

## REVIEW

# Emerging tropical diseases in Australia. Part 5. *Hendra virus*

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*Hendra virus* (HeV) was first isolated in 1994, from a disease outbreak involving at least 21 horses and two humans in the Brisbane suburb of Hendra, Australia. The affected horses and humans all developed a severe but unidentified respiratory disease that resulted in the deaths of one of the human cases and the deaths or putting down of 14 of the horses. The virus, isolated by culture from a horse and the kidney of the fatal human case, was initially characterised as a new member of the genus *Morbillivirus* in the family *Paramyxoviridae*. Comparative sequence analysis of part of the matrix protein gene of the virus and the discovery that the virus had an exceptionally large genome subsequently led to HeV being assigned to a new genus, *Henipavirus*, along with *Nipah virus* (a newly emergent virus in pigs).

The regular outbreaks of HeV-related disease that have occurred in Australia since 1994 have all been characterised by acute respiratory and neurological manifestations, with high levels of morbidity and mortality in the affected horses and humans. The modes of transmission of HeV remain largely unknown. Although fruit bats have been identified as natural hosts of the virus, direct bat–horse, bat–human or human–human transmission has not been reported. Human infection can occur via exposure to infectious urine, saliva or nasopharyngeal fluid from horses. The treatment options and efficacy are very limited and no vaccine exists.

Reports on the outbreaks of HeV in Australia are collated in this review and the available data on the biology, transmission and detection of the pathogen are summarized and discussed.

## HISTORY AND CASES

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In September 1994, an acute respiratory disease was reported in 21 horses on a property in the Brisbane suburb of Hendra (in Queensland, Australia). The equine illness was characterised by high rectal

temperatures, acute respiratory difficulty and influenza-like symptoms, including nasal discharge (Murray *et al.*, 1995). This disease resulted in the death or putting down of 14 of the horses within 2 weeks (Fig. 1). A trainer and a stable-hand who had had close contact with an infected, pregnant mare, that had died of similar symptoms 2 weeks prior to the main outbreak, developed symptoms of acute respiratory disease (Murray *et al.*, 1995). Although the stable-hand made a full recovery, the trainer died after 6 days of interstitial pneumonia. Virus was isolated, by culture, from lung tissue of one of the affected horses post-mortem and inoculated into two healthy horses to examine the aetiology of the infecting agent. Both inoculated horses became ill, with high fever and respiratory disease, after an incubation period of 6 or 10 days (Murray *et al.*, 1995). The virus involved was identified, by comparative sequence analysis of part of the matrix protein gene, as a member of the genus *Morbillivirus* (Murray *et al.*, 1995; St Georgiev, 2009). Although initially named *equine morbillivirus*, the pathogen was subsequently renamed as *Hendra virus* (HeV) after the suburb of Hendra, where the virus was first isolated (Field *et al.*, 2010). As the result of phylogenetic analysis and the observation of the structure and large size of the virus, Wang *et al.* (2000) placed HeV in a new genus, *Henipavirus*, within the family *Paramyxoviridae* (Fig. 2).

Although a retrospective study was performed, on laboratory records and stored specimens, including samples from rodents, marsupials, birds, amphibians and insects, no evidence of HeV infection prior to 1994 was found (Field *et al.*, 2001; Halpin and Mungall, 2007). The 1994 outbreak therefore probably signalled the emergence of a novel virus.

Since the initial outbreak in Hendra, there have been numerous other reports of HeV-related disease in Australian horses, with occasional transmission to humans. In 1995, a second, fatal, human case of HeV

was reported from the Queensland city of Mackay (Rogers *et al.*, 1996). This case was linked to two horses that had died, on the case's property, in 1994 — from what was initially believed to be avocado poisoning or snake-bite but retrospectively confirmed to be HeV infection. HeV activity was reported again in 1999 in Cairns, Queensland, when a thoroughbred mare suddenly died of respiratory illness (Field *et al.*, 2000). At death, the horse displayed a yellow, frothy, nasal discharge from which HeV was isolated. After no recorded activity for another 5 years, HeV caused morbidity in horses twice in 2004, once in Cairns (again) and once in Townsville, Queensland (Hanna *et al.*, 2006). Each of the incidents resulted in the death of a single horse. Although the veterinarian attending the Cairns case developed clinical illness, she recovered and, at the time the report of her illness was published, remained clinically well. Since 2006, HeV-related morbidity has been reported each year in Queensland, resulting in the deaths of two more humans and 15 more horses ([www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/nipah.htm](http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/nipah.htm)) and HeV-related disease becoming notifiable in Australia (Field, 2009; R. Simmons, unpubl. obs.).

