Nipah and Hendra Viruses

Vincent P. Hsu
Clinical Performance Improvement and Infection Control, Florida Hospital, 601 E. Rollins St. Orlando, FL 32803, USA

Introduction

Nipah and Hendra viruses are two zoonotic paramyxoviruses with an ability to cause fatal encephalitic and respiratory diseases in humans. Hendra virus was first identified in humans in Australia in 1994, with horses as the intermediate host. Nipah virus emerged in humans in 1998 in Malaysia, with pigs as the intermediate host. Nipah virus was later also identified in India and Bangladesh in 2001, with the flying fox (*Pteropus* spp.) as the natural host, although no intermediary animal host was found in more recent outbreaks there. A third zoonotic paramyxovirus, Menangle virus, was first identified in pigs, and will be discussed only briefly. Of the zoonotic paramyxoviruses, Nipah virus is responsible for the greatest number of human cases, with several hundred cases and at least 215 deaths reported, compared to Hendra virus, which has caused a handful of cases and 2 deaths, and Menangle, which has only caused self-limited illness in 2 individuals.

Classification, structure, and virology

Nipah and Hendra viruses are negative-sense, single-stranded RNA viruses in the *Paramyxoviridae* family, subfamily *Paramyxovirinae*. They are further categorized in the recently named genus *Henipavirus*, one of five genera in the subfamily (the others are *Respirovirus*, *Morbilivirus*, *Avulavirus*, and *Rubulavirus*) (Fig. 1). Other human pathogenic viruses exist in these other genera, such as measles, mumps, and parainfluenza viruses; Nipah and Hendra viruses, in the genus *Henipavirus*, and Menangle virus, in the genus *Rubalavirus*, are unique in that they are zoonotic and are viruses that have recently emerged in humans.

Nipah and Hendra viruses exhibit typical morphology of paramyxoviruses when examined by electron microscopy (EM), with a helical nucleocapsid structure...
surrounded by a membrane derived from the plasma membrane of the cell from which the viruses bud. Nucleocapsid filaments exhibit a typical ‘herringbone’ morphology, produced by the association of the nucleocapsid protein with genomic RNA (Murray et al., 1995b; Chua et al., 2000a; Halpin et al., 2000). In contrast to Nipah virus, which has only a single layer of surface projections, Hendra virus appears double-fringed, caused by projections on the surface of the viral envelope (Hyatt et al., 2001). Measurements by EM demonstrated that Nipah virus particles vary in size between 120 and 500 nm.

The determination of the nucleotide sequences of Nipah and Hendra viruses were completed soon after the Nipah outbreak in Malaysia in 1998 (Harcourt et al., 2000, 2001). Nipah and Hendra virus have 68–92% amino acid homology in the protein-coded regions and 40–67% nucleotide homology in the non-translated regions. Similar to other *Paramyxovirinae*, Nipah and Hendra viruses carry six genes that encode structural proteins, the nucleocapsid (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G), and the large polymerase (L), in that order. In addition, the P gene also encodes accessory proteins designated C, V,
and W. The C protein appears to regulate viral RNA synthesis and may play a role as a virulence factor. The addition of a single nucleotide, G, allows expression of the V protein, while the addition of two Gs allow expression of the W protein (Harcourt et al., 2000). The N, P, and L proteins are associated with genomic RNA and form part of the RNA polymerase complex, while the M protein serves to maintain the virion structure, assembling between the envelope and nucleocapsid core. The V and W proteins appear to be virulence factors that act by blocking activation of an interferon-inducible promoter (Park et al., 2003). The two membrane glycoproteins are the F and G proteins. The fusion protein, F0, is originally synthesized as an inactive precursor that requires cleavage by a host cell protease to become active subunits F1 and F2. These subunits mediate the fusion of the virion membrane with the plasma membrane of the host cell. The attachment protein, or glycoprotein, G, serves to mediate the binding of the virus to a cellular receptor, which has not yet been identified (Wang et al., 2001).

Strain variation Nipah viruses have been demonstrated. Genomic variation appears to be geographically distinct, with specific differences between human isolates from the outbreaks in Malaysia, India, and Bangladesh, and from bats in Cambodia (Harcourt et al., 2000; Harcourt, 2005; Reynes et al., 2005; Chadha et al., 2006). Although amino acid homologies between the Malaysia strain and Bangladesh strain were greater than 92%, the genome of the Bangladesh strain is 6 nucleotides longer than the Malaysian strain and demonstrated enough variation to be considered a new strain. Sequences obtained from the outbreak in India had a closer relation to the Bangladesh strain than the Malaysian strain, while the virus isolated in Cambodia demonstrated closer homology to the Malaysian strain. These observations support the finding that these viruses have natural reservoirs for evolving within distinct geographic areas.

