

Chapter 40

A Review of Hendra Virus and Nipah Virus Infections in Man and Other Animals

Kim Halpin and Paul Rota

Abstract Hendra virus (HeV) and Nipah virus (NiV) emerged in the last decade of the twentieth century. They were the cause of a number of outbreaks of respiratory and neurological disease infecting horses and pigs respectively. Transmission from infected domestic animal species resulted in human infections as well, with high case fatality rates a feature. Today they continue to cause outbreaks of human and animal disease. NiV causes yearly disease outbreaks in humans in Bangladesh, and HeV causes sporadic disease outbreaks in horses in north eastern Australia. Due to their zoonotic nature, they have been ideal candidates for collaborative projects in the One Health space, bringing public health and animal health professionals together. This has led to insightful epidemiological studies, which has resulted in practical disease prevention solutions including a horse vaccine for HeV and NiV spill-over prevention interventions in the field. As more surveillance is undertaken, their known distributions have expanded, as has the range of reservoir host species. The majority of bat species for which there is evidence of henipavirus infection belong to the group known as the Old World family of fruit and nectar feeding bats (Family *Pteropodidae*, Suborder *Megachiroptera*). This review of the bat borne henipaviruses discusses the epidemiology, pathology, transmission and disease symptoms in these closely related viruses which belong to the Genus *Henipavirus*, Family *Paramyxoviridae*.

40.1 Epidemiology of Hendra Virus in Animals

On the 1st August 1994, a heavily pregnant 10 year old thoroughbred brood mare died suddenly in a paddock in northern coastal Australia. Ironically the first of August is deemed the birthday of all thoroughbred horses in the southern hemisphere¹.

¹ All thoroughbreds have the same birthday so that their ages can be standardized for comparison. In the southern hemisphere the date is the 1st August.

K. Halpin (✉)
Australian Animal Health Laboratory, Geelong, Australia
e-mail: kim.halpin@csiro.au

P. Rota
Division of Viral Diseases Centres for Disease Control & Prevention, Atlanta, USA
e-mail: prota@cdc.gov

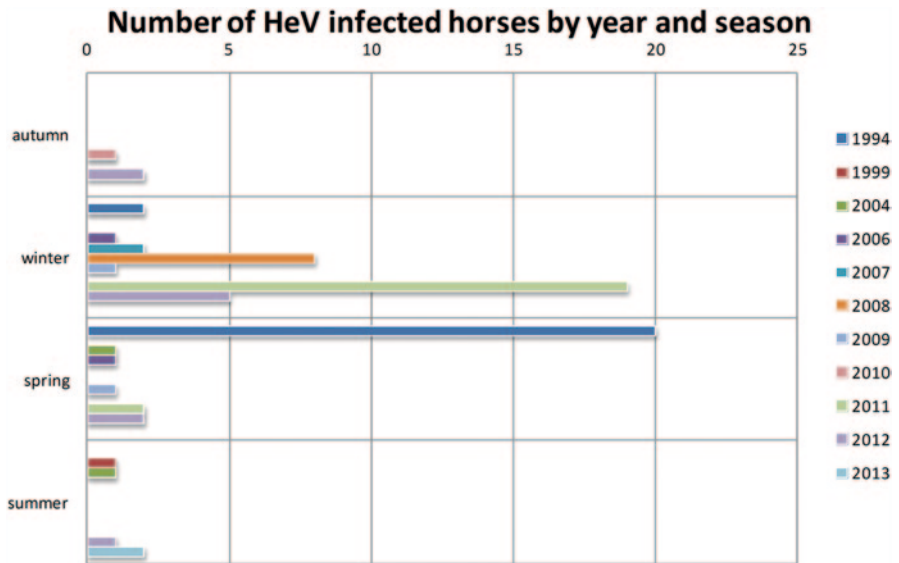


Fig. 40.1 Total number of HeV infected horses by year and season. Winter and spring are the months when three Australian species of pteropus bats are either pregnant or giving birth

However this date in 1994 would go down in history as the day Hendra virus (HeV) emerged.

Hendra virus was first discovered in horses, and horses remain by far the most commonly infected domestic species. The reservoir host of this virus are bats of the genus *Pteropus*. Finding the reservoir host for this virus resulted in the first reported isolation of a zoonotic paramyxovirus from bats (Halpin et al. 2000). To date, only horses have become directly infected from bats. Horses act as HeV amplifying hosts.

As of April 2013, there have been 42 spill-over events between horses and bats with most events involving only one infected horse. In total, 73 horses have been infected (Fig. 40.1). Only two outbreaks have involved more than three horses, and the spread of the virus between the stabled horses in these outbreaks was a result of close contact and assisted mechanical transmission of the virus. Aerosol transmission is unlikely as sneezing and coughing were not features of the syndrome and the spatial distribution of cases in the stables was not consistent with this form of spread (Baldock et al. 1996; Field et al. 2010).

The precise mortality rate in horses has not been possible to calculate as all live horses diagnosed with Hendra virus infection are euthanized according to national policy², presumably to prevent relapsing infection and possible further transmission. Experimentally we know that some horses can survive infection. In the outbreaks where there have been numerous horses infected this has also been the case.

² Australian veterinary emergency plan. Version 3.3; 2009 [cited 2013 Mar 28] <http://www.animalhealthaustralia.com.au/wp-content/uploads/2011/04/RPB3.3-05FINAL27Jul11.pdf>.

In the 1994 outbreak in Hendra, seven out of twenty horses apparently survived a lethal infection, and seroconverted before being euthanized (Murray et al. 1995) [refer to clarification]. In the 2008 Redlands outbreak, one horse survived for 42 days after clinical signs abated before being euthanized (Field et al. 2010).

Experimentally, cats can be infected and succumb to the virus (Westbury et al. 1995; Williamson et al. 1998). Anecdotally there was a story about a cat that became sick in the suburb of Hendra at the time of the first recognized outbreak. The cat was never identified and a diagnosis of the cat's condition was never elucidated. It perhaps remains an urban myth, but given the experimental evidence it seems likely that a cat could become infected if it came into close contact with high titres of virus which horses are able to generate (Williamson et al. 1998).

