

# WILDLIFE HEALTH AUSTRALIA – BAT HEALTH FOCUS GROUP

## INFORMATION DOCUMENT

### HENDRA VIRUS TESTING IN INDIVIDUAL FLYING FOXES AT NECROPSY

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#### **Purpose**

To provide advice regarding testing of individual flying foxes for Hendra virus (HeV). It covers current knowledge, available tests and their limitations, and challenges with interpretation of results. This document was prepared by the Wildlife Health Australia (WHA) Bat Health Focus Group in response to questions received by the group.

#### **Recommendations**

- Hendra virus testing of individual flying foxes should not be used for the purpose of assessing risk following contact between a flying fox and another animal.
- Risk assessment for these situations should be conducted on a case-by-case basis using the circumstances alone.
- Hendra virus testing of individual bats for research purposes may contribute to our understanding of the natural history of infection, the immune response, and virus distribution and dynamics in individual bats.

#### **Consultation and distribution**

This document was prepared by the WHA Bat Health Focus Group. Comments were sourced from State/Territory CVO-appointed WHA coordinators.

This document will be made available to State/Territory WHA coordinators, zoo/sentinel clinic coordinators, WHA focus groups and other stakeholders as considered appropriate by WHA, WHA Coordinators, and the Bat Health Focus Group.<sup>1</sup>

#### **Current knowledge**

##### *a) General*

There is a growing body of knowledge about the prevalence, epidemiology and pathogenesis of Hendra virus in flying fox populations in Australia, although there are still significant gaps in our understanding. Numerous publications discuss the epidemiological features of this disease including the observed spatial, temporal and species variation of infection. Evidence suggests that Hendra virus infections can occur in flying-fox populations across Australia, varying over space, time and species (Field *et al*, 2011; Breed *et al*, 2011; Field *et al*, 2015).

##### *b) Pathology*

It appears that Hendra virus is generally non-pathogenic in flying foxes. Experimental studies found minimal pathology associated with infection (Halpin *et al*, 2011; Williamson *et al*, 1998) and there is no evidence of clinical disease in free-living flying fox populations (Field *et al*, 2001). The impact of

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<sup>1</sup> <http://www.wildlifehealthaustralia.com.au/ProgramsProjects/BatHealthFocusGroup.aspx>

Hendra virus infection on flying fox populations is not fully known, however a significant negative effect appears unlikely.

### c) *Transmission to other species*

Flying foxes (*Pteropus* spp.) are the natural reservoir for Hendra virus (Halpin *et al*, 2000). There is evidence that black (*P. alecto*) and spectacled flying foxes (*P. conspicillatus*) are the main reservoir hosts, with grey-headed (*P. poliocephalus*) and little red flying foxes (*P. scapulatus*) playing a less important role in the epidemiology and transmission (Edson *et al*, 2015; Goldspink *et al*, 2015; Smith *et al*, 2014). The transmission of Hendra virus from flying foxes to horses is not completely understood, however mucous membrane contact with infected body fluids or excretions of flying foxes has been proposed (Field, 2005; Halpin *et al*, 2000; Williamson *et al*, 1998), and urine appears to be the most plausible source of infection (Edson *et al*, 2015). The role of stress in Hendra virus infection dynamics in flying-foxes is unclear. Nutritional and reproductive stress has previously been temporally associated with increased Hendra virus infection (Plowright *et al*, 2008), yet Edson *et al* (2015) found no association between roost disturbance and HeV excretion.

In a small number of cases the virus has been transmitted from horses to humans following contact with body fluids from an infected horse (Mahalingam *et al* 2012). Unlike the related Nipah virus, there is no evidence that Hendra virus is transmitted direct from flying foxes to humans. No cases of Hendra virus or detectable antibodies were identified in bat carers with regular contact with flying foxes (Selvey *et al*, 1996).

One dog in New South Wales was confirmed positive by PCR for Hendra virus infection in 2013. The dog was on a property with Hendra-infected horses, and evidence indicates that the route of transmission was from horse to dog (Kirkland *et al*, 2015). Hendra virus antibodies were detected in a dog in Queensland in 2011; this dog was also in contact with infected horses (QLD Government, 2015; Williamson, 2011).

### **Testing of flying foxes for Hendra virus**

Colonies: There is an established method for assessing and monitoring the Hendra status of flying fox colonies through testing of pooled urine samples collected from plastic sheeting placed under the colony (Field *et al*, 2011). This method has been undertaken in several states and territories, and has resulted in successful PCR detections and isolation of Hendra virus. However flying fox colonies are not closed populations; telemetry studies have demonstrated a high level of connectivity between roosts, and the status of any colony at any point in time is dynamic, reflecting the proportion of susceptible bats in the colony and the level of connectivity with currently infected colonies.

