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INTRODUCTION

Henipaviruses were not discovered until 1994, when a novel virus, later called Hendra virus (HeV), was identified as the etiological agent of a fatal viral encephalitis in Hendra, Australia. Nipah virus (NiV), another virus closely related to HeV, emerged in Malaysia in 1998. Subsequently, the genus Henipavirus was established within the family Paramyxoviridae as a taxonomic home for both HeV and NiV. HeV and NiV are high-consequence pathogens that are thought to be potential starting materials for biological weapons construction because they cause diseases with high case fatality rates, have the potential for aerosol transmission, are easily grown in cell culture to high titers, and have an unusually wide host range that encompasses humans and livestock, such as pigs and horses. The recent discovery of new henipaviruses of unknown virulence and spillover potential in bats emphasizes the need to understand this emergent group of viruses.

As disease agents posing a severe threat to both agricultural livestock and human health, HeV and NiV are considered “overlap” select agents regulated by both the US Animal and Plant Health Inspection Service and the Centers for Disease Control and Prevention. As is the case for most select agents, approved therapeutics for human henipavirus infections do not exist. Promisingly, however, there have been rapid advances in developing new therapies and in repurposing existing Food and Drug Administration (FDA)-approved drugs for use in henipavirus infection treatment.

HISTORY AND EMERGENCE OF HENIPAVIRUSES

Hendra Virus

Thoroughbred horse racing has been one of the great pastimes of Australia. The public imagination was struck in September 1994 when a prominent racehorse trainer, Vic Rail, 13 of his horses, and another horse from a neighboring property died of a sudden illness in Hendra, Australia. Rail’s stablehand also became ill, but recovered after an extended convalescence. The illness in both horses and humans was characterized by respiratory distress with neurological signs, and it culminated in blood-tinged, frothy nasal discharge in the horses.

A major outbreak investigation ensued resulting in the identification of a new “equine morbillivirus” from infected horse and human samples. Experimental inoculation of this virus into naïve horses reproduced the disease. Preliminary phylogenetic analysis resulted in the placement of this new agent in the genus Morbillivirus (of which measles virus is the prototype member) in the family Paramyxoviridae. As this “equine morbillivirus” had unique molecular and pathogenic features that distinguished it from other morbilliviruses, the agent was renamed Hendra virus and finally reclassified in a novel paramyxoviral genus, Henipavirus.

A year after the Hendra outbreak, a horse stud owner died of relapsed encephalitis and was retrospectively diagnosed with HeV infection, originating from an encephalitic illness that predated the Hendra outbreak by several weeks. This single infection, which had no known epidemiological link to the Hendra outbreak, occurred 970 km north of Hendra, near Mackay. As in the Hendra outbreak, transmission to the stud owner involved horses, two of which died. Since the 1994 outbreaks, HeV infection emerged only periodically, and briefly, in 1999 and 2004. Then HeV infection emerged yearly from 2006 and proceeded in a truly accelerated fashion from 2011, all in Australia and ranging up and down the east coast (Figure 22-1).

All disease outbreaks involving horses had an 84% case fatality rate, and of the seven human cases, including the 1994 outbreaks, four died. Considering that retrospective inspection of laboratory records and historical samples did not reveal signs of pre-1994 spillover of HeV to horses, HeV infection may have emerged from its natural reservoir in or around 1994. The lack of an epidemiological link between the outbreaks suggested potential spillover from another animal reservoir to the horses. However, extensive sampling of domestic animals and wildlife initially revealed no sign of HeV. A more targeted investigation based on outbreak characteristics (host should be present in both outbreak locales, be able to move or interchange between the locales, and plausibly have contact with horses) revealed that fruit bats of the genus Pteropus (Figure 22-2), otherwise known as “flying foxes,” had anti-HeV antibodies and sometimes were infected with HeV itself.

Nipah Virus

The emergence of NiV has several parallels to that of HeV, but with more dramatic consequences. NiV is named after Sungai Nipah, Malaysia, the home village of the patient from whom NiV was first isolated. NiV, like HeV, emerged from pteropid fruit bats, but in this case spilled over to domestic pigs (Sus scrofa domest-
Figure 22-1. The bats that harbor henipaviruses, including Hendra virus and Nipah virus, have widespread distribution. However, outbreaks of Hendra and Nipah disease have been limited to Australia and Malaysia/Bangladesh, respectively. Each outbreak shown on the timeline may represent more than one spillover event from the bat reservoir.


cus), which served as highly effective amplifying hosts. The first recorded potential NiV spillover occurred in 1997, with unusual pig deaths at pig farms near Ipoh, Malaysia, attributed to classical swine fever at the time. However, retrospective analysis of serum samples from several patients presenting with encephalitis in 1997 detected anti-NiV antibodies.17

