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Hendra Virus and Nipah Virus Animal Vaccines

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Abstract

Hendra virus (HeV) and Nipah virus (NiV) are zoonotic viruses that emerged in the mid to late 1990s causing disease outbreaks in livestock and people. HeV appeared in Queensland, Australia in 1994 causing a severe respiratory disease in horses along with a human case fatality. NiV emerged a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs which served as amplifying hosts. The key pathological elements of HeV and NiV infection in several species of mammals, and also in people, are a severe systemic and often fatal neurologic and/or respiratory disease. In people, both HeV and NiV are also capable of causing relapsed encephalitis following recovery from an acute infection. The known reservoir hosts of HeV and NiV are several species of pteropid fruit bats. Spillovers of HeV into horses continue to occur in Australia and NiV has caused outbreaks in people in Bangladesh and India nearly annually since 2001, making HeV and NiV important transboundary biological threats. NiV in particular possesses several features that underscore its potential as a pandemic threat, including its ability to infect humans directly from natural reservoirs or indirectly from other susceptible animals, along with a capacity of limited human-to-human transmission. Several HeV and NiV animal challenge models have been developed which have facilitated an understanding of pathogenesis and allowed for the successful development of both active and passive immunization countermeasures.

Keywords

Paramyxovirus; Henipavirus; Hendra; Nipah; Animal models; Pathogenesis; Vaccine; Antiviral; Monoclonal antibody

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Introduction

Hendra virus (HeV) and Nipah virus (NiV) are enveloped, single-stranded negative sense RNA viruses and the prototype members of the genus *Henipavirus* in the family *Paramyxoviridae* [1]. Recently, a third virus isolate Cedar virus (CedPV) has been added to the *Henipavirus* genus [2]. Whereas HeV and NiV are bat-borne disease-causing zoonoses, CedPV is not known to be zoonotic nor has it been shown to be pathogenic in any animal, including those susceptible to HeV and NiV, although it does reside in nature in the same bat species as HeV. To date, bats appear to be predominant natural reservoir hosts for henipaviruses [3]. Although nucleic acid based detection studies have identified related *Henipavirus* species, including complete genomic sequences [4, 5], HeV, NiV, and CedPV are the only virus isolates that have been reported.

In several disease-susceptible animal species, and in people, the major pathological observation of HeV and NiV infection is a severe systemic and often fatal neurologic and/or respiratory disease [6-8]. However, HeV and particularly NiV can also cause relapsed encephalitis which follows a recovery from an acute infection and appears to result from a recrudescence of virus replication in the central nervous system (CNS) [9, 10]. Outbreaks or spillovers of NiV in Bangladesh and India, since its first emergence in peninsular Malaysia, have continued to occur, as has HeV in Australia, making these henipaviruses important transboundary biological threats [11]. Both HeV and NiV are highly pathogenic in a number of mammalian species and possess several characteristics that distinguish them from all other known paramyxoviruses and are classified as Biosafety Level-4 (BSL-4) agents. Indeed, NiV is considered to have the potential to be a pandemic threat since it can infect humans directly from natural reservoirs (bats) or indirectly following amplification in a susceptible animal species (pig) and has a recognized capacity of, albeit limited, human-to-human transmission [12]. Since their discovery and recognition, a variety of approaches have been taken by multiple research groups to devise countermeasures as a means of addressing the transboundary threat issues brought about HeV and NiV. Several active and passive immunization approaches have been explored and some have led to successful deployment and human clinical trials. Concurrent with these developments, several animal challenge models of HeV and NiV infection and pathogenesis have been established which have provided insight into the nature of HeV and NiV disease [13, 14] and afforded the possibility of testing vaccine and therapeutic countermeasures [15-17].

Hendra virus and Nipah virus Emergence

In 1994 an outbreak of fatal cases of a severe respiratory disease in horses and humans occurred in the Brisbane suburb of Hendra, Australia. The infectious cause of this event was discovered to be a previously unknown paramyxovirus that was distantly related to certain morbilliviruses [18]. In all, 13 horses and a trainer succumbed to infection together with the non-fatal infection of 7 additional horses and a stable hand. In a separate incident that was retrospectively recognized, this same virus caused a brief aseptic meningitic illness in one person after he had cared for and assisted in the necropsies of two horses near Mackay in central Queensland ~1000 km north of Brisbane, and these animals were later shown to have died from this virus infection [19, 20]. Remarkably, 13 months following his recovery,

this individual suffered an episode of relapsed fatal encephalitis characterized by uncontrolled focal and generalized epileptic-activity caused by this virus [21]. Provisionally termed equine morbillivirus, this new paramyxovirus was later re-named Hendra virus (HeV), after the location of the first recognized outbreak. To date, HeV has appeared in Eastern Australia on 55 occasions, with the three most recent in 2015, causing the death or euthanasia of 97 horses, 2 HeV antibody positive and euthanized dogs, and 4 fatalities of 7 human cases [11, 22-29]. In all recognized HeV spillovers and those cases of confirmed human infections, the horse is the predominant target host acquiring infection from virus-shedding bats and is also the intermediate host from which humans have acquired infection. The epidemiological characteristics and the possible underlying mechanisms behind HeV spillovers have been examined [30, 31] and reviewed [32, 33]. In 2011, the first confirmed infection of HeV in a dog was reported. The dog was shown to be seropositive for HeV and was euthanized despite showing no clinical signs of disease. A postmortem was not performed and it was thought that transmission of infection had occurred by close contact with an infected horse [34]. A second confirmed HeV infection in a dog was reported in 2013. The dog tested positive to HeV by quantitative reverse transcription PCR (q-RT-PCR) during the apparent early stages of an acute infection and had appeared to have become naturally infected with HeV following close exposure to blood from a euthanized HeV infected horse [35]. This dog was euthanized and extensively analyzed via postmortem examination. Although the dog had appeared clinically healthy, histopathologic findings of widespread necrotizing vasculitis was noted, with the most severe lesions recorded in the kidney, brain and lymph nodes, with little involvement of the lungs. Additionally, HeV RNA was detected in multiple tissues [35]. Presently, the extent of HeV transmission from bats to dogs in Australia is an unknown concern and should be the focus of future studies.

A few years following the appearance of HeV there was a large outbreak of encephalitis among pig farmers in Peninsular Malaysia which began in the fall of 1998 and continued into the spring of the next year [36]. A virus was isolated from the cerebrospinal fluid (CSF) of two patients and shown to cross-react with antibodies against HeV [37]. Subsequent molecular genetic studies revealed a new paramyxovirus that was most closely related to HeV [38]. This Hendra-like virus was named Nipah virus (NiV) after the town of Nipah in the state of Perak, Malaysia where it was first isolated from a case of fatal encephalitis [39]. In total there were 265 cases of human infection with 105 fatalities in Malaysia along with an additional 11 cases and one fatality among abattoir workers in Singapore [38, 40]. The epidemiological features of this outbreak, its likely causes, and the factors that exacerbated it have been reviewed elsewhere [41, 42]. Although NiV has not re-emerged in Malaysia, nearly annual outbreaks of human cases of NiV infection have been recorded since 2001 in Bangladesh and a few in India. The most recent cases of human infections occurred in early 2015 with 9 human fatalities [43]. The outbreaks of NiV in Bangladesh and India have had lower total numbers of human cases but the fatality rates have been notably higher, ranging from 75-100%. In these instances, the direct transmission of NiV from bats to humans following the consumption of contaminated fruits or date palm sap has been noted along with several instances of significant human-to-human transmission of NiV infection being documented [44-47]. The epidemiological details of the spillovers of both HeV and NiV into

humans have been recently reviewed [48, 49]. In all, there have been ~620 cases of human NiV infection with 322 fatalities (reviewed in [43, 49, 50]).

Pteropus bat species appear to be the major natural reservoir hosts for henipaviruses and all bat isolates of HeV, NiV as well as CedPV have been derived from *Pteropus* bats [2, 51-54]. As natural reservoir hosts, overt disease has not been reported in wild bats, nor are there signs of clinical illness in experimentally infected pteropid bats [55-58]. Nevertheless, there continues to be ever expanding evidence of the presence of henipaviruses in a wide variety of other bat species in both Megachiroptera and Microchiroptera suborders [5, 59-65], as well as the existence of other henipa-like viruses in rodents [4]. Further, serological and/or nucleic acid evidence of henipaviruses in domestic livestock and in human populations have also been reported, providing evidence of sporadic henipavirus spillover events and also suggesting the existence of related henipaviruses [66-69].

The viruses, tropism and entry

HeV and NiV particles are enveloped and pleomorphic with spherical or filamentous forms observed by electron microscopy [70-72]. The viral envelope carries surface projections composed of the viral transmembrane anchored fusion (F) and attachment (G) glycoproteins which have been the major target of antiviral strategies. HeV and NiV are classified into the *Henipavirus* genus, *Paramyxoviridae* family [73] and their genomes are unsegmented, single-stranded, negative-sense RNA [1]. Genomic sequence analysis of many HeV isolates obtained from horses, a human case, and pteropid bats, have shown them to be ~99% identical [51, 72, 74]. In the initial Malaysian outbreak of NiV, both pig and human isolates were genetically highly similar to those obtained years later from bats [75-78]. However, some diversity among NiV isolates can be noted when comparing Malaysian isolates to NiV isolates from other areas of Southeast Asia, with NiV-Bangladesh sharing ~92% identity with NiV-Malaysia [79], and a third lineage of NiV was isolated from Lyle's flying fox (*P. lylei*) in Cambodia that is more closely related to NiV-Malaysia than to NiV-Bangladesh [53, 80].