Of particular relevance to the emergence of HeV was an outbreak of an acute respiratory illness in pigs on a farm in peninsular Malaysia in 1998, which resulted in the death of 105 people and the culling of over a million pigs (Chua *et al.*, 2000). Like HeV, the aetiological agent in this outbreak — named *Nipah virus* (NiV) — was characterised as a member of the family *Paramyxoviridae*. Several outbreaks of NiV-related disease have occurred in Central and South-east Asia since 1998 (St Georgiev, 2009). Since HeV and NiV display similar genomic and physical characteristics, including having fruit bats as natural hosts and causing a similar disease syndrome in humans, NiV was included as a member of the new genus *Henipavirus* by Chua *et al.* (2000).

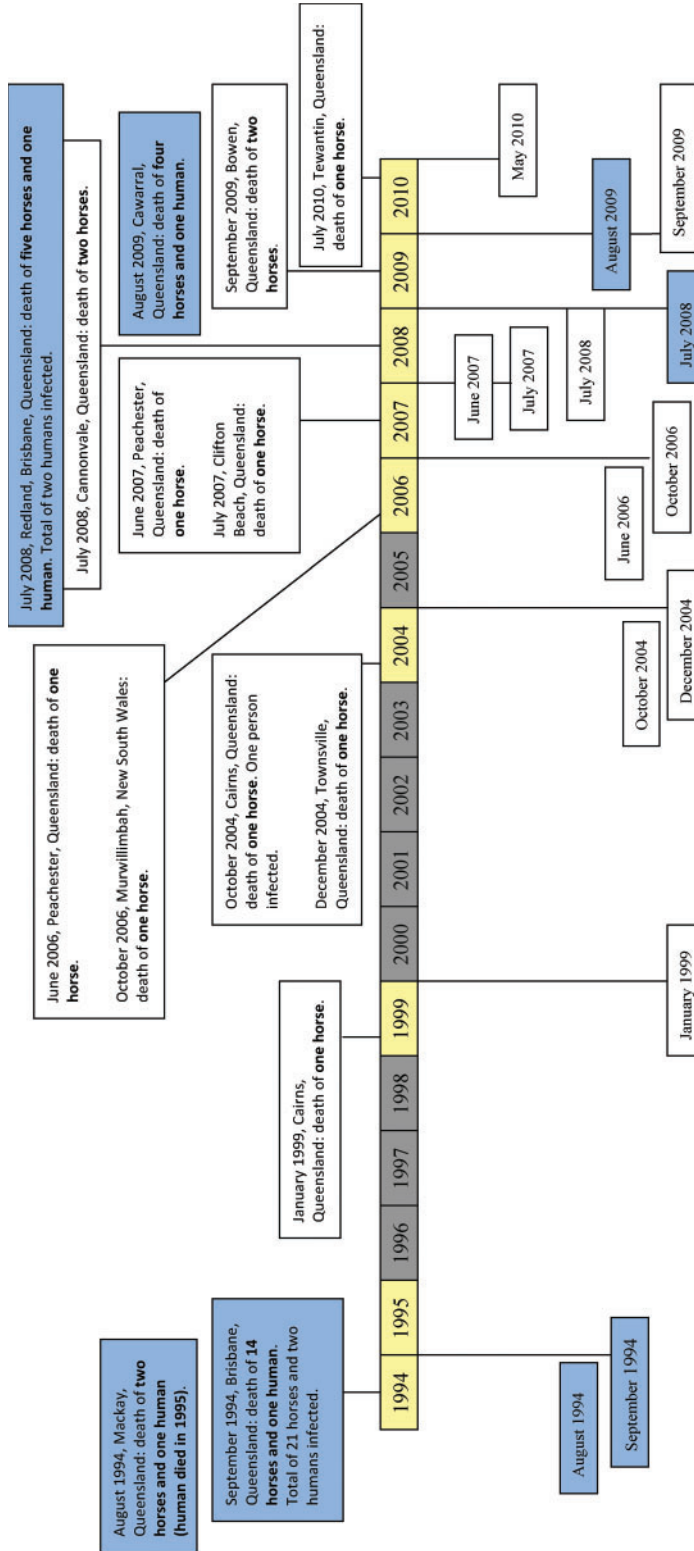


FIG. 1. The recorded *Hendra-virus* infections in horses and humans in Australia, the blue-shaded boxes indicating outbreaks with human fatalities.

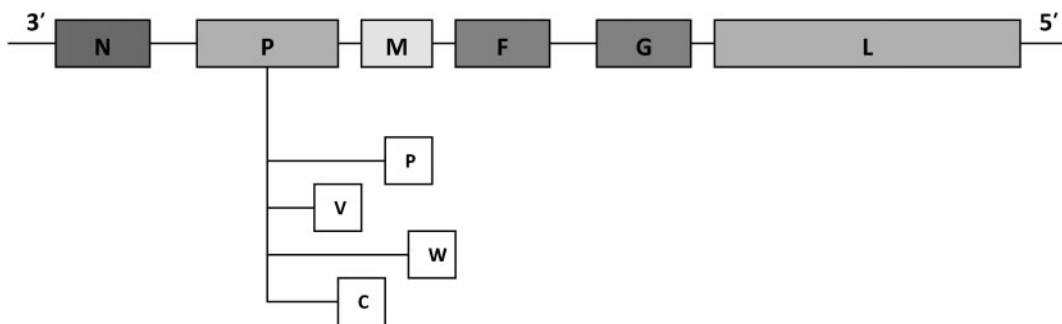


FIG. 2. The six transcription-unit genome of *Hendra virus*, showing the positions of the nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), glycoprotein (G) and large-polymerase (L) genes. The components, P, V and W, are P-gene products encoding the C protein.

