Queensland phenomenon, and highlighted the need for an acceleration of the research and expansion into NSW.

While the subsequent development and availability of a vaccine for horses offers effective risk mitigation if utilised, an understanding of the ecology of the virus in its natural host remains an essential part of an informed risk management strategy.

Methods used

The project incorporated epidemiological, ecological and social science components. The core component was a three-year observational study that monitored Hendra virus excretion in 50 flying fox roosts over 2300km and 20° of latitude from Cairns in northern Queensland to Batemans Bay in southern NSW, which spanned the locations of all known equine Hendra virus incidents, and yielded nearly 15,000 pooled urine samples and 3000 individual animal samples.

The study looked at Hendra virus infection and excretion in flying foxes over time, including

- an individual animal capture study examining routes of excretion in flying foxes, and molecular and serological patterns of infection
- retrospective studies of archived samples examining virus diversity and tissue tropism – the cells and tissues of a host which support growth of the virus
- modelling studies investigating recrudescence – the ability of dormant virus to reactivate in the flying fox – and the effect of temperature on virus survival in the environment
- attitudinal studies of horse owners and communities;
- telemetry – satellite tracking of flying foxes – and roost composition studies
- landscape utilisation studies of flying foxes and horses

Outcomes

Hendra virus was predominantly detected in the black flying fox (Pteropus alecto) and the closely related spectacled flying fox (P. conspicillatus). There was minimal or no detection in the grey headed flying fox (P. poliocephalus) and the little red flying fox (P. scapulatus).

Virus was most frequently detected in urine, less so in blood and faeces, and minimally in saliva and nasal discharge. Spleen and kidney were the tissues most likely to yield virus. Numerous diverse and previously unknown paramyxoviruses were detected, but no new henipaviruses.

Geographically, the prevalence of Hendra virus was highest in flying foxes in southern Queensland/northern NSW. Hendra virus was detected in all months of the year, but there was a marked peak in detection prevalence in winter in southern Queensland and central and northern NSW.

Satellite tracking studies illustrated the mobility of flying foxes and the connectivity of their roosts, with individuals
and groups constantly coming and going. Little red and grey headed flying foxes regularly moved many hundreds and sometimes thousands of kilometres, while the scale of black flying fox movements was more limited to a regional level.

Flying fox foraging was repetitious, with a preference for non-native plant species that results in increased activity around rural infrastructure. Horses used different areas of the paddock night and day, which combined with the observed flying fox foraging behaviour, could contribute to the risk of Hendra virus exposure. Further investigation is warranted.

A survey of horse owners showed that Hendra virus awareness and risk perception varied, suggesting a need for more effective or targeted communication strategies. Uptake of recommended risk minimisation strategies often lagged, even when these were readily implementable. Frustration was expressed that the recommended strategies were impractical, onerous and prohibitively expensive, and many doubted their effectiveness, posing a major barrier to adoption.

A follow-up interview study targeting owners of unvaccinated horses in known Hendra virus areas showed that perceived vaccine safety, cost and doubts over the effectiveness were the major reasons they did not vaccinate. Immediate threat of infection, a reduction in vaccination costs, and the advice of veterinarians were most likely to result in owners vaccinating and adopting other recommended strategies. Veterinarians were identified as critical mediators of horse owner behaviour and disease perceptions.

A community survey on urban flying fox management showed that only a minority of community members were directly impacted by urban roosts. Dispersal and culling were not seen as effective because of the nomadic nature of flying foxes. The mistaken belief by nearly a quarter of respondents that flying foxes pose a direct Hendra virus infection risk to humans indicates the need for additional communication strategies.

Simulation modelling indicated that

- Hendra virus could be maintained in isolated flying fox populations via periodic recurrence of dormant infection, as well as by the immigration of infected individuals.
- Hendra virus survival in the environment varied with latitude and season, and the effect of ambient temperature on survival could explain both the winter cluster of equine cases and sporadic cases at other times of year.
- The mobile nature of flying foxes underlines the need for a management strategy independent of state borders. The identification of two species as key reservoirs of Hendra virus allows additional risk communication to be strategically targeted within the geographic range of these species.

Understanding the factors driving the southern expansion of the black flying fox in NSW is also important in determining how risk profiles might change in eastern Australia in the future. As the range of the black flying fox includes the Northern Territory and parts of Western Australia, the potential for cases to occur in these regions should be appreciated, and the connectivity with eastern populations should be a focus of future study.

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**FURTHER INFORMATION**
Spatiotemporal Aspects of Hendra Virus Infection in Pteropid Bats (Flying Foxes) in Eastern Australia http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0144055


Flying Fox Species Density – A Spatial Risk Factor for Hendra Virus Infection in Horses in Eastern Australia http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0099965
Spikes in Hendra virus spillover: early warning through the bat urinary metabolome

The main objective of this project was to investigate virus dynamics in flying foxes to determine the prevalence of viral co-infections during Hendra virus spillover events. It found that peak periods of Hendra virus spillover from flying foxes are associated with a peak in other viral infections.

A secondary objective was to examine urinary metabolic profiles for flying foxes when they experience conditions that increase the replication of Hendra virus, such as nutritional stress, movement stress, pregnancy, birth or lactation.

This is believed to be the first metabolomics analysis performed on flying fox samples, with the aim of identifying biomarkers that could indicate periods of increased Hendra virus risk.

**Background**

Current monitoring of flying fox populations for Hendra virus focuses on detecting changes in Hendra virus viral ribonucleic acid (RNA) in their urine. However, by the time increased Hendra virus viral RNA is detected, the risk of exposure of susceptible species is already present. The prevalence of Hendra virus in pooled urine collected from flying fox colonies varies considerably over time; until recently, the prevalence in colonies near spillover events has remained below 5 per cent. In the 2011 spillover, the prevalence of the virus in urine samples collected from colonies spatially linked with Hendra virus outbreaks ranged from 3 per cent to 67 per cent at one location over two months. Preliminary studies have also provided evidence for an increase in the levels and number of other viruses coinciding with periods of high Hendra virus prevalence.
These results are consistent with the possibility that the increase in Hendra virus in flying fox populations is not simply due to the introduction of Hendra virus into a naïve population, but due to more complex events which lead to increased replication of a large number of viruses. It is also possible that increased viral co-infections predispose flying foxes to Hendra virus infection/replication. This project aimed to provide data that would assist in recognising periods of increased risk of Hendra virus replication in flying foxes, and inform wide-ranging practices involving horses and other animals at risk of infection. It is possible that Hendra virus is only one of many viruses that undergo a spike in prevalence, and flying foxes may be shedding a cocktail of viruses in both urine and faeces.