Transcription and replication of the henipaviruses have not been studied because of the high level of laboratory safety that is required, but the evidence to date suggests that these viruses follow the same replication mechanisms as the other Paramyxovirinae. After binding and fusion of the G and F proteins, respectively, the ribonucleoprotein is released into the cell cytoplasm. Transcription of the N, P, and L proteins then occurs prior to production of new proteins, while new membrane glycoproteins are transported to the surface. The newly produced proteins assemble at the cytoplasm and are released via viral budding.

Several unique features differentiate the henipaviruses from the other Paramyxovirinae viruses. Antigenic cross-reactivity occurs between Hendra and Nipah viruses, but not with other paramyxoviruses. The viruses exhibit a much longer genomic length compared to other members (18.2 kb vs. 15.5 kb), and have an unusually large P protein (Wang et al., 2000; Mayo, 2002). The cleavage of the F protein, a necessary step for all paramyxoviruses, occurs through a novel type of proteolytic cleavage that differs from that caused by known proteases (Moll et al., 2004). The G proteins of Nipah and Hendra virus do not have hemagglutinin and neuraminidase activity, features that are common to other paramyxoviruses (Yu et al., 1998; Wang et al., 2001). Lastly, it should be noted that Hendra and
Nipah viruses have a broad tropism, and are able to infect a broad range of animal species, a characteristic that is not typical of other paramyxoviruses.

**Epidemiology**

The epidemiology of Nipah and Hendra viruses has not been fully elucidated. A similarity exists between the epidemiology of each of these two viruses, such as the *Pteropus* fruit bat as the natural host for both viruses. For both Hendra and Nipah viruses, it is presumed that horses and pigs that have acted as an intermediary host to humans had been infected by indirect contact with pteropid bats endemic in these regions, although this has not been experimentally proven.

However, there are also differences in their epidemiology. Hendra virus was first described along the coastal regions of Australia, and to date has caused illnesses in 5 humans with 2 deaths, in infections that were acquired by close contact with ill horses infected with the virus (Table 1). Nipah virus infections in humans have been described in Malaysia, Singapore, Bangladesh, and India, and have been identified in bats in Cambodia and Thailand (Table 1). Until 2004, cases occurred only in clusters, but sporadic cases have been identified more recently through active surveillance (Anon., 2004b). Overall, Nipah virus has caused at least 215 human deaths to date. Direct contact with infected pigs was primarily responsible for the outbreak in Malaysia, although in Bangladesh the epidemiology was less well-defined, with some evidence for person-to-person transmission.

**Description of Hendra virus outbreaks**

In September 1994, a cluster of respiratory illnesses involving 18 horses and 2 humans was reported from the town of Hendra, a suburb of Brisbane, Australia. The first illness occurred in a pregnant mare that died, followed by 14 additional horse deaths. Within 1 week after the death of the index mare, a 49-year-old horse trainer and a 40-year-old stable hand who were closely involved in the care of the index mare (Murray et al., 1995a; Selvey et al., 1995) became ill with respiratory symptoms, and the trainer died after a 7-day illness. The stable hand recovered from mild respiratory symptoms after 6 weeks. An undescribed virus was isolated from several of the horses and from the kidney of the horse trainer who died; this virus was found to be distantly related to known morbilliviruses (Murray et al., 1995b). It was initially named equine morbillivirus and was subsequently renamed Hendra virus.

Sporadic cases of Hendra virus have continued to occur in horses and humans. In September 1995, a male farmer from Queensland, Australia was admitted to a hospital with fever, altered mental status, multiple seizures, and an initial diagnosis of meningitis (Anon., 1996; O'Sullivan et al., 1997). He died 25 days after admission. It was subsequently learned that the patient had been diagnosed with a self-limited episode of meningitis in August 1994, and that he had cared for two sick horses and assisted with their necropsies just prior to the onset of his first illness.
Subsequent testing of cerebral spinal fluid obtained from the patient’s first illness and from both ill horses confirmed that all were Hendra virus infections.

In 1999, a fatal case of Hendra virus infection occurred in a mare in Cairns, Queensland, Australia, but there was no recognized transmission to humans (Field et al., 2000; Hooper et al., 2000). In late 2004, two human cases of Hendra virus infection were reported in Cairns and Townsville, Queensland. Both illnesses were described as self-limited upper respiratory infections in female veterinarians who had each recently performed a postmortem examination of a horse that had been