HeV and NiV are also distinguished by their exceptionally broad species tropism and in addition to pteropid bats, natural or experimental infection has been documented in pigs, horses, cats, dogs, guinea pigs, mice, hamsters, ferrets, squirrel monkeys and African green monkeys and humans (reviewed in [13]). NiV can also productively infect chicken embryos [81] spanning 7 orders (6 mammalian and one avian).

The major determinant of species and cellular tropism of HeV and NiV is derived from the functions of the viral envelope glycoproteins (G and F) which are the mediators of virus attachment and host cell infection. The HeV and NiV G glycoprotein bind to the host cell membrane proteins ephrin-B2 and ephrin-B3 [82-85]. The ephrin-B2 and -B3 molecules are members of a large family of cell surface expressed glycoprotein ligands that bind to Eph receptors [86] and are highly sequence conserved across known susceptible hosts with amino acid identities ranging from 95-98% [87]. Ephrin-B2 expression is prominent in arteries, arterioles and capillaries in multiple organs and tissues [88] while ephrin-B3 is found predominantly in the nervous system and the vasculature [89, 90]). Their

identification as major receptors for HeV and NiV has helped clarify their broad species and tissue tropisms and pathogenic features observed in both animals and humans (reviewed in [9]).

The HeV and NiV G glycoprotein is a type II membrane glycoprotein consisting of a stalk and globular head domain which binds ephrin receptors. The native conformation of G is a tetramer comprised of a dimer of dimers [91]. The crystal structures of both NiV and HeV G globular head domains have been determined both alone and in complex with the ephrin-B2 and -B3 receptors, revealing the exact G-receptor interactions and identical receptor binding sites with four binding pockets in G for the residues in the ephrin-B2 and -B3 G-H loop that are highly conserved [92-96]. The second viral envelope glycoprotein is the fusion (F) glycoprotein that mediates the membrane fusion process between the virion and host cell. F is a type I membrane glycoprotein and is a class I viral fusion protein sharing several conserved features with other viral fusion glycoproteins [97]. F is initially expressed as a precursor (F₀) which forms an oligomeric homotrimer that is cleaved into two disulfide bond-linked subunits (F₁ and F₂) by the endosomal protease cathepsin L [98]. Following virus attachment to a ephrin receptor-bearing host cell, the fusion-promoting activity of G is initiated through its binding to receptor and then in turn facilitates the triggering of conformational changes in F, transitioning the molecule from its pre-fusion to post-fusion form driving the membrane fusion process between the virion and host cell, resulting in delivery of the viral nucleocapsid into the cytoplasm (reviewed in [99, 100]).

Clinical and pathological features of human HeV and NiV infection

Human HeV and NiV infections have an incubation period ranging from a few days to about 3 weeks [101, 102]. All human cases of HeV infection have been the result of exposure and transmission of the virus from infected horses to humans. Following an influenza-like illness (fever, myalgia, headaches, lethargy, vertigo, cough, pharyngitis, and cervical lymphadenopathy), the majority of human HeV cases developed severe disease and died; only 3 of 7 patients have survived (~60% mortality rate) [8, 102, 103]. The first human case presented as an acute severe respiratory disease but no clinical evidence of acute encephalitis. At autopsy, the lungs showed macroscopic evidence of congestion, hemorrhage and edema [103] associated with focal necrotizing alveolitis and evidence of syncytia and multinucleated giant cell formation, and viral inclusions. Although clinical encephalitis was apparently absent, the brain pathology clearly showed acute encephalitis characterized by mild meningitis, parenchymal and perivascular inflammation. More importantly, there was evidence of neuronal viral inclusions, vasculitis and necrotic/vacuolar plaques. A second fatality occurred in an individual who first experienced an aseptic meningitic illness associated with drowsiness caused by HeV infection acquired after assisting at the necropsies of two horses that were only later shown to have died from HeV infection. Approximately 13 months later this individual suffered a recurrence of severe encephalitis characterized by uncontrolled focal and generalized epileptic-activity. Inflammatory lesions were only found in the CNS, not in other organs obtained at autopsy [10]; this was the first case of relapsing henipavirus encephalitis. The other two fatal cases presented as an acute encephalitic syndrome characterized by drowsiness, confusion, ataxia, ptosis, dysarthria and seizures and the patients died soon after [8]. Of the 3 human Hendra virus survivors, there is

evidence in one patient who previously developed acute encephalitis in 2008 of the persistence of postencephalitic, high level cognitive deficits, but no evidence of relapse or of viral shedding in 2 of the survivors who were studied [104].

In contrast to human cases of HeV infection, there have been many more cases of human NiV infection. The main features of acute human NiV infection were fever, headache, dizziness and vomiting [101] and a majority of patients had reduced consciousness levels and signs of brainstem dysfunction. A small number, probably <10%, of patients with acute NiV infection developed a late-onset encephalitis or a relapsing encephalitis a few weeks later with a mortality rate of ~18%, considerably lower than that for acute encephalitis which was ~40% in the initial NiV outbreak in Malaysia [105]. The clinical features of late-onset encephalitis and relapsing encephalitis are similar to acute encephalitis. Although most NiV-infected human patients presented with acute encephalitis, ~25% of patients also presented with respiratory signs [41]. In addition, NiV infection could also take a quiescent course with neurological disease occurring later (>10 weeks) following a non-encephalitic or asymptomatic infection. Here, there appears to be a recrudescence of virus replication in the CNS. Most reported cases of relapsed encephalitis occurred between a few months to 2 years following the initial acute infection, however 2 cases were observed in 2003, 4 years later, [106-108] and one case of relapsed encephalitis 11 years later [109]. Persistent neurological deficits have been observed in >15% of NiV infection survivors [110]. In Bangladesh, the outcomes among 22 of 45 serologically confirmed cases of NiV infection revealed neurological sequelae in survivors, and patients who initially had encephalitis could continue to exhibit neurological dysfunction for several years [111]. Viral persistence and/or recrudescence within the CNS are suspected to be at play in these individuals, however, attempts at virus isolation from relapsed HeV and NiV patients have been unsuccessful, and there is no evidence to date indicating that relapsed patients are potentially infectious because of shedding [21, 104, 105, 109]. The mechanisms that allow NiV and HeV to escape immunological clearance for such an extended period and later result in disease are unknown, and this feature has important implications for therapeutics development.

In the first NiV outbreak in Malaysia and Singapore, autopsies were conducted on >30 individuals and the most distinctive microscopic feature was disseminated vasculitis in most organs examined, particularly in the CNS and lungs [112]. Extravascular necrotic lesions and inflammation in many organs were also reported. In the CNS parenchyma, distinct necrotic plaques as a result of vasculitis-induced vascular obstruction, ischemia and infarction and/or neuronal infection were commonly found. Viral inclusions in neurons in the CNS and other cells in non-CNS tissues were also observed. The pathological features in a few autopsy cases of NiV relapsing or late-onset encephalitis and the single case of HeV relapsing encephalitis were similar and confined mainly to the CNS [9, 105].

Clinical and pathological features of HeV and NiV infection in animals

Naturally acquired HeV infections have almost exclusively been observed in horses, and only recently have two dogs been reported HeV antibody positive. Whereas in addition to pigs, naturally acquired NiV infection was noted in dogs, cats and horses in the initial Malaysian outbreak [113]. Since their discovery, however, a large number of different

species have been examined under experimental conditions for the purposes of developing infection models and characterizing the viral pathogenic processes. Detailed reviews of the disease manifestations observed in natural and experimental infections of animals, including bats, with HeV and NiV have recently been reported [7, 13, 114, 115].

Animal models of HeV and NiV infection and pathogenesis have played a critical role in aiding our understanding of henipavirus pathogenesis and are also an absolute necessity for the testing and validation of potential vaccines and therapeutic countermeasures. There are now several well-established animal models of HeV and NiV infection and pathogenesis including the guinea pig [55, 57, 116, 117], hamster [118, 119], cat [56, 120, 121], pig [121-123], ferret [124, 125], African green monkey (AGM) [126, 127], squirrel monkey [128] and horse [129]. Among these models, the hamster, ferret and AGM best represent HeV and NiV pathogenesis observed in humans, whereas the most appropriate models for livestock are the pig and horse. Here, for the purposes of highlighting the findings to date on animal HeV and NiV vaccines and potential therapeutic modalities for people, a focus on the pig, horse and non-human primate models of infection will be summarized.

The pig

Experimental NiV infection of pigs has revealed an incubation period of ~5-7 days post-infection and the respiratory system serving as a major site of virus replication and pathology, with viral antigen and syncytia formation present in the respiratory epithelium (tracheal, bronchial, bronchiolar, and alveolar) and small blood and lymphatic vessels [113, 121, 123]. Virus was also observed in the kidneys and in endothelial and smooth muscle cells of small blood vessels [121]. CNS involvement is less common in the pig, with meningitis or meningoencephalitis observed as opposed to encephalitis [121]. NiV infection of piglets generally resulted in a mild clinical illness with respiratory signs and fever, along with virus replication in the respiratory system, lymphoid tissues and CNS [123]. Recoverable virus was seen in the respiratory, lymphatic and nervous systems, and there is virus shedding in nasal, pharyngeal and ocular fluids. HeV infection of pigs also presents primarily as a respiratory disease with possible CNS involvement observed in minipigs, along with similar patterns of virus shedding [122]. Overall, HeV appears to cause a more severe respiratory illness in pigs when contrasted to NiV in these experiments. However, the disease in pigs in Peninsular Malaysia in 1998-99 was characterized by a pronounced respiratory and neurological syndrome, sometimes accompanied by sudden death of sows and boars. It was referred to in Peninsular Malaysia as “barking pig syndrome” because of the characteristic cough [130]. Although HeV and NiV disease in pigs can be less severe in comparison to other animal models, the virus does replicate and disseminate to a variety of organs along with significant levels of virus shedding.