Methods used

The methodology for the virus identification and discovery part of the project included standard virus isolation techniques, polymerase chain reaction (PCR) and luminex technologies. Viruses were detected using both real-time and conventional PCR as well as next generation sequencing.

Luminex bead based multiplex PCR assays were also used to enable rapid and efficient screening of large numbers of samples for a variety of virus targets.

Mass spectrometry techniques were used to provide a broad overview of the serum and urinary metabolome of experimentally infected and uninfected flying foxes.

Urine and serum collected from captive female flying foxes experimentally infected with the paramyxovirus, Menangle virus, were used for a proof of principle study to examine the metabolic profile of infected and uninfected flying foxes.

Outcomes

Scientists found that flying fox colonies with a high Hendra virus prevalence contain a correspondingly high prevalence of other viruses, including paramyxoviruses from the genera henipavirus and rubulavirus. Analysis of urine collected from flying foxes during the 2011 Hendra virus spillover events has resulted in the isolation of more than 40 viruses, including a large number of new viruses which are yet to be classified. There was also a seasonal trend in the presence of viruses, indicating that environmental triggers may be associated with spillover events.

A clear difference in the metabolic profiles of Menangle virus-infected flying foxes compared to uninfected controls was detected in both urine and serum, supporting the use of metabolomics for the identification of biomarkers associated with viral infection in flying foxes. The metabolic profiles of pooled urine samples collected from the cages of uninfected flying foxes were tested to provide information on the profile expected from a mixed population consisting of both males and females. The metabolic profiles detected in the pooled uninfected samples were similar to those of samples collected from individual uninfected flying foxes. This analysis supports the feasibility of using pooled urine samples collected from wild flying fox colonies.

The metabolomics component of the work is now ready to be validated using field samples from wild colonies.

Separate to this project, a handheld device for use in the field is currently under development and could be used to detect metabolic biomarkers associated with increased viral replication.
Understanding of the mechanisms driving the emergence and transmission of Hendra virus to spillover hosts has been slow to develop, due to biosecurity restrictions and limited field data.

This project used modelling to quantify the environmental survival of Hendra virus and found that the length of virus survival did not influence the pattern of spillover events from flying foxes to horses.

It assessed whether survival could provide insight into the probable pathways of transmission, and concluded that transmission was likely to involve relatively direct contact of horses with flying fox excreta shortly after excretion.

However, it did not completely rule out indirect exposure through contaminated feed during some of the spillover events, especially where predicted 24-hour virus survival and moisture were high.

Background

The ability of a pathogen to survive outside its hosts can be indicative of the possible transmission pathways between hosts. For instance, influenza viruses may survive up to 2 months in water, and therefore can be transmitted by direct and indirect routes, while non-vector-transmitted pathogens such as human immunodeficiency virus (survival up to one week on smooth surfaces) are usually transmitted directly.

The clustering of flying foxes in tree canopies and subsequent exposure of many individuals to a mist of aerosolised urine might favour transmission routes that do not require long environmental survival.

Therefore transmission routes to horses may be similar to some of the transmission routes between flying foxes. If the virus survival is very short, exposure to flying fox excretions (urine, or birth/foetal fluids) shortly after excretion may be necessary for transmission.

Methods used

The project developed two models based on the Weibull and exponential cumulative distribution functions (CDFs). To run simulations, temperature profiles were generated to represent temperature changes between
MODELS THAT PREDICT RISK FOR HENDRA VIRUS TRANSMISSION FROM FLYING FOX TO HORSES

day and night. The profiles accounted for the variability and extremes observed at meteorological stations over the previous 20 years in order to emulate real-world conditions.

Simulations represented the survival over a period of at least 24 hours of an excreted viral population while temperature was the minimum. This method allowed researchers to assess the likelihood of virus accumulation in the environment on successive days.

Virus survival data used to fit the models was collected in experiments measuring Hendra virus survival, performed in the Australian Animal Health Laboratory under BSL4 conditions with the isolated Hendra virus strain in the index case. Survival of the virus was measured at three constant temperatures: 4, 22 and 56 degrees Celsius.

Environmental data used in simulations was downloaded from the Bureau of Meteorology and consisted of minimum and maximum daily air temperatures averaged from 1993 to 2012 from four locations where spillover events were recorded.

Researchers also generated a series of maps of the potential survival of Hendra virus across the geographical distribution of the black flying fox, which is the only flying fox species with a distribution covering all spillover sites and consistently found closer to spillover events.

The maps of Hendra virus survival were used to test whether survival was a good predictor of the spatial location of spillover in each season. Data for these simulations were obtained from the microclim dataset for soil temperatures and the Bureau of Meteorology for air temperatures.

Outcomes

The project concluded that the timing and geographical distribution of Hendra virus spillover events cannot be explained by virus survival in the environment, as they occurred when the suitability of temperatures for survival was intermediate to very low.

Given that urine is probably the most important route of Hendra virus excretion, this suggests that direct exposure of horses to infectious flying fox urine may be an important transmission pathway.

Behavioural studies of the interactions between flying foxes and horses would also assist in determining the route of transmission.

The winter-dominant seasonal pattern of Hendra virus transmission to horses in southern Queensland and northern New South Wales is likely driven by an additional seasonal factor (not virus survival).

For instance, flying foxes may spend more time feeding in agricultural landscapes (e.g. horse paddocks) in winter in the subtropics due to loss of winter feeding habitat, increasing the opportunities for horse exposure.

Present and past temperatures also affect the flowering status of many native Eucalyptus species, which then influences the distribution and abundance of flying foxes that feed on them. Temperature also influences pasture quality and the feeding behaviour of horses.

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FURTHER INFORMATION


LF Skerratt et al. The equine Hendra virus vaccine remains a highly effective preventative measure against infection in horses and humans: 'The imperative to develop a human vaccine for the Hendra virus in Australia'. http://www.infectionecologyandepidemiology.net/index.php/iee/article/view/31658
MicroRNAs are small ribonucleic acid molecules that are being adopted as biomarkers for the early detection of several diseases, including cancer and Alzheimer’s disease. Previous research has shown that levels of one particular microRNA are elevated in the blood of horses experimentally infected with Hendra virus several days prior to virus detection in blood. While this result suggests that microRNA profiling may have potential to aid early diagnosis of Hendra virus infection in horses, the present study developed methods to measure the expression levels of all detectable microRNAs in horse blood.

