HENDRA VIRUS DISEASE IN AUSTRALIA : THE TWENTY-YEAR ANNIVERSARY OF AN EMERGING ZOONOSIS

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ABSTRACT

Hendra virus is an emerging zoonotic virus first recognised when an outbreak of severe disease affected a racehorse stable in the Brisbane suburb of Hendra in 1994. The outbreak led to the death of the majority of the stable's horses and the horse trainer. Fruit bats were identified as the natural host of the virus and the transmission pathways between bats and horses and horses and people were elucidated.

Twenty years on from the original outbreak Hendra virus outbreaks continue to occur sporadically, having caused a further three human deaths and the deaths of many horses. While up to this point (January 2015) Hendra virus incidents have been confined to Queensland and northern New South Wales, Hendra virus is considered an emerging, endemic disease in Australia with the potential to affect horses and their handlers anywhere that the ranges of fruit bats and horses overlap.

Implications of the disease for the racehorse industry in Australia and strategies to minimise the disease risk are outlined. A vaccine against the disease in horses has been developed and provides a highly effective barrier to cross-species transmission of the virus.

Hendra virus is one of a multitude of recently discovered viruses for which bats are the natural reservoirs, many of which have proven zoonotic potential.

INTRODUCTION

Hendra virus (HeV) was first isolated from an outbreak of disease in September 1994, which occurred in a racing stable located in the Brisbane suburb of Hendra in south-east Queensland, Australia (Halpin et al., 1996). The outbreak resulted in the death of a horse trainer and 13 horses and serious illness in a stable employee. Another seven horses with evidence of exposure to the virus were humanely destroyed to avoid possible further spread of the disease.

The following year another person died of neurological disease, which was later identified to have been a relapse of an infection of HeV that he originally acquired while assisting in a post-mortem examination of a horse in August 1994 in Mackay, Queensland.

Scientific evidence suggests that HeV is carried by fruitbats (Halpin et al., 2000). Under unknown but rare circumstances, the virus spills over from these bats to susceptible horses, killing around 80% of the horses it infects. Under even rarer circumstances the virus then spreads to humans who have had very close contact with Hendra-infected horses. There is no evidence of bat-to-human, human-to-human or human-to-horse transmission of the virus.

HENDRA VIRUS INCIDENTS

Including the two outbreaks mentioned above, from 1994 until December 2014 there have been 51 identified incidents of HeV infection in horses, resulting in the death of 93 horses. In each of these incidents the index cases were housed in paddocks or yards, not stalls or stables. The majority of incidents were single-horse events, with infection identified in the index case alone. Where incidents involved infection of one or more companion horses, there was close contact with the index case. Four incidents involved both horses and humans. After close contact with infected horses, three people developed an influenza-like illness and recovered with variable degrees of residual symptoms. Another four people died from

influenza-like illnesses and encephalitis. The current human case fatality rate is therefore around 57%.

The incubation period of HeV in horses is 5 to 16 days. The course of the disease is generally rapid, with fatally infected horses dying on average two days after the first sign of infection. Clinical signs in horses are listed in Table 1. While approximately 20% of horses are thought to survive acute infection, the current national veterinary policy (AUSVETPLAN; Animal Health Australia 2013) requires these horses to be euthanased. The incubation period in humans is believed to be 5 to 14 days. Clinical signs in humans are listed in Table 2.

The mechanics of bat-to-horse transmission is unknown, but ingestion by horses of bat excreta, partially eaten food or reproductive fluids are plausible modes (Halpin et al., 2000; Field, 2005). Horse-to-horse transmission of the virus is plausible, as a proportion of incidents involved infection of both the index case and companion horses. In these cases, transmission of the virus appears to have been more efficient in horses housed in stables or stalls, probably due to the closer proximity of the horses. In at least one incident, there appears to have been fomite spread of virus between horses. In several other incidents of multiple horse infections, however, the possibility that companion horses were infected as a result of separate bat-to-horse transmissions cannot be ruled out.