Beginning in September 1998, also near Ipoh, pigs and pig farmers became ill in a major outbreak of febrile encephalitis with respiratory symptoms. The mosquito-borne Japanese encephalitis virus (JEV), endemic to Southeast Asia, was immediately suspected, especially since domestic pigs are known to serve as an amplifying host for JEV,18 and also because 4 of 28 initial human cases tested positive for anti-JEV antibodies.17 Despite extensive anti-JEV measures, however, including mosquito control and a JEV vaccination campaign,19 the outbreak continued unabated, spreading into neighboring districts and afflicting increasing numbers of pigs and humans. The outbreak also spread to Singapore via exported infected pigs.20 Furthermore, the outbreak was widespread with disease mainly afflicting adults,21 whereas Japanese encephalitis is asymptomatic in the vast majority of cases and more likely to cause disease in children.22 Also, cerebrospinal fluid from an infected patient gave rise to syncytia (multinucleated cells formed by cell-cell fusion) in Vero cell culture, indicative of a pH-independent fusion mechanism of the infectious agent.22 Such formation is uncharacteristic of JEV, which has a pH-dependent fusion mechanism. Finally, the Centers for Disease Control and Prevention
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determined by cross-reactive immunofluorescence, serology, and sequence analysis that the novel agent was closely related to HeV.\textsuperscript{23}

At the end of the 1998–1999 outbreak, at least 294 human infections resulting in clinical encephalitis were recorded in Malaysia and Singapore, of which 106 infections were fatal.\textsuperscript{17,20} The inclusion of milder nonencephalitic cases (as determined by exposure to infected pigs and seroconversion, a number of which later developed late onset encephalitis) increased the total number of cases to more than 360.\textsuperscript{24,25} More than a million pigs were culled in an effort to contain the outbreak, resulting in severe economic damage and widespread changes to the pig farming industry.\textsuperscript{26,27} Subsequent outbreaks of NiV infection have occurred in Bangladesh and adjacent areas of India on an almost yearly basis (Figure 22-1). In contrast to the Malaysian outbreak, these subsequent spillovers from pteropid bats have mainly occurred without involvement of a domestic animal intermediate. Generally these spillovers had higher case fatality rates, ranging from 40% to 100%.\textsuperscript{28} These differences may be the result of differences among disparate NiV strains, the standard of care in Bangladesh/India versus Malaysia, or

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image1}
\caption{One of the reservoirs of Hendra virus: a spectacled flying fox (Pteropus conspicillatus) near Cairns, Australia. Photograph: Courtesy of Pål A Olsvik, Bergen, Norway.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{image2}
\caption{(a) In Bangladesh, fresh date palm sap is collected by shaving the date palm tree, placing a tap, and collecting the sap in a clay pot overnight. Fruit bats are known to lick the sap stream and even urinate or defecate into the clay pots, thus potentially contaminating the raw sap with infectious Nipah virus. (b) Covering the sap stream with a bamboo skirt is highly effective at preventing contamination. Photographs: Courtesy of Nazmun Nahar, International Centre for Diarrhoeal Disease Research, Bangladesh.}
\end{figure}
sociocultural factors unique to Bangladesh/India. For example, a major route of spillover during the Bangladesh outbreaks is the consumption of fresh date palm sap, a seasonal delicacy that can become contaminated with fruit bat saliva or excreta during collection (Figure 22-3).

Newly Identified Henipaviruses

After nearly two decades of intensive investigation of HeV and NiV, evidence of novel henipaviruses in diverse wildlife species has created increased public health concern. For example, the recent discovery of divergent new clades of henipaviruses in indigenous bat populations across Africa and even Central/South America, at least another henipavirus endemic to Australia but distinct from HeV (Cedar virus), and a henipavirus in southeastern China (Mojia virus) from a putative rodent reservoir that may be linked to cases of fatal pneumonia in humans, all raise urgent questions about the possible global emergence of these zoonotic viruses. These new findings extended the potential geographic distribution of henipaviruses from Southeast Asia/Australia to Africa and Central/South America (Figure 22-1). At least one of these novel henipaviruses, Cedar virus, could be isolated in culture, but was nonpathogenic in small animal models. The discovery of a potential henipavirus spillover to humans in Cameroon, however, emphasizes the need to both increase vigilance and knowledge of this latent pool of henipaviruses. Human sera from Cameroon capable of neutralizing NiV were significantly associated with bat butchering in areas of intensive deforestation. This association highlights the role of environmental changes and specific human behaviors in determining the risk of zoonotic transmission. Although the pathogenicity and virulence of newly discovered henipaviruses remain to be determined, the repeated misdiagnosis of NiV as Japanese encephalitis in Southeast Asia remains a cautionary tale. Henipavirus-derived illness may often be ascribed to other encephalitic diseases known to occur in the affected area.

EPIZOOOTIOLOGY AND EPIDEMIOLOGY OF HENIPAVIRUSES

Pteropid (fruit) bats appear to be the major reservoir host for HeV and NiV. No appreciable signs of HeV and NiV infections have been found in other wildlife or domestic animals. Anti-HeV or anti-NiV antibodies are highly prevalent in pteropid bat populations throughout Southeast Asia and Australia; HeV and NiV have been isolated from Pteropus fruit bats throughout their geographic range; and all known outbreaks of HeV and NiV infection are linked to exposure of domestic animals or humans to fruit bats or their excretions. Furthermore, experimental infection of Pteropus bats belonging to different species with HeV or NiV did not result in clinical signs of infection, despite inconsistent signs of seroconversion, viral replication in tissues, and virion excretion in urine. These data support the hypothesis that HeV and NiV are not pathogenic in their natural Pteropus hosts, thus persisting subclinically in these bat populations. Despite their apparent long coevolutionary history with bats and likely endemic nature, HeV and NiV emerged to cause human disease suddenly and nearly simultaneously, likely signaling common factors driving their emergence. A retrospective study of environmental factors and man-driven changes in Malaysia identified slash-and-burn agriculture with concomitant loss of forest habitat, a resulting impenetrable smoke haze that led to crop failures, and severe drought as potential precipitating factors. Fruit bats were driven to populate cultivated fruit orchards, which were often located adjacent to pig farms, thus providing a means of transmission. In one plausible scenario, half-eaten fruits contaminated with NiV-infected bat saliva are dropped into a pig farm and then eaten by the pigs. Such half-eaten fruits were found in pig farms near the epicenter of the 1998–1999 NiV encephalitis outbreak. Similar environmental and manmade pressures facing fruit bat populations across Southeast Asia and Australia are likely behind the persistent upsurge in HeV and NiV spillovers in recent years.