<table>
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<tr>
<th>Virus</th>
<th>Country</th>
<th>Region (locality, if known)</th>
<th>Year</th>
<th>Affected species</th>
<th>Reference</th>
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<tr>
<td>Hendra</td>
<td>Australia</td>
<td>Queensland (Brisbane)</td>
<td>1994</td>
<td>Humans, horses</td>
<td>Murray et al. (1995a), Selvey et al. (1995)</td>
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<td></td>
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<td>Queensland, Northern Territories, New South Wales</td>
<td>1996, NA</td>
<td>Bats</td>
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<td>Queensland (Cairns, Townsville)</td>
<td>2004</td>
<td>Humans, horses</td>
<td>McCormack (2005)</td>
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<td>Nipah</td>
<td>Papua New Guinea</td>
<td>Madang (Madang)</td>
<td>NA</td>
<td>Bats</td>
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<td>Malaysia</td>
<td>Singapore</td>
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<td>Humans</td>
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<td>Singapore</td>
<td>West Bengal (Siliguri)</td>
<td>2001</td>
<td>Humans</td>
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<td>India</td>
<td>Meherpur (Chandpur)</td>
<td>2001</td>
<td>Bats</td>
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<td>Bangladesh</td>
<td>Naogaon (Chalksita, Biljoana)</td>
<td>2003</td>
<td>Humans, bats</td>
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<td>Rajbari (Goalando)</td>
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<td>Humans, bats</td>
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<td>Faridpur</td>
<td>2004</td>
<td>Humans</td>
<td>(Anon., 2004d)</td>
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NA = not available.
ill. Hendra virus was confirmed as the cause of death in one of those horses (McCormack, 2005).

**Risk factors and transmission of Hendra virus**

All reported human cases of Hendra virus infection have been associated with exposure to ill horses, most of which were also confirmed to have been infected with Hendra virus. The mode of transmission was attributed to exposure to horse respiratory droplets, but it appears that close and/or prolonged contact is necessary for transmission. After Hendra virus was first identified, subsequent surveillance among 296 other potential contacts failed to identify any other antibody positive individuals, suggesting that the threshold for infectivity is low (McCormack et al., 1999). There is no evidence for subclinical infection or human-to-human transmission of Hendra virus.

**Description of Nipah virus outbreaks**

**Malaysia and Singapore**

In late September 1998, a cluster of human illnesses characterized by encephalitic changes began appearing near the city of Ipoh in the Malaysian state of Perak, followed by a second cluster in December 1998 near the city of Sikamat in the state of Negri Sembilan. A third cluster, which ended up accounting for about 85% of all cases in Malaysia, began later that same month in the village of Sungai Nipah near the city of Bukit Pelandok, also in the state of Negri Sembilan, with a small number of cases confirmed from a third state, Selangor (Anon., 1999a). Most cases were in men who were working on pig farms, many in close contact with pigs. Some illnesses were observed in pigs 1–2 weeks before onset of the illness in humans. By February 1999, the outbreak in Perak had largely subsided, although it was not until early April that cases in Negri Sembilan began to decline. Altogether, a total of 265 Nipah virus encephalitis cases were confirmed, with 105 fatalities.

The outbreaks were facilitated by the movement of pigs between farms, and across the border with Singapore. In a 1-week period in March 1999, 11 abattoir workers in Singapore developed febrile illnesses that were confirmed to be due to Nipah virus infection, and all the affected workers had handled swine imported from Malaysia (Anon., 1999a). The outbreak in Singapore stopped after the pig importation from Malaysia was banned and the abattoirs were closed.

Because cases were associated with close contact with pigs, the disease was initially thought to be due to Japanese encephalitis, but another agent was sought after patients tested negative for that virus. Subsequent EM and immunofluorescence testing identified the etiologic agent as a Hendra-like virus. By early March 1999, the virus was determined to be a distinct paromyxovirus and was then named Nipah virus, after the village in Negri Sembilan where the first isolate was made from a fatal human case (Chua et al., 1999; Chua et al., 2000a). Measures taken to
control the outbreak focused on culling pigs in the affected states; over 1 million pigs were eventually culled, with estimated economic losses between US$350 and $400 million mainly due to animal losses (Anon., 2004a). Other measures that were undertaken included a ban on transporting pigs within the country, education, use of personal protective equipment by persons exposed to pigs, and establishment of a national surveillance and control system to detect infected animals (Anon., 1999b). The last confirmed fatal case was reported in May 1999, and no cases have been reported from Malaysia or Singapore since 1999.

Through December 1999, a total of 283 cases of viral encephalitis with 109 fatalities and a case fatality rate of 38.5% were reported to the Malaysia Ministry of Health (Chua, 2003). However, these numbers reflected only symptomatic cases; the true number of persons infected with Nipah virus, although uncertain, is higher due to the fact that asymptomatic patients were largely unrecognized.

**India**

In January and February 2001, an outbreak of febrile illnesses occurred in the city of Siliguri, in the West Bengal region of India. Although initially reported as atypical measles, it has been retrospectively confirmed that Nipah virus was the most likely cause of the outbreak, with IgM and IgG antibodies to Nipah Virus detected in serum in 9 of 18 patients and a positive PCR in urine from 5 patients (Chadha et al., 2006). A total of 66 cases of Nipah virus encephalitis were identified with at least 43 deaths; all cases occurred in individuals over 15 years of age. No clear animal exposure was identified, but there was some evidence suggesting person-to-person and nosocomial transmission.