The horse

Naturally acquired HeV infection in horses often results in severe disease, with fever and increased heart rate common, and a rapid deterioration with respiratory and/or neurological clinical signs. The case fatality rate is ~75%. In some naturally occurring cases HeV infection has presented with more subtle clinical signs including absence of fever. An

apparent incubation period in naturally infected horses appears to be 5-16 days based on the events of the two large outbreaks; the first in 1994, where the ill index equine case was brought to stable in Hendra and died two days later, with other horses becoming ill 8-11 days following the death of the first horse [103], and the second in 2008 at Redlands, Queensland [131].

Experimental infections have been essentially uniformly fatal [18, 56, 72, 129, 132]. Experimentally infected horses become ill ~3-11 days post-infection and animals initially present as anorexic and depressed with general uneasiness and ataxia, with the development of fever with sweating. Respiration becomes rapid, shallow and labored with pulmonary edema and congestion, along with nasal discharge 1-3 days following the onset of clinical signs typically just before death. In severe cases the airways of horses are often filled with a blood-tinged frothy exudate. Hemorrhage, thrombosis of capillaries, necrosis, and syncytial cells in the endothelium of pulmonary vessels are observed, and viral antigen within endothelial cells is seen in a wide variety of organs. Recoverable virus is present in a number of internal organs and also from saliva and urine. Neurologic clinical signs can also present [20], but in experimentally infected horses, meningitis (with vasculitis) is typically noted with viral antigen in the meninges [129]. In other experiments, HeV antigen was reported in blood vessels within the brain [15], but to date HeV antigen has not been reported in the neurons of infected horses, but this may be because of experimental limitations. However, the meningitis and inflammation of cerebral blood vessels in the experimentally infected horses may be sufficient explanation for the neurological clinical signs in naturally acquired cases of HeV infection (Deborah Middleton, personal communication). Experimental infection of horses with NiV has not been performed but the brain and spinal cord of one naturally infected horse was examined and immunohistochemical staining of viral antigen revealed non-suppurative meningitis [113].

Non-human primates

The only nonhuman primate model that has uniformly recapitulated human disease for both NiV and HeV infection was developed using the African green monkey (AGM) [126, 127]. Both NiV and HeV produce a uniformly lethal illness following low dose virus challenge by intratracheal inoculation within 7-10 days post-infection. NiV and HeV spread rapidly to numerous organ systems within the first 3-4 days following challenge, followed by the development of a progressive and severe respiratory disease ~7 days post-infection [126, 127]. The lungs become enlarged and high levels of virus replication, congestion, hemorrhage and polymerized fibrin are present. Wide-spread vasculitis with endothelial and smooth muscle cell syncytia with viral antigen and viral genome is detected in most organs and tissues with associated pathology. AGMs infected with either NiV or HeV also exhibit neurological disease signs with the presence of meningeal hemorrhaging and edema, and vascular and parenchymal lesions in the brain including infection of neurons with the brainstem particularly involved [126, 127].

The squirrel monkey was also found to be susceptible to experimental NiV infection via intravenous and intranasal routes demonstrating findings similar to AGM and human infection, but only some animals demonstrated NiV pathogenesis and not all challenged

animals exhibited clinical signs of disease and remained well, even after high dose administration of NiV [128].

Therapeutics and Vaccines

There are no approved HeV or NiV antiviral therapeutics or vaccines for human use, however, several countermeasure approaches have been evaluated in animal challenge models and have been recently reviewed in detail elsewhere [16]. Here, the focus will be an up to date summary of the HeV and NiV animal vaccine approaches that have been examined *in vivo* along with the one therapeutic approach for human use that has reached phase I clinical trial evaluation.

Active immunization strategies

There has been a variety of immunization strategies developed for HeV and NiV infection including live-recombinant virus platforms, protein subunit, virus-like particles and DNA vaccines; many of these approaches have only been examined for their ability to illicit HeV- or NiV-specific neutralizing antibody responses [133-136]. Some approaches, however, have been examined for both immune response and efficacy using a variety of animal challenge models (Table 1). The first vaccine approach tested utilized the attenuated vaccinia virus strain NYVAC, whereby recombinant viruses encoding either the NiV F or G glycoproteins were examined both individually and in combination to immunize hamsters [137]. This study revealed that complete protection from NiV-mediated disease was achievable following vaccination and that an immune response to the viral envelope glycoproteins could be an important mechanism of protection. A second poxvirus-based vaccine approach was examined as a potential livestock vaccine using recombinant canarypox virus in swine [138]. Similar to the recombinant vaccinia virus approach, the NiV F and G glycoprotein genes were inserted into canarypox virus (ALVAC) vectors and then used to immunize piglets (Table 1). The ALVAC vectors expressing either NiV F or NiV G were tested individually and in combination and following vaccination, piglets were challenged intranasally with NiV. This study showed that protection from NiV-mediated illness was achievable in immunized piglets by either ALVAC vector alone or in combination, and also that vaccinated animals shed only low levels of nucleic acid detectable virus and no recoverable virus was evident [138].

More recently, several additional viral vector-based NiV vaccines have been examined in animal challenge studies (Table 1). An adeno-associated virus (AAV) platform using the NiV G glycoprotein was examined in mice and revealed a strong antibody response following immunization and was then tested in the hamster model using NiV challenge, showing complete protection against NiV, however, only low level cross-protection (3 of 6 animals) against a HeV challenge [139]. Recombinant measles virus vectors encoding the NiV G glycoprotein have also been explored, using two different vectors based on the HL (rMV-HL-G) and Edmonston (rMV-Ed-G) measles virus strains [140] (Table 1). This study revealed that complete protection from NiV disease was achievable in the hamster challenge model using NiV after vaccination with either rMV-HL-G or rMV-Ed-G, and the rMV-Ed-G

was also used in a small nonhuman primate study showing 2 of 2 AGMs protected against NiV challenge (Table 1).

Several groups have also used the vesicular stomatitis virus (VSV) based platform using the NiV F or G envelope glycoproteins (Table 1). This was explored first in the ferret model and showed that a single immunization with either recombinant virus (NiV F or NiV G) afforded complete protection from NiV challenge [141]. Similarly, NiV G or F enveloped glycoproteins in recombinant VSV vectors were also successful in protection from NiV challenge in the hamster model [142, 143] (Table 1). One study also tested a NiV nucleoprotein (N) encoding recombinant VSV vector and revealed, not surprisingly, only partial protection from challenge but that cell-mediated immunity to NiV infection can also be relevant [142]. All these studies demonstrated that a single dose of vaccine (recombinant VSV-based) could induce strong neutralizing antibody responses and could afford protection from NiV challenge, highlighting their potential usefulness as either a livestock vaccine or one suitable in an emergency-use or outbreak scenario.

The most extensively examined vaccine approach against HeV or NiV has been a recombinant protein subunit strategy, primarily because of the inherent safety of such vaccine. Soluble, secreted, oligomeric forms of the G glycoprotein (sG) from both NiV and HeV were developed early on as a possible vaccine [91]; HeV-sG was the most extensively studied because of its utility in eliciting the more potent cross-protective immune response [120] (Table 1). The HeV-sG glycoprotein is a secreted version of the molecule with a genetically deleted transmembrane and cytoplasmic tail that is produced in mammalian cell culture systems and is properly N-linked glycosylated [144] (Figure 1). HeV-sG has been shown to retain many native characteristics including oligomerization and the ability to bind ephrin receptors [84, 91], and is capable of eliciting potent cross-reactive (HeV and NiV) neutralizing antibody responses in a variety of animals including mice, rabbits, cats, ferrets, monkeys and horses. The first studies using the HeV-sG subunit immunogen in the cat model demonstrated that it could elicit a completely protective immune response against a lethal subcutaneous NiV challenge [120], showing that a single subunit vaccine (HeV-sG) could be effective against both HeV and NiV. Follow-up studies in the cat model demonstrated that pre-challenge virus neutralizing antibody titers as low as 1:32 were completely protective from a high dose oronasal challenge of NiV (50,000TCID₅₀) [145] (Table 1). Additional studies with the HeV-sG vaccine in the ferret model using either 100 µg, 20 µg or 4 µg doses of HeV-sG formulated in the vaccine adjuvants CpG and Allhydrogel™ could all afford complete protection from a 5,000 TCID₅₀ dose of HeV (100 times the minimal lethal dose) with no disease or evidence of virus or viral genome in any tissues or body fluids in the 100 µg and 20 µg vaccine groups; only a low level of HeV genome was detected in the nasal washes from 1 of 4 animals in the 4 µg vaccine group, and no infectious HeV could be recovered from any immunized ferrets [124]. In a similar immunization study carried out in ferrets but utilizing the NiV-Bangladesh strain, all animals challenged with NiV following vaccination remained disease free, and virus or viral genome was undetectable in all tissues and fluids examined, with no lesions and no viral antigen observed in all examined tissues. Further, there was no increase in antibody titer following challenge, consistent with failure of virus replication and lack of stimulation of a humoral memory response. The study also revealed good durable immunity with other ferrets

challenged 434 days post-vaccination; 5 of 5 animals were disease free following challenge and viral genome was detected only from the nasal secretions of one ferret and the bronchial lymph nodes of another ferret that were given an intermediate vaccine dose [146].