Hendra Virus

All seven known human cases of HeV infection resulted from intimate contact with sick horses. HeV is highly virulent in horses, and infection often culminates in copious production of infectious respiratory secretions. Human infections were traced back to efforts to save these horses without the use of personal protective equipment. A horse trainer, for example, attempted to force-feed a sick mare with abraded bare hands. However, such cases of horse-to-human transmission remain the exception. Many other people who were also highly exposed to contaminated horse bodily fluids, even to fluids from horses that were implicated in HeV transmission to other humans, did not develop signs of HeV infection. With increased public awareness of the risk of HeV transmission and the corresponding increase in proper use of personal
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protective equipment, the risk of spillover to humans has been mitigated. The introduction of an HeV vaccine for horses in 2012, Equivac HeV (Pfizer Animal Health, Brisbane, Australia), will hopefully further minimize human transmission risk.

How horses initially become infected remains to be elucidated. Equine cases of infection may result from horses grazing on pastures contaminated with bat excreta or remains of half-eaten fruit. Infected horses inefficiently transmit the virus, even to other horses, but some horse-to-horse transmission may occur through licking infectious nasal discharge. Respiratory transmission of HeV has never been demonstrated experimentally or during natural infection. The inefficiency of HeV transmission is buttressed by findings that despite a continuous low prevalence of HeV in Pteropus bats in Queensland and New South Wales, people having extensive contact with Pteropus bats in these areas, with bat bites and exposures to bat blood in many cases, had no sign of HeV exposure. Direct comparisons of NiV and HeV infection in some rodent models suggest that HeV may be less efficient in infecting animals through the intranasal route, although this lower efficiency remains to be verified in a more relevant nonhuman primate model.

Nipah Virus

Malaysia Outbreak, 1998–1999

During the large Malaysia outbreak, pigs served as a highly effective amplifying reservoir, contracting infection and transmitting NiV so efficiently that the infection rate among pigs at affected farms approached 100%. The risk to humans posed by this spread was exacerbated by the mild illness NiV caused in the pigs, with a lethality of less than 1% to 5% and often presenting asymptomatically. Unsuspecting farmers thus moved asymptomatic pigs to other farms and slaughterhouses, quickly spreading the virus. A subset of infected pigs developed febrile illness, respiratory signs such as labored breathing and harsh nonproductive cough, and neurological signs such as myoclonus and uncoordinated gait.

Nearly all human cases during the Malaysia NiV encephalitis outbreak can be attributed to direct contact with or proximity to infected pigs, and most cases were pig farmers. One person who denied any recent proximity to pig farms repaired pig cages, suggesting that the virion-containing secretions remained infectious on surfaces for extended periods. A few human infections may have resulted from secondary transmission through dogs, which were commonly infected during the outbreak. Evidence of human-to-human transmission is limited. A large cohort study of 393 healthcare workers intimately involved in caring for NiV-infected patients identified only three nurses who seroconverted and had potential illness, despite many reported high-risk exposures. Risk may have been minimized by precautions taken by the healthcare workers, because patients clearly shed infectious NiV in respiratory secretions and urine, especially during the early phase of illness. Human-to-human transmission possibly may have played a role in the NiV outbreak, although the epidemiological record does not provide unambiguous evidence. Finally, retrospective investigations appear to show that NiV was causing disease in pigs as early as 1996, but that the mild symptoms and rough similarity to other diseases (e.g., classical swine fever) did not raise suspicion of anything unusual. The advent of increasingly inexpensive, high-throughput sequencing may make disease surveillance and agent identification more likely to catch emerging pathogens such as NiV in the future.

Bangladesh and India, 2001 to Present

In comparison to the Malaysia NiV encephalitis outbreak, subsequent outbreaks in Bangladesh and India are of greater concern because of evidence of clear chains of human-to-human transmission. As in Malaysia, infected patients shed NiV in their bodily fluids including saliva. The intimate care for sick family members, involving being in physical contact, sharing utensils and food, and sleeping in the same bed to provide them comfort, greatly increased the risk of transmission. The longest documented transmission chain involved five generations, with the third generation involving a religious leader who infected 22 family members and followers. A major 2001 outbreak in Siliguri, India, was also characterized by hospital-associated transmission: one admitted patient became the source of more than 40 subsequent infections within a hospital and nursing homes. As with the outbreak in Malaysia, Japanese encephalitis was initially suspected, and the causative agent was not identified as NiV until samples were retrospectively analyzed several years later.