**Bangladesh**

In April and May 2001, a cluster of febrile neurologic illnesses with nine deaths was reported in a village in Meherpur District, Bangladesh. Preliminary testing of sera collected from survivors soon after the outbreak suggested that a Nipah-like virus might have been the cause. A similar outbreak of encephalitis was reported in January 2003 in Naogaon District with eight reported deaths. A later investigation concluded that these 17 deaths were probably due to Nipah virus encephalitis, with an additional eight encephalitis survivors having antibody to Nipah virus (Hsu et al., 2004). Clustering of cases occurred in several households, suggesting limited person-to-person transmission. No clear animal exposure was identified as a possible source for the disease, although two *Pteropus* bats in Naogaon were found to have antibodies to Nipah virus.

In January and February 2004, a cluster of encephalitic illnesses occurred in the Bangladesh district of Rajbari, followed by reports of other Nipah virus-associated illness in various other districts through March 2004 (Anon., 2004b). Altogether, 22 of 29 patients died. Nipah virus was isolated in this outbreak, which demonstrated a 95% homology with the Malaysian strain. A fourth outbreak occurred between
February and April 2004 in Faridpur District, with 27 fatalities from 36 total cases (Anon., 2004d). In this outbreak, it was observed that clusters of these cases occurred in households.

**Risk factors and mechanisms of transmission of Nipah virus**

Given the epidemiologic differences between cases in Malaysia, Bangladesh, and India, it is apparent that various factors play a role in the transmission of Nipah virus, including close exposure to intermediate zoonotic hosts, indirect contact with infected pteropid bats or exposure to their body secretions, and person-to-person transmission. In Malaysia and Singapore, direct contact with pigs, especially activities involving close contact, was the primary source of human Nipah virus infection (Parashar et al., 2000). Zoonotic transmission from pigs to humans probably occurred through respiratory droplets, given that infected pigs demonstrate both upper and lower respiratory vasculitis and have been shown in experimental studies to infect one another through the oral and respiratory route (Hooper and Williamson, 2000; Mohd Nor et al., 2000). Zoonotic transmission also appears to occur via close handling of infected tissue, as seen in the cases of infected abattoir workers (Paton et al., 1999).

Exposure to infected pigs accounted for most cases of Nipah virus in humans, but 8% of case patients stated had no direct contact with pigs (Parashar et al., 2000). Furthermore, no obvious zoonotic source of transmission has been found in any of the Bangladesh outbreaks. The observation that many infected individuals in Bangladesh were under 19 years of age and had no exposure to pigs or other animals, in contrast to the Malaysia outbreak has led to the hypothesis that infection might have occurred by indirect contact with fruit bats or their secretions. It was observed that in Goalanda, boys ate fruit collected from trees where fruit bats were presumably foraging (Anon., 2004c). Further epidemiologic studies and animal surveys of these outbreaks are currently ongoing.

Nipah virus has been isolated from urine and respiratory secretions of humans with Nipah virus infection during the Malaysia outbreaks, suggesting the possibility of person-to-person transmission. In Malaysia, no evidence of person-to-person transmission was found despite extensive searching; but several households in both the 2001 and 2004 outbreaks in Bangladesh exhibited family clustering of cases, suggesting that limited person-to-person transmission might have occurred (Hsu et al., 2004; Anon., 2004d). Person-to-person transmission was strongly suspected during the most recent outbreak in Faridpur District, of which studies are ongoing. Testing of high-risk health care workers with patient contact during the Malaysia and Bangladesh outbreaks revealed no evidence of nosocomial transmission (Mounts et al., 2001; Hsu et al., 2004). During the Siliguri outbreak, encephalitis cases developed among some hospital staff several days after the admission of patients with Nipah virus encephalitis, suggesting possible nosocomial transmission, but specific exposures were not assessed in affected individuals. Despite the high use of standard and respiratory precautions in Malaysia, only about 40% of health care
workers in Bangladesh during the outbreaks used any type of barrier precaution. These findings taken together suggest that person-to-person transmission of Nipah virus can occur, but that the transmission is rather inefficient, and probably requires prolonged close contact.

**Animal reservoirs**

Fruit bats (order Chiroptera), specifically bats of the genus *Pteropus*, have been shown to be the natural reservoir for Nipah and Hendra virus. About 60 species of pteropid bats, also known as flying foxes, are known to exist; they are native to Asia (including throughout China) and Australia, ranging as far west as the east coast of Africa and as far east as the Pacific Islands (Koopman, 1992). The bats develop subclinical disease due to Nipah virus and are assumed to be the intermediate hosts for infections of humans, but this has not been experimentally shown (Williamson et al., 1998, 2000). Suspicion of bats as natural hosts for the zoonotic paramyxoviruses began after neutralizing antibodies to Hendra virus were found in 4 species of pteropid bats in Australia (Young et al., 1996). Hendra virus has since been isolated from reproductive tissue from *P. poliocephalus* and *P. alecto* (Halpin et al., 2000).

Hendra virus has been demonstrated in Australian bats from the northern city of Darwin down to Melbourne as well as in Papua New Guinea (Paterson et al., 1998). Extensive animal surveillance among other animals has not shown evidence of natural Hendra virus infection among horses or farm animals, or among more than 40 species of wildlife tested from Queensland (Rogers et al., 1996; Ward et al., 1996).