Common signs may include any one or combination of the following:	
Acute onset of illness	
Increased body temperature	
Increased heart rate	
Discomfort/weight shifting between legs	
Rapid deterioration with respiratory and neurological signs	
Respiratory signs	**Neurological signs**
Congestion and fluid on lungs	Altered consciousness
Dyspnoea	Muscle twitching
Nasal discharge, clear to frothy/blood-stained	Urinary incontinence Head tilting
	Weakness and incoordination, collapse

Table 1 : Clinical signs of Hendra virus infection in horses

Influenza-like symptoms	**Neurological signs**
Pyrexia	Encephalitis with headache
Coughing	Drowsiness
Pharyngitis	

Table 2: Clinical signs of Hendra virus infection in humans

Transmission of HeV from horses to humans is rare. The greatest risk of human infection appears to be through direct physical contact with the body fluids of ill, dying or dead horses. As evidence suggests that horses have a potential to excrete virus through nasal secretions up to two days before showing signs of infection, contact with apparently healthy horses in the early stages of disease may also pose a real but lesser risk of infection.

IDENTIFICATION OF HENDRA VIRUS

After the outbreak in Brisbane in September 1994, quarantine and movement restrictions were immediately implemented and the horseracing industry in south-east Queensland was temporarily shut down. Epidemiological investigations focusing on possible toxic agents and known viruses that produce similar symptoms in horses were negative. Samples taken from sick and dying horses were then analysed for an unknown virus.

As the suspected virus had caused a human death, the virus was cultured in a Physical Containment Level 4 (PC4) laboratory, providing the highest level of biological containment. Within weeks of the outbreak, the virus was isolated from cell culture. Identical viruses were also isolated from cell cultures of samples taken from the first human case and from horses experimentally infected with the virus.

Visualisation by electron microscopy in affected equine and human tissues confirmed that virus was the causative agent of the outbreak, infecting a wide range of cells but predominantly endothelial cells. Combined with genome sequencing data, this work led to the reclassification of the virus as a member of the Paramyxoviridae — a diverse family of large enveloped RNA viruses including mumps and measles viruses. The provisional name for the virus, equine morbillivirus, was subsequently discarded and the virus was renamed Hendra after the Brisbane suburb involved during the September 1994 outbreak. Rapid molecular tests to detect the virus were developed using the sequence data.

Further molecular analysis justified the creation of a new genus within the Paramyxoviridae family. This new genus, the Henipaviruses, initially comprised two species: Hendra virus and Nipah virus. A third, Cedar virus, was recently added.

RESERVOIR HOSTS

After HeV was isolated and specific diagnostic tests were developed, extensive investigations were undertaken

to establish the source of the virus in nature. Serum sampling conducted throughout eastern Queensland involving over 5000 samples from some 60 species of rodents, marsupials, birds, amphibians and insects found no evidence of antibodies in species other than the horses and humans involved in incidents, as well as all four mainland pteropid bat species — the black (*Pteropus alectus*), grey-headed (*P. poliocephalus*), little red (*P.* scapulatus) and spectacled (*P.* conspicullatus) fruitbats (see Figure 1 for distribution of these bat species).

Ongoing research supports fruitbats to be the natural reservoir of the virus, with live HeV being repeatedly isolated from various pteropid bat species and evidence of HeV in pooled urine samples collected under fruitbat roosts. Extensive sero-surveys of Australian fruitbats show evidence of exposure to HeV in up to 47% of the bats sampled.

This high frequency of Hendra antibodies suggests transmission of the virus between these bats is efficient. Fruitbat camps often consist of thousands of bats roosting together in the tree canopy. In these dense roosts, bats excrete urine and faeces throughout the day and a fine mist of urine is commonly observed. Given the bats' regular grooming activities, transmission from one bat to another in the roost is highly plausible under these conditions.

Computer modelling of fruitbat populations suggests that HeV does not persist as a constant endemic infection in discrete populations, but persists throughout the range of bats in a pulsing pattern. In this pattern of infection, a nomadic individual or small group of bats from an infected colony may make contact with a colony susceptible to infection either because they have not yet been exposed to the virus or their immunity has waned. These nomadic bats then introduce (or reintroduce) the virus to the susceptible colony, resulting in an increase (or pulse) of infection followed by a period of waning immunity.