The outbreaks in Bangladesh and India are also notable for the apparent lack of a domestic animal intermediate between the Pteropus bat reservoir and humans. Although a few incidents appear to involve domestic animals such as cows, pigs, or goats, the major route of spillover has been the consumption of contaminated fresh date palm sap. Date palm trees are tapped for their sweet sap in the winter, and bats often lick the sap stream. Defecation and urination...
into or near the collection pots, or even drowned dead bats, have been observed.\textsuperscript{31,63} Fortunately, the use of a simple bamboo skirt to cover and protect the sap stream, a method local to northwest Bangladesh that has not been consistently or widely used, appears to be highly effective in preventing contamination (Figure 22-3).\textsuperscript{64} More widespread use of this method and other interventions to minimize risk would help the affected regions of the Indian subcontinent break out of its cycle of yearly NiV encephalitis outbreaks.

CLINICAL PRESENTATION OF HENIPAVIRUS INFECTIONS

The incubation period of HeV or NiV infection and illness typically ranges from a few days to 14 days.\textsuperscript{65} Although information on HeV infection is limited because of the few human cases, the clinical signs and pathology of HeV and NiV infections are similar.\textsuperscript{66} The hallmarks of henipavirus pathogenesis are extensive vasculopathy, respiratory distress, and encephalitic disease with corresponding neurological symptoms. Respiratory and encephalitic symptoms may appear to varying degrees. During the first Malaysia NiV disease outbreak, clinical signs were mainly encephalitic in nature with minor pulmonary involvement, whereas in subsequent outbreaks in Bangladesh, encephalitis was more commonly joined by severe respiratory distress.\textsuperscript{29,62} Researchers are actively investigating whether these differences result from genetic differences among viral variants, the route of transmission, or other factors.

Clinical Signs and Symptoms

Neurological signs and symptoms may include fever, headache, confusion, myocloni, seizures, meningism, and motor deficits including areflexia and hypotonia.\textsuperscript{5,29,48,59,67,68} Brain stem involvement, a poor prognostic factor, may be evidenced by a reduced level of consciousness, vomiting, abnormal pupillary and doll’s eye reflex, hypertension, and tachycardia.\textsuperscript{65}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image}
\caption{(a) Multinucleated endothelial syncytium (arrow) in brain with perivascular hemorrhage, hematoxylin and eosin stain. (b) Necrotic plaque in cerebral parenchyma with adjacent thrombosis (arrow), hematoxylin and eosin stain. (c) Typical magnetic resonance imaging for acute henipavirus encephalitis, with discrete, hyperintense lesions. Photographs: Courtesy of KT Wong, University of Malaya, Kuala Lumpur, Malaysia, with permission from Elsevier (a and b) and Neurology Asia (c).}
\end{figure}
Cerebrospinal fluid is characterized by elevated white blood cell counts and/or protein concentration in a substantial proportion of cases.\(^{58,69}\)

A minority of survivors (<10\%) of HeV and NiV infection may experience relapsing encephalitis after apparent recovery or even initial asymptomatic or apparently nonencephalitic infection. Relapse may occur soon after apparent recovery or long after, with an average of 8 months and up to 11 years documented.\(^6\) Even without relapsing or progressive disease, a substantial proportion of survivors may experience long-term neurological deficits.\(^{70,71}\)

Although the Malaysia outbreak of NiV disease was mainly characterized by encephalitic signs, a substantial proportion of patients still developed pulmonary signs such as cough and abnormal chest radiographs.\(^{20,48,68}\) More severe pulmonary symptoms, seen in subsequent NiV disease outbreaks and also some cases of HeV infection (eg, a horse trainer who developed progressive respiratory failure\(^5\)), may also include atypical pneumonia, breathing difficulty, and acute respiratory distress syndrome.\(^{25,63}\)

Pathology

Infection of microvascular endothelial cells leads to systemic vasculitis, thrombosis, and resultant microinfarction, especially in major organs such as kidneys, heart, lungs, and brain.\(^{65,72}\) Focal perivascular necrosis or hemorrhage is seen in highly vascular organs such as the spleen.\(^72\) Syncytia (giant multinucleated cell) formation is occasionally seen in the endothelium (Figure 22-4a) and among parenchymal cells of major organs. In the brain, discrete plaque-like lesions with varying degrees of necrosis, edema, and inflammation (Figure 22-4b) likely correspond to the small hyperintense lesions in both grey and white matter seen by magnetic resonance (Figure 22-4c).\(^6\) The dual pathology of vasculitis with associated microinfarction and direct infection of parenchyma of major organs is a distinguishing feature of henipavirus pathogenesis.\(^{68}\) Tissue damage in the central nervous system from both microinfarction and direct infection of neuronal cells distinguishes henipavirus infections from other viral encephalitic diseases.\(^74\) Relapse encephalitis appears to result from recrudescent infection, with extensive parenchymal necrosis, edema, and inflammation corresponding to confluent lesions seen in magnetic resonance scans (as opposed to the more discrete foci usually seen during acute encephalitis during early illness). Pathology associated with relapsing encephalitis is only found in the central nervous system, and no vasculopathy is present, even in the brain.\(^{25,75}\)

MOLECULAR BIOLOGY OF HENIPAVIRUSES

Virus Structure

Henipaviruses are negative-sense ribonucleic acid (RNA) viruses that produce enveloped virions. Henipaviruses are currently classified as members of the genus Henipavirus, family Paramyxoviridae, order Mononegavirales.

