Initial experimental characterisation of HeV (Redland Bay 2008) infection in horses

D Middleton, CSIRO AAHL

Introduction

Since its emergence in Queensland in 1994, Hendra virus (HeV) infection in horses has recurred on a regular basis with at least two disease events recorded in four of the past five years. Of the 11 equine outbreaks, four have extended to involve infection of humans. Of the six known human cases three have died, the most recent of these in August 2008. Clearly, HeV is an unmanaged emerging infectious disease.

Since the zoonotic potential of HeV was confirmed, clinical and laboratory evaluation of affected field cases has been very limited. In particular, optimal biological samples for rapid confirmation of the diagnosis in the live animal, and the relationship between the onset of clinical signs and duration of viral shedding have not been determined. In terms of laboratory-based infection studies, the understandably few equine experimental infections that were carried out in the mid-nineties also yielded limited data for the purpose of informing effective management of the exposure risk to people.

An additional complexity has arisen with respect to the recent HeV outbreak (Redland Bay 2008) in that the major presenting signs in horses were attributable to disease of the central nervous system. Nervous signs have been associated with previous outbreaks of HeV and material used for public education has been drafted accordingly, but HeV is commonly considered to induce primarily a respiratory syndrome in horses. In the case of Redland Bay 2008 credible alternate provisional diagnoses, and thus delay in definitive diagnosis, likely contributed to an increased HeV exposure risk to attending staff and to in-contact horses.

The aim of this work is to provide data that will contribute to reduction of the exposure risk to people, and the cross-transmission risk to other horses, from horses acutely infected with HeV. The specific objectives of this study are to describe the associated clinical syndrome(s) with particular emphasis on the early phases of disease, to document the optimal biological samples to be collected for rapid diagnosis in clinical suspects, and to assess the relative transmission risk posed by horses at various time points during acute infection. This will permit recommendations to be made with respect to reducing the transmission risk to people and other horses, for incorporation into advisory and outbreak management strategies.

Methods

Animals

Three adult mares were used. Animal husbandry methods and experimental design were endorsed by the CSIRO AAHL Animal Ethics Committee and aligned with the Code of Practice for the Welfare of Horses (Bureau of Animal Welfare, Victoria). Animals were housed in single pens within one room at Biosafety Level 4, and fed a mixture of lucerne and grass hay, horse nuts, and specified fruit and vegetables. Pressure matting was placed on the floor of each pen. Various behavioural enrichment items were also provided and regular grooming was carried out. One side of each pen was modified for movement along a track that permitted staff to work alongside each animal without the need to enter the pen itself. Room temperature was maintained at 22^0C with 15 air changes per hour; humidity varied between 40 and 60%.

Staff wore fully encapsulating suits with breathing apparatus while in the animal room.

The horses were acclimatised to the facility for one week prior to exposure to HeV. Immediately prior to viral challenge, each mare was fitted with an intra-uterine (transcervical) temperature transponder for the purpose of continuous recording of core body temperature. An indwelling catheter was placed in the jugular vein of each animal and sutured in position.

In addition to clinical assessment carried out during husbandry and sample collection procedures, continual video-monitoring of horses was done including infra-red enabled image capture overnight.

When horses reached predetermined humane disease endpoints they were euthanased by intravenous barbiturate following intravenous sedation with detomidine and butorphanol. The humane endpoint was defined as fever accompanied by dyspnea, depression, ataxia or other sign consistent with involvement of the nervous system. Allowing animals to survive to this point allowed documentation of incubation period, duration and route of viral shedding from the time of virus exposure to readily detectable disease, and time for localisation of virus to the various body tissues. Observations from field cases suggest that animals that progress to moderately severe signs of disease usually do not survive. Should an animal become cast in the BSL4 facility, this would present a considerable risk to operators in an already hazardous environment, as well as a substantial welfare impact: the level of disease severity proposed for intervention struck a balance of allowing the scientific objectives to be achieved with due consideration of these other aspects. Animals that did not reach the humane endpoint were to be kept for at least 21 day post-challenge to monitor for sero-conversion.

Virus

An isolate of HeV (Redland Bay 2008) derived from field samples supplied by the Queensland Department of Primary Industries was used. Noting that the Redland Bay isolate of HeV showed 99.6% genetic similarity at the amino acid level to the classical Hendra isolate, the virus challenge was designed to mimic previous experimental studies as closely as possible (H_0 HeV Redland Bay ≡ classical HeV). Accordingly, the virus was administered at equivalent dose rates and routes of challenge as for earlier studies with HeV in horses, and an isolate from spleen tissue was selected for this purpose.

Virus was administered to each horse oronasally; $1x10^6$ $TCID_{50}$ by each route.