In one of the 2011 outbreaks, a dog on an infected property was found to have antibodies to the virus. It most likely had close contact with HeV laden material from an infected horse, but was clinically well and remained so until it was euthanized in accordance with national policy². Since this time, experimental infections in dogs have been conducted at the Australian Animal Health Laboratory, to better understand the role that dogs might play in the epidemiology of the disease. In these studies dogs could be reliably infected with HeV. Consistent with the field observation, few if any clinical signs were noted during the acute stage of infection. Viral shedding from the oral cavity occurred for a relatively short period of time, and oral secretions collected from dogs during this period were capable of transmitting infection to naïve ferrets (Middleton 2013). Neutralising antibody titres generated in these dogs were similar to that observed in the single recorded canine field case of HeV infection. In dogs, the key site of virus replication within the oral cavity was the tonsil. Middleton (2013) concludes that it is feasible for Hendra virus to be transmitted to people from acutely infected dogs. Further studies are planned to test the repeatability of these observations as well as to assess the duration of the period of maximum transmission risk.

Infection in horses most likely occurs after close contact with bat urine, spats and birthing material which contain sufficiently high enough titres of virus to infect a horse. Luckily for horses, these bat samples rarely contain high titres of virus. The risk of transmission to horses was found to be increased during pteropus bat reproductive periods (especially late pregnancy) and at times when the colonies were undergoing nutritional stress (such as during lactation), presumably leading to higher viral loads (Plowright et al. 2008; Breed et al. 2011). The reproductive cycle in other bat species has been linked to seropositivity and viral activity of other viruses including filoviruses, coronaviruses, lyssaviruses and astroviruses (Pourrut et al. 2007; Drexler et al. 2011; Turmelle et al. 2010).

Hendra virus is present in all four mainland *Pteropus* species of bats in Australia, namely the black flying fox (*Pteropus alecto*), the grey headed flying fox (*P. poliocephalus*), the spectacled flying fox (*P. conspicillatus*) and the little red flying fox (*P. scapulatus*).

It appears that the reservoir host co-exists with this virus in complete harmony. The virus spreads quite easily amongst bats, with the HeV seroprevalence in bat colonies fluctuating over time and geographical spread. In one bat colony,

seroprevalence steadily increased from 45 to 69% over a 2 year period supporting a model of endemic infection in the population (Breed et al. 2011). Absence of disease attributable to HeV infection is supported by experimental observations (Halpin et al. 2011). This is consistent with the observation that many viruses do not cause disease in their reservoir host. The long-term coexistence of viruses and their reservoir hosts has given co-evolution a good chance to reach a relative equilibrium (Domingo 2010). The theory of viral co-evolution with chiropteran hosts has been previously suggested, and all field observations and experimental evidence to date supports this (Halpin et al. 2007).

40.2 Epidemiology of Nipah Virus in Animals

Since HeV was detected in fruit bats of the *Pteropus* genus, these bats were among the first species investigated as possible reservoirs for Nipah virus (NiV) after its emergence in 1999 (Halpin et al. 2000). Neutralizing antibodies to NiV were detected in *Pteropus hypomenalus* and *Pteropus vampyrus* during wildlife surveillance following the initial NiV outbreak in Malaysia in 1999, but NiV was not isolated at this time (Yob et al. 2001). The first NiV isolates from bats were obtained from colonies of *Pteropus hypomenalus* on Tioman Island, Malaysia (Chua et al. 2002). Since then, antibodies to NiV have been detected in other *Pteropus* species (*Pteropus lylei*, *Pteropus giganteus*) and less frequently in other species of bats including *Hipposideros larvatus* and *Scotophiilus kuhli* from Cambodia, Thailand, Indonesia, and Bangladesh (Hsu et al. 2004; Reynes et al. 2005; Sendow et al. 2006; Wacharapluesadee et al. 2005). In 2000, NiV was isolated from a urine sample collected underneath the roost of *Pteropus lylei* bats in Cambodia (Reynes et al. 2005). More recently, Nipah virus was isolated from *Pteropus vampyrus* in Malaysia (Sohayati et al. 2011). Serologic evidence of Nipah infection was also obtained from *Rousettus leschen* and *Cynoptera sphinx* in Vietnam (Hasebe et al. 2012). Several species of Chinese bats also contained antibodies to Nipah or Nipah-like viruses (Li et al. 2008). A very thorough study of the presence of henipaviruses in Australasia indicated that NiV was present in East Timor and that non-NiV, non-HeV henipaviruses were present in Sumba, Sulawesi, and possibly Papua New Guinea (Breed et al. 2013). The authors suggested that NiV can be detected in areas where *Pteropus vampyrus* is present. In Madagascar, seropositive *Pteropus rufus* and *Eidolon dupreahum* bats have been found, and 39% of *Eidolon helvum* from Ghana had NiV reactive antibodies (Hayman et al. 2008; Iehle et al. 2007). Henipavirus-like sequences were obtained from *Eidolon helvum* in Ghana (Drexler et al. 2009). The detection of antibodies to and sequences of henipaviruses in African bats suggests that the range of potential NiV infections may be wider than previously thought, though no human cases of NiV have been reported from any region other than Southeast Asia.

Experimentally infected *Pteropus* bats develop subclinical NiV infection with only sporadic viral excretion in urine. Some animals seroconvert and some show evidence of infection by detection of viral antigen in tissues (Middleton et al. 2007; Halpin et al. 2011).

With regard to domestic species affected by NiV, pigs featured in the first outbreak in Malaysia (Chua et al. 2000). Pigs presumably became infected from bats, and the disease spread throughout piggeries with pigs serving as an amplifying host. Most of the human infections occurred in people with direct contact to sick pigs. Serologic studies demonstrated evidence of infection among other domestic species of animals in Malaysia, including horses, dogs and cats (Chua et al. 2000; Hooper and Williamson 2000). In the outbreak in Meherpur, Bangladesh in 2001, close contact to both infected patients as well as to sick cows was associated with NiV infection in humans, although samples from cows were not available for testing (Hsu et al. 2004).

40.3 Epidemiology of Hendra Virus in Humans

It has been estimated that over 700 people have come into contact with Hendra virus infected horses, however, to date it has only been those who have had very close contact with infected bodily fluids through performing invasive procedures, and/or have not worn fully protective gear who have become infected with HeV.