Antibodies to Nipah virus have been found in 9–25% of pteropid bats in Malaysia, Cambodia, Thailand, and Bangladesh, (Yob et al., 2001; Hsu et al., 2004; Reynes et al., 2005; Wacharapluesadee et al., 2005). Neutralizing antibodies to Nipah virus were found in 4 species of pteropid bats in Malaysia; in Cambodia, antibodies were found in a third species, *P. lylei*; while in Thailand, antibodies were present in all three species. In Bangladesh, *P. giagnteus* bats, a more common species in that region, were found to have neutralizing antibodies to Nipah virus. Neutralizing antibodies to Nipah virus have also been found in other frugivorous and insectivorous bat genera including *Eonycteris*, *Cynopterus*, *Scotophilus*, and *Hipposideros*, although in a lower proportion than in *Pteropus* spp. (Yob et al., 2001; Wacharapluesadee et al., 2005; ) whether these bats are also considered natural hosts and the significance of these findings have yet to be determined. Isolation of Nipah virus from the bats has proven to be difficult, but the virus was isolated from 3 of 263 pooled bat urine samples in Malaysia, and 2 of 769 urine samples in Cambodia (Chua et al., 2002; Reynes et al., 2005).

**Menangle virus**

Menangle virus is a third zoonotic paramyxovirus, described only in New South Wales, Australia. Between April and September 1997, the number of live piglet
births at a pig farm near Sydney was noted to decrease dramatically, accompanied by an increase in the number of deformed and stillborn piglets (Philbey et al., 1998). A novel virus, named Menangle, was isolated from affected piglets, characterized, and found to be in the genus *Rubulavirus*, a genus whose viruses are distantly related to Nipah and Hendra viruses (Bowden and Boyle, 2005) (Fig. 1). Of more than 250 persons with potential exposure to the infected pigs, 2 had antibodies to the virus, both of whom had a self-limited illness consisting of malaise, chills, and fever. Extensive serologic investigations ruled out other viruses (Chant et al., 1998). The entire Menangle virus genome was subsequently sequenced (Bowden and Boyle, 2005). As with Hendra and Nipah viruses, fruit bats are thought to be the primary reservoirs for Menangle virus.

**Pathogenesis and clinical characteristics**

**Pathogenesis of Hendra virus infection**

The incubation period of Hendra virus is not known, but illness onsets for the first two human cases began between 5 and 8 days after the known contact with the index case mare. Autopsies of these two human cases revealed disease in lung and brain tissue. One patient, with symptoms primarily of pneumonitis, had focal necrotizing alveolitis with giant cells, syncytial formation, and viral inclusions (Selvey et al., 1995). The other patient, with predominantly encephalitic symptoms, had leptomenigitis with lymphocyte and plasma cell infiltration (O'Sullivan et al., 1997). Necrosis of the neocortex, basal ganglia, and cerebellum, was seen, but the subcortical white matter was not affected. It is unclear how Hendra virus enters the CNS, but a guinea-pig model suggested evidence of invasion via the choroid plexus (Williamson et al., 2001). Multinucleated endothelial cells have been seen in the liver and spleen from patients with Hendra virus infections. Hendra virus has been detected by PCR in serum and CSF from humans, but it has only been isolated from kidneys.

In addition to natural Hendra virus infection in horses and humans, Hendra virus has been experimentally transmitted to cats and guinea pigs. In horses, the predominant pathological findings are in lungs, with pulmonary edema and congestion; histologically, interstitial pneumonia has been found with focal necrotizing alveolitis, along with syncytial formation affecting the vascular endothelium. In horses, cats, and guinea pigs, the virus has been isolated from spleen, kidney, urine, and serum (Westbury et al., 1996; Williamson et al., 2000).

**Pathogenesis of Nipah virus infection**

The exact incubation period of Nipah virus is uncertain; however, the period from last contact with pigs to onset of symptoms during the Malaysian outbreaks was <2 weeks in 92% of patients (mean = 10 days) (Chong et al., 2000; Goh et al., 2000). However, incubation periods up to 2 months have been reported, and in one
case study, presumed Nipah virus encephalitis developed 4 months after exposure (Wong et al., 2001). It is unclear whether such unusual cases represent prolonged incubation periods or cases with late-onset encephalitis in initially asymptomatic individuals. The recent outbreaks in Bangladesh have not yielded further data regarding the incubation period.