### CHARACTERISTICS AND TAXONOMY OF THE VIRUS

HeV belongs to the family *Paramyxoviridae*, the members of which are characterised by having a non-segmented, negative-strand RNA (NNS) genome (Wang *et al.*, 2000). Both members of the genus *Henipavirus* (i.e. HeV and NiV) have a linear ribonucleoprotein core (RNP) that is characteristic of other paramyxoviruses. Structurally, the RNP core consists of a single-stranded genomic RNA molecule to which nucleocapsid proteins (N) are bound. The genomic RNA strand exhibits negative polarity, with one N bound to the genome for every six nucleotides (Calain and Roux, 1993). Each henipavirus is spherical or filamentous, measuring 150–200 nm in diameter and up to 10,040 nm in length, and has surface projections (St Georgiev, 2009). The *Henipavirus* genome is markedly longer than that of other members of the *Paramyxoviridae* (typically 15.1–15.9 kb; Wang *et al.*, 2000). The genome of HeV is 18,234 nucleotides long and arranged as 3'-N-P/V/C-M-F-G-L-5' (Fig. 3; Wang *et al.*, 2000). Nucleoproteins (N), smaller phosphoproteins (P) and a large polymerase protein (L) constitute the RNP core, which is enveloped by a receptor-binding glycoprotein (G) and a fusion protein (F) (Bishop *et al.*, 2007). The G and F proteins appear as the characteristic spikes on the surface of

the RNP core and, combined with the RNP core, are responsible for the transcription of genomic RNA to messenger RNA (St Georgiev, 2009). The matrix protein (M), which lies beneath the viral envelope and is known to be released into host cells from the RNP core, completes the architecture of HeV.