There has been no human-to-human spread of the virus. The first person to become infected and die from Hendra virus was assisting his wife, a veterinarian, to perform an autopsy on a horse that had died suddenly in a paddock (Rogers et al. 1996). This patient recovered from a short illness, but went on to die 13 months later after a relapse with encephalitis (O'Sullivan et al. 1997). In the second outbreak of Hendra virus, the horse trainer and a strapper, who both had very close contact to infected horses in their racing stables, became infected (Selvey et al. 1995). The next person to become infected was a veterinarian who had performed an autopsy on a horse who had died from colic-like symptoms. At the time, colic-like symptoms had never been associated with HeV infection in horses. The veterinarian came down with a flu-like illness, but recovered and to this day has neutralizing antibodies to the virus (Taylor et al. 2012). The next two people to become infected were a male veterinarian who had performed a nasal lavage on a horse which had respiratory symptoms, and the veterinary nurse who had assisted with the procedure (Playford et al. 2010). The veterinarian went on to die. The last person to become infected with Hendra virus was a male veterinarian who cared for a horse which was also diagnosed with Hendra virus (Field et al. 2010).

The human case fatality rate stands at 57%, with four deaths and three survivors. Interestingly to date only male patients have died, however with such a small sample size this should not be over-interpreted.

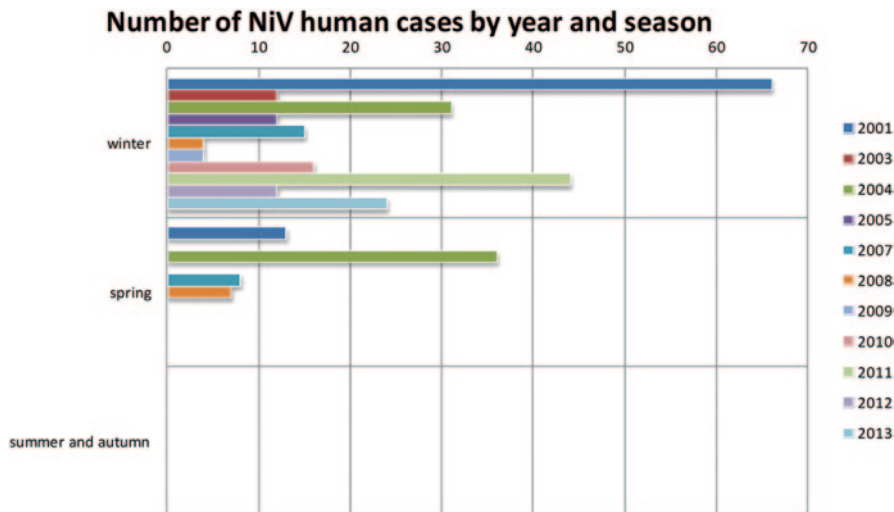


Fig. 40.2 Total number of NiV infected people by year and season in Bangladesh and India. Winter and spring are the seasons when date palm sap is harvested in the region where NiV infections occur. (Source: Luby et al. 2006, Data taken from <http://www.iedcr.org> and http://www.searo.who.int/entity/emerging_diseases/links/nipah_virus_outbreaks_sear/en/index.html)

40.4 Epidemiology of Nipah Virus in Humans

The first detected outbreak of NiV occurred in Malaysia and lasted 9 months. Overall, 276 cases were reported which included 106 deaths (Chua et al. 2000). Nipah virus was transmitted to pigs and spread rapidly among swine herds causing primarily respiratory symptoms in pigs. Pig-to-human transmission resulted in acute febrile encephalitis mostly among adult males who worked in the pig industry. The outbreak spread to Singapore via the transport of infected pigs (Chua et al. 2000; Paton et al. 1999). Culling of more than 1 million pigs was undertaken in an attempt to control the outbreak (Chua et al. 2000).

Since the outbreak in Malaysia, outbreaks have been reported almost annually in Bangladesh and India from 2001 (Fig. 40.2) (Chadha et al. 2006; Luby and Gurley 2012; Luby et al. 2009a). The epidemiologic characteristics of the outbreaks in Bangladesh differed from the Malaysia outbreaks in several respects. Most notably, the case fatality rate in Bangladesh (2001–2010) ranged from 38% to as high as 100%, with an average mortality rate of 73%, while the mortality rate for Malaysia was approximately 38% (Luby and Gurley 2012). Infected individuals in Bangladesh were more likely to have respiratory symptoms, and there was evidence of human-to-human spread.

Luby et al. 2009b showed in their study that Nipah case-patients who had difficulty breathing were more likely than those without respiratory difficulty to transmit Nipah. Although a small minority of infected patients transmit Nipah virus,

more than half of identified cases result from person-to-person transmission. In these cases, virus was spread during close contact while caring for sick individuals or preparing bodies for burial (Blum et al. 2009; Chadha et al. 2006; Luby et al. 2009b).

All confirmed Nipah outbreaks in Bangladesh have occurred in the same central and northwestern regions (Luby et al. 2009b). Notably, the only two outbreaks that have been reported from India have been in regions within 50 km of the border with Bangladesh and immediately contiguous with the affected areas in Bangladesh (Chadha et al. 2006).

40.5 Evidence of Animal to Human Transmission of Hendra Virus

While bats are the reservoir host of the virus, humans have only become infected after close contact to infected horses. In Australia there are many bat carers who have close contact to sick and injured bats. They get bitten and scratched and come into contact with urine and faecal material, as well as placenta and birthing fluids. However, no bat carer has ever been diagnosed with infection. An extensive serological survey of bat carers in Queensland was performed in the mid 1990s, and there was no serological evidence of exposure to the virus (Selvey et al. 1996). However, bat carers are at risk of becoming infected with Australian bat lyssavirus.

In one HeV experiment, a small amount of viral RNA was detected in the nasal secretions of HeV infected horses two days after exposure to the virus and at least 2 days before the onset of clinical signs, suggesting that transmission of the virus from the infected horse may be possible before it is obviously unwell (Marsh et al. 2011). However at this early stage of infection, the amount of viral genome detected was very low and it is unlikely that this would be enough to infect another host. The findings also supported the observation in experimentally infected pteropus bats that a local mucosal infection, from days two to approximately six post exposure precedes a systemic infection (Halpin et al. 2011). Only after the systemic infection has been established does it become possible to isolate infectious virus from urine and blood.

Sequence analysis of different isolates from both horses and pteropus bats reveals extreme conservation at the genome and protein levels (Marsh et al. 2010; Smith et al. 2011). In one study comparing five horse isolates from five locations which spanned almost 2000 km, across three time points, to the original 1994 isolate, less than 1% variation at both the nucleotide and amino acid levels was shown across the 18.2-kb genome (Marsh et al. 2010). This genetic stability supports the theory of co-evolution where HeV is well adapted to its host resulting in minimal pressure to change over time (Halpin et al. 2007; Smith et al. 2011).