It found that the expression levels of many microRNAs change within 24 hours of a horse being infected with Hendra virus, despite the horse showing no outward signs of the virus. Measuring these molecules would be relevant for field testing in both the early and latter stages of infection, but more work must be done to develop tests to rapidly streamline the detection process.
Background

Early and rapid diagnosis of infection in horses is important for controlling the spread of Hendra virus. Horses infected with Hendra virus may not display signs of the disease, such as elevated heart rate and temperature, or detectable Hendra virus genome until the disease is full blown, which can be up to 16 days after exposure.

Host molecules known as microRNAs are produced during the early stages of virus infection. MicroRNAs can be detected from easily collected bodily fluids such as blood and urine using common lab techniques. Previously, researchers identified a single microRNA that was up-regulated in horses infected with Hendra virus. A single molecule is not specific enough to act alone as a diagnostic tool, so the purpose of this work was to assess a microRNA profile associated with Hendra virus in horses, and to find out whether this profile appears before other measures of infection.

Methods used

Blood samples from healthy horses and existing field samples of infected horses were tested, as well as horses infected with Hendra virus under strict controls at the Australian Animal Health Laboratory. About 600 microRNAs were measured and researchers found significant changes in levels in a subset of microRNAs, including from samples taken in the very early stages of controlled infection. Some changes occurred within 24 hours of infection, when horses were showing absolutely no sign of illness.

Outcomes

This research shows clear differences in microRNA responses between healthy horses and horses infected with Hendra virus, meaning microRNA profiling could be used to assess early and late stage infection status in horses.

There is also scope for future work in developing a test for the detection of select microRNAs, and analysing the microRNA profiles of horses infected with other viruses.
Assessing the risks posed by Hendra virus antibody positive animals

This project assessed the likelihood that Hendra virus could persist in animals that had clinically recovered from the disease, and the subsequent risk of re-infection and transmission to other animals or humans at a later stage.

Ferrets were experimentally infected then treated with monoclonal antibody and monitored for up to four weeks during convalescence for virus replication and shedding. Mice were infected with Hendra virus and assessed for longer term virus persistence in the central nervous system, recurrence of disease in the form of encephalitis and the likelihood of production of infectious virus particles at that time.

There was no evidence that Hendra virus persists in convalescent animals in a form that could lead to production of infectious virus at a later time.

Background

Hendra virus induces an overwhelming infection with high mortality rate in naturally infected horses, as well as various species under experimental conditions (horses, cats, ferrets). Consequently, there are few opportunities in the field to assess the duration of viral replication and shedding as a host recovers.

In humans, Hendra virus disease may return after a long period of apparent recovery, although there is no evidence that affected patients are infectious to others. Ferrets respond to Hendra virus infection in a similar way to horses, and have been used to model both the equine and the human disease. In addition, ferrets can recover from the virus after being treated with a single dose of the human monoclonal antibody directed against the Hendra virus G glycoprotein – the protein that allows the virus to attach itself to a host cell – thereby providing a source of animals in which the virus replicates, leading to milder or even subclinical disease, after which they convalesce.

Mice are the only species of animal that can feasibly be held over a lifespan under the BSL4 containment conditions required for Hendra virus (BSL4 being the highest level of biosafety requirements). They provide a unique opportunity for research into longer term persistence of the virus in the central nervous system, recurrence in the form of encephalitis, and the likelihood of production of infectious virus particles at that time.
Methods used

Under BSL4 containment conditions, 16 ferrets were exposed to a recent isolate of Hendra virus (Redlands 2008) via the mouth and nose. Twelve hours later, ferrets were re-anaesthetised and human monoclonal neutralising antibody was administered to them. Afterwards, daily clinical observations were made and play activity scores were recorded for each animal with collection of blood, nasal washes, oral and rectal swabs for assessment of virus load.

Four animals were euthanised on each of days 22, 24, 29 and 35 but animals reaching a pre-determined progression of disease during the study period were humanely euthanised at that time. Post mortem examinations were carried out. A wide range of fluid and tissue samples were collected and tested for viral genome by polymerase chain reaction (PCR) and virus isolation or examined by histology and immunohistochemistry. Based on the outcome of these tests, whole genome sequencing (Illumina Next Gen sequencing) was attempted on selected tissues.

Ninety-six clinically healthy female BalbC mice including 67 mice aged eight weeks and 29 mice aged 12 months were used in the second component of this study. Four days prior to exposure to Hendra virus, mice were anaesthetised and baseline blood samples collected. LifeChip Bio-Thermo® implantable microchips were also placed under the interscapular skin. Under BSL4 containment conditions, the mice were exposed to a recent isolate of Hendra virus (Redlands 2008) via nose drops. Daily clinical observations were made as well as thrice weekly records of temperature and body weight. Mice were randomly allocated for elective euthanasia on post-exposure days 7, 14, 21, 28, 35, 42, 49, and 169, and post mortem examinations carried out.

Outcomes

Hendra virus infection was established in 16 ferrets, and convalescence was induced in 14 of these by administration of monoclonal antibody 12 hours after exposure to virus. Clearance of live virus was associated with the onset of the adaptive immune response and generation of antibody to the Hendra virus G protein. In samples of brain tissue collected from convalescent ferrets up to seven weeks after viral challenge the Hendra virus genome coverage was poor and incomplete. This was in contrast to the outcome of sequencing spleen tissue from one of the two ferrets which had succumbed to acute infection, where sufficient reads were obtained to confirm the presence of the complete Hendra virus genome.

Chronic Hendra virus encephalitis was confirmed in 29 mice. The infection was subclinical in 26 mice, while three mice showed signs of neurological disease including two which exhibited late-onset encephalitis at 27 and 122 days post infection. Infectious virus was not recovered from any of these mice at post mortem examination. Moreover, there was no evidence of persistence of infectious virus in Hendra virus infected but recovered animals, or in those exhibiting late-onset or recrudescent CNS disease, in a form that might pose a risk for viral shedding and subsequent onward transmission of Hendra virus.

Overall, the study found no evidence for virus persistence in convalescent animals in a form that could lead to production of infectious virus at a later time.
This project aimed to develop new diagnostic tests to differentiate between horses infected with Hendra virus and horses vaccinated against it. None of these proved to be as specific or sensitive as the tests that are currently in use.

Several tests were developed which can be used if necessary, but they are not suitable for mass screening of horse samples. The Australian Animal Health Laboratory (AAHL) in Victoria will keep these as internal tests, with state laboratories forwarding samples for testing if necessary.
**Background**

At the time the project began, it was a legal requirement that any horse shown to be positive to the Hendra virus had to be euthanised, to prevent spreading of the virus. This situation changed when a vaccine was developed for Hendra virus, as a vaccinated horse produces antibodies to the virus which will be detected in routinely used serological tests.