The HeV-sG subunit vaccine has also been evaluated in nonhuman primates (AGMs). In the first study, a range of doses (10, 50, or 100 µg of HeV-sG) were mixed with Allhydrogel™ and CpG and the vaccine was given to three animals in each dosing group twice, three weeks apart. These animals were challenged 21 days later by intratracheal administration with a 10-fold lethal dose of NiV (1×10^5 TCID₅₀) (Table 1). Complete protection was observed in all vaccinated animals with some having pre-challenge NiV neutralizing titers as low as 1:28. No evidence of clinical disease, virus replication, or pathology was observed in any vaccinated animals. A second study also examined HeV-sG vaccination and protection from a HeV challenge in AGMs, but evaluated the HeV-sG subunit (100 µg doses) in Allhydrogel™ and CpG or in Allhydrogel™ alone [147]. Similarly, animals were vaccinated twice, 3 weeks apart, and challenged intratracheally with a 10-fold lethal dose of HeV ($\sim 5 \times 10^5$ plaque-forming units) 21 days after the booster immunization. All vaccinated animals remained disease-free and there was no evidence of virus replication or pathology observed. Importantly, this study also clearly demonstrated that HeV-sG-Allhydrogel™ alone is capable of providing complete protection from a HeV challenge in a nonhuman primate providing support for its preclinical development as a HeV and NiV vaccine for use in people.

The inherent safety and simplicity of the HeV-sG subunit vaccine approach along with the numerous successful vaccination and challenge studies that have been carried out in multiple animal models, provided the information that justified the evaluation of the HeV-sG subunit vaccine as a possible equine vaccine to prevent not only HeV infection of horses, but also as the means to reduce the risk of HeV transmission to people. HeV-sG was licensed by Zoetis™ (formerly Pfizer Animal Health) and developed as an equine vaccine for use in Australia. Horse HeV-sG vaccination and HeV challenge studies were conducted at the BSL-4 facilities of the Australian Animal Health Laboratory (AAHL) in Geelong [15] (Table 1). Here, the purified recombinant HeV-sG glycoprotein was formulated in an equine vaccine adjuvant (Zoetis, Inc.). In two initial efficacy studies in horses, either a 50 µg or 100 µg dose of the same sourced HeV-sG glycoprotein which was used in all the HeV-sG animal challenge studies described above was used to immunize horses. Two additional studies used 100 µg of recombinant HeV-sG produced from clarified CHO cell culture supernatant (Zoetis, Inc.). Immunizations were two 1-mL doses administered intramuscularly 3 weeks apart. All horses in these efficacy studies were exposed oronasally to 2×10^6 TCID₅₀ of HeV. Seven horses were challenged 28 days, and three horses were challenged 194 days, after the second immunization. All vaccinated horses remained clinically healthy following challenge, showing protection with HeV neutralizing titers as low as 1:16 or 1:32 pre-challenge. There was no gross or histologic evidence of HeV infection in any of the vaccinated horses at study completion and all tissues examined were negative for HeV antigen by immunohistochemistry. Additionally, there was no detectable viral genome in any tissue. In 9 of 10 vaccinated horses, HeV nucleic acid was not detected in daily nasal, oral, or rectal swab specimens or from blood, urine, or feces samples collected before euthanasia, and no recoverable virus was present. Only in 1 of 3 horses challenged 6 months after

vaccination was viral nucleic acid detected in nasal swab samples collected on post-challenge days 2, 4 and 7, a finding consistent with either self-limiting local replication or possibly remnant challenge virus nucleic acid, but again no recoverable virus was present [15]. The equine vaccine against HeV (Equivac® HeV) was launched in November 2012 initially on a Minor Use Permit by the regulatory authority, the Australian Pesticides and Veterinary Medicines Authority (APVMA), and is the first commercially developed and deployed vaccine as a countermeasure for a BSL-4 agent, and is the only licensed antiviral approach for henipavirus infection. A database was established for all vaccinated horses by microchip use, and in August 2015 Equivac® HeV received full registration by the APVMA. To date, ~400,000 doses of Equivac® HeV vaccine have been administered to approximately 120,000 horses, and since vaccine release, laboratory confirmed HeV infections in horses (n=15) have only occurred in unvaccinated horses. Injection site reaction calculated by the APVMA at 342,282 doses of vaccine administered from launch to 31 March 2015 was 0.18%, probable plus possible reaction incidence was <0.25% [148]. In the vast majority of horses there is no observable adverse clinical reaction to vaccination reported by veterinarians in the field.

The passive immunization approach

The first passive immunization studies were conducted in the hamster NiV-challenge model and demonstrated that antibody immunotherapy against NiV infection through the targeting of the viral envelope glycoproteins was possible. Protective passive immunotherapy using either NiV G and F-specific polyclonal antisera, or mouse monoclonal antibodies (mAbs) specific for the NiV or HeV G or F glycoproteins has been demonstrated [118, 137, 149]. These studies demonstrated the importance of viral glycoprotein specific antibody in protection from NiV- and HeV-mediated disease (reviewed in [17]).

Previously, using recombinant antibody technology, HeV- and NiV-neutralizing human mAbs reactive to the G glycoproteins of both HeV and NiV were identified [150]. One mAb, m102, possessed strong cross-reactive neutralizing activity against HeV and NiV and was affinity matured and converted to an IgG1 format (m102.4) and produced in a CHO-K1 cell line [151]. The m102.4 mAb epitope maps to the receptor binding site of G and engages G in a similar fashion as the ephrin receptors [152] (Figure 1). The m102.4 mAb can neutralize all HeV and NiV isolates tested, including HeV-1994, HeV-Redlands, NiV-Malaysia and NiV-Bangladesh [125] (Table 1). In a post-exposure NiV challenge experiment in the ferret, a single dose of m102.4 mAb administered by intravenous infusion 10 hrs following a lethal virus challenge could provide complete protection [125]. The therapeutic efficacy of mAb m102.4 has also been examined in monkeys against both NiV and HeV challenge in a study design reflective of a potential real life virus exposure scenario that would call for a post-exposure therapeutic modality [153, 154] (Table 1). In one study, animals were challenged intratracheally with HeV and following virus administration they were infused twice with m102.4 (~15 mg/kg) beginning at 10 hrs, 24 hrs or 72 hrs post-infection followed by a the second infusion ~48 hrs after the first. In this study, all subjects became infected following challenge, and all animals that received m102.4 survived whereas all controls succumbed to severe systemic disease eight days following virus exposure. Animals in a 72 hrs treatment group did exhibit neurological signs but all recovered by day

16. There was no evidence of HeV-specific pathology in any of the m102.4-treated animals, and no infectious HeV could be recovered from any tissues from any m102.4-treated subjects at the termination of the study. A later study evaluated the efficacy of m102.4 against NiV challenge in AGMs, also at several time points after virus exposure by intratracheal challenge including as late as the onset of clinical illness [154]. In this study, animals were infused twice with m102.4 (15 mg/kg) beginning at either 1, 3, or 5 days following NiV challenge and again 48 hrs after the first dose. All animals became infected following virus exposure, and again all subjects that received m102.4 therapy survived the infection, whereas the untreated control subjects succumbed to disease between days 8 and 10 following virus exposure. Animals in the day 5 treatment group exhibited clinical signs of disease, but all recovered by day 16. Taken together, these studies have shown that m102.4 mAb therapy can prevent HeV or NiV disease in exposed subjects with a remarkably long window of therapeutic opportunity and the mAb is the only effective post-exposure therapeutic tested *in vivo* in nonhuman primates and one with clear potential for use in people.

Conclusions and future directions

Because of the potential environmental accessibility of HeV and NiV, their highly pathogenic characteristics and ability to infect a broad range of mammalian hosts including people, the development of effective countermeasures against these biothreats has been a major area of research focus. These efforts have led to the development and testing of potential vaccine candidates and antiviral therapeutics. In 2010, the m102.4 mAb producing cell line was provided to the Queensland Government, Queensland Health, Australia to produce the m102.4 mAb for compassionate emergency use basis in the event of future high-risk human HeV exposure. Queensland Health Authorities announced in 2013 plans for the first phase 1 clinical safety trial of m102.4 in human subjects, which is now underway in Australia [155]. In addition, since 2010, there have been 11 individuals that have received high-dose m102.4 therapy on an emergency use basis because of high-risk exposure to HeV in Australia (10 people) or NiV in the United States (one person), and all have remained well with no associated adverse events. In addition, the horse vaccine against HeV (Equivac® HeV) is expected to provide a substantial health benefit to humans, and fits well within the concept of the 'One Health' approach for addressing infectious diseases of both human and animal populations.