Although the genome of HeV is in many ways similar to those of other paramyxoviruses, several features distinguish HeV. The most obvious of these is the large genome size of HeV, which is primarily attributable to the P and L proteins (Wang *et al.*, 1998, 2000). Sequencing of the L protein of HeV has revealed a conserved intergenic trinucleotide sequence, 3'-GAA-5', and a stop signal that is similar to those of members of the genera *Respirovirus* and *Morbillivirus* (Wang *et al.*, 2000). It was on the basis of the phylogenetic analysis of RNA polymerase (L) genes that a new genus, *Henipavirus*, was created within the sub-family Paramyxovirinae to accommodate the two 'new' viruses, HeV and NiV (Gould, 1996; Yu *et al.*, 1998; Wang *et al.*, 2000).

The complete genome of NiV has still to be reported but this virus appears to be fairly closely related to HeV, showing antigenic similarities, similar amino-acid sequences in its N (92% identity), P (71%), and L (87%) proteins (Halpin *et al.*, 2007) and sequence homology in its N, P, M, F and G regions

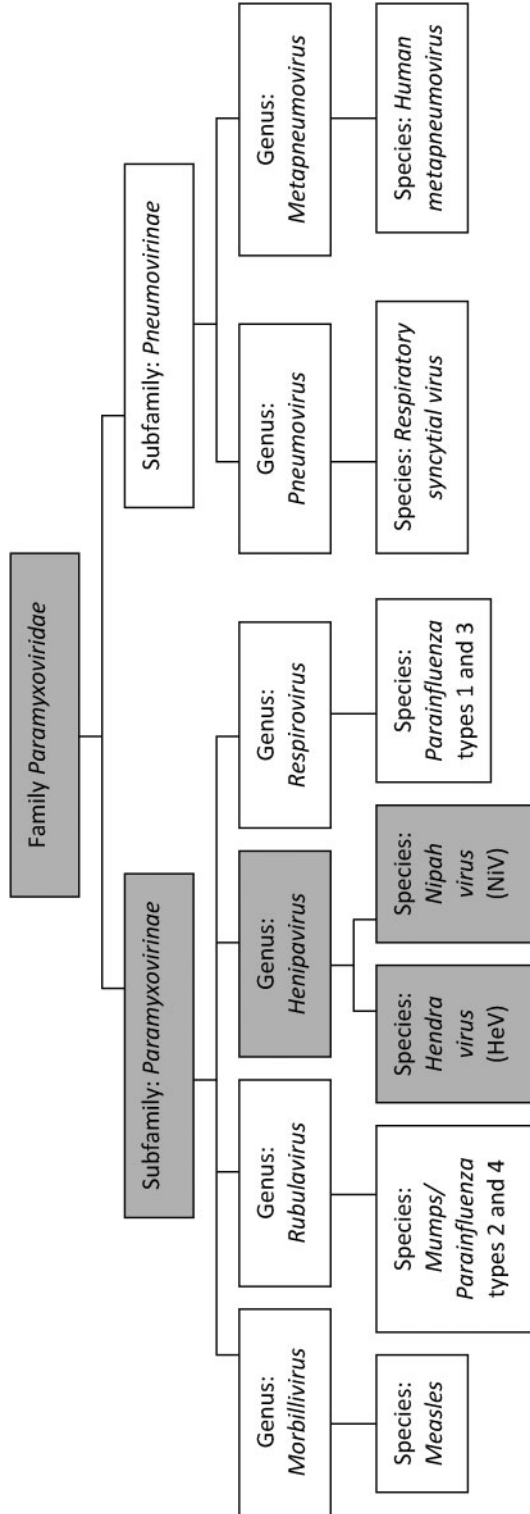


FIG. 3. The phylogenetic position of *Hendra virus* within the family *Paramyxoviridae* and genus *Henipavirus* (Wang *et al.*, 2000).

(Harcourt *et al.*, 2000). There are, however, considerable differences between the genomes of HeV and NiV. Among 1301 bp in a region from the M gene, for example, the HeV originally isolated in 1994 was found to differ by only five base pairs from an HeV isolated, from another horse, in 1999 but by 408 bp from an NiV isolate (Chua *et al.*, 2000). In addition, an open reading frame (ORF) believed to encode a short basic protein in the P gene of HeV is absent from NiV (Wang *et al.*, 1998).