The gold standard of Hendra virus detection is the ‘virus neutralisation test’, which identifies that an animal is carrying antibodies specific to the virus (but this does not distinguish horses exposed and horses vaccinated).

In this project different expression systems that produce proteins that identify antibodies to the Hendra virus were tested, but failed to give as strong a response as the current test.

**Methods used**

Researchers used serum collected over many years at AAHL from both field cases and experimental animal infections (horses and ferrets) to test for antibodies to Hendra virus and compare the results to the current test.

Each test provided evidence of Hendra virus infection. They were then assessed using a panel of field horses that had previously tested negative to Hendra virus.

Of more than 100 samples tested, approximately 15 per cent responded positively to at least one of the Hendra virus proteins, with some giving positive reaction with multiple proteins. This was considered to be due to cross-reacting antibodies to viruses closely related to Hendra, such as the Cedar virus, which is also found in Australian flying foxes.

**Outcomes**

It is recommended that the tests developed in this project are maintained at AAHL to be used if necessary, although this is not likely to be common. Following the release of the Hendra virus vaccine, policy is changing both within Australia and internationally to consider vaccinated horses to be protected from infection. Therefore if an owner can prove a horse has been vaccinated, there may be no need for serological testing.
This project aimed to identify the natural features of Hendra virus infection in dogs and to determine whether there is a risk of dogs, as well as horses, infecting humans with Hendra virus. The study concluded that there is the potential for transmission of Hendra virus to people from acutely infected dogs. Of concern is the finding that most infected trial dogs did not show any signs of illness at a time when they were shedding virus.

Background

Hendra virus causes a severe infection with a high mortality rate in several species of animal as well as in people, while other animals appear to be resistant. In nature, severe infection has been identified in horses and humans, and has also been induced under laboratory conditions in non-human primates, ferrets, guinea pigs, golden hamsters and cats. By contrast, experimental rats and chickens appear to be resistant.

Shortly after Hendra virus was discovered in 1994, a very limited amount of preliminary research was carried out on the susceptibility of laboratory dogs to the virus. In that study, one animal showed an immune response to the virus without any signs of illness and in the other there was no evidence of infection at all. No data was gathered on whether or not the dogs shed the virus in any of their secretions.

More recently, a dog which was resident on a property that was experiencing a Hendra virus outbreak in horses was shown to have had an immune response to the virus, consistent with it having been infected.

Considering the close relationship between dogs and people, the confirmation of natural infection in a dog raised the possibility that dogs as well as horses may pose a source of infection for people. A particular concern around the potential for dogs as infection sources is that they may not appear to be ill at a time when they are shedding virus, so there will be no indication that infection control procedures should be put in place.
Methods used

The project had two main objectives – to identify the natural features of Hendra virus infection in dogs, the areas of the body from which the virus was shed, the amount of shedding, and the number of days during which shedding occurred; and to determine which tissues and organs supported virus growth, and the timeframe for virus growth after the dogs were exposed to the virus.

All dogs were exposed to Hendra virus via the nose and mouth, as the most plausible routes of infection in the field. Biological samples including body fluids such as blood and saliva were tested for viral shedding over the course of infection. After the dogs were euthanised, tissues and organs were also tested for virus content and signs of disease.

Researchers also assessed whether the virus being shed from the mouth of dogs could transmit infection to ferrets, since ferrets are susceptible to infection and develop a similar Hendra virus-associated disease to people.

Outcomes

Most dogs did not show any clinical signs of infection. Mild disease signs developed in some dogs, such as loss of appetite for dry kibble for one or two days, although softer food was still eaten. Researchers concluded that it is unlikely a dog infected with Hendra virus would come to the attention of a veterinarian, and in some cases may not be noticed by even the most observant of owners.

The dogs developed an acute infection, followed by the development of neutralising antibody to the Hendra virus which was associated with clearance of the virus. This infection pattern is similar to that for the cat, horse, and ferret. There was no evidence of persistent infection.

Hendra virus genetic material was recovered from the mouth of dogs between two and 10 days after they had been exposed to infectious virus. The levels in oral fluids typically peaked on day four. Using tissue culture detection systems, Hendra virus was recovered from the mouth but not the nasal secretions of infected dogs on days two and four. Virus was not detected from rectal swabs or from urine; however, there was evidence of infection in the kidney. Accordingly, urine should not be ruled out as a potential source of Hendra virus in infected dogs in addition to oral fluids.

Canine oral fluids collected from infected dogs on days four and six were infectious for ferrets, while samples collected on days two and eight did not transmit infection to ferrets.

Hendra virus growth was confirmed in a limited range of tissues in the dogs, including the tonsil, lung and associated lymphoid nodes, kidney and spleen. There was no evidence of virus, viral genome, or virally-induced lesions in the brain. Given these results, there is the potential for transmission of Hendra virus to people from acutely infected dogs. Under the exposure conditions in this study, the window for potential transmission extended at least from days two to six after exposure of dogs to Hendra virus.

It should be noted that so far no human infection with Hendra virus has been attributed to contact with an infected dog. The prevalence of field infection in the Australian canine population is also unknown. The significance of field infection of dogs with Hendra virus has yet to be fully clarified.

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This project investigated whether the disturbance of flying fox roosts leads to an increase in stress levels and Hendra virus infection and excretion in dispersing animals, which might potentially increase the risk of spillover of the virus to horses.

It found no evidence that roost disturbance results in increased Hendra virus excretion by flying foxes. This finding does not change the ‘background’ risk of horses being exposed to the virus, and the need to manage that risk.

Background

Flying foxes are the natural host of Hendra virus which periodically causes fatal disease in horses and humans in Australia. Flying foxes are increasingly found in urban areas where people sometimes react negatively to them, prompting calls for the dispersal of roosts.

It has been suggested that disturbance of roosts might result in an increase in stress and subsequent Hendra virus excretion by flying foxes, potentially causing an increase in Hendra virus transmission and infection if the animals move to another established camp.

Methods used

Researchers targeted flying fox colonies that had been approved for dispersal under state legislation in Queensland and New South Wales, and measured pre and post-disturbance stress levels, pre and post-disturbance Hendra virus levels, and tracked the movement of dispersed flying foxes.

Urine samples were collected under the roosts. Urinary cortisol levels were used as a measure of stress and urinary Hendra virus genetic material as a measure of virus excretion. Movement of flying foxes after dispersal was monitored visually and through satellite tracking.
Outcomes

Researchers found no association between disturbance of the roosts and Hendra virus excretion, indicating that roost dispersal does not cause increased Hendra virus infection and excretion in dispersing flying foxes.