Like other paramyxovirus particles, Hendra and Nipah virions have pleomorphic shapes, usually more spherical although sometimes filamentous as well (Figure 22-5a), and range in size from less than 200 nm to more than 1,000 nm in diameter.\(^{76,77}\) The virions contain helical ribonucleocapsids with the herringbone appearance characteristic of, and unique to, paramyxoviruses (Figure 22-5b). A unique feature of Hendra virions is the frequent presence of a double fringe surrounding the particle,\(^77\) which may result from differing lengths or conformations of the envelope proteins; in contrast, Nipah virions predominantly display a single fringe.

Virus Genome

Like all mononegaviruses, henipaviruses have a linear, monopartite, single-stranded RNA genome of negative polarity. The overall structure of henipavirus genomes is similar to those of other paramyxoviruses, with 3′ leader and 5′ trailer sequences at the termini of the genomes that act as virus-specific promoters, 5′ and 3′ untranslated regions flanking each gene, and a conserved intergenic signal between each gene.\(^78\) With a few recently described exceptions, henipaviruses have the longest known paramyxoviral genomes (≈18 kb). The especially long 3′ untranslated regions are unique features of henipavirus genomes and account for much of the extra length compared to other paramyxoviruses (≈15 kb).\(^79\) The functional relevance of these long untranslated regions remains to be determined.

Viral Proteins

Henipavirus genomes contain six genes, which encode a nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), attachment glycoprotein (G), and large RNA-dependent RNA polymerase (L). N encapsulates the genome, thereby forming the ribonucleoprotein complex. Like the genomes of other paramyxoviruses, henipavirus genomes have nucleotide lengths that are evenly divisible by six, a feature likely resulting from the periodicity of N protein encapsidation of
the genome. P and L form the polymerase complex, which replicates the viral genome and transcribes viral mRNAs.

M organizes the assembly and budding of virions and underlies the viral envelope. M also transits through the nucleus, although the ultimate significance of this transit for henipavirus replication and pathogenesis remains unclear. G binds to the cellular receptor(s), and F catalyzes the membrane-membrane fusion responsible for viral entry. G and F can also catalyze cell-cell fusion, leading to syncytium (giant multinucleated cell) formation, which is a hallmark of henipavirus infection (Figure 22-5c). The henipavirus attachment protein is unique among paramyxoviruses in that it does not possess any hemagglutinin activity (although morbilliviruses such as measles virus will only agglutinate red blood cells from primates of certain species).80

The tissue tropism of HeV and NiV is determined by entry receptor use. The receptor tyrosine kinase ephrin-B2 serves as an entry receptor for all known henipaviruses, but at least HeV and NiV can also use the related ephrin-B3 as an alternative receptor.83,84 As cellular factors that are critical during embryogenesis, ephrin-B2 and -B3 are highly evolutionarily conserved. This high level of conservation contributes to the unusually wide potential host range of HeV and NiV, which have been shown to infect mammals spanning six orders.85 Ephrin-B2 and -B3 from a wide range of mammals allow viral entry, chicken embryos are susceptible to NiV infection, and even zebrafish ephrin-B2 can serve as an entry receptor.72 The tropism of henipaviruses is also not restricted by the cellular protease required for F protein cleavage, a maturation step required to render it fusion-competent. Unlike some paramyxoviral F proteins that require a protease of limited tissue distribution, the henipavirus F protein uses the ubiquitously expressed endosomal protease cathepsin L, thereby further extending tissue and possibly host tropism.

Figure 22-5. (a) Nipah virions produced from infected Vero E6 cells. (b) Nipah virus nucleocapsid, with the herringbone appearance characteristic of paramyxoviruses. (c) Multinucleated Nipah virus-induced syncytium in human umbilical vein endothelial cells. Blue represents nuclei; red represents actin filaments and illustrates cell boundaries; and green represents Nipah virus matrix protein.

Photographs: (a and b) Courtesy of Cynthia Goldsmith, Centers for Disease Control and Prevention, Atlanta, Georgia. (c) Courtesy of Arnold Park, Icahn School of Medicine at Mount Sinai, New York, New York.
Henipaviruses

PATHOGENESIS

The pathogenesis of henipavirus encephalitis has been examined predominantly using animal models. The oronasal route of infection is used most often during such experiments as it reflects at least one type of natural transmission and might also simulate exposure during a biological attack.\textsuperscript{90} Similar to results obtained in vitro, in vivo tropism is predominantly determined by the absence or presence of henipavirus receptors on potential target cells.