### INFECTION AND DISEASE SYNDROMES

So far, there have been only 14 independent incidents of equine and/or human HeV infection detected in Australia (Fig. 1). Horses infected with HeV display severe respiratory and/or neurological symptoms in most cases and, as such, the HeV-related disease is colloquially known as equine respiratory syndrome or equine *Morbillivirus* pneumonia (St Georgiev, 2009). The incubation period of HeV in horses seems to range between 48 h and 16 days (Baldock *et al.*, 1996; St Georgiev, 2009; Field *et al.*, 2010). Although some variation in the clinical manifestations of HeV infection — which may be attributable to differences in the virus strain circulating in each outbreak (Field *et al.*, 2001) — has been observed, respiratory and/or neurological distress can be ascribed as the primary manifestations of HeV infection in both horses and humans. In the original outbreak of 1994, acute respiratory disease with high fevers was reported in the affected horses while influenza-like symptoms were reported in the affected humans (Murray *et al.*, 1995). In the outbreak that occurred in 2008, however, the main manifestations in the affected horses and humans were neurological (Field *et al.*, 2010). In parallel with the respiratory and neurological illness, HeV infection in horses is associated with facial swelling, a frothy nasal discharge and ataxia (Hooper

*et al.*, 1997). Meningitis has occurred in at least one human infected with HeV (O'Sullivan *et al.*, 1997) and late-onset encephalitis, which may develop up to 8 months post-exposure, has been reported in humans infected with NiV (Tan *et al.*, 2002).

### TRANSMISSION DYNAMICS

Direct infection of humans and horses with HeV appears to occur as a result of contact with infectious material from horses. HeV has been isolated from the nasopharyngeal secretions, saliva, urine, foetal material and organs of horses (Daniels *et al.*, 2001) and humans or horses coming into contact with such (presumably) infectious materials are at risk of infection (Field *et al.*, 2007). Transmission from fruit bats of the genus *Pteropus* — which are believed to be a natural host of HeV (Halpin *et al.*, 2000) — to horses probably occurs but has not been confirmed. Direct human–human transmission has also not been reported. In challenge trials in animal models, HeV has been found in the urine and saliva of experimentally infected cats, and a horse held near the experimentally infected cats showed signs of HeV infection (Williamson *et al.*, 1998). In the same trials, attempts to show horse–cat and fruit-bat–horse transmission were, however, unsuccessful.

To explore the possibility of transmission by vectors, blood-fed mosquitoes (from the properties affected by HeV outbreaks in Brisbane and Mackay) and *Cyclopodia albertisii* (obligate ectoparasites of fruit bats) have been surveyed for HeV (Field *et al.*, 2001). Although one pooled sample of mosquitoes was found PCR-positive for the virus, all subsequent investigations of potential vectors have given negative results.

HeV has been detected in all four species of fruit bat found on mainland Australia (*Pteropus conspicillatus*, *P. alecto*, *P. poliocephalus* and *P. scapulatus*) and isolated from three of the species (Mackenzie, 1999;

Halpin *et al.*, 2000; Field *et al.*, 2001, 2007). Remarkably, when Field *et al.* (2001) checked >1000 fruit bats for anti-HeV antibodies, almost one in every two of the bats (47%) was found seropositive. Caution when handling fruit bats is therefore warranted, as is the adherence to strict universal precautions when in contact with an infected human.

Given the high seroprevalence of HeV in fruit bats and the presence of spatial and temporal clustering in the known outbreaks of HeV in Australian horses and humans, viral spill-over from fruit bats to horses seems a plausible explanation of the equine infections (Field *et al.*, 2007). All known outbreaks of HeV in horses and humans have occurred in tropical or sub-tropical regions of Australia where fruit bats are common, either in Queensland or northern New South Wales. There appears to be some evidence of seasonality in the known outbreaks, all but four of which have occurred in June, July, August or September (Fig. 1), and this may be indicative of a seasonal cycle in viral excretion by infected fruit bats (Field *et al.*, 2007).