They found no association between roost disturbance and concentration of the stress hormone cortisol, but found an underlying association between cortisol concentration, season and region, suggesting that other factors – possibly biological or environmental – play a role in determining levels of cortisol in flying foxes.

Researchers detected an apparent link between the presence of Hendra virus in the urine and the concentration of cortisol in the urine, but finding a cause was beyond the scope of the study.

The study highlighted the need for a ‘best practice’ approach to dispersal of flying fox roosts, as the nature or timing of the activity had a clear impact on the level of behavioural distress exhibited by the animals. While flying foxes have some capacity to escape roost disturbance, their increasing urban presence may make them the target of ongoing harassment, with unknown consequences.

The findings are also potentially relevant to Nipah virus, a virus closely related to Hendra that is responsible for regular outbreaks in Bangladesh and India, and more broadly will be of interest in parallel scenarios, where there is an intersection of urban wildlife, humans and emerging zoonoses (diseases which can be transmitted from animals to humans).
Previous research found that a prototype vaccine could prevent Hendra virus in horses, and this project provided the additional data required by regulatory authorities to register the vaccine for routine use.

The study confirmed that the commercial formulation of the vaccine stimulated equivalent immune responses in ferrets in the lab and horses in the lab and in the field, and that horses were protected from Hendra virus for at least six months after vaccination.

**Background**

An effective Hendra virus vaccine for horses can both protect them from disease and reduce the possibility of their transmitting the virus to people. Testing of a promising prototype at the Australian Animal Health Laboratory confirmed that Hendra virus disease in horses could be prevented by vaccination and that viral shedding could be eliminated in immunised horses.

However additional data was required using the commercial formulation of the prototype vaccine, to support licensing of the vaccine for routine use. Some of this data had to be derived from the target species (horse) while other data was able to be acquired from laboratory animal studies.

**Methods used**

Researchers tested the prototype on five groups of four ferrets and collected blood samples for antibody analysis. Five horses received two doses of vaccine and were exposed to Hendra virus at different intervals, with clinical observations recorded twice daily.

**Outcomes**

Vaccination of horses against Hendra virus was proven to be a direct and straightforward means by which the horse owning community, and those involved in horse health, can protect their animals and themselves from the impacts of this serious zoonotic infection.
ADDITIONAL RESEARCH REQUIRED IN RELATION TO THE DEVELOPMENT OF THE HORSE HENDRA VIRUS VACCINE

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FURTHER INFORMATION
Longitudinal cohort study of horse owners

This study used surveys and interviews to assess how horse owners perceive the risk of Hendra virus, their uptake of risk mitigation practices such as the Hendra virus vaccine, and their engagement with government and industry stakeholders such as veterinarians.

After initial increases in vaccination, it revealed increasing intentions to withdraw from the booster program and delay boosters; poor uptake of recommended property management practices; and a decreasing interest in Hendra virus generally.

The findings identify challenges for future engagement between horse owners and authorities, and for the valuable relationship between horse owners and veterinarians.

**Background**

A sudden upsurge in cases of Hendra virus in 2011 triggered a public outcry, media frenzy, and a political response. Funding was made available through the National Hendra Virus Research Program to prioritise and fast-track Hendra virus research.

Minimising horse exposure to flying foxes and their excretions remains a fundamental risk management strategy for the virus, and understanding the uptake of recommended practices over time is important for those involved in horse owner communication and engagement.

**Methods used**

This study involved 1149 horse owners, who shared their views on related issues such as uptake of the Hendra vaccine, compulsory vaccination, property management to minimise risk of the virus, perceived risk of Hendra virus and engagement with stakeholders.

The study was conducted in two phases: a series of five online surveys conducted at six-monthly intervals, and an in-depth interview, with both phases involving horse owners or those who care for or manage horses. Participants were from all industry sectors and all Australian states and territories.
Researchers assessed the relationships between Hendra virus risk mitigation practices and a number of other factors, such as risk perception and awareness, knowledge, demographics, and types of horse involvement/ownership.

The interviews complemented and expanded on these issues.

Outcomes

Perceived likelihood of Hendra virus infection did not change over time, but there was a marked decline in concern about horses and self/family becoming infected by the virus over the course of the study. This was interpreted as positive, indicating lower anxiety and a more measured response to the low risk of infection.

However, the implications of this finding include lower vaccine uptake rates over time, low uptake of property management practices recommended to reduce Hendra virus risk, decreased interest in Hendra virus as an issue generally and therefore challenges for future engagement. It is recommended that a more nuanced and balanced dialogue is adopted by stakeholders, as horse owners are disengaging from stakeholders who ‘talk up the risk’, viewing them with scepticism.

The uptake of property management practices such as reducing horses’ access to areas under trees and covering food and water was generally low, around 20 per cent, because owners regarded such precautions as too hard, too expensive, too impractical, and/or ineffective. This means a large proportion of horses and their owners are exposed to a greater risk of Hendra virus transmission, especially when overall uptake of vaccination is also low. Recommendations include promoting uptake of property management practices through specifically addressing these concerns.

Uptake of the Hendra virus vaccine was much higher in the HHALTER study sample, which due to its concentration in Queensland and New South Wales, was probably more at risk, more engaged, and more pro-vaccination. However, vaccination levels have peaked and owners are reporting delays and withdrawal from the recommended vaccination regime.

Horse owners urgently need to be reassured about the safety and efficacy of the vaccine, approval for less frequent boosters is needed to meet owner expectations, and the pursuit of mandatory vaccination in the current climate (lower perceived Hendra virus risk) is counter-productive and is driving actions to undermine the vaccine.

There is also evidence of increasing tensions in the veterinarian-horse owner relationship caused by lack of uptake of the vaccine, mis-communication, and more recently changes in policy in which some veterinarian practices are refusing to treat unvaccinated horses.

There is an urgent need to protect and preserve the veterinarian-horse owner relationship, through encouraging an open and collaborative approach to decision-making to mitigate the risk of Hendra virus; creating a safe context for debate in this area at a broad/national level; and raising the profile of government veterinarians as independent voices in Hendra virus engagement and communication with horse owners.
The replicative cycle of henipaviruses, the group that Hendra virus belongs to, is poorly understood in molecular and structural terms. This project aimed to improve molecular understanding of the way in which henipaviruses reproduce or copy themselves, to provide scientists with laboratory tools for functional assays and antibody generations that are in scarce supply in Australia.

Structured in two parts, the project has established new research on Hendra virus replication machinery; characterised a potential drug target to block replication; and is working towards providing a molecular ‘movie’ of the replicative machinery of the virus, based on snapshots of the key proteins N, P and L working in concert.
**Background**

The discovery of other potentially lethal viruses closely related to Hendra virus worldwide and in Australia highlights how little we know about the pathogenic and epidemic potentials of paramyxoviruses, the family to which henipaviruses belong.