Individual flying foxes: Table 1 outlines the tests currently available in Australia for Hendra virus testing. The limitations of these tests for individual flying foxes are outlined in the following section. Note: Hendra virus is not a standard test conducted on flying foxes submitted to the Australian Animal Health Laboratory (AAHL), and will generally only be conducted if specifically requested by the submitter.

In the research context, serosurveillance of individual flying foxes has been conducted (e.g. Field, 2005; Breed *et al*, 2011; Plowright *et al*, 2008), however, with the notable exception of Edson *et al*

(2015), there is less published research on testing of individual flying foxes for the presence of Hendra virus. A summary of published literature on Hendra virus PCR detections and virus isolations is provided in Appendix 1.

Other species: There are significant differences between species in relation to samples and testing for HeV. The information below relates specifically to flying foxes. For information on testing of other species including horses, see the Hendra Response Policy Brief, AHA (2013).

**Table 1: HeV tests for flying foxes available in Australia**

Test	Samples	Comments
PCR – real time (TaqMan)	<ul style="list-style-type: none"> <li>- Submitting multiple tissues may increase the likelihood of detection.</li> <li>- Spleen and urine appear to be the optimal samples for testing. PCR positive results have been obtained from spleen, kidney, urine, uterine fluid, foetal tissues, saliva, lung, liver, swabs, blood (Appendix 1).</li> <li>- Detection is lower from swabs (nasal, oral, rectal, urogenital) than from urine. Unlike in horses (AHA, 2013), viral shedding from mucous membranes appears to be low.</li> </ul>	<ul style="list-style-type: none"> <li>- Conducted by AAHL and some state/territory DPI laboratories. Results generally available within 24 hours.</li> <li>- Optimal tissues and circumstances for HeV detection not completely understood.</li> <li>- The protocol for HeV diagnosis in flying foxes using solid tissue samples is the same as for other species.</li> </ul>
Immunohistochemistry (IHC)	<ul style="list-style-type: none"> <li>- Incomplete understanding of which samples are the most appropriate.</li> <li>- Antigen is very rare, but has been found in vascular tissue and lung.</li> </ul>	<ul style="list-style-type: none"> <li>- Optimal tissues and circumstances for HeV detection not completely understood.</li> <li>- The sensitivity of IHC is likely to be lower than for PCR and isolation.</li> </ul>
Virus isolation	<ul style="list-style-type: none"> <li>- Incomplete understanding of which samples are the most appropriate for virus isolation.</li> <li>- Virus has been isolated from: urine, uterine fluid, foetal liver/lung, kidney, blood, heart, spleen (Appendix 1).</li> </ul>	<ul style="list-style-type: none"> <li>- Can only be undertaken at AAHL under PC4 conditions.</li> </ul>
Serology – ELISA/VNT/ Luminex	<ul style="list-style-type: none"> <li>- Conducted on serum.</li> </ul>	<ul style="list-style-type: none"> <li>- ELISA only validated in horses.</li> <li>- Virus Neutralisation Test (VNT) can only be undertaken at AAHL.</li> </ul>

## Interpretation of results from individual flying foxes

Interpretation of Hendra virus test results from individual flying foxes is complex, and should take into account the history, epidemiology and temporal pattern; sample type, process and transport; and test type, process, characteristics, and sensitivity and specificity. Table 2 demonstrates the challenges associated with interpretation of HeV test results from individual flying foxes.

**Table 2: Interpretation of HeV test results**

Test	Comments	Positive means...	Negative means...
PCR	<ul style="list-style-type: none"> <li>- PCR detects virus genome OR a genome fragment.</li> <li>- Viral RNA extraction from solid organs can be problematic. Sensitivity is higher for urine samples.</li> <li>- Infected flying foxes may only excrete virus intermittently.</li> </ul>	<ul style="list-style-type: none"> <li>- The flying fox is currently infected or was recently infected.</li> <li>- The flying fox may or may not have been infectious, as detection of virus genome in solid organs may not equate to excretion of viable virus (AHA, 2013).</li> </ul>	<ul style="list-style-type: none"> <li>- No genome/ fragment was detected in that particular sample at that particular time.</li> <li>- The flying fox could therefore be:               <ul style="list-style-type: none"> <li>a) uninfected;</li> <li>b) infected but not infectious i.e. not shedding;</li> <li>c) infected and infectious i.e. false negative.</li> </ul> </li> </ul>
Virus isolation	<ul style="list-style-type: none"> <li>- Virus isolation is less sensitive than PCR for detecting current infection.</li> </ul>	<ul style="list-style-type: none"> <li>- The flying fox is currently infected.</li> </ul>	<ul style="list-style-type: none"> <li>- The flying fox could be:               <ul style="list-style-type: none"> <li>a) uninfected;</li> <li>b) infected i.e. false negative.</li> </ul> </li> </ul>
Serology	<ul style="list-style-type: none"> <li>- A single serology result gives no information about current infection status.</li> <li>- Immune responses to HeV in flying foxes are incompletely understood and may differ between species.</li> <li>- Antibodies may take time to be produced in response to infection (AHA, 2013).</li> </ul>	<ul style="list-style-type: none"> <li>- The flying fox could be:               <ul style="list-style-type: none"> <li>a) currently infected and infectious (shedding virus);</li> <li>b) previously infected, immune, and not infectious.</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>- The flying fox could be:               <ul style="list-style-type: none"> <li>a) uninfected;</li> <li>b) currently infected and shedding virus without having seroconverted.</li> </ul> </li> </ul>