Ephrin-B2 is highly expressed on the endothelial and smooth muscle cells that line arterial vessels, lungs, and brain. The human airway epithelium expresses both ephrin-B2 and -B3,\textsuperscript{91} and upon infection, the virus likely crosses the epithelium via limited basolateral virion release, while disruption of the epithelium via cell-cell fusion may also facilitate systemic entry. In many animal models of Nipah encephalitis, the alveolar epithelium tests immunopositive for NiV antigens, and the alveolar wall frequently undergoes fibrinoid necrosis.\textsuperscript{92} Interestingly, in pigs, an increased number of alveolar macrophages is a consistent histological feature. In human lung tissue, viral antigen is found in multinucleated giant cells located in the alveolar space.\textsuperscript{73} Multinucleated giant cell formation is a product not only of alveolar macrophage fusion, which is generally a prominent feature of chronic inflammatory conditions, but also of NiV-induced syncytia formation. Transmigration of infected alveolar macrophages may serve as a “Trojan horse” for the virus to gain systemic access via the microvasculature. Cell-free and cell-associated viremia then result in systemic infection.\textsuperscript{93}

Ephrin-B3 expression is mostly restricted to the central nervous system (eg, brainstem), which correlates with the observation of antemortal brainstem dysfunction noted during henipavirus encephalitis.\textsuperscript{45,93} Henipaviruses likely gain access to the brain via basolateral release from brain microvascular endothelial cells, disruption of the blood-brain barrier as a result of cell-cell fusion and vasculitis, and transmigration of “Trojan horse” inflammatory macrophages. It is also possible that henipaviruses access the central nervous system more directly via infection of the olfactory epithelium and spread along the olfactory neurons.\textsuperscript{54,95}

Clear differences exist between HeV and NiV infections regarding localization of initial infection in animal models despite their use of the same entry receptors,\textsuperscript{46,47} which has yet to be understood. The mechanism of foodborne NiV transmission also is unclear since digestive tract epithelium does not express ephrin-B2 or -B3.\textsuperscript{72} Virus infection most likely occurs via accessible and susceptible cells in the oropharyngeal mucosa, such as those of tonsillar tissues and salivary glands.

Henipaviruses partly owe their virulence to effective antagonism of host immune responses. Interferons (messengers of innate immunity that trigger extensive antiviral responses) bind extracellularly to interferon receptors, which usually trigger an intracellular signal cascade that includes the critical immune signaling factor signal transducer and activator of transcription 1 (STAT1). Like other paramyxoviruses, HeV and NiV encode several nonstructural proteins from their P genes, that is, the C, V, and W proteins, which antagonize innate immune signaling. The P, V, and W proteins have identical N-termini that bind and inhibit STAT1, partly by sequestering STAT1 and thus inhibiting transcription of interferon-inducible genes.\textsuperscript{96–99} The P gene products also antagonize signaling from other intracellular sensors of infection, such as TLR3 and Mda-5,\textsuperscript{100–102} which sense double-stranded RNA (an intermediate of viral replication) in endosomes and the cytoplasm, respectively.

In contrast to the host species-specific inhibition of interferon signaling induced by some paramyxoviruses,\textsuperscript{103} NiV can inhibit interferon signaling in cells from a number of tested mammals,\textsuperscript{104} consistent with the ability to cause disease in a wide range of hosts. The high virulence of henipaviruses in the “wrong” host may therefore be a function of their broad tissue tropism in vivo, their ability to gain systemic access, and their inhibitory effect on immune responses.

HENIPAVIRUSES AND BIOLOGICAL WEAPONS

HeV and NiV are classified as Centers for Disease Control and Prevention Category C Bioterrorism agents because of the following:

- their availability,
- ease of production and dissemination, and
- potential for high morbidity and lethality.

Availability

HeV and NiV are readily available from tissues or fluids from patients during frequent, recurring disease outbreaks as well as by isolation from their natural bat reservoirs. Furthermore, reverse genetics systems are available in biosafety level 4 laboratories for the rescue
of henipaviruses directly from plasmids. De novo access to, and modification of, henipaviruses can be accomplished by a determined hostile group with access to resources and the relevant technical expertise.

**Ease of Production and Dissemination**

Henipaviruses can be grown to very high titers, up to $10^8$ TCID$_{50}$/mL or PFU/mL, in a wide range of cell lines. Although henipaviruses are highly sensitive to temperature variation and dessication, henipaviruses can persist for days under certain conditions. Therefore, under optimal conditions henipaviruses could possibly be maintained at high titer for extended periods of time. Henipaviruses infect a wide range of hosts, including domestic animals such as dogs and livestock, that could serve as amplifiers of infection. Although NiV and HeV clearly have the potential to infect through aerosol, this route has not been conclusively shown, either experimentally or from the natural history; however, limited epidemiological evidence suggests that some human cases of NiV infection in Bangladesh may have resulted from exposure to coughing. Also restricting the biological weapons potential of henipaviruses is the lack of sustained human-to-human transmission: the longest documented chain of transmission was five generations. A naturally occurring or intentionally mutated strain with higher transmission efficiency would be required to sustain an epidemic; an expanding pandemic, however, may not be a required or desired goal of a bioterror attack.

**Potential for High Morbidity and Lethality**

HeV and NiV cause disease with very high case fatality rates, ranging from 40% to 100% in recent outbreaks. Survivors of disease or asymptomatically infected people may present years later with relapsing or late onset encephalitis, indicating occasional persistence of viral infection.