Clinical data and biological sample collection and analysis

Clinical observations were recorded twice daily commencing three days prior to virus challenge. These included comment on general demeanour, as well as rectal temperature measurements (to augment records from the implanted transponders) and collection of heart rate data using an electronic monitor. The character of the respiratory pattern and effort was also noted on each occasion; resting respiratory rate had previously been assessed as highly variable in each animal from time to time and so was not routinely recorded.

Daily biological samples collected included swabs from the nasal mucosa, oral and rectal swabs, and blood. Urine and feces were collected from the pen floor, and equipment was available for mid-stream urine collection on an opportunistic basis. All biological samples apart from blood for serology were placed immediately on wet ice, and then stored at -80C until processed. Serum was stored at -20C until tested for the presence of antibody to HeV. Quadruplicate samples were collected and archived for possible future use in pen-side test development and preliminary validation.

Nasal, oral, and rectal swabs, urine and feces, and blood were analysed for the presence of HeV by attempted virus isolation (Vero cells), and for HeV genome by Taqman PCR. Two Taqman assays were used. The first assay (P-gene) was designed by staff from Queensland Health Scientific Services and is regularly used for routine Hev diagnosis. The second N-gene TaqMan assay was designed by CSIRO AAHL staff for Henipah virus detection and is also used for routine HeV diagnosis.

Following euthanasia, a post mortem examination was carried out on each horse. Diverse tissues were collected and samples retained for virus isolation, Taqman PCR, histopathology and immunohistochemical examination.

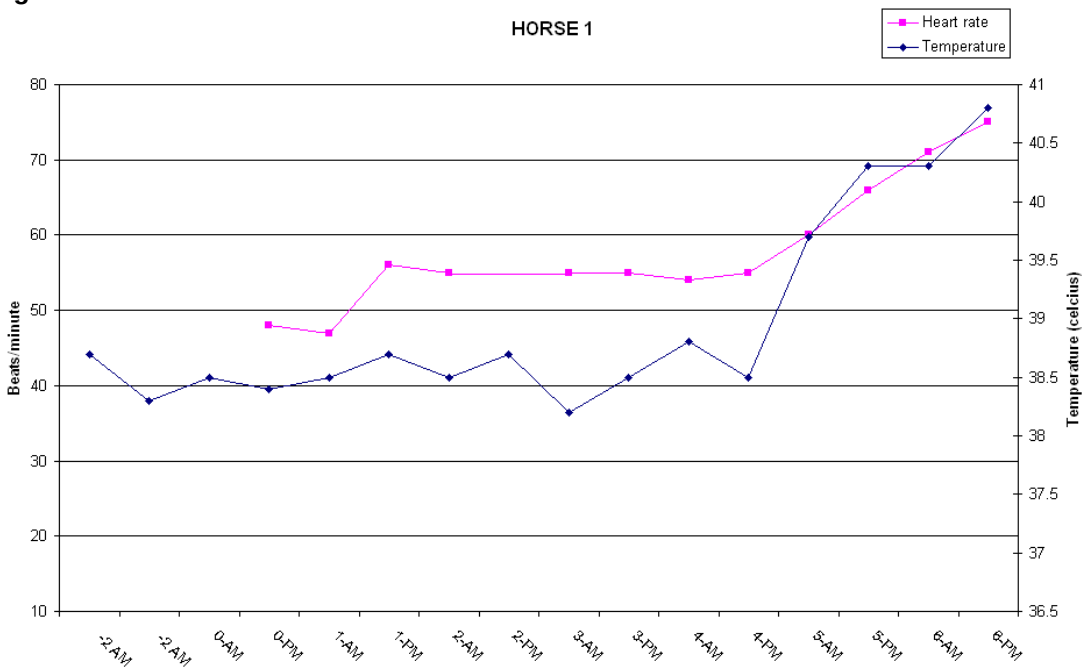
Results

Clinical observations

Horse 1

The horse remained well until Day 5 post challenge when a rise in body temperature above baseline was noted in the morning, rising further in the afternoon of the same day (Fig 1). This was paralleled by a steady rise in resting heart rate. At this time the horse was eating well and otherwise appeared clinically normal.