## PATHOGENESIS AND TREATMENT

The molecular mechanisms that lie behind the pathogenicity and virulence of HeV have been investigated in some detail. Generally, pathogenicity in the *Henipavirus* genus is dependant on the ability of the viruses to circumvent the host's cellular interferon response (Guillaume *et al.*, 2004), which is believed to be controlled by the P-gene products: P, V, W and C (Eaton *et al.*, 2005; Shaw *et al.*, 2005). As indicated by their names, the cell-attachment glycoprotein (G) and the fusion protein (F) play a significant role in the attachment and fusion of the viruses to host-cell receptors (Bossart *et al.*, 2005). Interestingly, both HeV and NiV display attachment properties akin to those of the *Pneumovirinae*, with a type-II membrane G glycoprotein that does not

haemagglutinate or show neuraminidase activity (Yu *et al.*, 1998). Unlike the *Pneumovirinae*, however, henipaviruses can use cell-surface protein receptors to bind in the absence of the N-acetyl neuraminic acid that is usually found on the outer membrane of mammalian cells (Eaton *et al.*, 2004).

The features of HeV and NiV pathogenicity, along with the interferon system, have offered significant targets in the development of vaccines against the viruses. Despite a focus on NiV vaccines (Guillaume *et al.*, 2004; Tamin *et al.*, 2009), an HeV vaccine has been trialled in laboratory ferrets and is expected to be released within the decade ([www.csiro.au/science/Hendra-Virus.html](http://www.csiro.au/science/Hendra-Virus.html)). An antiviral drug, ribavirin, has recently been demonstrated to reduce viral load in HeV infection (Williamson and Torres-Velez, 2010) although the drug's use to treat patients infected with HeV has met with varied success (Wright *et al.*, 2004; Playford *et al.*, 2010). Prophylactic immunoglobulins (Ig) are now available in Queensland and are soon to be offered to individuals considered to be at-risk of HeV infection (R. Simmons, unpubl. obs.). In the absence of reliable drug therapy, rapid detection and careful adherence to disease-control procedures are currently the most efficient strategies in the management of HeV outbreaks.

## DIAGNOSIS AND DETECTION

The isolation of the virus from a fluid or tissue sample is the definitive method of confirming HeV infection (Hyatt *et al.*, 2001). Clinical samples can be inoculated onto Vero or RK13 cells (Halpin *et al.*, 2000; Rockx *et al.*, 2010), with the subsequent formation of syncytia and punctate holes in the surface of the cell monolayer indicative of the presence of HeV (or NiV). Each syncytium contains at least 20 nuclei and the arrangement of the nuclei within a syncytium can be used to differentiate between HeV and NiV (Hyatt *et al.*,

2001). HeV has been isolated from several different tissues but attempts at viral isolation are often supported by serological, enzymatic and genetic procedures (Daniels *et al.*, 2001). In fatal infections, the virus can be cultured from tissue samples collected post-mortem, including liver, spleen and kidney necropsies. In non-fatal cases, HeV may be isolated from throat swabs, urinary-tract swabs and/or serum (Williamson *et al.*, 1998; Field *et al.*, 2010).

Given the high mortality associated with HeV infection in humans and horses and the lack of an effective vaccine or treatment, samples suspected of containing the virus should only be cultured in laboratories with the highest biosafety classification (i.e. level 4; Williamson and Torres-Velez, 2010). In outbreak situations in Australia, serological testing, such as ELISA, can be performed at level-3 facilities or even, if the serum has been pre-treated to inactivate the virus, at level-2 facilities (Daniels *et al.*, 2001). Once the virus is isolated, characterisation and confirmation tests may be performed by the inoculation of uninfected animals such as golden hamsters (Williamson and Torres-Velez, 2010) as well as by immunohistopathology (Guillame *et al.*, 2009), serology (Black *et al.*, 2001) and using PCR (Smith *et al.*, 2001).

### **Immunohistochemistry (IHC) and Electron Microscopy**

One of the most successful and basic tests for the detection of HeV post-mortem involves the use of a *Hendra*-specific monoclonal or polyclonal antiserum on formalin-embedded tissue, to detect HeV-specific antigen (Daniels *et al.*, 2001). Many different tissue samples can be used for such IHC, including spleen, lung, brain, mediastinal lymph nodes, kidney, uterus, placenta, and foetal matter. Although primary infection occurs in the vascular endothelium, HeV antigens may be cleared from the lung relatively soon after infection and tissue sampling should therefore not be confined to the lung (Hooper *et al.*, 2001).