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References

1. Wang, LF.; Mackenzie, JS.; Broder, CC. Henipaviruses. In: Knipe, DM.; Howley, PM., editors. Fields Virology. Philadelphia: Lippincott Williams & Wilkins; 2013. p. 1070-85.
2. Marsh GA, de Jong C, Barr JA, Tachedjian M, Smith C, Middleton D, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. PLoS Pathog. 2012; 8:e1002836. [PubMed: 22879820]
3. Clayton BA, Wang LF, Marsh GA. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. Zoonoses Public Health. 2013; 60:69–83. [PubMed: 22709528]

4. Wu Z, Yang L, Yang F, Ren X, Jiang J, Dong J, et al. Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis.* 2014; 20:1064–6. [PubMed: 24865545]
5. Drexler JF, Corman VM, Muller MA, Maganga GD, Vallo P, Binger T, et al. Bats host major mammalian paramyxoviruses. *Nature communications.* 2012; 3:796.
6. Abdullah S, Tan CT. Henipavirus encephalitis. *Handbook of clinical neurology.* 2014; 123:663–70. [PubMed: 25015510]
7. Wong KT, Ong KC. Pathology of acute henipavirus infection in humans and animals. *Patholog Res Int.* 2011; 2011:567248. [PubMed: 21961078]
8. Playford EG, McCall B, Smith G, Slinko V, Allen G, Smith I, et al. Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis.* 2010; 16:219–23. [PubMed: 20113550]
9. Wong KT, Tan CT. Clinical and pathological manifestations of human henipavirus infection. *Curr Top Microbiol Immunol.* 2012; 359:95–104. [PubMed: 22427144]
10. Wong KT, Robertson T, Ong BB, Chong JW, Yaiw KC, Wang LF, et al. Human Hendra virus infection causes acute and relapsing encephalitis. *Neuropathol Appl Neurobiol.* 2009; 35:296–305. [PubMed: 19473296]
11. Broder CC, Xu K, Nikolov DB, Zhu Z, Dimitrov DS, Middleton D, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral Res.* 2013; 100:8–13. [PubMed: 23838047]
12. Luby SP. The pandemic potential of Nipah virus. *Antiviral Res.* 2013; 100:38–43. [PubMed: 23911335]
13. Geisbert TW, Feldmann H, Broder CC. Animal Challenge Models of Henipavirus Infection and Pathogenesis. *Curr Top Microbiol Immunol.* 2012; 359:153–77. [PubMed: 22476556]
14. de Wit E, Prescott J, Falzarano D, Bushmaker T, Scott D, Feldmann H, et al. Foodborne transmission of nipah virus in Syrian hamsters. *PLoS Pathog.* 2014; 10:e1004001. [PubMed: 24626480]
15. Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis.* 2014; 20:372–9. [PubMed: 24572697]
16. Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. *Curr Opin Virol.* 2012; 2:176–87. [PubMed: 22482714]
17. Broder CC, Geisbert TW, Xu K, Nikolov DB, Wang LF, Middleton D, et al. Immunization Strategies Against Henipaviruses. *Curr Top Microbiol Immunol.* 2012; 359:197–223. [PubMed: 22481140]
18. Murray K, Rogers R, Selvey L, Selleck P, Hyatt A, Gould A, et al. A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis.* 1995; 1:31–3. [PubMed: 8903153]
19. Hooper PT, Gould AR, Russell GM, Kattenbelt JA, Mitchell G. The retrospective diagnosis of a second outbreak of equine morbillivirus infection. *Aust Vet J.* 1996; 74:244–5. [PubMed: 8894044]
20. Rogers RJ, Douglas IC, Baldock FC, Glanville RJ, Seppanen KT, Gleeson LJ, et al. Investigation of a second focus of equine morbillivirus infection in coastal Queensland. *Aust Vet J.* 1996; 74:243–4. [PubMed: 8894043]
21. O'Sullivan JD, Allworth AM, Paterson DL, Snow TM, Boots R, Gleeson LJ, et al. Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet.* 1997; 349:93–5. [PubMed: 8996421]
22. ProMED-mail. Hendra virus, equine - Australia (12): (QL) vaccine. Pro-med: International Society for Infectious Diseases; 2012. <http://www.promedmail.org>, archive no. 20121104.1390394
23. ProMED-mail. Hendra virus, equine - Australia (09): New South Wales dog affected. Promed: International Society for Infectious Diseases; 2013. <http://www.promedmail.org>, archive no. 20130721.1837123
24. ProMED-mail. Hendra virus, equine - Australia: (08) Queensland, New South Wales. Promed: International Society for Infectious Diseases; 2013. <http://www.promedmail.org>, archive no. 20130712.1820724

25. ProMED-mail. Hendra virus, equine - Australia (02): New South Wales. Pro-med: International Society for Infectious Diseases; 2014. <http://www.promedmail.org>, archive no. 20140621.2557020
26. ProMED-mail. Hendra virus, equine - Australia (03): Queensland. Pro-med: International Society for Infectious Diseases; 2014. <http://www.promedmail.org>, archive no. 20140721.2626012
27. Hendra virus case confirmed after horse dies in North Queensland. Australian Broadcasting Corporation; 2015. http://www.abc.net.au/news/2015-07-24/horse-dies-from-hendra-virus-northqueensland/6646178?WT.ac=statenews_qld
28. ProMED-mail. Hendra virus, equine - Australia: (New South Wales). Pro-med: International Society for Infectious Diseases; 2015. <http://www.promedmail.org>, archive no. 20150625.3463123
29. ProMED-mail. Hendra virus, equine - Australia (04): (New South Wales). Pro-med: International Society for Infectious Diseases; 2015. <http://www.promedmail.org>, archive no. 20150905.3625268
30. Plowright RK, Foley P, Field HE, Dobson AP, Foley JE, Eby P, et al. Urban habituation, ecological connectivity and epidemic dampening: the emergence of Hendra virus from flying foxes (*Pteropus* spp.). *Proceedings Biological sciences / The Royal Society*. 2011; 278:3703–12. [PubMed: 21561971]
31. Plowright RK, Eby P, Hudson PJ, Smith IL, Westcott D, Bryden WL, et al. Ecological dynamics of emerging bat virus spillover. *Proceedings Biological sciences / The Royal Society*. 2015; 282:20142124. [PubMed: 25392474]
32. Field H, Crameri G, Kung NY, Wang LF. Ecological aspects of hendra virus. *Curr Top Microbiol Immunol*. 2012; 359:11–23. [PubMed: 22476530]
33. Field HE, Breed AC, Shield J, Hedlefs RM, Pittard K, Pott B, et al. Epidemiological perspectives on Hendra virus infection in horses and flying foxes. *Aust Vet J*. 2007; 85:268–70. [PubMed: 17615038]
34. ProMED-mail. Hendra virus, equine - Australia (21): (Queensland) canine. Pro-med: International Society for Infectious Diseases; 2011. <http://www.promedmail.org>, archive no. 20110802.2324
35. Kirkland PD, Gabor M, Poe I, Neale K, Chaffey K, Finlaison DS, et al. Hendra Virus Infection in Dog, Australia, 2013. *Emerg Infect Dis*. 2015; 21:2182–5. [PubMed: 26583697]
36. Outbreak of Hendra-like virus--Malaysia and Singapore, 1998-1999. *MMWR Morbidity and mortality weekly report*. 1999; 48:265–9. [PubMed: 10227800]
37. Chua KB, Goh KJ, Wong KT, Kamarulzaman A, Tan PS, Ksiazek TG, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet*. 1999; 354:1257–9. [PubMed: 10520635]
38. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science*. 2000; 288:1432–5. [PubMed: 10827955]
39. Farrar JJ. Nipah-virus encephalitis--investigation of a new infection. *Lancet*. 1999; 354:1222–3. [PubMed: 10520625]
40. Paton NI, Leo YS, Zaki SR, Auchus AP, Lee KE, Ling AE, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet*. 1999; 354:1253–6. [PubMed: 10520634]
41. Chua KB. Nipah virus outbreak in Malaysia. *J Clin Virol*. 2003; 26:265–75. [PubMed: 12637075]
42. Pulliam JR, Epstein JH, Dushoff J, Rahman SA, Bunning M, Jamaluddin AA, et al. Agricultural intensification, priming for persistence and the emergence of Nipah virus: a lethal bat-borne zoonosis. *Journal of the Royal Society, Interface / the Royal Society*. 2012; 9:89–101.
43. Nipah encephalitis, human - Bangladesh (02). Pro-med: International Society for Infectious Diseases; 2015. <http://www.promedmail.org>, archive no. 20150407.3280088
44. Rahman MA, Hossain MJ, Sultana S, Homaira N, Khan SU, Rahman M, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector Borne Zoonotic Dis*. 2012; 12:65–72. [PubMed: 21923274]
45. Homaira N, Rahman M, Hossain MJ, Nahar N, Khan R, Podder G, et al. Cluster of Nipah virus infection, Kushtia District, Bangladesh, 2007. *PLoS One*. 2010; 5:e13570. [PubMed: 21042407]
46. Homaira N, Rahman M, Hossain MJ, Epstein JH, Sultana R, Khan MS, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. *Epidemiol Infect*. 2010; 138:1630–6. [PubMed: 20380769]