40.6 Evidence of Animal-to-Human Transmission of Nipah Virus

In contrast to Malaysia where pigs clearly served as the amplifying host that facilitated spread of the virus from bats to humans, no intermediate animal host was identified in Bangladesh. Several routes of transmission of NiV from bats to humans have been identified by studying the nearly annual outbreaks in Bangladesh and the single outbreak in India. Consumption of contaminated date palm sap or contaminated fruit has been linked to a number of cases and outbreaks in Bangladesh (Rahman et al. 2012). Case-patients reported no history of physical contact with bats, though community members often reported seeing bats. Infrared camera photographs have shown that *Pteropus* bats frequently visited date palm trees in those communities where sap was collected for human consumption. This provided an opportunity for intervention in an attempt to prevent NiV spillover to humans. It has been shown that skirts (made from bamboo, dhoincha, jute stick and/or polythene) covering the sap producing areas of a tree effectively prevented bat-sap contact (Khan et al. 2012).

Genetic analysis of NiV isolates and sequences obtained from clinical samples indicated that the outbreaks in Bangladesh were the result of multiple, independent introductions of virus into the human population (Harcourt et al. 2005; Luby et al. 2009b). Sequences of NiV isolates from human outbreaks in India and Bangladesh showed more heterogeneity than the sequences obtained in the initial Malaysian outbreak (AbuBakar et al. 2004; Arankalle et al. 2011; Chadha et al. 2006; Harcourt et al. 2005; Lo et al. 2012) and phylogenetic analysis indicated there are at least two distinct lineages of NiV circulating in Southeast Asia. Sequences obtained from Malaysia and Cambodia are designated as genotype M, while sequences obtained from Bangladesh and India are designated genotype B. Genotypes can be assigned based on the sequence of a 729 nucleotide window in the N-terminal region of the N gene ORF. Levels of nucleotide variation among full-length ORFs between genotypes M and B ranged from 6 to 9% and between the complete genomes nucleotide variation is approximately 8% (Lo et al. 2012). It is not clear if there are biologic differences between the genotypes and this question is the subject of ongoing investigation (Clayton et al. 2012; DeBuysscher et al. 2013).

40.7 Principles of Pathogenesis

These two paramyxoviruses, NiV and HeV, enter cells by binding to the receptor Ephrin-B2, which is expressed on neurons, smooth muscle, and endothelial cells surrounding small arteries (Bonaparte et al. 2005; Negrete et al. 2005). Ephrin-B3 serves as an alternative receptor for NiV, but not HeV (Negrete et al. 2006). After receptor binding by the attachment protein, G, the fusion protein (F) which is cleaved to create two linked polypeptides, F₁ and F₂, fuses to the host cell mem-

brane, initiating endocytosis (Wang et al. 2001). Following fusion between the viral envelope and the host cell membrane, the viral ribonucleocapsid is released into the cytoplasm (Lamb and Parks 2007). The polymerase complex composed of the polymerase (L) and phosphoprotein (P) initiates transcription of viral mRNAs. As translation of viral mRNA occurs, viral proteins accumulate in the cell, and the polymerase switches from transcription to genome replication.

Newly made genomes are encapsidated by the nucleoprotein (N) and polymerase complexes become associated with packaged nucleocapsids. The glycoproteins are synthesized in the endoplasmic reticulum (ER), mature through the Golgi network and are transported to the cell membrane. The processing of the fusion (F) glycoprotein occurs in the endosome (Diederich et al. 2005). The cytoplasmic tails of the F and G glycoproteins play a role in the interaction with the matrix (M) protein, which initiates virus maturation and budding (Ciancanelli and Basler 2006; Lamb and Parks 2007; Ong et al. 2009; Patch et al. 2007, 2008).

This tropism for endothelial cells results in a pathology characterized by vasculitis, thrombosis, ischaemia, necrosis and CNS parenchymal infection (Wong et al. 2002, 2009; Weingartl et al. 2009).

A post-mortem study of human NiV infection determined that a systemic multi-organ vasculitis associated with infection of endothelial cells was the main pathologic feature, with infection being most pronounced in the central nervous system (CNS) (Wong et al. 2002). In the CNS vascular endothelium, immunohistochemical analysis showed intense staining of endothelial, parenchymal, and multinucleate giant cells which are characteristic of paramyxovirus infection. Evidence of endothelial infection and vasculitis was also observed in other organs, including lung, heart, spleen, and kidney. NiV has been isolated from cerebrospinal fluid, tracheal secretions, throat swabs, nasal swabs, and urine specimens of patients (Chua et al. 2001; Goh et al. 2000; Wong et al. 2002) and detection of viral RNA by RT-PCR in urine and throat swabs samples is routinely used to confirm NiV infection.

40.8 Disease Symptoms in Humans and Animals

Early cases of Hendra virus infection in horses had clinical signs of an acute respiratory disease (Murray et al. 1995). However, as more cases appeared, the spectrum of clinical signs widened to include colic-like symptoms and neurological manifestations. The incubation period is between 4 and 16 days (Baldock et al. 1996), after which time clinical signs such as fever, tachycardia, inappetence, depression, dyspnea and restlessness may be observed (Marsh et al. 2011). Associated with the laboured breathing, a nasal discharge which may be frothy or blood-tinged, develops. Ataxia and myoclonus may also be seen (Rogers et al. 1996).

The first fatal human cases of Hendra virus infection died of an acute respiratory illness (Selvey et al. 1995). The second fatal human case suffered from relapsing encephalitis (O'Sullivan et al. 1997) with the third and fourth cases succumbing to encephalitis (Field et al. 2010; Playford et al. 2010). Two of the surviving human

cases suffered from a self-limited influenza-like illness at the time of Hendra virus infection (Hanna et al. 2006). The third survivor showed development of an influenza-like illness that progressed to acute encephalitis and suffered a long and debilitating neurological illness which to this day has not fully resolved (Playford et al. 2010). To date, this patient remains seropositive (Taylor et al. 2012).