Fig 1

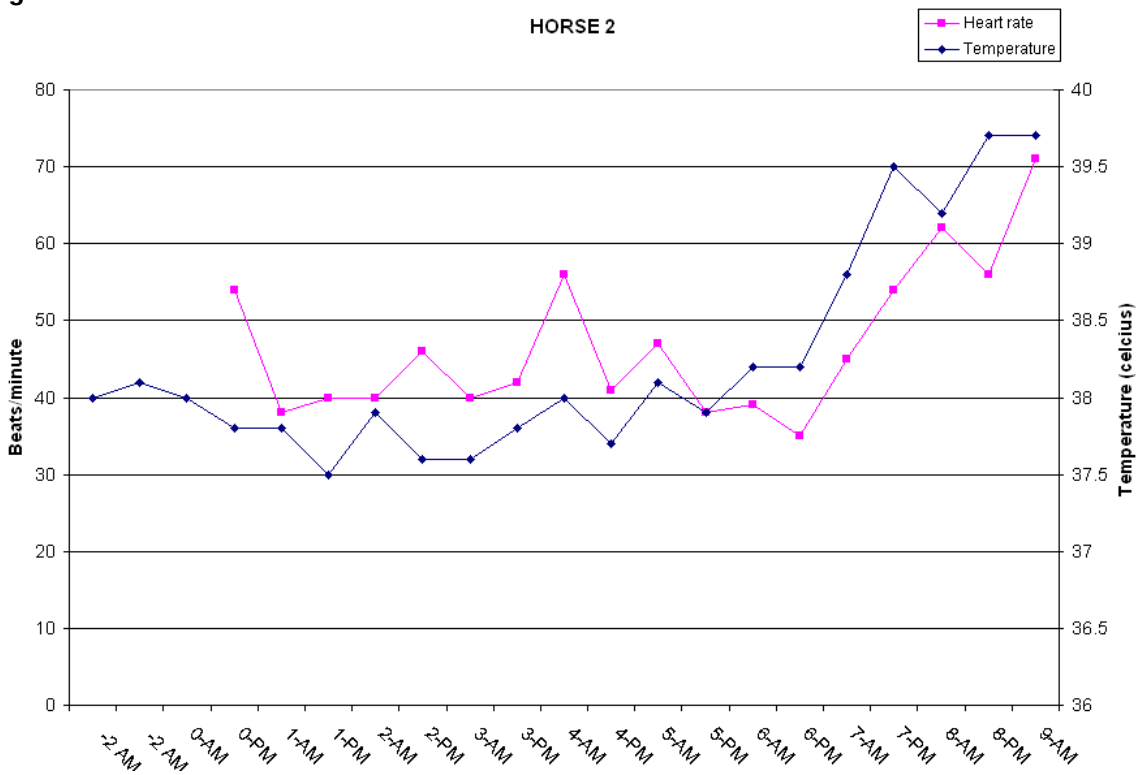


On Day 6, the horse was clinically depressed with reduced appetite. Temperature and heart rate had continued to rise and over the next few hours the horse exhibited continuous restlessness, with constant shifting of weight between all four limbs especially the hind limbs. By the afternoon it was disinterested in its surroundings and had begun to stand with its head facing up against the side of the pen. In view of the rapid clinical deterioration the horse was euthanased early that evening, and post mortem examination was carried out.

Horse 2

A slight bilateral serous nasal discharge was observed in this horse 2 Days PI. The horse remained otherwise well until Day 7 PI when it developed a rise in temperature above baseline that was paralleled by a rise in resting heart rate (Fig 2). At this time the horse was otherwise clinically normal. The following day, the horse was slightly depressed and the elevated heart rate and temperature appeared to have stabilised. However, on Day 9 PI the heart rate had continued to rise and the horse was exhibiting mild dyspoea with a prolonged expiratory phase. This normally quiet mare also became quite agitated on being approached. Accordingly, she was euthanased on the afternoon of Day 9 PI and post mortem examination carried out.

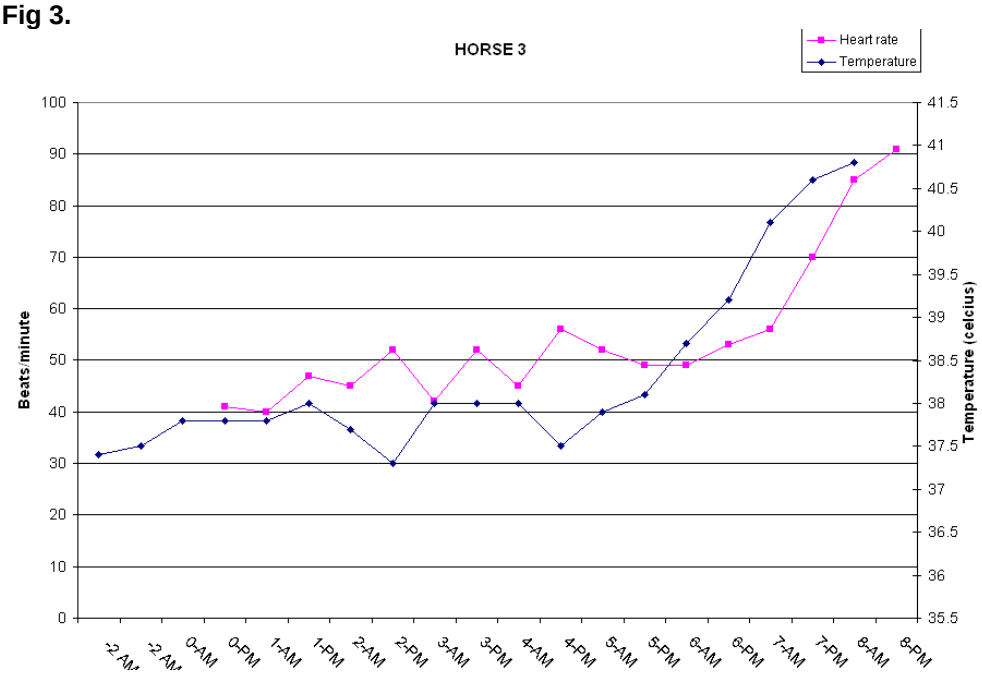
Fig 2.