47. Luby SP, Hossain MJ, Gurley ES, Ahmed BN, Banu S, Khan SU, et al. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. *Emerg Infect Dis.* 2009; 15:1229–35. [PubMed: 19751584]
48. Luby SP, Gurley ES. Epidemiology of henipavirus disease in humans. *Curr Top Microbiol Immunol.* 2012; 359:25–40. [PubMed: 22752412]
49. Luby, SP.; Broder, CC. Paramyxoviruses: Henipaviruses. In: Kaslow, RA.; Stanberry, LR.; Le Duc, JW., editors. *Viral Infections of Humans, Epidemiology and Control.* New York: Springer Science +Business Media; 2014. p. 519-36.
50. Nipah encephalitis, human - Bangladesh (02). Pro-med: International Society for Infectious Diseases; 2014. <http://www.promedmail.org>, archive no. 20140118.2181682
51. Halpin K, Young PL, Field HE, Mackenzie JS. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J Gen Virol.* 2000; 81:1927–32. [PubMed: 10900029]
52. Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect.* 2002; 4:145–51. [PubMed: 11880045]
53. Reynes JM, Counor D, Ong S, Faure C, Seng V, Molia S, et al. Nipah virus in Lyle's flying foxes, Cambodia. *Emerg Infect Dis.* 2005; 11:1042–7. [PubMed: 16022778]
54. Rahman SA, Hassan SS, Olival KJ, Mohamed M, Chang LY, Hassan L, et al. Characterization of Nipah virus from naturally infected *Pteropus vampyrus* bats, Malaysia. *Emerg Infect Dis.* 2010; 16:1990–3. [PubMed: 21122240]
55. Middleton DJ, Morrissy CJ, van der Heide BM, Russell GM, Braun MA, Westbury HA, et al. Experimental Nipah virus infection in pteropid bats (*Pteropus poliocephalus*). *J Comp Pathol.* 2007; 136:266–72. [PubMed: 17498518]
56. Williamson MM, Hooper PT, Selleck PW, Gleeson LJ, Daniels PW, Westbury HA, et al. Transmission studies of Hendra virus (equine morbillivirus) in fruit bats, horses and cats. *Aust Vet J.* 1998; 76:813–8. [PubMed: 9972433]
57. Williamson MM, Hooper PT, Selleck PW, Westbury HA, Slocombe RF. Experimental hendra virus infection in pregnant guinea-pigs and fruit Bats (*Pteropus poliocephalus*). *J Comp Pathol.* 2000; 122:201–7. [PubMed: 10684689]
58. Halpin K, Hyatt AD, Fogarty R, Middleton D, Bingham J, Epstein JH, et al. Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg.* 2011; 85:946–51. [PubMed: 22049055]
59. Hayman DT, Suu-Ire R, Breed AC, McEachern JA, Wang L, Wood JL, et al. Evidence of henipavirus infection in West African fruit bats. *PLoS ONE.* 2008; 3:e2739. [PubMed: 18648649]
60. Peel AJ, Baker KS, Crameri G, Barr JA, Hayman DT, Wright E, et al. Henipavirus neutralising antibodies in an isolated island population of African fruit bats. *PLoS One.* 2012; 7:e30346. [PubMed: 22253928]
61. Peel AJ, Sargan DR, Baker KS, Hayman DT, Barr JA, Crameri G, et al. Continent-wide panmixia of an African fruit bat facilitates transmission of potentially zoonotic viruses. *Nature communications.* 2013; 4:2770.
62. Hasebe F, Thi Thu Thuy N, Inoue S, Yu F, Kaku Y, Watanabe S, et al. Serologic Evidence of Nipah Virus Infection in Bats, Vietnam. *Emerging Infectious Diseases.* 2012; 18:536–7. [PubMed: 22377109]
63. Wacharapluesadee S, Lumlertdacha B, Boongird K, Wanghongsa S, Chanhom L, Rollin P, et al. Bat Nipah virus, Thailand. *Emerg Infect Dis.* 2005; 11:1949–51. [PubMed: 16485487]
64. Li Y, Wang J, Hickey AC, Zhang Y, Wu Y, Zhang H, et al. Antibodies to Nipah or Nipah-like viruses in bats, China. *Emerg Infect Dis.* 2008; 14:1974–6. [PubMed: 19046545]
65. Drexler JF, Corman VM, Gloza-Rausch F, Seebens A, Annan A, Ipsen A, et al. Henipavirus RNA in African bats. *PLoS One.* 2009; 4:e6367. [PubMed: 19636378]
66. Ching PK, de Los Reyes VC, Sucaldito MN, Tayag E, Columna-Vingno AB, Malbas FF Jr, et al. Outbreak of henipavirus infection, Philippines, 2014. *Emerg Infect Dis.* 2015; 21:328–31. [PubMed: 25626011]
67. Pernet O, Schneider BS, Beaty SM, LeBreton M, Yun TE, Park A, et al. Evidence for henipavirus spillover into human populations in Africa. *Nature communications.* 2014; 5:5342.

68. Chowdhury S, Khan SU, Crameri G, Epstein JH, Broder CC, Islam A, et al. Serological evidence of henipavirus exposure in cattle, goats and pigs in Bangladesh. *PLoS Negl Trop Dis*. 2014; 8:e3302. [PubMed: 25412358]
69. Hayman DT, Wang LF, Barr J, Baker KS, Suu-Ire R, Broder CC, et al. Antibodies to henipavirus or henipa-like viruses in domestic pigs in Ghana, West Africa. *PLoS One*. 2011; 6:e25256. [PubMed: 21966471]
70. Hyatt AD, Zaki SR, Goldsmith CS, Wise TG, Hengstberger SG. Ultrastructure of Hendra virus and Nipah virus within cultured cells and host animals. *Microbes Infect*. 2001; 3:297–306. [PubMed: 11334747]
71. Goldsmith CS, Whistler T, Rollin PE, Ksiazek TG, Rota PA, Bellini WJ, et al. Elucidation of Nipah virus morphogenesis and replication using ultrastructural and molecular approaches. *Virus Res*. 2003; 92:89–98. [PubMed: 12606080]
72. Murray K, Selleck P, Hooper P, Hyatt A, Gould A, Gleeson L, et al. A morbillivirus that caused fatal disease in horses and humans. *Science*. 1995; 268:94–7. [PubMed: 7701348]
73. Wang LF, Yu M, Hansson E, Pritchard LI, Shiell B, Michalski WP, et al. The exceptionally large genome of Hendra virus: support for creation of a new genus within the family Paramyxoviridae. *J Virol*. 2000; 74:9972–9. [PubMed: 11024125]
74. Marsh GA, Todd S, Foord A, Hansson E, Davies K, Wright L, et al. Genome sequence conservation of Hendra virus isolates during spillover to horses, Australia. *Emerg Infect Dis*. 2010; 16:1767–9. [PubMed: 21029540]
75. AbuBakar S, Chang LY, Ali AR, Sharifah SH, Yusoff K, Zamrod Z. Isolation and molecular identification of Nipah virus from pigs. *Emerg Infect Dis*. 2004; 10:2228–30. [PubMed: 15663869]
76. Chan YP, Chua KB, Koh CL, Lim ME, Lam SK. Complete nucleotide sequences of Nipah virus isolates from Malaysia. *J Gen Virol*. 2001; 82:2151–5. [PubMed: 11514724]
77. Chua KB, Lek Koh C, Hooi PS, Wee KF, Khong JH, Chua BH, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect*. 2002; 4:145–51. [PubMed: 11880045]
78. Harcourt BH, Tamin A, Ksiazek TG, Rollin PE, Anderson LJ, Bellini WJ, et al. Molecular characterization of Nipah virus, a newly emergent paramyxovirus. *Virology*. 2000; 271:334–49. [PubMed: 10860887]
79. Harcourt BH, Lowe L, Tamin A, Liu X, Bankamp B, Bowden N, et al. Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg Infect Dis*. 2005; 11:1594–7. [PubMed: 16318702]
80. Wacharapluesadee S, Boongird K, Wanghongsa S, Ratanasetyuth N, Supavonwong P, Saengsen D, et al. A longitudinal study of the prevalence of Nipah virus in *Pteropus lylei* bats in Thailand: evidence for seasonal preference in disease transmission. *Vector Borne Zoonotic Dis*. 2010; 10:183–90. [PubMed: 19402762]
81. Tanimura N, Imada T, Kashiwazaki Y, Sharifah SH. Distribution of viral antigens and development of lesions in chicken embryos inoculated with nipah virus. *J Comp Pathol*. 2006; 135:74–82. [PubMed: 16956618]
82. Negrete OA, Wolf MC, Aguilar HC, Enterlein S, Wang W, Muhlberger E, et al. Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. *PLoS Pathog*. 2006; 2:e7. [PubMed: 16477309]
83. Negrete OA, Levroney EL, Aguilar HC, Bertolotti-Ciarlet A, Nazarian R, Tajyar S, et al. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature*. 2005; 436:401–5. [PubMed: 16007075]
84. Bonaparte MI, Dimitrov AS, Bossart KN, Crameri G, Mungall BA, Bishop KA, et al. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc Natl Acad Sci U S A*. 2005; 102:10652–7. [PubMed: 15998730]
85. Bishop KA, Stantchev TS, Hickey AC, Khetawat D, Bossart KN, Krasnoperov V, et al. Identification of Hendra virus G glycoprotein residues that are critical for receptor binding. *J Virol*. 2007; 81:5893–901. [PubMed: 17376907]
86. Drescher U. Eph family functions from an evolutionary perspective. *Curr Opin Genet Dev*. 2002; 12:397–402. [PubMed: 12100883]