The incubation period for NiV ranges from 6 days to 2 weeks; after symptom onset patients deteriorated rapidly usually requiring hospitalization (Eaton et al. 2007; Hossain et al. 2008). In a subset of 14 secondary patients who had well defined exposure to another case, the incubation period was 6–11 days (Luby et al. 2009b). In humans, NiV causes acute febrile encephalitis including fever, headache, drowsiness, dizziness, myalgia, and vomiting with reduced consciousness and evidence of brainstem involvement being a poor prognostic factor. Some patients with NiV initially present with pulmonary symptoms such as cough, atypical pneumonia and acute respiratory distress. The percentage of NiV patients presenting with respiratory disease was higher in Bangladesh (69%) than in Malaysia (25%) (Luby et al. 2009b; Tee et al. 2009). Some NiV cases experienced relapse of disease or late onset encephalitis after initial infection, which occurred on average approximately 8 months after initial infection (range: 9 days—22 months) and both syndromes have similar clinical manifestations (Goh et al. 2000; Tan et al. 2002; Tyler 2009). Upon post mortem examination viral antigen was found in the brains of patients with relapse and late onset encephalitis indicating viral replication took place in these tissues. Unlike acute NiV encephalitis cases, relapse and late onset encephalitis cases did not show vasculitis in the CNS (Tan and Chua 2008; Tan et al. 2002; Tyler 2009). A number of NiV infected individuals also experienced residual neurological symptoms that ranged from mild cognitive or cerebellar disabilities to more severe cognitive impairment, with some remaining in a vegetative state (Goh et al. 2000).

In the NiV outbreak in Malaysia, a newly identified porcine respiratory and neurologic syndrome developed in some pigs infected with NiV. This syndrome was characterized by fever, barking cough, behavioral changes, uncoordinated gait, spasms, and myoclonus (Mohd Nor et al. 2000).

40.9 Unresolved Issues

Both viruses are designated biosafety level (BSL) 4 agents which makes it difficult for researchers to work with these viruses. Furthermore, diagnostic tests requiring the use of live virus are restricted. However these tests are very important because of the nonspecific nature of clinical signs associated with henipavirus infections. Molecular detection of viral genome is currently the central arm of henipavirus infection diagnosis. Expanding the surveillance and laboratory capacity for rapid diagnosis of encephalitis outbreaks is crucial to early detection and containment in areas at risk for NiV and HeV.

There are currently no vaccines, passive immunoprophylaxes, or antiviral chemoprophylaxes approved for human henipavirus infections and it will be difficult to interrupt the transmission of viruses from the natural reservoir to horses, pigs, or other amplifying hosts. Numerous studies have identified potentially valuable vaccines and antiviral compounds (Broder et al. 2012). At present, human infections with NiV can potentially be prevented by early recognition of NiV infection of animals and the use of appropriate barrier precautions when exposed to potentially infectious material or persons.

Clearly HeV poses a serious threat to the veterinary profession. Five of the seven (71%) people infected with Hendra virus were associated with this profession. Fortunately for horse owners and breeders, there have been no infections in this cohort to date. However, any horse which is infected with Hendra virus poses a serious threat to all who come in contact with the animal, and this includes dogs, cats, ferrets and possibly other animals. This situation prompted the development of a vaccine for horses which was released at the end of 2012 (Broder et al. 2013). It remains to be seen what the uptake of this vaccine will be like, and how it will impact the epidemiology of the disease. A successful equine vaccination program has the potential to reverse the current trend of veterinarians exiting equine practice in HeV-endemic regions due to the perceived personal risk and workplace liability (Mendez et al. 2012).

The discovery of HeV in pteropus bats in 1996 (Halpin et al. 2000) marked the beginning of a new wave of research activities, which led to the association of bats with some of the most notable viral pathogens to emerge in recent history, including NiV (Chua et al. 2002), severe acute respiratory syndrome-like coronaviruses (Li et al. 2005), Ebola virus (Leroy et al. 2005), and Marburg virus (Towner et al. 2009). Two recently published studies from Papua New Guinea identified an increased henipavirus seroprevalence from less than 10% in the period 1996–1999 to 55% in 2010, suggesting that the dynamics of HeV or NiV or another closely related virus is changing (Breed et al. 2010; Field et al. 2013). Additional studies are needed to determine the ecologic, environmental and epidemiologic circumstances that favor transmission of NiV and HeV from their natural reservoir host to humans and other domestic species. Pteropid bat cell lines have recently been developed (Cramer et al. 2009) and future studies should help improve our understanding of how NiV and HeV persist in various bat species.

We know at least one other henipavirus exists in south east Asia. Cedar virus is the most recently discovered henipavirus (Marsh et al. 2012). It remains to be seen if Cedar virus has the capacity to spill over from its reservoir host, the pteropus bat, to other species and cause disease. The question is not, will other henipaviruses be discovered? The question is, how many other henipaviruses will be discovered and which ones will pose a threat to the health of humans and other animals?