Horse 3

A slight bilateral serous nasal discharge was observed in this horse 2 Days PI. The horse remained otherwise well until Day 6 PI when its temperature started to rise above baseline. Fever was established by Day 7 PI and a concomitant rise in heart rate was also noted (Fig 3). The serous nasal discharged had resumed, but the horse was otherwise well and eating normally. On Day 8 PI, temperature and heart rate were continuing to rise and small amounts of blood were seen in the feces that were also coated in mucus. The mare developed a rigid forelimb stance alternating with general restlessness and constant shifting of weight from limb to limb, difficulty in prehension of food, frequent head shaking, and irritability with attempts to bite handlers. A panting type of respiration was noted. The horse was euthanased on the afternoon of Day 8 PI.

Fig 3.



Post-mortem and histopathological findings

Horse 1

Significant post mortem abnormalities comprised enlarged and edematous sub-mandibular lymph nodes and several 2 x 0.5cm subpleural hemorrhages on the left lung. A small (6x6cm) area of brownish-pink consolidation was present on the ventral border of the left lung posterior to the cardiac notch. A 2.5cm follicle was noted in the left ovary.

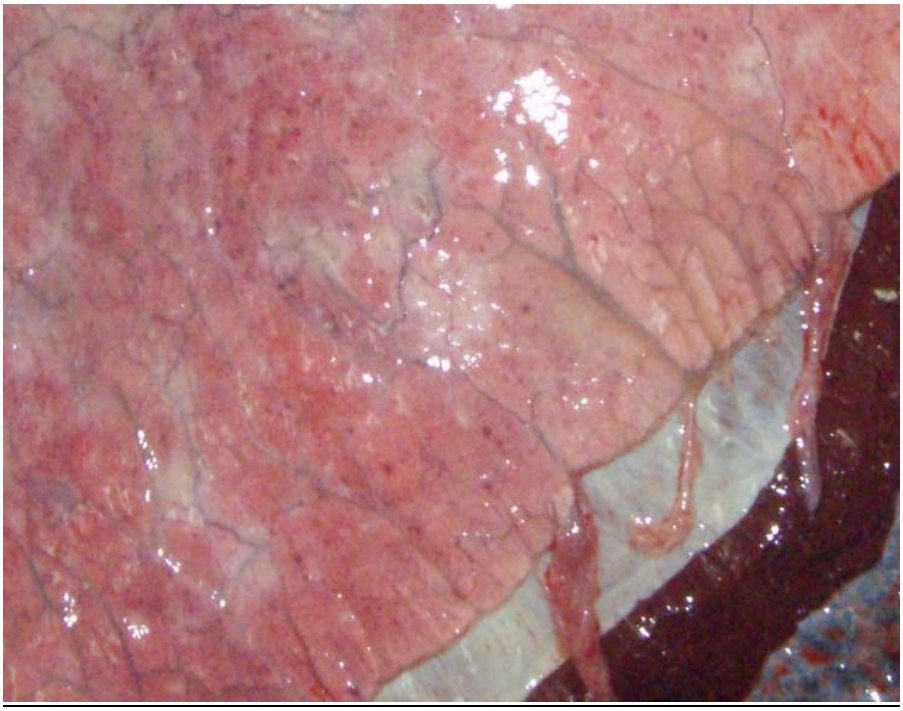
On histological examination, systemic vasculitis was observed affecting meninges, nasal mucosa, trachea, lung, diverse lymph nodes, spleen, kidney, heart, uterus, ovary and intestine. Edema, syncytial cells, viral inclusion bodies and alveolitis were seen in lung sections. Focal necrosis of the adrenal gland was identified, together with glomerulitis and syncytial cell formation in the kidney. HeV antigen was present in tissues and organs including meninges, alveolar walls, lymph nodes, renal glomeruli, and adrenal gland; and in blood vessels supplying each of these, plus the nasal mucosa, trachea, spleen, heart, uterus, ovary and intestine.