Molecular models are crucially lacking, not only for the replicative process of Hendra virus but also those of most non-segmented, negative-strand RNA viruses, despite concerted efforts worldwide.

While there is knowledge of the entry process of the virus into cells, where crystal structures of the receptor-binding and fusion proteins can be used to guide vaccine and antiviral development, there is limited understanding of the molecular and structural facets of the replication process of henipaviruses.

The ultimate goal is to determine the structure of these crucial components of the replicative machinery, setting the basis for rational drug design and enabling scientists to identify the molecular determinants of virulence.

**Methods used**

Scientists aimed to produce recombinant proteins for the L, N and P proteins, to provide a pipeline allowing the rapid test of multiple constructs for their biochemical properties and ability to crystallise. The final procedure will be structure determination of available crystals.

The project used a combination of advanced structural biology techniques including X-ray crystallography and cryoEM within the Australian Synchrotron facility and the newly established Ramaciotti Centre for Structural Cryo-Electron Microscopy at Monash University.

**Outcomes**

This project comprised two research components. The first built capacity to be able to perform the subsequent structural studies of the second, established a protein production pipeline, and made a biophysical characterisation of the protein that coats the viral genome (including electron microscopy).

With the second component of the project due for completion in late 2017, scientists have established new research on the structural analysis of Hendra virus replication machinery.

They have determined the structure of a precursor of the ribonucleoprotein (i.e. the genome coated by the N protein), providing insights into the assembly of the virus. This represents a potential drug target to block replication.

Work is proceeding towards the ultimate goal of providing a molecular movie of the replicative machinery of the virus, based on high-resolution models of the proteins N, P and L working in concert.
Viruses cannot cause infection without ‘hijacking’ elements from the host cell. This project investigated which host pathways or molecules Hendra virus relies upon, to enable it to infect and make copies of itself.

The study found that Hendra virus infection causes host cells to up-regulate levels of a particular host molecule, the microRNA miR-146a, which actually promotes the infection. By studying how miR-146a promotes Hendra virus infection, scientists found that virus replication is actually boosted by the triggering of an altered antiviral immune response in the host.

They also discovered that Hendra virus, in addition to multiple other viruses from the same family (Nipah, measles, mumps) is critically reliant on a host protein called fibrillarin for infection. Inhibiting fibrillarin blocked Hendra virus infection in human cells by more than 1000-fold. This study not only increased understanding of the Hendra virus infection cycle in human cells, but identified candidates for new antiviral therapies.
**Background**

Viruses rely upon host pathways in order to infect a cell and make more copies of themselves. Knowing which pathways are used by viruses for replication can inform the development of novel antivirals and increase knowledge of how viruses cause disease. While host pathways required for some virus infection cycles are known, such as influenza virus and HIV-1, such information has not been available for Hendra virus, perhaps due to the difficulty performing such large scale experiments at biosafety level 4.

**Methods used**

Over two years researchers performed a genome-wide screen, where every gene from the human genome was knocked down or removed one by one within a cell, then the cell infected with Hendra virus to determine whether the virus flourished or waned.

It revealed that Hendra virus makes use of a microRNA, miR-146a, to replicate itself and that blocking miR-146a reduces Hendra virus replication.

Researchers discovered one mechanism that allows this to occur – the targeting of a host gene by miR-146a – and set out the host pathways responsible for triggering miR-146a production during Hendra virus infection.

**Outcomes**

This represents the first genome-wide screen identifying host factors required for infection by the Hendra virus. Future projects will investigate other host microRNA responses and the host factors associated with Hendra virus infection, based on knowledge from the genome-wide screen.

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**FURTHER INFORMATION**
Promotion of Hendra Virus Replication by microRNA 146a [http://jvi.asm.org/content/87/7/3782.full](http://jvi.asm.org/content/87/7/3782.full)

The urgent need for therapeutics for the growing number of henipaviruses, such as Hendra and Nipah virus, prompted this research into how Hendra virus interacts with its host on a molecular level, compared to its close, non-pathogenic relative, the Cedar virus.

Protein interactions were identified that may provide attractive drug targets to interfere with the assembly of the virus and gene regulation in the host cell.

This knowledge is also important for filoviruses such as Ebola virus, as they are distantly related to henipaviruses.
**Background**

The henipaviruses Hendra virus in Australia and Nipah virus in Southeast Asia are indigenous to flying foxes and cause zoonotic disease outbreaks, where the virus is spread from animals to humans.

To find antidotes for these viruses it is essential to understand the host-virus interactions, particularly the cellular pathways used by the virus to switch off the host’s immune system and replicate itself.

This project made comparative studies between Hendra virus and the related Cedar virus, which is also carried by flying foxes but does not seem to be harmful to humans.

**Methods used**

Studies revealed that the ability of Cedar virus P protein to counteract the antiviral or immune response in the host is compromised, compared to the Hendra virus P protein.

A key strategy of Hendra virus infections appears to be the blocking of the signalling pathway that alerts the antiviral response in cells – in much the same way as a blocked phone line prevents messages from getting through – but this is not as pronounced in the Cedar virus.

To shed more light on the process of viral morphogenesis, researchers investigated the cellular pathways of matrix (M) proteins. The M proteins are essential organisers of the viral assembly process.

The project demonstrated that the M proteins from pathogenic and non-pathogenic henipaviruses travel through the cell nucleus, to finally engage the cell membrane to facilitate viral assembly.

Cellular factors that assist the trafficking of the Hendra virus M protein were identified, and will provide an in-depth understanding of the life cycle of the emerging viral group.

**Outcomes**

The interactions between host factors and the viral M protein could provide targets for therapeutic drugs to interfere with the life cycle of the virus at the level of virus assembly, or at the level where it interferes with host gene expression.
Using a newly established mouse model, this project aimed to better understand Hendra virus-specific immune responses following infection.

Preliminary indications are that researchers have been able to modify this largely encephalitic model to now show signs of virus dissemination to other mouse organs. This is the first step in developing a fulminating murine model of disease observed in some humans and horses.

The objective is to better understand the biology of henipaviruses and the type of immune response they elicit in infected hosts.
Background

Hendra virus is a highly virulent virus, with around 60 per cent mortality in humans, and has developed immune evasion mechanisms. Seven humans in total have had clinically confirmed Hendra virus infection. Of the four fatalities, one was attributed to respiratory illness and the remaining three to encephalitis. Acute and relapsing encephalitis represent the two most life-threatening complications of Hendra virus infection. The need to better understand host-pathogen interactions is urgent, given continued urban expansion and the ever present risk to humans and their companion animals. Hendra virus requires specialised facilities for its growth and handling (including animal infection studies). This project employed a newly-established mouse model and utilised unique, state of the art infrastructure to investigate Hendra virus infection.