Widespread vasculitis seen in patients is consistent with the viremia that appears to be the mechanism for spread to various organ systems. In humans and in the hamster animal model (Wong et al., 2002, 2003), it has been proposed that the virus enters the CSF as a result of vascular wall damage. In a porcine model, the data suggest that the virus invades the CNS directly through the cranial nerves (Weingartl et al., 2005). CNS involvement was seen in >90% of autopsies in the Malaysia outbreak, with both parenchymal necrosis and thrombotic vasculitis in the CNS, typically of the small vessels, characterized by varying degrees of segmental endothelial destruction, necrosis, and karyorrhexis (Wong et al., 2002). The lungs were the second most involved organ, with vasculitis seen in the lungs in 62% of cases, along with varying degrees of alveolar hemorrhage and pulmonary edema. Renal, cardiac, and splenic involvement is seen in lesser degrees, each associated with vasculitis, thrombosis, and necrosis (Chua et al., 2001). The occasional observation of syncytial multinucleated giant endothelial cells in the CNS and other organs is a distinct finding not usually seen in other types of viral encephalitis.

Nipah virus has been isolated from human CSF, throat and nasal swabs, and urine (Goh et al., 2000; Chua et al., 2001). Nipah virus has been shown experimentally to infect a variety of tissues from pigs, cats, dogs, and hamsters (Mohd Nor et al., 2000; Middleton et al., 2002; Wong et al., 2003). Most studies of the pathogenesis of Nipah virus have been conducted in pigs. Nipah virus infection is milder in pigs than in humans, often asymptomatic, with mortality from <1% to 5% (Mohd Nor et al., 2000). However, clinical disease in pigs can involve the respiratory system and CNS, and is known as porcine respiratory and encephalitis syndrome. Mild-to-severe lung injury is often present, with emphysema or hemorrhage, and evidence of consolidation. Histology of the lungs reveals interstitial pneumonia and syncytial cell formation with vasculitis, fibrinoid necrosis, and hemorrhage. In experimental infection of pigs, virus is present in nasal turbinates, trachea, lungs, cranial nerves, and olfactory epithelial cells (Weingartl et al., 2005).

Clinical manifestations

Clinical features of Hendra virus infection

It is difficult to delineate the clinical manifestations of a disease for which, to date, only three cases have been reported with detailed clinical information. No asymptomatic human infections have been observed, although asymptomatic Hendra virus infections have been noted in horses (Murray et al., 1995b).

The clinical features of Hendra virus infection involve the respiratory system or CNS, which range from a mild influenza-like illness to fatal pneumonia or
encephalitis. In two of the three cases reported with detailed clinical descriptions, presenting symptoms included myalgia, headaches, lethargy, and vertigo (Selvey et al., 1995). One of the two was characterized by 6 weeks of lethargy, and otherwise normal physical and laboratory examinations. The other patient went on to develop nausea, vomiting, and respiratory failure. His chest X-ray had bilateral alveolar and interstitial infiltration. Initial laboratory abnormalities included thrombocytopenia, liver enzyme elevation, acidosis, and hypoxemia, but the white count and differential remained in the normal range. The third case developed a recurrent neurologic syndrome that began as a self-limited meningitis lasting about 2 weeks, followed 1 year later by an encephalitic syndrome consisting of fever, altered mental status (unconscious by day 7 of the encephalitis), focal and generalized tonic-clonic seizures, and death (O'Sullivan et al., 1997). Initial blood count, electrolytes, and liver function tests were normal, but there was a mono-nuclear pleocytosis in the CSF. MRI of the brain showed gray matter abnormalities that worsened as the illness progressed. Of the patients infected with Hendra virus who recovered, there have been no reports of residual neurologic or other clinical deficits.

Clinical features of Nipah virus infection

Nipah virus can cause asymptomatic infections in some patients; in the Malaysia and Singapore outbreaks between 17% and 45% of infections were asymptomatic. There has not been any evidence for asymptomatic infection in outbreaks in Bangladesh (Hsu et al., 2004). In a study of Malaysian house holds with symptomatic family members infected by Nipah virus, 6 of the 36 (17%) antibody positive individuals were asymptomatic (Tan et al., 1999). Parashar found that among symptomatic pig farmers and their families in Malaysia, 30 of 110 (27%) were asymptomatic (Parashar et al., 2000). In another study designed specifically to compare symptomatic versus asymptomatic Nipah virus infection among high risk groups in Singapore, 10 of 22 (45%) of those with Nipah virus antibodies were asymptomatic, with no neurologic or respiratory symptoms (Chan et al., 2002).

Nipah virus infection produces an encephalitic syndrome predominantly characterized by fever, headache, and neurologic signs. Fever is almost universal, followed by headache in 65–88% of patients (Chong et al., 2000; Goh et al., 2000). A reduced level of consciousness was seen in 55% of all infected individuals during the Malaysia outbreaks (Goh et al., 2000) and in >90% in the Bangladesh outbreaks (Hsu et al., 2004; Anon., 2004c). Vomiting and dizziness are reported as prominent clinical features, which could be secondary to neurologic dysfunction. Some neurological signs reflected brain stem abnormalities, including reduced or absent reflexes, variable reactive pupils, and doll’s-eye reflexes. Other specific neurologic signs noted include myoclonus, tonic-clonic seizures, and nystagmus.