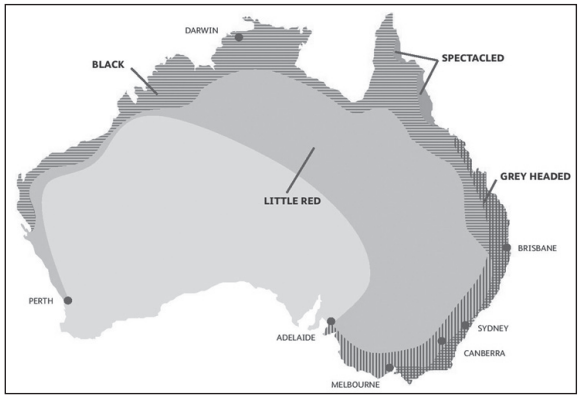

Figure 1 : Distribution of pteropid bats in Australia (adapted from Richards & Hall 2012)

The exact mechanics of HeV transmission from bats to horses is not known, as no virus has been isolated from fruitbats during incidents. However, transmission of the virus to horses is thought to be through ingestion of grass or partially eaten fruit contaminated with bat urine, saliva or other bodily fluids.

The timing of HeV infection in horses may then be linked to the pattern of pulsing endemicity in fruitbats. A period of peak virus excretion may follow the introduction of infection to a susceptible bat colony, likely to correspond with increased risk of exposure and infection of susceptible horses in the vicinity. Contact with Hendra-infected bat birthing products may also be a significant route of infection for horses, suggested by the coincident timing of a number of HeV incidents with the birthing seasons of Australian fruitbats and the isolation of the virus from the uterine fluid and aborted foetuses of fruitbats.

Reproduction and nutritional stress in bats are important drivers of HeV infection, plausibly associated with immune system compromise during these periods. Habitat loss or alteration, roost disturbance, urbanisation and hunting pressure lead to disturbances in food availability and nutritional stress, which may increase the risk of transmission of HeV from bats to horses.

Diversity and distribution of fruitbats in Australia (Figure 1) are two other factors that may contribute to transmission of the virus from bats to horses. Known incidents of HeV infection have been limited to north-eastern Australia. While there may be a risk of bat-to-horse transmission wherever the two are found in close proximity, the geographical range of fruitbats with proximity to horses is much broader than north-eastern Australia. This suggests that HeV infection in fruitbats, and/or the risk of spillover to horses, may be related to the type or mix of fruitbats in this region.

Further investigation of both the dynamics of HeV infection in bats and the mode of transmission to horses is clearly required to determine which factors play a role in fruitbat infection, and the timing and location of virus spillover from bats to horses.

IMPROVED LABORATORY TESTS FOR HENDRA VIRUS

There have been significant advances in test development for human and animal surveillance since the first serological and molecular tests were developed to detect HeV. The original molecular test developed for HeV detection used a particular combination of

polymerase chain reaction (PCR) techniques, reverse transcription and nested PCR. New tests still employ reverse transcription, but the innovative use of real-time PCR makes these tests much faster while requiring less viral material.

A number of HeV isolates have been fully sequenced. Prior to 2006, the gene sequences of these viral isolates were virtually identical, irrespective of the species from which they were recovered, the location of the incident, or the time of recovery. In more recent incidents, genetic differences have been detected between the isolates and those sequenced previously. While it is possible that the virus may be changing, it is more likely that fruitbats have carried this virus for a long time and variants have evolved over the years. As more isolates are identified and sequenced, the likelihood of detecting these variants increases. These genetic variations could explain the range of clinical signs observed in infected horses across different HeV incidents. However, recent experimental studies suggest these variations are more likely to be due to differences in the route of Hendra infection or the system that is first compromised in an infected horse.

Molecular tests for Hendra are fast, accurate and sensitive, but viruses are usually cleared from infected individuals within a few days of infection, making PCR tests useful only during the acute stages of an infection. Serological assays are employed for detecting viral exposure over longer periods.