Methods used

Experiments were performed in a new mouse model of Hendra virus infection in the recently opened National Collaborative Research Infrastructure Strategy BSL4 facility at CSIRO’s Australian Animal Health Laboratory, augmented by PC3/4 flow cytometry capabilities. Researchers aimed to identify innate and adaptive immune checkpoints and pathways involved in controlling Hendra virus infection, through the use of knockout mouse lines. Multiple strains of mice were infected intranasally with Hendra virus (Redlands 2008), monitored daily and then euthanised, after which tissue samples and organs were processed for immunological analysis.

Outcomes

It is anticipated that the results of this research will enable scientists to better understand host immune responses involved in pathogenesis and therefore contribute to future vaccine design or therapeutic intervention. Moreover these studies have provided the first step in developing a fulminating murine (pertaining to mice or rodents) model of disease observed in some humans and horses.
Can the bat immune response to Hendra virus inform drug and vaccine development in other species?

The aim of this project was to examine the immune response to Hendra virus in its natural host, the flying fox, which rarely shows clinical signs of the disease, compared to the response of susceptible hosts, in which Hendra virus causes severe disease and death (humans and horses).

The project has made the first thorough characterisation of the flying fox’s adaptive immune response to the virus. Experience with Hendra virus has led to the development of a technology platform that will allow a rapid response to other zoonotic viruses that may spill over to humans and livestock in the future.

**Background**

Flying foxes are a major reservoir of emerging and re-emerging infectious diseases, including SARS-like coronaviruses, henipaviruses and Ebola virus. While highly pathogenic to their spillover hosts, flying foxes harbour these viruses and a large number of other viruses with little or no clinical signs of disease.

How they asymptptomatically co-exist with these viruses is unknown. In particular, little is known about flying fox adaptive immunity. This study has carefully probed both the innate and adaptive immune responses of flying foxes and provides fundamental information on these pathways and how they differ in susceptible organisms. This information is critical to understand pathogenesis and potentially will highlight new therapeutic avenues for these deadly viruses.

**Methods used**

In a major component of the study, scientists infected tissue from flying foxes and ferrets with the Hendra virus and using proteomics investigated the difference between cellular responses in the different hosts. The flying fox does not respond vigorously to the incursion by the virus, which is then shed leaving the
host relatively unaffected. In contrast, in ferrets and therefore inferred in humans, there is a very strong immune response which causes immunopathology and potential mortality.

The second component of the project focussed on understanding how the adaptive immune system works in flying foxes. This involved a detailed study of major histocompatibility complex (MHC) encoded molecules, a set of proteins that reside within the cell and capture fragments of the virus (peptides) to take to the surface and display to the immune system, to galvanise it into action.

**Outcomes**

This is the first study to characterise the peptide cargo of MHC molecules from a flying fox species. It demonstrated that flying foxes employ a similar strategy to capture these peptides as that described for humans and other mammals. This study provides fundamental insights into the adaptive immune system of an understudied but extremely important mammal, as well as a technology platform that expands the potential to quickly assess viruses that spill over into the human population or livestock, and enables the fast-tracking of therapeutic development and overall response. By furthering understanding of the antiviral responses of the flying fox, science may begin to grasp how these animals are able to control viral levels and avoid the onset and development of viral diseases that can spread to humans with often devastating consequences.

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**FURTHER INFORMATION**

Characterization of the Antigen Processing Machinery and Endogenous Peptide Presentation of a Bat MHC Class I Molecule http://www.jimmunol.org/content/early/2016/04/27/jimmunol.1502062.abstract
The broad aim of this research was to test a delivery system that could lead to a safe intranasal vaccine for Hendra virus, stopping the virus before it enters the body. Hendra virus is most likely transmitted from infected horses to humans by means of droplet exposure via the mouth and/or nose, and then replicates in the body.

The study used mannan, a sugar that can be isolated from yeast. This sugar was linked to proteins then injected into mice, where it stimulated an efficient response to kill the Hendra virus. This may lead to the development of a self-administered vaccine that humans, particularly those working with horses or after exposure to a Hendra virus outbreak, can apply via the mouth or nose.

Background

A Hendra virus vaccine is available for horses – delivered through intramuscular injection – and a human monoclonal antibody (mAb) shows great promise for increasing survival when administered soon after virus exposure. However, administration of mAb post-exposure does not significantly influence Hendra virus replication in infected ferrets and may not fully suppress the neurological effect of the disease.

Development of a mucosal vaccine would suppress Hendra virus replication in the upper respiratory tract directly after entry and reduce the risk of further infection. A vaccine that can be administered to humans, particularly those working with horses or post exposure to an outbreak, and that can be inhaled via the mouth or nose, would be an ideal addition to the regimen.

If mannan is chemically linked to proteins from infectious disease causing organisms and injected intranasally into mice, it stimulates an immune response to generate killer cells and antibodies that recognise the organism in mucosal sites, as well as in the blood.
Methods used

Synthetic G and F proteins – those proteins that Hendra virus uses to attach or bind onto a cell to infect it, and to fuse with the cell – were mixed or conjugated with mannan and injected intranasally into mice, to determine whether they could generate antibodies to block the proteins binding to target cells and hence the virus. Mice were injected three times every fortnight with a mix of the proteins and mannan, or the proteins and mannan conjugated (linked chemically), then two weeks later euthanised to assess the antibody response using ELISA assay, a common laboratory technique that measures the concentration of antibodies or antigens in solution.

Hendra virus-F conjugated with mannan generated mucosal antibodies in the lung whilst Hendra virus-F alone did not. When two rather than three immunisations are given, conjugates are better than the mixture. Mice that had only protein did not give a good response. Mucosal washings from the lungs found that proteins with mannan gave a mucosal response, but this was lacking in mice given only the protein.

The sera from mice immunised with the various compositions were tested to measure whether they could neutralise the ability of the virus to infect. Antibodies in sera from mice injected with mixtures of mannan with either Hendra virus-F or Hendra virus-G are more efficient than conjugates in virus neutralisation assays. Surprisingly, sera from mice immunised with mannan conjugates of Hendra virus-F were inactive in the virus neutralisation assay.