## Hendra virus testing and risk management

A common situation where a risk assessment for HeV is conducted is following known or suspected contact between a flying fox and a horse or another animal, and associated contact with a person. Inappropriate reliance on tests for the purpose of risk assessment could result in: a) a real risk going unrecognised (with a potentially high level of consequence), or b) inappropriate euthanasia or management burden for domestic animals. The results of individual flying fox testing will not usefully inform this risk:

- Negative HeV result: A negative PCR result in a flying fox does not rule out the potential for transmission (Table 2), and therefore could falsely convey a lack of risk for an in-contact animal. In addition, the presence of a flying fox indicates that other flying foxes are likely to be in the vicinity, with associated risk of HeV transmission to the horse or other susceptible animal.
- Positive HeV result: A positive PCR result may not equate to an infectious state (Table 2), although from a risk perspective it is reasonable to consider the flying fox as infectious. A positive virus isolation is more definitive, particularly if isolated from urine. However it is still difficult to interpret the extent of the risk to an in-contact horse or other susceptible animal, due to incomplete understanding of HeV transmission from flying foxes to other species.

In addition, in situations where there has been direct human contact with a flying fox, the act of testing the flying fox for HeV could foster a public perception that HeV can be transmitted directly from flying foxes to people, which is not supported by available evidence.

Due to the limitations of the tests, the difficulty in interpretation of the results, and the gaps in our knowledge of HeV epidemiology, HeV testing of individual flying foxes should not be used to inform risk to in-contact susceptible animals. Appropriate precautions and management should instead be determined on a case-by-case basis using information regarding the circumstances of the case.

## Summary

- There are established methods for assessing the Hendra status of a flying fox colony through testing of pooled urine samples collected below the colony.
- For individual flying foxes, available tests for HeV include PCR, IHC, virus isolation and serology, all of which have limitations. PCR is the most sensitive test, however a positive result may not equate to an infectious state, and a negative result does not rule out current HeV infection. Serology does not differentiate between past exposure and current infection.
- There are gaps in knowledge of Hendra virus epidemiology, including transmission of HeV from flying foxes to other species.
- Given the problems associated with interpretation of the results and gaps in our knowledge of HeV epidemiology, HeV testing of individual flying foxes should not be used to inform risk. Risk management should be determined on a case-by-case basis, based only on the circumstances of the case.

## APPENDIX 1: Summary of published literature – Hendra virus detection in flying foxes

### PCR

Reference	Tissue/Fluid	Species
Edson <i>et al</i> (2015)	Urine, urogenital swabs, serum, packed haemocytes, rectal swabs, nasal swabs, oral swabs	<i>P. alecto</i>
Goldspink <i>et al</i> (2015)	Spleen, kidney, liver, lung, placenta, blood	<i>P. alecto</i> , <i>P. conspicillatus</i> <i>P. poliocephalus</i>
Breed <i>et al</i> (2013)	Blood, urine, saliva	<i>P. alecto</i>
Field <i>et al</i> (2011)	Pooled urine	Various
Halpin <i>et al</i> (2011) [ <i>Experimental infection</i> ]	Blood, urine, throat & rectal swabs Lung (2 x flying foxes) Spleen (5) Liver (2) Kidney	<i>P. alecto</i>

### Virus Isolation

Reference	Tissue/Fluid	Species
Halpin <i>et al</i> (2000)	Uterine fluid (recently aborted) Pooled liver & lung (aborted foetuses of above) Lung of a foetus	<i>P. poliocephalus</i> <i>P. poliocephalus</i> <i>P. alecto</i>
Halpin (unpublished) – from Daniels <i>et al</i> (2007)	Kidneys (3) Blood Lung (neonate)	<i>P. scapulatus</i> <i>P. poliocephalus</i> <i>P. poliocephalus</i>
Smith <i>et al</i> (2011)	Pooled urine	<i>P. alecto</i> <i>P. poliocephalus</i> <i>P. conspicillatus</i>
Williamson <i>et al</i> (1999) [ <i>Experimental infection</i> ]	Heart, buffy coat Spleen, kidney (foetus of above) Kidney, heart, spleen	<i>P. poliocephalus</i>



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