RISKS TO THE RACING INDUSTRY

Hendra virus poses multiple potential risks to the racing industry:

Hendra virus outbreaks in horses

Even though HeV does not cause explosive outbreaks like equine influenza virus (when exposed to a naïve population of horses), depending on circumstances and locations, potentially significant numbers may be involved in outbreaks centred in large horse population centres, as a result of the disease-monitoring and quarantine restrictions imposed (see point 4 below). Hendra virus causes serious disease in horses, with a natural case mortality rate of around 80% of infected horses but with losses of 100% due to the national euthanasia policy (see point 3 below).
Given that fruitbat populations all around Australia carry HeV, and given the great overlap of the ranges of fruitbats and horses, the geographical range of the disease is likely to increase outside the current Queensland to mid-coast New South Wales area.

Hendra virus infection in people — Workplace Health and Safety

Hendra virus has on seven occasions spread from infected horses to people. In people it causes neurological disease linked to the virus's ability to localise in the brain and spinal cord with a mortality rate of around 57%. In recovered individuals the disease can result in long-term neurological deficits, or cause relapse (but not recrudescence) of disease after an apparent recovery.
In these cases of relapsing encephalitis there are no signs of extraneural infection, and the disseminated, vasculitis-associated thrombosis and microinfarction, which are hallmarks of acute Henipavirus infection, are also absent (Tan et al., 2002; Wong et al., 2009). Furthermore, no virus is able to be isolated from the brain tissue of such cases, suggesting that in relapsing Henipavirus infection, as in the measles virus (another paramyxovirus) in subacute sclerosing panencephalitis, the virus could have undergone mutations involving a matrix protein gene, resulting in failure of viral morphogenesis at the cell membrane and thus failure to produce infective virions (Cattaneo et al., 1986).

People most at risk of contracting the infection are those dealing with sick and dead horses. However, as HeV can be found in many of the body fluids of an infected horse (blood, saliva, respiratory secretions and urine), even before clinical signs of disease are apparent, all people coming into close contact with equine body fluids (e.g. sample collection officers, trainers, strappers, race-day farriers and veterinarians) are potentially at risk of HeV infection during the course of their normal work. Racing regulatory bodies in Australia (particularly in state jurisdictions within the current Hendra outbreak zone) thus face significant Workplace Health and Safety (WH&S) obligations in relation to HeV.

National euthanasia policy for Hendra virus-exposed horses

There is a national policy in place in Australia to euthanase all horses showing evidence of exposure to HeV (seroconversion). During past outbreaks, all HeV seropositive horses, regardless of whether they have shown signs of clinical disease, have been subjected to euthanasia. There is a significant ongoing risk of high-value racehorses being subjected to this policy during future HeV outbreaks.

While relapsing encephalitis has been a feature in humans infected by both HeV and the closely related Nipah virus, these infections have been shown to be confined to the central nervous system and to be non-

contagious. It is likely that horses that have recovered from acute infection have completely cleared the virus from their bodies; or, if virus remains to later cause relapsing infection, it persists in a similarly mutated form, allowing it to continue to evade the horse's immune system but rendering it unable to form budding virions able to spread infection to other individuals (Cattaneo et al., 1986).

Quarantine restrictions

Hendra virus is a notifiable disease in Australia. When a new HeV outbreak is detected, the affected property is immediately quarantined. All horses on the affected property undergo tests (PCR and serology) to determine their HeV status. Trace-back and trace-forward investigations are then undertaken to establish the risk of virus spread to recent in-contact horses. Any additional horse properties with potentially positive tracings are also quarantined. Horses on all quarantined properties are subject to repeated HeV testing. Different types of tests are used to establish the HeV status of a horse and each type has limitations, meaning that often the results of multiple tests are required before a horse's HeV status can be fully established.

The minimum quarantine period imposed is 32 days (twice the longest-known incubation period of HeV in horses). This quarantine period will recommence every time a new case of HeV is detected on a given property. The above limitations in the interpretation of individual HeV test results may result in prolongation of the overall quarantine period on any given property.

While horses with confirmed HeV infection may be relatively small in number during an outbreak, the effect of quarantine and testing requirements on an enterprise may be significant in terms of lost training days and racing opportunity, additional staffing and other quarantine costs. A significant industry-wide impact would result from the quarantining of large training/racing centres.