Outcomes

Conjugates or a mixture of mannan with Hendra virus-F or G antigens that was administered nasally in mice induced systemic and mucosal antibody responses that were G or F-specific; however, the mixtures of mannan with F-induced antibody were more effective in neutralising the virus. The mannan/antigen mixtures will have to be further optimised and eventually tested in larger animal models and compared to the current Hendra virus horse vaccine, before they can be considered for a potential human vaccine.
New Hendra virus treatments

The overall aim of this project was to develop an RNA-based therapy for the treatment of Hendra virus. Small interfering siRNA, sometimes known as silencing RNA, work to turn off genes to prevent the virus replicating. In layman’s terms, they enter a cell where the virus is replicating and bind to the virus sequence and turn it off, so that the cell cannot make the proteins it needs to replicate the virus, and dies. RNA is a useful, robust and safe technology that has been used in cancer research and for viral protection. A range of drugs using siRNA were developed for Hendra virus and 15 were tested, with the most effective treatment stopping the virus replicating by 98 per cent. The second half of the study aimed to test the drugs on animals as a prerequisite for use in humans.

This study was designed to develop a platform for the rapid development of antiviral therapies to newly arising infections such as Hendra, SARS and Zika virus. One therapy was found to reduce Hendra virus by up to 98 per cent, but a lack of appropriate animal models and limited capacity in high security facilities impacted on the success of the program.

However, this research has obvious public health benefits illustrating the potential benefit from continued research support from relevant funding agencies.
Methods used

The aims were reduced to testing short-interfering siRNA loaded stealth lipoplexes in cell and animal models of Hendra virus, investigating both pre and post-exposure therapies. Cell-based studies commenced in 2012 and demonstrated that siRNA-based targeting of the abundantly expressed viral genes N, P and M of Hendra virus resulted in over 95 per cent reduction of Hendra virus. Researchers also undertook biodistribution studies of their nanoparticles in the lung, showing delivery of the siRNA to the target cells Hendra virus infected. From this they chose to proceed with the P18 Hendra virus siRNA as the most effective therapy.

Animal studies were conducted at the Australian Animal Health Laboratory (AAHL) under PC4 conditions. Overall six studies were performed using the mouse BALB/c model developed at AAHL with the Redlands 09 Hendra virus strain and the P18 Hendra virus siRNA.

Study 1: Intranasal siRNA pre-treatment with 20µL dose
Study 2: Intravenous siRNA pre-treatment with 100µL dose
Study 3: Innocuity (Safety) Study
Study 4: Intravenous siRNA pre-treatment with 200µL dose
Study 5: Intravenous siRNA pre-treatment with 200µL dose
Study 6: Intravenous siRNA post-treatment with 200µL dose

Treatment was well tolerated and safe. Overall these studies indicated some possible therapeutic effect in the P18 Hendra virus siRNA treatment group; however, significant variations within the groups made it difficult to quantify the observations. Overall the mouse model proved to be highly variable making it impractical for testing of Hendra virus therapies. It will only be possible to determine this with a more robust and reproducible animal model of infection. The study concluded that P18 Hendra virus siRNA is highly effective in vitro, but the in vivo (live animal) therapeutic benefit of P18 Hendra virus siRNA treatment was not proven.

Outcomes

The project, while delayed due to PC4 laboratory availability and personnel recruitment in Geelong, provides proof that siRNA-based therapies have potential for the rapid development of antivirals. The in vitro protection of cells from viral infection was excellent. The weakness of the work was the use of the mouse model, which proved to be highly variable, even in control groups, such that valid conclusions could not be drawn. While the application had planned to use the more robust ferret model, there was insufficient funding for this to occur and the AAHL team felt that the mouse model would first have to be tried before the more resource-intensive ferret studies could be contemplated.

Overall there are grounds to believe that there is therapeutic potential to be gained from anti-Hendra virus P18 siRNA treatment. It will only be possible to determine this with a more robust and reproducible animal model of infection. It may also benefit from use of improved siRNA in vivo delivery systems for siRNA therapeutics developed in the last few years.

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FURTHER INFORMATION
Potent Inhibition of Hendra Virus Infection via RNA Interference and Poly I:C Immune Activation
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0064360
Development and testing of a monoclonal antibody

The purpose of this project is to have a supply of a safe monoclonal antibody readily available for the prevention and treatment of Hendra virus in humans.

The Hendra virus monoclonal antibody mAb m102.4 is a laboratory-produced molecule that is carefully engineered to attach to cells infected with the Hendra virus, thereby alerting the body’s immune system to the presence of the virus and marking it for destruction.

Forty healthy adult volunteers have participated in the phase 1 clinical safety trial; 30 have received mAb m102.4 and 10 placebo. Adverse reactions have been minor and to date, all participants remain well.
Background

At present there is no licensed or readily available prophylactic or therapeutic treatment for humans exposed to the deadly Hendra virus – which has a fatality rate of more than 50 per cent. Antivirals have shown limited effectiveness to date.

The Hendra virus monoclonal antibody, developed by Professor Chris Broder, is an experimental drug product that has been shown to prevent death in ferrets and African Green monkeys that were infected with the Hendra virus.

As of May 2016, the mAb m102.4 had been administered to 11 Australian patients known to have had significant exposure to the blood and body fluids of Hendra virus infected horses. To date, 10 of the patients have remained well and have had no ill effects from treatment. The eleventh patient was not expected to survive when treated, due to the advanced stage of the illness.

The lack of effective treatments available and the strong preliminary evidence of efficacy in non-clinical models and clinical cases has prompted further clinical development of mAb m102.4.

Methods used

Scientists at the University of Queensland’s Australian Institute for Bioengineering and Nanotechnology have produced high quality clinical grade mAb m102.4 and completed bioactivity and efficacy testing and design and development of a test to detect antibodies to mAb m102.4.

Pre-clinical toxicity testing of mAb m102.4 has been completed. Studies to determine the shelf life of the monoclonal antibody are ongoing, as are clinical safety trials of mAb m102.4 in healthy human volunteers.

The human trial is being performed at the Q-Pharm Pty Ltd clinics located within the Clive Berghofer Cancer Researcher Centre, QIMR Berghofer Medical Research Institute, Queensland and supervised by renowned Hendra virus specialist, Dr Geoffrey Playford from the Princess Alexandra Hospital in Brisbane.

The main objective of the clinical trial – due to finish in June 2016 – is to evaluate the safety and tolerability of mAb m102.4 in 40 healthy adults.

Outcomes

Safe use of the mAb m102.4 should result in improved health outcomes for people who are at risk of death and/or severe disability due to Hendra virus infection.

The mAb m102.4 may also be effective against Nipah virus, which is closely related to Hendra virus and has infected hundreds of people in Malaysia, Singapore, Bangladesh and India.