87. Bossart KN, Tachedjian M, McEachern JA, Crameri G, Zhu Z, Dimitrov DS, et al. Functional studies of host-specific ephrin-B ligands as Henipavirus receptors. *Virology*. 2008; 372:357–71. [PubMed: 18054977]
88. Gale NW, Baluk P, Pan L, Kwan M, Holash J, DeChiara TM, et al. Ephrin-B2 selectively marks arterial vessels and neovascularization sites in the adult, with expression in both endothelial and smooth-muscle cells. *Dev Biol*. 2001; 230:151–60. [PubMed: 11161569]
89. Poliakov A, Cotrina M, Wilkinson DG. Diverse roles of eph receptors and ephrins in the regulation of cell migration and tissue assembly. *Dev Cell*. 2004; 7:465–80. [PubMed: 15469835]
90. Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. *Cell*. 2008; 133:38–52. [PubMed: 18394988]
91. Bossart KN, Crameri G, Dimitrov AS, Mungall BA, Feng YR, Patch JR, et al. Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J Virol*. 2005; 79:6690–702. [PubMed: 15890907]
92. Bowden TA, Aricescu AR, Gilbert RJ, Grimes JM, Jones EY, Stuart DI. Structural basis of Nipah and Hendra virus attachment to their cell-surface receptor ephrin-B2. *Nature structural & molecular biology*. 2008; 15:567–72.
93. Bowden TA, Crispin M, Harvey DJ, Aricescu AR, Grimes JM, Jones EY, Stuart DI. Crystal structure and carbohydrate analysis of Nipah virus attachment glycoprotein: a template for antiviral and vaccine design. *J Virol*. 2008 Dec; 82(23):11628–36. [PubMed: 18815311]
94. Bowden TA, Crispin M, Harvey DJ, Jones EY, Stuart DI. Dimeric architecture of the Hendra virus attachment glycoprotein: evidence for a conserved mode of assembly. *J Virol*. 2010; 84:6208–17. [PubMed: 20375167]
95. Xu K, Rajashankar KR, Chan YP, Himanen JP, Broder CC, Nikolov DB. Host cell recognition by the henipaviruses: crystal structures of the Nipah G attachment glycoprotein and its complex with ephrin-B3. *Proc Natl Acad Sci U S A*. 2008; 105:9953–8. [PubMed: 18632560]
96. Xu K, Chan YP, Rajashankar KR, Khetawat D, Yan L, Kolev MV, et al. New insights into the Hendra virus attachment and entry process from structures of the virus G glycoprotein and its complex with ephrin-B2. *PLoS One*. 2012; 7:e48742. [PubMed: 23144952]
97. Bossart KN, Fusco DL, Broder CC. Paramyxovirus entry. *Advances in experimental medicine and biology*. 2013; 790:95–127. [PubMed: 23884588]
98. Pager CT, Dutch RE. Cathepsin L is involved in proteolytic processing of the hendra virus fusion protein. *J Virol*. 2005; 79:12714–20. [PubMed: 16188974]
99. Aguilar HC, Iorio RM. Henipavirus membrane fusion and viral entry. *Curr Top Microbiol Immunol*. 2012; 359:79–94. [PubMed: 22427111]
100. Lee B, Ataman ZA. Modes of paramyxovirus fusion: a Henipavirus perspective. *Trends Microbiol*. 2011; 19:389–99. [PubMed: 21511478]
101. Goh KJ, Tan CT, Chew NK, Tan PS, Kamarulzaman A, Sarji SA, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med*. 2000; 342:1229–35. [PubMed: 10781618]
102. Mahalingam S, Herrero LJ, Playford EG, Spann K, Herring B, Rolph MS, et al. Hendra virus: an emerging paramyxovirus in Australia. *Lancet Infect Dis*. 2012; 12:799–807. [PubMed: 22921953]
103. Selvey LA, Wells RM, McCormack JG, Ansford AJ, Murray K, Rogers RJ, et al. Infection of humans and horses by a newly described morbillivirus [see comments]. *Med J Aust*. 1995; 162:642–5. [PubMed: 7603375]
104. Taylor C, Playford EG, McBride WJ, McMahon J, Warrilow D. No evidence of prolonged Hendra virus shedding by 2 patients, Australia. *Emerg Infect Dis*. 2012; 18:2025–7. [PubMed: 23171522]
105. Tan CT, Goh KJ, Wong KT, Sarji SA, Chua KB, Chew NK, et al. Relapsed and late-onset Nipah encephalitis. *Ann Neurol*. 2002; 51:703–8. [PubMed: 12112075]
106. Wong SC, Ooi MH, Wong MN, Tio PH, Solomon T, Cardoso MJ. Late presentation of Nipah virus encephalitis and kinetics of the humoral immune response. *J Neurol Neurosurg Psychiatry*. 2001; 71:552–4. [PubMed: 11561048]

107. Chong HT, Tan CT. Relapsed and late-onset Nipah encephalitis, a report of three cases. *Neurol J Southeast Asia*. 2003; 8:109–12.
108. Tan CT, Wong KT. Nipah encephalitis outbreak in Malaysia. *Ann Acad Med Singapore*. 2003; 32:112–7. [PubMed: 12625108]
109. Abdullah S, Chang LY, Rahmat K, Goh KT, Tan CT. Late-onset Nipah virus encephalitis 11 years after the initial outbreak: A case report. *Neurology Asia*. 2012; 17:71–4.
110. Bellini WJ, Harcourt BH, Bowden N, Rota PA. Nipah virus: an emergent paramyxovirus causing severe encephalitis in humans. *Journal of neurovirology*. 2005; 11:481–7. [PubMed: 16287690]
111. Sejvar JJ, Hossain J, Saha SK, Gurley ES, Banu S, Hamadani JD, Faiz MA, Siddiqui FM, Mohammad QD, Mollah AH, Uddin R, Alam R, Rahman R, Tan CT, Bellini W, Rota P, Breiman RF, Luby SP. Long-term neurological and functional outcome in Nipah virus infection. *Ann Neurol*. 2007 Sep; 62(3):235–42. [PubMed: 17696217]
112. Wong KT, Shieh WJ, Kumar S, Norain K, Abdullah W, Guarner J, et al. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol*. 2002; 161:2153–67. [PubMed: 12466131]
113. Hooper P, Zaki S, Daniels P, Middleton D. Comparative pathology of the diseases caused by Hendra and Nipah viruses. *Microbes Infect*. 2001; 3:315–22. [PubMed: 11334749]
114. Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. *Pathogens (Basel, Switzerland)*. 2013; 2:264–87.
115. Middleton DJ, Weingartl HM. Henipaviruses in their natural animal hosts. *Curr Top Microbiol Immunol*. 2012; 359:105–21. [PubMed: 22476529]
116. Williamson MM, Hooper PT, Selleck PW, Westbury HA, Slocombe RF. A guinea-pig model of Hendra virus encephalitis. *J Comp Pathol*. 2001; 124:273–9. [PubMed: 11437503]
117. Westbury HA, Hooper PT, Selleck PW, Murray PK. Equine morbillivirus pneumonia: susceptibility of laboratory animals to the virus. *Aust Vet J*. 1995; 72:278–9. [PubMed: 8534238]
118. Guillaume V, Wong KT, Looi RY, Georges-Courbot MC, Barrot L, Buckland R, et al. Acute Hendra virus infection: Analysis of the pathogenesis and passive antibody protection in the hamster model. *Virology*. 2009; 387:459–65. [PubMed: 19328514]
119. Wong KT, Grosjean I, Brisson C, Blanquier B, Fevre-Montange M, Bernard A, et al. A golden hamster model for human acute Nipah virus infection. *Am J Pathol*. 2003; 163:2127–37. [PubMed: 14578210]
120. Mungall BA, Middleton D, Crameri G, Bingham J, Halpin K, Russell G, et al. Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. *J Virol*. 2006; 80:12293–302. [PubMed: 17005664]
121. Middleton DJ, Westbury HA, Morrissy CJ, van der Heide BM, Russell GM, Braun MA, et al. Experimental Nipah virus infection in pigs and cats. *J Comp Pathol*. 2002; 126:124–36. [PubMed: 11945001]
122. Li M, Embury-Hyatt C, Weingartl HM. Experimental inoculation study indicates swine as a potential host for Hendra virus. *Vet Res*. 2010; 41:33. [PubMed: 20167195]
123. Weingartl H, Czub S, Copps J, Berhane Y, Middleton D, Marszal P, et al. Invasion of the central nervous system in a porcine host by nipah virus. *J Virol*. 2005; 79:7528–34. [PubMed: 15919907]
124. Pallister J, Middleton D, Wang LF, Klein R, Haining J, Robinson R, et al. A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. *Vaccine*. 2011; 29:5623–30. [PubMed: 21689706]
125. Bossart KN, Zhu Z, Middleton D, Klippel J, Crameri G, Bingham J, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS Pathog*. 2009; 5:e1000642. [PubMed: 19888339]
126. Rockx B, Bossart KN, Feldmann F, Geisbert JB, Hickey AC, Brining D, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. *J Virol*. 2010; 84:9831–9. [PubMed: 20660198]
127. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, Smith MA, Chan YP, Wang LF, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLoS One*. 2010; 5:e10690. [PubMed: 20502528]