References

- AbuBakar S, Chang LY, Ali AR, Sharifah SH, Yusoff K, Zamrod Z (2004) Isolation and molecular identification of Nipah virus from pigs. *Emerg Infect Dis* 10(12):2228–2230
- Arankalle VA, Bandyopadhyay BT, Ramdasi AY, Jadi R, Patil DR, Rahman M, Majumdar M, Banerjee PS, Hati AK, Goswami RP, Neogi DK, Mishra AC (2011) Genomic characterization of Nipah virus, West Bengal, India. *Emerg Infect Dis* 17(5):907–909
- Baldock FC, Douglas IC, Halpin K, Field H, Young PL, Black PF (1996) Epidemiological investigations into the 1994 equine morbillivirus outbreaks in Queensland, Australia. *Sing Vet J* 20:57–61
- Blum LS, Khan R, Nahar N, Breiman RF (2009) In-depth assesment of an outbreak of Nipah encephalitis with person-to-person transmission in Bangladesh: implications for prevention and control strategies. *Am J Trop Med Hyg* 80(1):96–102
- Bonaparte MI, Dimitrov AS, Bossart KN, Cramer G, Mungall BA, Bishop KA, Choudhry V, Dimitrov DS, Wang LF, Eaton BT, Broder CC (2005) Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc Natl Acad Sci U S A* 102(30):10652–10657
- Breed AC, Yu M, Barr JA, Cramer G, Thalmann CM, Wang L-F (2010) Prevalence of henipavirus and rubulavirus antibodies in pteropid bats, Papua New Guinea. *Emerg Infect Dis* 16:1997–1999
- Breed AC, Breed MF, Meers J, Field HE (2011) Evidence of endemic Hendra virus infection in flying-foxes (*Pteropus conspicillatus*)-implications for disease risk management. *PLoS ONE* 6(12):e28816
- Breed AC, Meers J, Sendow I, Bossart KN, Barr JA, Smith I, Wacharapluesadee S, Wang L, Field HE (2013) The distribution of henipaviruses in Southeast Asia and Australasia: is Wallace's line a barrier to Nipah virus? *PLoS ONE* 8(4):e61316
- Broder CC, Geisbert TW, Xu K, Nikolov DB, Wang LF, Middleton D, Pallister J, Bossart KN (2012) Immunization strategies against henipaviruses. *Curr Top Microbiol Immunol* 359:197–223
- Broder CC, Xu K, Nikolov DB, Zhu Z, Dimitrov DS, Middleton D, Pallister J, Geisbert TW, Bossart KN, Wang LF (2013) A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral Res* 100(1):8–13
- Chadha MS, Comer JA, Lowe L, Rota PA, Rollin PE, Bellini WJ, Ksiazek TG, Mishra A (2006) Nipah virus-associated encephalitis outbreak, Siliguri, India. *Emerg Infect Dis* 12(2):235–240
- Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Shieh W, Goldsmith CS, Gubler DJ, Roehrig JT, Eaton B, Gould AR, Olson J, Field H, Daniels P, Ling AE, Peters CJ, Anderson LJ, Mahy BW (2000) Nipah virus: a recently emergent deadly paramyxovirus. *Science* 288(5470):1432–1435
- Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, Chan YP, Lim ME, Lam SK (2002) Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 4(2):145–151
- Chua KB, Lam SK, Goh KJ, Hooi PS, Ksiazek TG, Kamarulzaman A, Olson J, Tan CT (2001) The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia. *J Infect* 42(1):40–43
- Ciancanelli MJ, Basler CF (2006) Mutation of YMYL in the Nipah virus matrix protein abrogates budding and alters subcellular localization. *J Virol* 80(24):12070–12078
- Clayton BA, Middleton D, Bergfeld J, Haining J, Arkinstall R, Wang L, Marsh GA (2012) Transmission routes for Nipah virus from Malaysia and Bangladesh. *Emerg Infect Dis* 18(12):1983–1993
- Cramer G, Todd S, Grimley S, McEachern JA, Marsh GA, Smith C, Tachedjian M, De Jong C, Virtue ER, Yu M, Bulach D, Liu JP, Michalski WP, Middleton D, Field HE, Wang LF (2009) Establishment, immortalisation and characterisation of pteropid bat cell lines. *PLoS ONE* 4(12):e8266

- DeBuyscher BL, de Wit E, Munster VJ, Scott D, Feldmann H, Prescott J (2013) Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. *PLoS Negl Trop Dis* 7(1):e2024
- Diederich S, Moll M, Klenk HD, Maisner A (2005) The Nipah virus fusion protein is cleaved within the endosomal compartment. *J Biol Chem* 280(33):29899–29903
- Domingo E (2010) Mechanisms of viral emergence. *Vet Res* 41:38
- Drexler JF, Corman VM, Gloza-Rausch F, Seebens A, Annan A, Ipsen A, Kruppa T, Müller MA, Kalko EK, Adu-Sarkodie Y, Oppong S, Drosten C (2009) Henipavirus RNA in African bats. *PLoS ONE* 4(7):e6367
- Drexler J, Corman V, Wegner T, Tateno A, Zerbinati R, Gloza-Rausch F, Seebens A, Müller MA, Drosten C (2011) Amplification of emerging viruses in a bat colony. *Emerg Infect Dis* 17:449–456
- Eaton BT, Mackenzie JS, Wang L-F (2007) Paramyxoviridae: Henipaviruses, vol 2. In: DM Knipe and PM Howley (eds) *Fields Virology*, 5th edn. Lippincott Williams & Wilkins, Philadelphia. pp 1587–1600
- Field H, de Jong CE, Halpin K, Smith CS (2013) Henipaviruses and fruit bats, Papua New Guinea. *Emerg Infect Dis* 19(4):670–671
- Field H, Schaaf K, Kung N, Simon C, Waltisbuhl D, Middleton D (2010) Hendra virus outbreak with novel clinical features, Australia. *Emerg Infect Dis* 16:338–340
- Goh KJ, Tan CT, Chew NK, Tan PS, Kamarulzaman A, Sarji SA, Wong KT, Abdullah BJ, Chua KB, Lam SK (2000) Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med* 342(17):1229–1235
- Halpin K, Young PL, Field HE, Mackenzie JS (2000) Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J Gen Virol* 81(Pt 8):1927–1932
- Halpin K, Hyatt AD, Fogarty R, Middleton D, Bingham J, Epstein JH, Rahman SA, Hughes T, Smith C, Field HE, Daszak P, Henipavirus Ecology Research Group (2011) Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission. *Am J Trop Med Hyg* 85(5):946–951
- Halpin K, Hyatt AD, Plowright RK, Epstein JH, Daszak P, Field HE, Wang L, Daniels PW, Henipavirus Ecology Research Group (2007) Emerging viruses: coming in on a wrinkled wing and a prayer. *Clin Infect Dis* 44:711–717
- Hanna JN, McBride WJ, Brookes DL, Shield J, Taylor CT, Smith IL (2006) Hendra virus infection in a veterinarian. *Med J Aust* 185:562–564
- Harcourt BH, Lowe L, Tamin A, Liu X, Bankamp B, Bowden N, Rollin PE, Comer JA, Ksiazek TG, Hossain MJ, Gurley ES, Breiman RF, Bellini WJ, Rota PA (2005) Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg Infect Dis* 11(10):1594–1597
- Hasebe F, Thuy NT, Inoue S, Yu F, Kaku Y, Watanabe S, Akashi H, Dat DT, Mai leTQ, Morita K (2012) Serologic evidence of Nipah virus infection in bats, Vietnam. *Emerg Infect Dis* 18(3):536–537
- Hayman DT, Suu-Ire R, Breed AC, McEachern JA, Wang L, Wood JL, Cunningham AA (2008) Evidence of Henipavirus infection in West African fruit bats. *PLoS ONE* 3(7):e2739
- Hooper PT, Williamson MM (2000) Hendra and Nipah virus infections. *Vet Clin North Am Equine Pract* 16(3):597–603
- Hossain MJ, Gurley ES, Montgomery JM, Bell M, Carroll DS, Hsu VP, Formenty P, Croisier A, Bertherat E, Faiz MA, Azad AK, Islam R, Molla MA, Ksiazek TG, Rota PA, Comer JA, Rollin PE, Luby SP, Breiman RF (2008) Clinical presentation of Nipah virus infection in Bangladesh. *Clin Infect Dis* 46(7):977–984
- Hsu VP, Hossain MJ, Parashar UD, Ali MM, Ksiazek TG, Kuzmin I, Niezgodka M, Rupprecht C, Bresee J, Breiman RF (2004) Nipah virus encephalitis reemergence, Bangladesh. *Emerg Infect Dis* 10(12):2082–2087
- Iehle C, Razafitrimo G, Razainirina J, Andriaholinirina N, Goodman SM, Faure C, Georges-Courbot MC, Rousset D, Reynes JM (2007) Henipavirus and Tioman virus antibodies in Pteropodid bats, Madagascar. *Emerg Infect Dis* 13(1):159–161