Horse 2

Significant post mortem abnormalities comprised enlarged and edematous submandibular and bronchial lymph nodes, and heavy lungs that oozed fluid from the cut surface. There were numerous petechial hemorrhages over the surface of the diaphragmatic regions of the lung (Fig 4). The liver was also smaller than normal with an irregular finely nodular surface.

On histological examination, systemic vasculitis was observed affecting meninges, nasal mucosa, trachea, lung, diverse lymph nodes, spleen, liver, kidney, heart, uterus, ovary and intestine. Edema, syncytial cells, viral inclusion bodies and alveolitis were seen in lung sections. Focal necrosis and syncytial formation within lymph nodes was identified, together with glomerulitis and syncytial cell formation in the kidney. There was also acute myocarditis and focal necrosis of corpus luteum tissue. HeV antigen was present in tissues and organs including meninges, alveolar walls, lymph nodes, renal glomeruli, myocardium and ovary; and in blood vessels supplying each of these, plus the nasal mucosa, liver, spleen, adrenal gland, uterus, and intestine. Hepatic amyloidosis was an incidental finding.

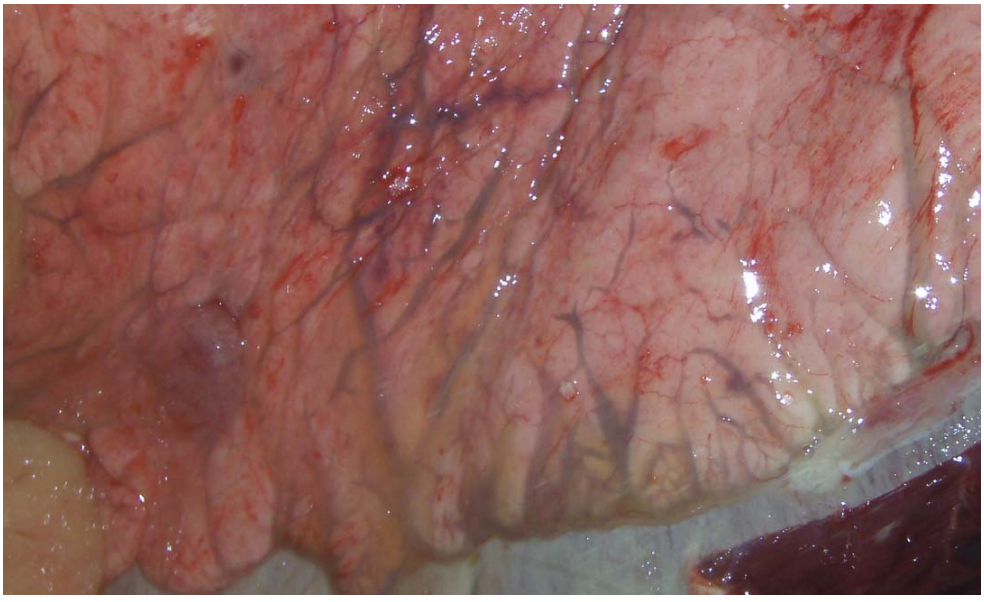
Fig 4



Horse 3

At post mortem examination there were swollen and edematous submandibular, sternal and bronchial lymph nodes; dilation of lymphatic vessels at ventral lung lobe margins (Fig 5); and endometrial edema with purplish discoloration of the serosal surface of the uterus.

Fig 5



On histological examination, systemic vasculitis was observed affecting meninges, nasal mucosa, trachea, lung, diverse lymph nodes, spleen, liver, kidney, heart, uterus, ovary and intestine. Edema, syncytial cells, viral inclusion bodies and alveolitis were seen in lung sections. Focal necrosis and syncytial formation within lymph nodes was identified, together with glomerulitis and syncytial cell formation in the kidney. There was also acute myocarditis and focal necrosis of adrenal and corpus luteum tissue. HeV antigen was present in tissues

and organs including alveolar walls, lymph nodes, tonsil, renal glomeruli, myocardium and ovary; and in blood vessels supplying each of these, plus the nasal mucosa, liver, spleen, adrenal gland, uterus, and intestine. Hepatic amyloidosis was an incidental finding.