128. Marianneau P, Guillaume V, Wong T, Badmanathan M, Looi RY, Murri S, et al. Experimental infection of squirrel monkeys with nipah virus. *Emerg Infect Dis.* 2010; 16:507–10. [PubMed: 20202432]
129. Marsh GA, Haining J, Hancock TJ, Robinson R, Foord AJ, Barr JA, et al. Experimental infection of horses with hendra virus/australia/horse/2008/redlands. *Emerg Infect Dis.* 2011; 17:2232–8. [PubMed: 22172152]
130. Mohd Nor MN, Gan CH, Ong BL. Nipah virus infection of pigs in peninsular Malaysia. *Rev Sci Tech.* 2000; 19:160–5. [PubMed: 11189713]
131. Field H, Schaaf K, Kung N, Simon C, Waltisbuhl D, Hobert H, et al. Hendra virus outbreak with novel clinical features, Australia. *Emerg Infect Dis.* 2010; 16:338–40. [PubMed: 20113576]
132. Hooper PT, Ketterer PJ, Hyatt AD, Russell GM. Lesions of experimental equine morbillivirus pneumonia in horses. *Vet Pathol.* 1997; 34:312–22. [PubMed: 9240840]
133. Kong D, Wen Z, Su H, Ge J, Chen W, Wang X, et al. Newcastle disease virus-vectored Nipah encephalitis vaccines induce B and T cell responses in mice and long-lasting neutralizing antibodies in pigs. *Virology.* 2012; 432:327–35. [PubMed: 22726244]
134. Kurup D, Wirblich C, Feldmann H, Marzi A, Schnell MJ. Rhabdovirus-Based Vaccine Platforms against Henipaviruses. *J Virol.* 2015; 89:144–54. [PubMed: 25320306]
135. Wang X, Ge J, Hu S, Wang Q, Wen Z, Chen H, et al. Efficacy of DNA immunization with F and G protein genes of Nipah virus. *Ann N Y Acad Sci.* 2006; 1081:243–5. [PubMed: 17135518]
136. Walpita P, Barr J, Sherman M, Basler CF, Wang L. Vaccine potential of Nipah virus-like particles. *PLoS One.* 2011; 6:e18437. [PubMed: 21494680]
137. Guillaume V, Contamin H, Loth P, Georges-Courbot MC, Lefevre A, Marianneau P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. *J Virol.* 2004; 78:834–40. [PubMed: 14694115]
138. Weingartl HM, Berhane Y, Caswell JL, Loosmore S, Audonnet JC, Roth JA, et al. Recombinant nipah virus vaccines protect pigs against challenge. *J Virol.* 2006; 80:7929–38. [PubMed: 16873250]
139. Ploquin A, Szecsi J, Mathieu C, Guillaume V, Barateau V, Ong KC, et al. Protection against henipavirus infection by use of recombinant adeno-associated virus-vector vaccines. *J Infect Dis.* 2013; 207:469–78. [PubMed: 23175762]
140. Yoneda M, Georges-Courbot MC, Ikeda F, Ishii M, Nagata N, Jacquot F, et al. Recombinant measles virus vaccine expressing the Nipah virus glycoprotein protects against lethal Nipah virus challenge. *PLoS One.* 2013; 8:e58414. [PubMed: 23516477]
141. Mire CE, Versteeg KM, Cross RW, Agans KN, Fenton KA, Whitt MA, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J.* 2013; 10:353. [PubMed: 24330654]
142. DeBuysscher BL, Scott D, Marzi A, Prescott J, Feldmann H. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. *Vaccine.* 2014; 32:2637–44. [PubMed: 24631094]
143. Lo MK, Bird BH, Chattopadhyay A, Drew CP, Martin BE, Coleman JD, et al. Single-dose replication-defective VSV-based Nipah virus vaccines provide protection from lethal challenge in Syrian hamsters. *Antiviral Res.* 2014; 101:26–9. [PubMed: 24184127]
144. Colgrave ML, Snelling HJ, Shiell BJ, Feng YR, Chan YP, Bossart KN, et al. Site occupancy and glycan compositional analysis of two soluble recombinant forms of the attachment glycoprotein of Hendra virus. *Glycobiology.* 2011; 22:572–84. [PubMed: 22171062]
145. McEachern JA, Bingham J, Cramer G, Green DJ, Hancock TJ, Middleton D, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine.* 2008; 26:3842–52. [PubMed: 18556094]
146. Pallister JA, Klein R, Arkinstall R, Haining J, Long F, White JR, et al. Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. *Virol J.* 2013; 10:237. [PubMed: 23867060]
147. Mire CE, Geisbert JB, Agans KN, Feng YR, Fenton KA, Bossart KN, et al. A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. *J Virol.* 2014; 88:4624–31. [PubMed: 24522928]

148. Australian Government. Australian Pesticides and Veterinary Medicines Authority – Summary of adverse experience reports made to the APVMA about Hendra virus vaccine. 2015. Available: <http://apvma.gov.au/node/15786>
149. Guillaume V, Contamin H, Loth P, Grosjean I, Courbot MC, Deubel V, et al. Antibody Prophylaxis and Therapy against Nipah Virus Infection in Hamsters. *J Virol*. 2006; 80:1972–8. [PubMed: 16439553]
150. Zhu Z, Dimitrov AS, Bossart KN, Crameri G, Bishop KA, Choudhry V, et al. Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. *J Virol*. 2006; 80:891–9. [PubMed: 16378991]
151. Zhu Z, Bossart KN, Bishop KA, Crameri G, Dimitrov AS, McEachern JA, et al. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. *J Infect Dis*. 2008; 197:846–53. [PubMed: 18271743]
152. Xu K, Rockx B, Xie Y, Debuysscher BL, Fusco DL, Zhu Z, et al. Crystal structure of the hendra virus attachment g glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS Pathog*. 2013; 9:e1003684. [PubMed: 24130486]
153. Bossart KN, Geisbert TW, Feldmann H, Zhu Z, Feldmann F, Geisbert JB, et al. A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Sci Transl Med*. 2011; 3:105ra3.
154. Geisbert TW, Mire CE, Geisbert JB, Chan YP, Agans KN, Feldmann F, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med*. 2014; 6:242ra82.
155. Government Q. World-first Hendra treatment one step closer. 2013
156. Prescott J, DeBuysscher BL, Feldmann F, Gardner DJ, Haddock E, Martellaro C, et al. Single-dose live-attenuated vesicular stomatitis virus-based vaccine protects African green monkeys from Nipah virus disease. *Vaccine*. 2015; 33:2823–9. [PubMed: 25865472]
157. Bossart KN, Rockx B, Feldmann F, Brining D, Scott D, Lacasse R, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. *Sci Transl Med*. 2012; 4:146ra07.
158. Kelley LA, Sternberg MJ. Protein structure prediction on the Web: a case study using the Phyre server. *Nat Protoc*. 2009; 4:363–71. [PubMed: 19247286]

Fig. 1.

Model of the Hendra virus soluble G glycoprotein subunit vaccine (HeV-sG) and its complex with the HeV- and NiV-neutralizing human mAb. The related mAb m102.3, featuring an identical heavy chain and a similar light chain, was used in place of the m102.4 in the structural solution of the complex [152]. The HeV-sG subunit vaccine consists of the entire ectodomain (amino acids 76-604) of the HeV G glycoprotein. HeV-sG is shown as dimer with one monomer colored blue and the other magenta. Secondary structure of the two globular head domains of HeV-sG are derived from the crystal structure [96, 144], and the stalk regions of each G monomer (residues 77-136) are modeled [158], and the N-linked glycosylation sites are gray spheres. The HeV-sG head (magenta) is shown in complex with the m102.3 Fab antibody, where the light chain is yellow and the heavy chain is red and modeled based on the crystal structure [152]. An overlaid m102.3 heavy chain CDR-H3 sequence (red) is shown in complex with the HeV-sG head (blue) identifying the location of

both the m102.3 and the m102.4 mAb epitope which overlaps the ephrin receptor binding site.

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Table 1

Advanced active vaccination and passive immunization platforms tested in Hendra virus and/or Nipah virus animal challenge models.

Platform	Viral antigen target or immunogen	Animal challenge model
Active vaccination		
Recombinant vaccinia virus	Nipah F and/or G glycoprotein	Hamster ^a (NiV)
Recombinant canarypox virus	Nipah F and/or G glycoprotein	Pig ^b (NiV)
Recombinant VSV	Nipah F and/or G glycoprotein	Ferret ^c (NiV), Hamster ^d (NiV), nonhuman primate ^e (NiV)
Recombinant AAV	Nipah G glycoprotein	Hamster ^f (NiV, HeV)
Recombinant measles virus	Nipah G glycoprotein	Hamster and nonhuman primate ^g (NiV)
Recombinant subunit	Hendra soluble G glycoprotein	Cat ^h (NiV), Ferret ⁱ (HeV, NiV), nonhuman primate ^j (HeV, NiV) Horse ^k (HeV)
Passive immunization		
Human monoclonal antibody m102.4	Hendra / Nipah G glycoprotein	Ferret ^l (NiV) Nonhuman primate ^m (HeV, NiV)

^aHamsters immunized with NiV F and/or G glycoprotein encoding recombinant vaccinia viruses were protected against disease following intraperitoneal challenge with 10^3 PFU of NiV [137].

^bPigs immunized with NiV F and/or G glycoprotein encoding recombinant canarypox viruses were protected against intranasal challenge with 2.5×10^5 PFU of NiV [138].

^cFerrets immunized with NiV F and/or G glycoprotein encoding recombinant vesicular stomatitis virus (VSV) vectors were protected against lethal intranasal challenge with 5×10^3 PFU of NiV [141].

^dHamsters immunized with NiV F and/or G glycoprotein encoding recombinant vesicular stomatitis virus (VSV) vectors were protected against lethal intraperitoneal challenge with 10^5 TCID₅₀ of NiV [143]; or 6.8×10^4 TCID₅₀ of NiV [142].

^eAfrican green monkeys immunized with a NiV G encoding recombinant VSV vector were protected against lethal intratracheal challenge with 10^5 TCID₅₀ of NiV [156].

^fHamsters immunized with a NiV G encoding recombinant adeno-associated virus (AAV) vector were protected against lethal intraperitoneal with 10^4 PFU of NiV [139].

^gHamsters and African green monkeys immunized with a NiV G encoding recombinant measles virus vector were protected against lethal intraperitoneal challenge with 10^3 TCID₅₀ of NiV (hamsters) or 10^5 TCID₅₀ of NiV (monkeys) [140].

^hHendra virus soluble G glycoprotein (HeV-sG) used to immunize cats protects against lethal subcutaneous (500 TCID₅₀) [120] or oronasal (5×10^4 TCID₅₀) NiV challenge [145].

ⁱHeV-sG used to immunize ferrets protects against lethal oronasal challenge with 5×10^3 TCID₅₀ of HeV [124] or 5×10^3 TCID₅₀ of NiV challenge [146].

^jHeV-sG used to immunize African green monkeys protects against lethal intratracheal challenge with 10^5 TCID₅₀ of NiV [157] or 5×10^5 PFU of HeV [147].

^kHeV-sG used to immunize horses protects against lethal oronasal challenge with 2×10^6 TCID₅₀ of HeV [15].

^l A NiV and HeV cross-reactive G glycoprotein specific neutralizing human mAb (m102.4) protects ferrets against lethal oronasal challenge with 5×10^3 TCID₅₀ of NiV [125] or 5×10^3 TCID₅₀ of HeV (J. Pallister and C. Broder, unpublished) by post-exposure infusion.

^m Human mAb m102.4 protects African green monkeys by post-exposure infusion following lethal intratracheal challenge with 4×10^5 TCID₅₀ of HeV[153] or lethal intratracheal challenge with 5×10^5 PFU of NiV [154].