- Khan SU, Gurley ES, Hossain MJ, Nahar N, Sharker MA, Luby SP (2012) A randomized controlled trial of interventions to impede date palm sap contamination by bats to prevent Nipah virus transmission in Bangladesh. *PLoS ONE* 7(8):e42689
- Lamb RA, Parks GD (2007) Paramyxoviridae: the viruses and their replication. In: Knipe DMHP (ed) *Fields virology*, vol 1, 5th edn. Lippincott Williams & Wilkins, Philadelphia, pp 1449–1496
- Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, et al (2005) Fruit bats as reservoirs of Ebola virus. *Nature* 438:575–576
- Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, et al (2005) Bats are natural reservoirs of SARS-like coronaviruses. *Science* 310:676–679
- Li Y, Wang J, Hickey AC, Zhang Y, Li Y, Wu Y, Zhang H, Yuan J, Han Z, McEachern J, Broder CC, Wang LF, Shi Z (2008) Antibodies to Nipah or Nipah-like viruses in bats, China. *Emerg Infect Dis* 14(12):1974–1976
- Lo MK, Lowe L, Hummel KB, Sazzad HM, Gurley ES, Hossain MJ, Luby SP, Miller DM, Comer JA, Rollin PE, Bellini WJ, Rota PA (2012) Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. *Emerging Infect Dis* 18(2):248–255
- Luby SP, Gurley ES (2012) Epidemiology of Henipavirus disease in humans. *Curr Top Microbiol Immunol* 359:25–40
- Luby SP, Gurley ES, Hossain MJ (2009a) Transmission of human infections with Nipah virus. *Clin Infect Dis* 49:1743–1748
- Luby SP, Hossain MJ, Gurley ES, Ahmed B-N, Banu S, Khan SU, Homaira N, Rota PA, Rollin PE, Comer JA, Kenah E, Ksiazek TG, Rahman M (2009b) Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007. *Emerg Infect Dis* 15(8):1229–1235
- Luby SP, Rahman M, Hossain MJ, Blum LS, Husain MM, Gurley E, Khan R, Ahmed BN, Rahman S, Nahar N, Kenah E, Comer JA, Ksiazek TG (2006) Foodborne transmission of Nipah virus, Bangladesh. *Emerg Infect Dis* 12:1888–1894
- Marsh GA, de Jong C, Barr JA, Tachedjian M, Smith C, Middleton D, Yu M, Todd S, Foord AJ, Haring V, Payne J, Robinson R, Broz I, Crameri G, Field HE, Wang LF (2012) Cedar virus: a novel Henipavirus isolated from Australian Bats. *PLoS Pathog* 8(8):e1002836
- Marsh GA, Haining J, Hancock TJ, Robinson R, Foord AJ, Barr JA, Riddell S, Heine HG, White JR, Crameri G, Field HE, Wang LF, Middleton D (2011) Experimental infection of horses with Hendra virus/Australia/horse/2008/Redlands. *Emerg Inf Dis* 17(12):2232–2238
- Marsh GA, Todd S, Foord A, Hansson E, Davies K, Wright L, Morrissy C, Halpin K, Middleton D, Field HE, Daniels P, Wang LF (2010) Genome sequence conservation of Hendra virus isolates during spillover to horses, Australia. *Emerg Infect Dis* 16(11):1767–1769
- Mendez DH, Judd J, Speare R (2012) Unexpected result of Hendra virus outbreaks for veterinarians, Queensland, Australia. *Emerg Infect Dis* 18(1):83–85
- Middleton DJ (2013) Hendra virus update. *Proceedings of the Australian Veterinary Association Annual Conference*. Cairns, Australia
- Middleton DJ, Morrissy CJ, van der Heide BM, Russell GM, Braun MA, Westbury HA, Halpin K, Daniels PW (2007) Experimental Nipah virus infection in pteropid bats (*Pteropus poliocephalus*). *J Comp Pathol* 136(4):266–272
- Mohd Nor MN, Gan CH, Ong BL (2000) Nipah virus infection of pigs in peninsular Malaysia. *Rev Sci Tech* 19:160–165
- Murray K, Selleck P, Hooper P, Hyatt A, Gould A, Gleeson L, Westbury H, Hiley L, Selvey L, Rodwell B, Ketterer P (1995) A morbillivirus that caused fatal disease in horses and humans. *Science* 268(5207):94–97
- Negrete OA, Levroney EL, Aguilar HC, Bertolotti-Ciarlet A, Nazarian R, Tajyar S, Lee B (2005) EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature* 436(7049):401–405
- Negrete OA, Wolf MC, Aguilar HC, Enterlein S, Wang W, Muhlberger E, Su SV, Bertolotti-Ciarlet A, Flick R, Lee B (2006) Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. *PLoS Pathog* 2(2):e7