Analysis of biological specimens

Virus was not reisolated from any sample collected from any of the horses prior to post mortem examination. Blood samples collected during acute disease were highly toxic to tissue cultures and it is possible that virus was present at low titre in some of these samples. Similar observations were made for urine and fecal samples. It should also be noted that virus isolation from swab material in particular may be a less sensitive means of detecting viral replication than Taqman PCR, and so a negative isolation result may not reflect absence of virus.

Not unexpectedly, all horses were seronegative at the time of post-mortem examination.

Horse 1

Results of Taqman assays for the P and N genes on clinical samples are illustrated below in Fig 6.

Fig 6: Ct values Horse 1 (Day 0 = day of challenge)**

Horse 1	0	1	2	3	4	5	6
Blood Heparin Ribo-pure RNA extraction P-gene	U	U	U	U	39.4	33	31.2
Blood Heparin Ribo-pure RNA extraction N-gene	U	U	U	U	37.9	31.3	29.8
Urine P-gene	N/A	U	U	41.9/U	U	41.6/U	36.2
Urine N-gene	N/A	U	U	U	40.3/U	38.7	34.2
Rectal swab P-gene	U	U	U	U	U	U	U
Rectal swab N-gene	U	U	U	U	U	U	42
Nasal swab P-gene	U	U	37.5	34.7	35.9	29.5	32.8
Nasal swab N-gene	U	U	35.9	33.1	34.3	28.2	31.7
Oral swab P-gene	U	U	U	U	U	41.2	38.5
Oral swab N-gene	U	U	U	U	U	38.2	34.7
Faeces P-gene	N/A	U	U	U	U	40.7	36.1
Faeces N-gene	N/A	U	U	U	40.6/U	39.8	33.2

Ct : 0-40 = positive
Ct: 40-45= indeterminate
Ct: 45 + = negative
NO amplification= U

**** A fall in Ct value of one unit is equivalent to a doubling of the target gene. A fall in Ct value of 3.3 units is equivalent to a 10-fold increase in the target gene.**

Viral genetic material was consistently detected in nasal swabs from 2 days post-exposure to virus. The steady fall in Ct values over time is consistent with viral replication and shedding into the nasal cavity in nasal secretions. From day 5 PI, Ct values in nasal swabs and blood were of the order associated with successful recovery from virus from tissue samples (solid organs) using traditional virus isolation techniques. Genome was detected in blood the day prior to the onset of fever (D 4). When fever was established (Day 5) but while the horse was otherwise clinically normal, viral genome was detected additionally in oral swabs, urine and feces but not rectal swabs. It is possible that fecal material on the floor of the pen may have been contaminated by urine containing viral genetic material; it is also possible that the smaller amount of material collected on the rectal swab influenced the sensitivity of the test. Similar results were obtained on Day 6 when clinical disease was readily apparent. The Ct scores from the N-gene Taqman PCR were consistently lower than those from the P-gene

test, raising the possibility that the N-gene test may have a slightly higher diagnostic sensitivity.

Following post mortem examination, virus was reisolated from kidney, lung and sub-mandibular, inguinal and renal lymph nodes.

Taqman assays for the P and N genes were positive for all tissues sampled at post mortem with the exception of the P gene assay on spinal cord which provided an indeterminate result. Generally, the highest levels of target genes were associated with tissues from which live virus was also recovered.

Horse 2

Results of Taqman assays for the P and N genes on clinical samples are illustrated below in Fig 7.

Fig 7: Ct values Horse 2 (Day 0 = day of challenge)

Horse 2	0	1	2	3	4	5	6	7	8	9
Blood Heparin Ribo-pure RNA extraction P-gene	U	U	U	U	U	U	37.3	32.2	31.4	29.9
Blood Heparin Ribo-pure RNA extraction N-gene	U	U	U	U	U	40.6/U	34.8	30.6	30.3	28.6
Urine P-gene	N/A	U	U	U	N/A	U	U	36.2	36.3	33.5
Urine N-gene	N/A	U	U	U	N/A	U	38.7/U	33.9	34	30.1
Rectal swab P-gene	U	U	U	U	U	U	U	U	38.2	40.5
Rectal swab N-gene	U	U	U	U	U	U	U	43.6	36.4	36.5
Nasal swab P-gene	U	U	36.3	32.4	38.9	34.3	31.1	28.1	29.2	35.2
Nasal swab N-gene	U	U	34	31.7	35.8	31.8	30	27.5	27.8	33.1
Oral swab P-gene	U	U	U	U	U	U	U	36.6	35.8	34.5
Oral swab N-gene	U	U	U	U	U	U	40.0/U	33.6	33.4	32.1
Faeces P-gene	N/A	U	U	U	U	U	40.7/U	37	35	35.2
Faeces N-gene	N/A	U	U	U	U	U	40.5	34.9	34.2	33