**DIAGNOSIS**

In a bioweapons attack or other mass casualty scenario, rapid diagnostic methods must be used to identify the causative agent(s). These methods may be differentiated by whether or not the specific agent must be suspected before testing and by how quickly the results can be obtained. Under normal circumstances, henipavirus etiology would not be suspected without exposure to risk factors (contact with bats, ill persons, or domestic animals, or consumption of raw date palm sap) in the currently affected areas of Southeast Asia and Australia. Henipavirus etiology may also be considered throughout the known geographic range of bats known to harbor henipaviruses (Figure 22-1) if the responsible agent is unknown, with the initial misdiagnosis of NiV as Japanese encephalitis virus remaining a cautionary tale. These factors do not apply in a bioweapons or bioterrorism event, however. The clinical presentation of henipaviruses with encephalitic and/or respiratory symptoms cannot be readily distinguished from other viral and non-viral causes of encephalitis, and diagnosis requires epidemiological and laboratory investigation. Given the broad host range of henipaviruses, the involvement of sick domestic or local animals can suggest potential henipavirus etiology.

Detailed recent reviews of henipavirus diagnosis may be found elsewhere; the available methods are discussed below.

**Reverse Transcription-Polymerase Chain Reaction**

If henipaviruses are suspected, henipavirus-specific polymerase chain reaction or real-time polymerase chain reaction should be performed on RNA extracted from patient samples. Prospective samples include serum, whole blood (detectable viremia may be cell-associated), urine, nasopharyngeal aspirates, throat swabs, cerebrospinal fluid, or tissue samples from highly affected tissues such as the brain, lungs, kidneys, or spleen.

**Detection of Henipavirus Antigens**

Characterized anti-henipavirus antibodies can be used to detect viral antigens in formalin-fixed tissues. Similarly, immunofluorescence with anti-henipavirus antibodies can be performed on infected cell cultures.

**Detection of Anti-henipavirus Immune Responses**

A number of methods exist for detection of anti-henipavirus antibodies in sera of infected patients. Specific immunoglobulin M responses develop in virtually all patients within the first week postexposure, and the slower immunoglobulin G response encompasses virtually all patients after 2 weeks of infection. Detection of serum antibodies is useful for diagnostics because antibodies are more stable than viral RNA over time and under different conditions. Furthermore, viremia
can be difficult to detect when patients are symptomatic. Serum neutralization of replicating virus is considered the gold standard serological test, although this test requires biological safety level 4 containment.\textsuperscript{107} Surrogate neutralization tests, which can be performed at biological safety level 2 conditions, offer a combination of high sensitivity and high specificity. Such tests include the use of vesicular stomatitis Indiana virus pseudotyped with the henipavirus envelope proteins\textsuperscript{115–117} or a Luminex (Luminex Corporation, Austin, TX) platform-based assay assessing the ability of sera to inhibit the binding of the henipavirus receptor to microbeads coated with henipavirus attachment protein.\textsuperscript{118} Enzyme-linked immunosorbent assay variations on this test using infected cell lysate or recombinant henipavirus proteins have been commonly used as frontline assays because of their simplicity and affordability, but typically have a relatively high false-positive rate.\textsuperscript{107}

**Virus Isolation**

Viral etiology may be suspected if cells incubated with filtered patient samples develop cytopathic effects. Henipaviruses grow efficiently in a wide range of cell lines, including Vero E6 cells.\textsuperscript{119} Syncytia formation in cell culture would implicate enveloped viruses with a pH-independent fusion mechanism, which include henipaviruses (Figure 22-5c). Electron microscopy on viral preparations and infected cells could implicate a henipavirus as the potential agent. New or modified henipaviruses may have different characteristics, however, so any final diagnosis requires multiple routes of confirmation. In contrast to sequencing, virus isolation is a days-long process (2–5 days usually pass until cytopathic effects become visible), and two rounds of 5 days each are recommended before virus recovery is judged unsuccessful.\textsuperscript{107,112} Furthermore, for an outbreak suspected to be caused by a highly virulent agent, virus isolation attempt should be performed under high-level biosafety containment. Nevertheless, isolation and characterization of the agent remain the most conclusive demonstration of etiology.

**Next-generation Sequencing**

Next-generation sequencing (NGS) is slowly becoming more widely available and will enable the identification of known and unknown henipaviruses. As the cost and speed of NGS continue to decrease, a rapid and routine measure using NGS in a suspected outbreak is increasingly possible following RNA extraction and reverse transcription polymerase chain reaction.\textsuperscript{107} Since henipaviruses only rarely infect humans, detection of henipavirus sequences would not normally be expected, and an appreciable presence of henipavirus sequence in multiple samples would suggest potential henipavirus etiology. Henipavirus-specific NGS is already becoming routinely used during postoutbreak investigations to determine the characteristics of new henipavirus isolates.\textsuperscript{107,120}

**MEDICAL MANAGEMENT**

Licensed therapeutics to treat henipavirus infection in humans are not available. Thus, medical management of henipavirus infections is supportive. Mechanical ventilation is required if the patient becomes comatose or develops acute respiratory distress syndrome. Appropriate measures should be taken as for any potentially highly contagious pathogen, including quarantine and use of personal protective equipment and engineering controls such as negative air flow, if available; careful handling of clinical specimens; and rapid epidemiological investigation (with particular attention to potential spread via domestic animals) and identification of high-risk contacts. Despite the lack of specifically recommended therapeutics, several potential treatments with varying levels of supporting evidence should be considered in the event of a biowarfare or mass casualty event. Some of the proposed therapeutic interventions discussed below may also be appropriate in the case of accidental exposure or as prophylaxis for frontline responders to a potential outbreak.