- Ong ST, Yusoff K, Kho CL, Abdullah JO, Tan WS (2009) Mutagenesis of the nucleocapsid protein of Nipah virus involved in capsid assembly. *J Gen Virol* 90(Pt 2):392–397
- O’Sullivan JD, Allworth AM, Paterson DL, Snow TM, Boots R, Gleeson LJ, Gould AR, Hyatt AD, Bradfield J (1997) Fatal encephalitis due to novel paramyxovirus transmitted from horses. *The Lancet* 349(9045):93–95
- Patch JR, Cramer G, Wang LF, Eaton BT, Broder CC (2007) Quantitative analysis of Nipah virus proteins released as virus-like particles reveals central role for the matrix protein. *Virol J* 4:1
- Patch JR, Han Z, McCarthy SE, Yan L, Wang LF, Harty RN, Broder CC (2008) The YPLGVG sequence of the Nipah virus matrix protein is required for budding. *Virol J* 5(1):137
- Paton NI, Leo YS, Zaki SR, Auchus AP, Lee KE, Ling AE, Chew SK, Ang B, Rollin PE, Umaphathi T, Sng I, Lee CC, Lim E, Ksiazek TG (1999) Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet* 354(9186):1253–1256
- Playford EG, McCall B, Smith G, Slinko V, Allen G, Smith I (2010) Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis* 16:219–223
- Plowright RK, Field HE, Smith C, Divljan A, Palmer C, Tabor G, Daszak P, Foley JE (2008) Reproduction and nutritional stress are risk factors for Hendra virus infection in little red flying foxes (*Pteropus scapulatus*). *Proc Biol Sci* 275(1636):861–869
- Pourrut X, Délicat A, Rollin P, Ksiazek T, Gonzalez J, Leroy EM (2007) Spatial and temporal patterns of Zaire ebolavirus antibody prevalence in the possible reservoir bat species. *J Infect Dis* 196:S176–183
- Rahman MA, Hossain MJ, Sultana S, Homaira N, Khan SU, Rahman M, Gurley ES, Rollin PE, Lo MK, Comer JA, Lowe L, Rota PA, Ksiazek TG, Kenah E, Sharker Y, Luby SP (2012) Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector Borne Zoonotic Dis* 12(1):65–72
- Reynes JM, Counor D, Ong S, Faure C, Seng V, Molia S, Walston J, Georges-Courbot MC, Deubel V, Sarthou JL (2005) Nipah virus in Lyle’s flying foxes, Cambodia. *Emerging Infect Dis* 11(7):1042–1047
- Rogers RJ, Douglas IC, Baldock FC, Glanville RJ, Seppanen KT, Gleeson LJ, Selleck PN, Dunn KJ (1996) Investigation of a second focus of equine morbillivirus infection in coastal Queensland. *Aust Vet J* 74(3):243–244
- Selvey LA, Wells RM, McCormack JG, Ansford AJ, Murray K, Rogers RJ, Lavercombe PS, Selleck P, Sheridan JW (1995) Infection of humans and horses by a newly described morbillivirus. *Med J Aust* 162(12):642–645
- Selvey LA, Taylor R, Arklay A, Gerrard J (1996) Screening of bat carers for antibodies to equine morbillivirus. *Commun Dis Intell* 20:477–478
- Sendow I, Field HE, Curran J, Morrissy C, Meehan G, Buick T, Daniels P, Darminto (2006) Henipavirus in *Pteropus vampyrus* bats, Indonesia. *Emerg Infect Dis* 12(4):711–712
- Smith I, Broos A, de Jong C, Zeddeman A, Smith C, Smith G, Moore F, Barr J, Cramer G, Marsh G, Tachedjian M, Yu M, Kung YH, Wang LF, Field H (2011) Identifying Hendra virus diversity in pteropid bats. *PLoS ONE* 6(9):e25275
- Sohayati AR, Hassan L, Sharifah SH, Lazarus K, Zaini CM, Epstein JH, Shamsyul Naim N, Field HE, Arshad SS, Abdul Aziz J, Daszak P, Henipavirus Ecology Research Group (2011) Evidence for Nipah virus recrudescence and serological patterns of captive *Pteropus vampyrus*. *Epidemiol Infect* 139(10):1570–1579
- Tan CT, Chua KB (2008) Nipah virus encephalitis. *Curr Infect Dis Rep* 10(4):315–320
- Tan CT, Goh KJ, Wong KT, Sarji SA, Chua KB, Chew NK, Murugasu P, Loh YL, Chong HT, Tan KS, Thayaparan T, Kumar S, Jusoh MR (2002) Relapsed and late-onset Nipah encephalitis. *Ann Neurol* 51(6):703–708
- Taylor C, Playford EG, McBride WJ, McMahon J, Warrilow D (2012) No evidence of prolonged Hendra virus shedding by 2 patients, Australia. *Emerg Infect Dis* 18(12):2025–2027
- Tee KK, Takebe Y, Kamarulzaman A (2009) Emerging and re-emerging viruses in Malaysia, 1997–2007. *Int J Infect Dis* 13(3):307–318
- Towner JS, Amman BR, Sealy TK, Carroll SA, Comer JA, Kemp A et al (2009) Isolation of genetically diverse Marburg viruses from Egyptian fruit bats. *PLoS Pathog* 5:e100053

- Turmelle AS, Allen LC, Jackson FR, Kunz TH, Rupprecht CE, McCracken GF (2010) Ecology of rabies virus exposure in colonies of Brazilian free-tailed bats (*Tadarida brasiliensis*) at natural and man-made roosts in Texas. *Vector Borne Zoonotic Dis* 10:165–175
- Tyler KL (2009) Emerging viral infections of the central nervous system: part 2. *Arch Neurol* 66(9):1065–1074
- Wang L, Harcourt BH, Yu M, Tamin A, Rota PA, Bellini WJ, Eaton BT (2001) Molecular biology of Hendra and Nipah viruses. *Microbes Infect* 3(4):279–287
- Wacharapluesadee S, Lumlerdacha B, Boongird K, Wanghongsa S, Chanhom L, Rollin P, Stockton P, Rupprecht CE, Ksiazek TG, Hemachudha T (2005) Bat Nipah virus, Thailand. *Emerg Infect Dis* 11(12):1949–1951
- Weingartl HM, Berhane Y, Czub M (2009) Animal models of henipavirus infection: a review. *Vet J* 181(3):211–220
- Westbury HA, Hooper PT, Selleck PW, Murray PK (1995) Equine morbillivirus pneumonia: susceptibility of laboratory animals to the virus. *Aust Vet J* 72(7):278–279
- Williamson MM, Hooper PT, Selleck PW, Gleeson LJ, Daniels PW, Westbury HA, Murray PK (1998) Transmission studies of Hendra virus (equine morbillivirus) in fruit bats, horses and cats. *Aust Vet J* 76:813–818
- Wong KT, Shieh WJ, Kumar S, Norain K, Abdullah W, Guarner J, Goldsmith CS, Chua KB, Lam SK, Tan CT, Goh KJ, Chong HT, Jusoh R, Rollin PE, Ksiazek TG, Zaki SR (2002) Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol* 161(6):2153–2167
- Wong KT, Robertson T, Ong BB, Chong JW, Yaiw KC, Wang LF, Ansford AJ, Tannenberg A (2009) Human Hendra virus infection causes acute and relapsing encephalitis. *Neuropathol Appl Neurobiol* 35(3):296–305
- Yob JM, Field H, Rashdi AM, Morrissy C, van der Heide B, Rota P, bin Adzhar A, White J, Daniels P, Jamaluddin A, Ksiazek T (2001) Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. *Emerg Infect Dis* 7(3):439–441