The respiratory system is the second most commonly affected system in Nipah virus infection. Cough, cold-like symptoms and dyspnea were the most common respiratory symptoms reported. Respiratory symptoms and abnormal chest
x-rays were reported at a higher rate in the Bangladesh outbreaks compared with the Malaysia outbreaks (Anon., 2004c, d). This finding may explain why person-to-person transmission was found in Bangladesh but not in Malaysia. The gastrointestinal system was much less commonly affected, with some reporting symptoms of abdominal pain, diarrhea, and constipation.

**Laboratory and radiographic findings**

Common hematologic abnormalities in Nipah virus infection include thrombocytopenia (30%) and leukopenia (11%). Elevated liver function tests are also seen in about 40% of patients, and hyponatremia is sometimes found. Hemoglobin, renal indices, and electrolytes other than sodium are usually normal. CSF white count and protein are elevated in about 75% of cases, although normal CSF white counts were reported in all cases in one outbreak (Chadha et al., 2006).

An initial IgM anti-Nipah virus antibody response was noted in about half of patients on day one of symptoms, rising to 100% from day 3–9 (Ramasundrum et al., 2000). Over half of patients exhibited positive IgG antibody after 2 weeks, and all became positive by day 17–25. However, the presence of antibody in serum or CSF did not influence the rate of isolation of virus from CSF, nor did it correlate with decreases in morbidity or mortality (Ramasundrum et al., 1999; Chua et al., 2000b).

Computed tomography of the head is normal, but MRI findings on T1-weighted imaging include multiple widespread small lesions in the white matter, mostly in the frontal and parietal lobes (Lee et al., 1999; Goh et al., 2000). The pons and cerebellum have also been affected (Lim et al., 2002). T2-weighted imaging demonstrates hyperintense lesions in gray matter and on fluid-attenuated inversion recovery sequences. Chest X-ray is abnormal in a varying number of patients, ranging from 6% to 72% in the Malaysia and Singapore outbreaks, consisting of mild interstitial infiltrates or alveolar consolidation in one or both lung fields (Paton et al., 1999; Chong et al., 2000; Goh et al., 2000; ). In Bangladesh, a chest X-ray pattern seen in acute respiratory distress syndrome was interpreted and reported (Anon., 2004d).

**Complications, relapse, and mortality**

The exact prevalence of individuals with neurologic or psychiatric sequelae of Nipah virus encephalitis is uncertain, as study results vary and sample sizes are generally small. The largest study found 15% (14 of 110 individuals) with residual neurologic deficits (Goh et al., 2000). Higher percentages of residual neurologic, psychiatric, or cognitive symptoms have been reported in smaller studies (Lim et al., 2003; Ng et al., 2004). Neurologic sequelae have included residual cognitive deficits, verbal impairment, cranial nerve palsy, cerebellar abnormalities, and persistent vegetative state. Neuropsychiatric sequelae have included personality
changes and major depression. Chronic fatigue syndrome has sometimes also been reported as a sequela of Nipah virus encephalitis.

Relapse occurs in 3–8% of patients, occasionally causing more severe clinical symptoms than the initial manifestation (Goh et al., 2000; Tan et al., 2002). Relapse occurs at a mean of 8 months after initial presentation. Late-onset encephalitis occurs in 3% of infected patients who were initially asymptomatic or non-encephalitic, and has been reported to occur as late as 4 months after initial infection (Goh et al., 2000; Wong et al., 2001). Clinical symptoms of relapsed and late-onset encephalitis are similar to those of initial encephalitis. However, focal MRI abnormalities of the cortical gray matter have also been present in patients with relapsed or late-onset encephalitis.

The overall mortality rate of symptomatic Nipah virus infection differs by country: 40% (105/265) in Malaysia, 9% (1/11) in Singapore, 74% (exact figures uncertain) in India, and 76% (66/87) in Bangladesh. It is uncertain whether these differences are due to virulence factors in the virus or whether they reflect the level or availability of supportive care in each country. Factors that predicted mortality included the presence of doll’s-eye reflexes, tachycardia, high fever, hypertension, and a positive viral culture of the CSF (Chong et al., 2000; Chua et al., 2000b; Goh et al., 2000).

**Laboratory diagnosis**

Only a few laboratories worldwide have the capability for testing and confirming the presence of Hendra and Nipah viruses by virus isolation, immunohistochemistry, and molecular amplification. In addition, these viruses are classified as biosafety level 4 (BSL-4) agents and must be handled under strictest physical containment standards.

Viral isolation in cell culture from affected tissue is an important diagnostic methodology for these viruses, particularly when determining the etiology of a new outbreak (Daniels et al., 2001). Both Hendra and Nipah viruses grow well in Vero cells, and a cytopathic effect is usually noted within 3 days. Nipah virus has been isolated from human CSF, nasal and throat swabs, and urine (Chua et al., 1999; Goh et al., 2000). The virus has also been isolated from pigs and cats in a variety of tissue including lung, spleen, serum, and kidneys (Daniels et al., 2001; Middleton et al., 2002). Although Hendra virus has been isolated in humans only from kidney tissue, it has been isolated from serum, lung, spleen, and CNS tissue from a variety of animals including horses, cats, and guinea pigs (Murray et al., 1995a; Williamson et al., 1998, 2000, 2001). Immunostaining, neutralization techniques, PCR, and EM, including immunoelectron microscopy, are utilized for further identification of the virus.