Viral genetic material was consistently detected in nasal swabs from 2 days post-exposure to virus. The steady fall in Ct values over time is consistent with viral replication and shedding into the nasal cavity in nasal secretions. From day 7 PI, Ct values in nasal swabs and blood were of the order associated with successful recovery from virus from tissue samples (solid organs) using traditional virus isolation techniques. Genome was detected in blood the day prior to the onset of fever (D 6). When fever was established (Day 7) but while the horse was otherwise clinically normal, viral genome was detected additionally in oral swabs, urine and feces but at an indeterminate level in the rectal swab. It is possible that fecal material on the floor of the pen may have been contaminated by urine containing viral genetic material; it is also possible that the smaller amount of material collected on the rectal swab influenced the sensitivity of the test. Similar results were obtained on Days 8 and 9 when clinical disease was readily apparent. The data from this horse also suggest that the Taqman PCR for the N gene may have a slightly higher diagnostic sensitivity than the Taqman PCR for the P gene.

Following post mortem examination, virus was reisolated from guttural pouch; pharynx; sub-mandibular, inguinal, bronchial and renal lymph nodes; lung; spleen, kidney, heart, large intestine, spinal cord, brain and intrathoracic sympathetic chain. Interestingly, virus was not reisolated from urine collected at post mortem.

Taqman assays for the P and N genes were positive for all tissues (and urine) sampled at post mortem. Generally, the highest levels of target genes were associated with tissues from which live virus was also recovered.

Horse 3

Results of Taqman assays for the P and N genes on clinical samples are illustrated below in Fig 8.

Fig 8: Ct values Horse 3 (Day 0 = day of challenge)

Horse 3	0	1	2	3	4	5	6	7	8
Blood Heparin Ribo-pure RNA extraction P-gene	U	U	U	U	U	39.1	36.3	32.8	31.6
Blood Heparin Ribo-pure RNA extraction N-gene	U	U	U	U	U	36.5	33.6	31.7	29.9
Urine P-gene	N/A	U	U	U	U	U	40.7	39	34.3
Urine N-gene	N/A	U	U	U	U	U	39.4	38.1	32.8
Rectal swab P-gene	U	U	U	U	U	U	U	U	U
Rectal swab N-gene	U	U	U	U	U	U	U	42.9	42.8
Nasal swab P-gene	U	U	42	N/A	41.4	43	U	37.9	32.9
Nasal swab N-gene	U	U	38.8	N/A	U	41.2	40.0/U	34.6	31.5
Oral swab P-gene	U	U	U	U	U	41.8	40.3/U	39.5	38.5
Oral swab N-gene	U	U	U	U	U	U	38.9	35.6	34.4
Faeces P-gene	N/A	41.1/U	U	U	42.2	U	39.2	39	34.5
Faeces N-gene	N/A	U	U	U	U	U	35.5	32	32.8

Viral genetic material was not detected consistently in nasal swabs from this horse until Day 7 PI when fever was established, although a positive Taqman for the N-gene was observed on Day 2 PI at the time of the transient serous nasal discharge. The steady fall in Ct values thereafter is consistent with viral replication and shedding into the nasal cavity in nasal secretions. From day 7 PI, Ct values in blood were of the order associated with successful recovery from virus from tissue samples (solid organs) using traditional virus isolation techniques. Genome was detected in blood at least one full day (Day 5) prior to the onset of fever. When fever was established (Day 7) but while the horse was otherwise clinically normal, viral genome was detected additionally in oral swabs, urine and feces but at an indeterminate level in the rectal swab. It is possible that fecal material on the floor of the pen may have been contaminated by urine containing viral genetic material; it is also possible that the smaller amount of material collected on the rectal swab influenced the sensitivity of the test. Similar results were obtained on Day 8 when clinical disease was readily apparent. The data from this horse also suggest that the Taqman PCR for the N gene may have a slightly higher diagnostic sensitivity than the Taqman PCR for the P gene.

Following post mortem examination, virus was reisolated from guttural pouch; sub-mandibular, inguinal, bronchial and renal lymph nodes; lung; kidney, heart, adrenal gland, spinal cord, brain, cerebrospinal fluid and meninges.

Taqman assays for the P and N genes were positive for all tissues sampled at post mortem. Generally, the highest levels of target genes were associated with tissues from which live virus was also recovered.

Summary comments and recommendations

- Systemic HeV infection was established in 3/3 horses challenged by the oronasal route with 2 x 10^6 $TCID_{50}$ HeV (Redland Bay 2008). In 2/3 horses, the experimental data strongly suggested that systemic spread was preceded by local viral replication in the nasal cavity or nasopharynx.