Following the post-mortem isolation of virus, electron microscopy can be used to demonstrate HeV in viral cultures. At a minimum density of  $10^8$  plaque-forming units/ml, viral cultures can be viewed either directly after negative staining or after specific labelling using immuno-electronmicroscopy techniques (Hyatt *et al.*, 2001).

### **Serology**

The serum neutralization test is the 'gold standard' reference test for HeV. The neutralization test works on the principle that the usual cytopathogenic effect (CPE) of the virus, on a cell monolayer, will be blocked by the HeV-specific antibodies present in serum from an animal that, or patient who, is seropositive for HeV. In the standard assay, a test serum, which may be diluted 1:2 or even 1:5 if the serum sample is small (Halpin *et al.*, 2000), is incubated with HeV before the virus is added to a Vero-cell monolayer in culture. The cultures are checked for a CPE 3 days later. In the last decade, the standard serum neutralization test has been modified to produce a rapid, immune plaque assay that takes advantage of the development of syncytia in HeV-infected Vero-cell cultures within 24 h of culture inoculation and allows viral load to be quantified (Cramer *et al.*, 2002). This plaque assay can be used to test sera after any viruses present have been inactivated with methanol and gives a result after just 1 day of incubation. In situations requiring urgent, rapid detection, such as an outbreak, ELISA and real-time PCR are, however, more commonly used. Although an indirect ELISA for the detection of anti-HeV antibodies is the test that is most routinely employed, antigen-capture ELISA are rapidly gaining credence and Luminex-based tests are also being used (Daniels *et al.*, 2001; Hayman *et al.*, 2008; Chiang *et al.*, 2010).

### **Real-time PCR**

A real-time assay based on a TaqMan PCR has been used for the detection of HeV in Australia (Halpin *et al.*, 2000; Smith *et al.*,



2001). For this test, RNA is extracted, either using a commercial, viral-RNA extraction kit or manually (Smith *et al.*, 2001). Complementary DNA (cDNA) is generated from the viral RNA template before the cDNA is amplified using primers and probes targeting the M gene of HeV (Smith *et al.*, 2001). The sensitivity of this assay, which Smith *et al.* (2001) described as 1000 times better than that of conventional PCR, can be improved by the use of additional primers targeting different regions of the HeV genome (such as the P gene), to the point where direct culture becomes unnecessary (Feldman *et al.*, 2009). Several other, modified PCR-based assays, including some based on TaqMan or SYBR Green, are in use at diagnostic facilities in Australia (R. Simmons, unpubl. obs.). Of these modified assays, one targeted at the N gene and based on SYBR-Green chemistry appears the most sensitive, with a detection limit of just 200 HeV virions (Feldman *et al.*, 2009).

## CONCLUSIONS

The mechanisms of HeV emergence are largely unknown, as are the modes of the pathogen's transmission between fruit bats, horses and humans. Some evidence exists for spill-over from fruit bats to horses during outbreaks (Turmelle and Olivial, 2009) but such transmission remains to be demonstrated (Williamson *et al.*, 1998). From an epidemiological perspective, an understanding of the role of possible secondary or intermediate hosts, such as cats, must also be considered (Hyatt *et al.*, 2004). There is currently no vaccine for this highly pathogenic virus and treatment is restricted to general antiviral drugs or prophylactic immunoglobulin therapy, with limited success. The handling of infectious HeV specimens must therefore be restricted to laboratories with high-level biosafety containment and outbreak situations must be treated with great concern by the relevant

public-health and veterinary authorities. Although HeV is only known to have caused sporadic and relatively minor outbreaks in the past, it is a virus of significant veterinary, medical and biosecurity importance, especially given the high mortality associated with equine and human infections. It must be acknowledged that HeV is a newly emergent virus, with a known history of only 16 years. The transmission routes of the virus need to be identified and the tests for the diagnosis of HeV infection and the strategies for the management of such infection need to be improved, ideally before further, possibly major, outbreaks occur.

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