Immunohistochemistry can be performed on preserved tissues allowing a diagnosis to be made retrospectively. It also has the advantage that testing can be done in the absence of a BSL-4 facility. A range of polyclonal and monoclonal antisera are used, but they are not available commercially. PCR methods and
sequencing are necessary for genetic characterization of these viruses, especially with a suspected new outbreak in a geographically distinct area, as occurred in the Bangladesh outbreak (Harcourt, 2005). Nested primers coding for the M or N genes are most commonly used at present, although primers for the P gene were used in the initial outbreak in Malaysia and Singapore (Chua et al., 2000a; Daniels et al., 2001).

Serologic methods include neutralization tests and enzyme-linked immunosorbent assays (ELISA). The serum neutralization test is the accepted standard for serology, but it requires BSL-4 facilities, as cell cultures must be used to determine whether a cytopathic effect has occurred. The ELISA utilizes both indirect formats for IgG and antibody capture for detecting IgM antibodies for Hendra and Nipah viruses. To perform these tests, preparation of viral antigen has been used, but research is being done to express antigen utilizing individual viral proteins such as the N protein of both viruses (Bellini et al., 2002). These newer techniques allow ELISA preparation and testing to be done at facilities with BSL-2. This gives ELISA the advantage of being able to quickly detect antigen in a wider range of laboratory settings; the test is also more useful for rapid diagnosis for many cases in a suspected outbreak setting compared to serum neutralization. However, the sensitivity and specificity of the ELISA test are slightly inferior to serum neutralization tests.

Treatment, prevention, and control

Treatment for Hendra virus is supportive only. No effective antiviral therapy is known for Hendra virus. However, in vitro, ribavirin has been shown to have an inhibitory effect against RNA synthesis and the yield of Hendra virus (Wright et al., 2005).

Ribavirin and acyclovir have been used to treat Nipah virus infection. In Malaysia, ribavirin was administered orally or intravenously to 140 persons with Nipah virus encephalitis and compared to a group of 54 control patients who did not receive ribavirin. A total of 45 deaths in the treated group (32%) compared to 29 deaths in the control group (54%) suggested a 36% reduction in mortality with ribavirin administration (Chong et al., 2001). In Singapore, acyclovir was administered to all encephalitis patients during the Nipah outbreak (Paton et al., 1999; Bellini et al., 2002). Only one fatality occurred in Singapore, but the effect that the drug had on the course of disease is unclear.

Nipah and Hendra virus infections can theoretically be prevented by avoiding direct or indirect contact with fruit bats or fruit bat urine or droppings. Using precautionary measures such as gloves and masks may be considered in locations with fruit bats. Fruit or other products from trees where fruit bats roost should be carefully washed.

Because it is assumed that these viruses can spread through respiratory droplets or by contact, caution should be used when caring for an infected individual, including frequent handwashing, avoidance of direct contact with urine or salivary
secretions, and wearing a mask. Although nosocomial transmission of the viruses is unlikely, contact and droplet precautions seem prudent when taking care of any patient with suspected Nipah or Hendra virus infection.

Surveillance is an important tool for early detection for illnesses caused by the henipaviruses. Surveillance for Hendra virus illness has been established in Australia, and surveillance for encephalitic disease has been implemented in Malaysia, Thailand, and Bangladesh. Clusters of respiratory or encephalitic illness in humans or in certain animals, occurring in geographic locations where Pteropus bats are known to be endemic, should raise awareness of the possibility of henipavirus infection. Thus, surveillance in animals such as horses and pigs is also important for the early detection of Nipah and Hendra virus infections.

No specific vaccine is available against Nipah or Hendra virus. However, active immunization against Nipah virus and passive transfer of antibody to Nipah virus have shown promising results in hamster models (Guillaume et al., 2004).

Ecologic aspects and future considerations

Ecologic changes, human demographics, and behavior patterns such as international travel, technology and industry, and microbial adaptation have all been factors that are thought to play a role in infectious disease emergence (Morse, 1995). For the henipaviruses, speculation has focused on environmental changes such as deforestation and hunting, which subsequently affected the roosting habitats of flying foxes and placed them in closer proximity to humans (Field et al., 2001; Daszak et al., 2004; Breed et al., 2005). It should be noted also that in Bangladesh, located at a more subtropical latitude, all Nipah outbreaks occurred in the first half of the year, suggesting that perhaps climate change or bat activities affected by seasonal change may also have played a role. Until a better understanding of the causes of these newly emergent viruses is obtained, future outbreaks are likely to recur. Research is continuing to identify the factors that have led to the emergence of Hendra and Nipah viruses in domestic animal and human populations.

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References