**Passive Immunotherapy**

Active vaccination is highly effective in animal models\textsuperscript{83} and was the basis for the recently approved Equivac HeV vaccine for horses. This vaccine contains a soluble version of the HeV attachment envelope protein, which stimulates the production of neutralizing anti-HeV antibodies and provides protection against HeV infection.\textsuperscript{121} However, an active vaccination approach for henipaviruses is unlikely to be a practical strategy on a population-wide basis in humans for several reasons. Compared to veterinary vaccines, human vaccines have higher regulatory hurdles. In addition, such vaccines would be truly useful only for a few people. Few cases have occurred during natural outbreaks, and the likelihood that populations outside of affected locations will be exposed to a pathogenic henipavirus is low. However, the risk–benefit calculations may be different for frontline responders to suspected outbreaks.
Passive immunotherapy may be highly efficacious as postexposure treatment. In recent postexposure prophylaxis studies, ferrets and grivets received 1 to 2 doses of a human monoclonal antibody with neutralizing activity against HeV and NiV attachment proteins 10 to 72 hours after virus exposure, which protected the animals from disease. The antibody has been offered to individuals with high risk of HeV exposure, and human clinical trials started in May 2015 in Australia. Although further development of this strategy may not be economically viable if left to the market, such monoclonal antibodies warrant serious consideration as a stockpiled resource that can be used in a limited outbreak or bioweapons attack.

Ribavirin

Ribavirin, a guanosine analog first synthesized in 1970, has been known to have broad-spectrum activity against many RNA and DNA viruses. Ribavirin is mainly used against human respiratory syncytial virus infections and to treat persistent hepatitis C virus infections. Ribavirin has several potential antiviral properties, which may differ in importance for different viruses. Because of its broad-spectrum effect, ribavirin was used in an off-label, nonrandomized, unblinded trial during the first Malaysia outbreak of NiV encephalitis, despite the known adverse effects (primarily hemolytic anemia at high dose). The lethality in the treated group was reduced by 40%, without affecting the rate of anemia. Follow-up studies revealed that ribavirin inhibits HeV and NiV replication in vitro. Ribavirin has several potential antiviral properties, which may differ in importance for different viruses.

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Current treatment of chronic hepatitis C involves the combination of type I interferon (discussed further in the next section) and ribavirin, which act synergistically. Ribavirin may therefore be evaluated in combination with other promising therapeutics in the case of henipavirus infection.

Innate Immunotherapy

Henipavirus inhibition of cellular production of interferon and of cellular responses to exogenous interferon is incomplete. Therefore, a clear opportunity is available to inhibit HeV and NiV pathogenesis by augmenting the innate immune response in vivo. The investigational double-stranded RNA compound poly(I)-poly(C), which stimulates type I interferon production, is highly effective against NiV infection in the hamster model when administered immediately after exposure. However, follow-up studies need to address the postexposure therapeutic window. Furthermore, despite undergoing phase III clinical trials for treatment of chronic fatigue syndrome, poly(I)-poly(C) (Rintatolimod, Hemispherx Biopharma, Philadelphia, PA) has not been FDA approved.

Recombinant and modified type I interferons (eg, PEGylated interferon-α), have been approved by the FDA for multiple uses, including the treatment of chronic hepatitis B and C. Exogenous interferon inhibits henipavirus replication in vitro and would be a more direct approach to treat henipavirus infections than stimulating interferon production with compounds like poly(I)-poly(C). However, these compounds have yet to be evaluated against henipavirus infections.

“Off-the-Shelf” Therapies Evaluated In Vitro

Numerous potential therapies to treat henipavirus infections are in varying stages of development (reviewed elsewhere). Promising therapies that are already available for off-label use against henipaviruses, but have not been evaluated for efficacy in vivo, are briefly described below.

As NiV M protein requires ubiquitinylation as part of its intracellular trafficking pathway, proteasome inhibitors, which deplete the intracellular pool of free ubiquitin, are potent inhibitors of NiV replication in vitro. Next-generation proteasome inhibitors with improved pharmacokinetics, such as the FDA-approved carfilzomib or orally bioavailable analogs such as oprozomib (currently in phase I/II oncology trials), have even greater efficacy against henipaviruses in vitro. If these effects on henipaviruses can be translated into in vivo potency, the possibility of using potential FDA-approved proteasome inhibitors for off-label use will be a significant and realistic option for exposed or infected frontline responders.

Many clinically available drugs inhibit henipavirus replication in vitro in the low micromolar range, including the alpha blocker phenoxybenzamine as well as the beta blocker propranolol, the antifungal clotrimazole, and the estrogen receptor antagonist tamoxifen. However, it is unclear whether any of these therapies can achieve viral inhibitory concentrations in vivo.
SUMMARY

The recent emergence, high virulence, and pandemic potential of HeV and NiV have fueled public concern and even lead to repeated public calls in Australia for culling of the reservoir hosts, the flying foxes. The worldwide discovery of numerous henipaviruses of unknown pathogenicity, including evidence of a potential spillover event to humans in Africa, further emphasizes the need for continued investigations into all aspects of henipavirus ecology, molecular biology, and pathogenesis. Although HeV and NiV do not appear efficiently transmissible at this time, vigilance for variants (or other henipaviruses) with enhanced transmissibility should be maintained. Significant progress in the development and identification of effective therapeutics for henipaviruses will mitigate the risks involved in managing future outbreaks.

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