Potential loss of horse export earnings/markets

Following previous HeV outbreaks, some of Australia's trading partners have temporarily prevented imports of Australian horses while quarantine restrictions and freedom-from-disease testing has taken place. China currently prevents the import of any horses from within a 200 km radius of any previous HeV outbreak. The recent advent of a vaccine to prevent HeV infection in horses has entailed new considerations regarding the export of vaccinated horses, as detailed below.

HENDRA VIRUS VACCINE FOR HORSES

On 1 November 2012, pharmaceutical company Zoetis was issued with a minor-use permit (MUP) for HeV vaccine by the Australian Pesticides and Veterinary Medicines Authority (APVMA). Under this MUP, vaccine is available to be administered to horses by accredited vets. This is a sub-unit vaccine**,** consisting of a recombinant soluble version of the virus's attachment glycoprotein (sG). Vaccine-induced antibodies neutralise HeV by making it unable to attach to host cells, thereby preventing infection.

In considering the use of HeV vaccination in racehorses, the following issues need to be considered.

Vaccine's duration of immunity. All horses commencing the HeV vaccination course receive two initial injections 21–42 days apart, eliciting strong immunity by 3 weeks after the second dose. In order to stop this immunity waning over time, regular vaccine boosters are needed. Currently, the vaccine is approved for a 6-monthly booster interval. Studies to evaluate a 12-month duration of immunity interval following the first 6-monthly booster have been completed and are currently before the APVMA.

Racing industry access to vaccination data. A database recording details of all vaccinations is being maintained by Zoetis as part of the conditions of the MUP. Read-only access to this database is freely available, allowing horse vaccination status to be verified prior to competition, and to facilitate control in the case of a Hendra virus outbreak**.** Regular large-scale vaccination data uploads into existing racing industry databases would allow for efficient integration of vaccination into other routine racing administrative processes.

Export of Hendra virus-vaccinated horses. Currently, HeV-vaccinated horses can be freely exported to Australia's major racehorse export markets, including Hong Kong, Singapore, the EU, Japan, New Zealand, USA and Canada.

At this stage, China, Indonesia, Malaysia and the United Arab Emirates require Australian horses to be seronegative for HeV, making vaccinated horses ineligible for export. Further negotiations between governments are required to resolve these differences in export protocols.

Vaccine side-effects. With over 300,000 vaccine doses now administered to over 150,000 horses in Australia

(December 2014), HeV vaccine has shown a favourable safety profile, with an overall adverse reaction rate of 0.7%, largely made up of minor injection site reactions or self-limiting malaise. It is anticipated that, as vaccine usage continues, the full spectrum of local and systemic reactions displayed by other vaccines will be seen. A Local Rule of Racing has been introduced in Queensland and New South Wales preventing use of the vaccine within 7 clear days of competition to minimise the possibility of a vaccine reaction affecting performance.

The arrival of an efficient vaccine against HeV is welcomed, as vaccination is widely accepted as being the single most effective defence against the significant impact of HeV on the horse industry. Given the likelihood of continued HeV outbreaks and the potential effects on the racing industry as detailed above, recommendations have been made to introduce mandatory vaccination for all horses racing in Queensland.

Australian federal and state government biosecurity policies for HeV are being reviewed in view of the increasing uptake of vaccine by horse owners. It is likely there will be changes for vaccinated horses in an outbreak situation in terms of quarantine requirements and the application of the national horse euthanasia policy.

CONCLUSIONS

Hendra virus causes sporadic disease usually involving small numbers of horses due to its very low transmissibility (close contact with infectious fluids is required for transmission).

Hendra virus vaccination is highly effective (prevents disease and stops multiplication and shedding of virus) and safe (sub-unit vaccine).

Safe export of horses from Australia can be assured through certification of premises freedom from disease (Hendra virus is a notifiable disease in Australia), pre-export quarantine (the disease is rapidly fatal in 80% of infected horses), vaccination against Hendra virus, and/or pre-export serological and PCR testing (seroconverted horses do not pose a transmission risk).

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