- The Redland Bay 2008 isolate of HeV showed 98% similarity at the nucleotide level to the reference Genbank sequence (an isolate from a horse obtained during the '94 outbreak). At the amino acid level, this increases to 99.6% similarity. Observations made during experimental infection of horses with Redland Bay 2008 were generally comparable to those recorded in earlier studies using the original Hendra isolate. Together, these data suggest that there has been no significant change in the virus over intervening years, at least in respect of infectivity and tissue tropism in horses. The prominent neurological signs that were noted in field cases of HeV Redland Bay 2008 may reflect part of the normal spectrum of HeV in horses, a spectrum that might include the influence both of the infectious dose and, particularly, the route of exposure.

- The mode and critical control points of HeV spillover to horses from flying foxes remain uncertain. On current knowledge, the risk of human infection and spread to

additional horses is best managed by controlling viral spread from the known infected horse. Essential elements of such control include early consideration of HeV in the differential diagnosis of horses that are unwell, accompanied by adoption of infection control practices that may be adjusted on the basis of assessed exposure risk.

- Early diagnostic consideration will be facilitated by emphasis on the pro-dromal and early clinical signs of HeV in infected horses within guidelines and educational materials prepared for the veterinary profession and also the general public. It is noteworthy that none of the three animals in this study fulfilled the currently available case definition for even a “possible” case of HeV in horses. In particular, more conservative estimates on elevated temperature and heart rate should be advised as part of an early warning strategy. Current emphasis on later, nigh terminal, clinical signs to compose the case definition is insufficient to prevent future exposure of veterinarians or animal owners.

- The route of natural infection of horses by HeV is not known, but it is likely that primary exposure occurs via the upper respiratory tract and/or the oropharynx. In previous experimental studies using HeV, either route has been used to successfully establish infection. The data from this study indicate that a transmission risk may be posed by the nasal secretions of asymptomatic horses during the early disease phase that precedes systemic infection. The data also suggest that the risk provided by these animals is relatively low, compared to animals in the immediate pre-symptomatic and symptomatic stages of infection. On the other hand, duration of exposure also contributes to infection risk, and there may be certain routine contacts or procedures that may increase operator risk at this stage of infection, such as nasal intubation or possibly routine dental procedures. Practical strategies for infection control in these situations should be developed for incorporation into published guidelines.

- The experimental data confirm that the febrile, and then the symptomatic , horse likely poses a greater transmission risk, and from a variety of its excretions. Additional detail on optimal samples (including blood, oral and nasal swabs, urine and feces) for diagnostic confirmation might usefully be incorporated into published guidelines for handling suspect cases, including collection and handling processes that reduce the exposure risk to veterinarians and animal attendants. Most febrile horses will of course not be infected with HeV but they may require ongoing veterinary interventions for their underlying disease. This will need to be taken into account in development of infection control guidelines for such cases, so that ongoing compliance is encouraged until the HeV diagnosis is confirmed or excluded.

- Not surprisingly, the activity that is likely to pose the highest transmission risk is post mortem examination, on account of the virus load present in the animal at the time, the opportunity for gross contamination of operator and assistants with infective material, and the inherent risks associated with the handling of sharps. Complete post mortem examination of affected animals remains of particular value when atypical disease is suspected or in other situations where a diagnosis is essential, such as where human exposure is suspected or transmission may have occurred to other animals. This can be carried out safely by suitably experienced and equipped operators with strategies and infrastructure for personnel and environmental decontamination. The most cost-effective strategy will likely be trained diagnostic teams that are maintained nationally for this work. For inexperienced operators or those ill-prepared to safely manage such a procedure, and especially where diagnostic confirmation of a typical HeV case is sought, limited collection of tissues such as superficial lymph nodes may provide least exposure risk.

- Horses in the prodromal stages of infection, i.e. febrile but otherwise well, are unlikely to have come to the attention of a veterinarian. However, there is clearly the potential for case confirmation early in the symptomatic phase of disease and still prior to the period when transmission risk is likely to be highest. Availability of such a test at the stall-side would assist decisions associated with infection control and also

subsequent handling of suspect cases with respect to administration of veterinary care. Determination of the most suitable technologies for this purpose and their application to a sensitive and specific stall-side test remain key challenges for the future.

Acknowledgments:

Thanks are given to the Queensland Department of Primary Industries, and to the following staff of CSIRO AAHL: Jessica Klippel, Timothy Hancock, John Muschialli, Rachel Robinson, Adam Foord, Hans Heine, Jennifer McEachern, Gary Crameri, Shane Riddell and John White.

This work was supported by funds supplied by the Australian Biosecurity CRC for Emerging Infectious Disease.

.