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Vaccines to Emerging Viruses: Nipah and Hendra

Moushimi Amaya and Christopher C. Broder

Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814, USA; email: christopher.broder@usuhs.edu

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Abstract

Hendra virus (HeV) and Nipah virus (NiV) are bat-borne zoonotic paramyxoviruses identified in the mid- to late 1990s in outbreaks of severe disease in livestock and people in Australia and Malaysia, respectively. HeV repeatedly re-emerges in Australia while NiV continues to cause outbreaks in South Asia (Bangladesh and India), and these viruses have remained transboundary threats. In people and several mammalian species, HeV and NiV infections present as a severe systemic and often fatal neurologic and/or respiratory disease. NiV stands out as a potential pandemic threat because of its associated high case-fatality rates and capacity for human-to-human transmission. The development of effective vaccines, suitable for people and livestock, against HeV and NiV has been a research focus. Here, we review the progress made in NiV and HeV vaccine development, with an emphasis on those approaches that have been tested in established animal challenge models of NiV and HeV infection and disease.

INTRODUCTION

Nipah virus (NiV) and Hendra virus (HeV) are bat-borne viral zoonoses that were discovered in the mid- to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia [NiV-Malaysia (NiV-M)] (1). They are the prototype members of the genus *Henipavirus* in the family *Paramyxoviridae* (2). NiV outbreaks have also been recorded in Bangladesh and India by a closely related strain, NiV-Bangladesh (NiV-B) (3). Three other henipaviruses are also recognized: Cedar virus (CedV) as an isolate and Ghana virus (GhV) and Mojiang virus (MojV) known only from sequence data (4–7). Both NiV and HeV are highly pathogenic in a broad range of mammalian hosts that are capable of infecting and causing severe disease in humans, monkeys, pigs, horses, cats, dogs, ferrets, hamsters, and guinea pigs and that span six mammalian orders including bats, although bats do not exhibit disease when infected (8–21). In contrast, CedV is nonpathogenic in well-characterized models of HeV and NiV disease including ferrets and hamsters (4, 22). The pathogenic potential of GhV and MojV is unknown.

Several species of *Pteropus* fruit bats are the natural reservoir hosts of NiV, HeV, and CedV (4, 23–27). NiV- or HeV-mediated disease has not been reported in wild or experimentally infected bats (13, 28–30). NiV and HeV infections in people and many animals manifest as severe systemic and often fatal neurologic and/or respiratory diseases (31–33). Both NiV and HeV are regarded as transboundary biological threats to both human and animal health and are classified as biosafety level 4 (BSL-4) select agents (34, 35). NiV and henipaviral diseases are included in the World Health Organization (WHO) R&D Blueprint list of priority pathogens with epidemic potential that need research attention (36). This review summarizes the important characteristics of the NiV and HeV pathogens, the modes of virus transmission, and the immunization strategies being developed against them.

Emergence and Outbreaks of Hendra and Nipah Viruses

In 1994 in the Brisbane suburb of Hendra, Australia, an outbreak of severe respiratory disease resulted in the deaths of 14 horses and their trainer, along with the nonfatal infection of 7 other horses and 1 other person. This led to the discovery of a novel paramyxovirus initially termed equine morbillivirus, now known as HeV (37–39). The first known cases of HeV in horses and a human actually occurred a few months prior, where one person became ill after assisting in the necropsies of two horses later shown to have died from HeV (40, 41). This individual experienced a relapsed fatal encephalitis caused by HeV 13 months later (42). HeV has since re-emerged in Australia 62 times with a total of 104 horse deaths (fatal or euthanized), along with 4 human fatalities of 7 cases (43). Every recorded occurrence of HeV in Australia has involved horses, all resulting in a severe or fatal disease, and all cases of human infection were acquired from virus-shedding horses (31, 44).

In 1998, an outbreak of encephalitis among pig farmers in Peninsular Malaysia occurred and a virus was isolated from samples of cerebrospinal fluid (CSF) of two patients who had died; cells infected with this virus cross-reacted with antibodies against HeV (45). Genetic studies revealed a new paramyxovirus that was closely related to HeV, and it was named Nipah after the village in Malaysia where one of the patients had lived (45). There were 265 cases of human infection with 105 fatalities in Malaysia and 11 cases and 1 fatality among abattoir workers in Singapore (46, 47). This outbreak was controlled through the culling of more than 1 million pigs, resulting in significant economic impacts to the region (48, 49).

A genetically similar but distinct strain of NiV was identified as the causative agent of fatal encephalitis in people in Bangladesh (NiV-B) (3, 50). Since 2001, nearly annual occurrences of human NiV-B infections have occurred in Bangladesh, and there have been three outbreaks in

India (51–54). The recent 2018 NiV outbreak in Kerala, India, was significant, having occurred in a new geographic region far from locations in Bangladesh and India where all prior outbreaks had occurred and with a case fatality rate of 91% (51). In 2014, an outbreak of NiV-M encephalitis occurred in the Philippines with 9 fatalities of 11 human cases of acute encephalitis and influenza-like illness or meningitis in another 6 individuals (55). Altogether, there have been over 650 cases of human NiV infection (combined ~60% fatality rate) in South Asia and Southeast Asia in five countries (54, 56).

Transmission of Hendra and Nipah Viruses

The routes of transmission of virus infection to humans from animals are different for HeV and NiV, with horses the only spillover host of HeV in Australia, while for NiV it was pigs in Malaysia and horses in the Philippines (**Figure 1**). However, human NiV infections in Bangladesh, India, and the Philippines also include bat-to-human and human-to-human transmission (57–60). Transmission routes of HeV and NiV to animals are likely urine from infected bats contaminating pastures or pigsties and/or virus-contaminated fruit spat from bats that is ingested (61, 62) (**Figure 1**). Recoverable virus is shed in the urine of experimentally infected bats and can also be detected in throat and rectal swabs (13, 28–30). Pooled urine samples from flying foxes are also routinely used to detect and isolate henipaviruses (4, 13, 23, 27, 63–65).

It was previously suggested that infected horses could transmit HeV to people during the feeding of ill animals (38). Also, the majority of all HeV-infected horse cases have involved a single animal, suggesting that HeV is not readily transmitted between horses, and multiple horse outbreaks are likely via contamination of fomites (43, 66). The transmission risk of HeV from infected horses to humans appears to be virus-contaminated fluids or tissues during examination procedures and/or the necropsy of horses (31, 67) (**Figure 1**). Indeed, all cases of human HeV infection have been associated with postmortem examination of horses or close contact with ill horses (31, 38, 42, 68). In Malaysia, it was contact with infected pigs or fresh infected pig products that was required for transmission of virus to humans (45, 69, 70) (**Figure 1**). NiV shedding in respiratory fluids of infected pigs suggested that it probably spread among farmed animals by aerosol droplets or direct contact (16, 71, 72). In Bangladesh, the transmission of NiV from bats to people has been linked to the consumption of virus-contaminated fresh date palm sap, and bats will consume sap during its collection (57, 73, 74). Domestic animals have also been linked to NiV infection in people in Bangladesh from unwell animals (cows and goats) and pigs (50, 59). Human-to-human transmission of NiV has been well documented in Bangladesh and India (52, 58–60, 75) (**Figure 1**). A study of human NiV-B cases in Bangladesh spanning 14 years reported that of 248 cases studied, one-third were caused by human-to-human transmission (56). Human-to-human transmission of NiV-M was not apparent in Malaysia (76, 77), whereas in the Philippines' NiV-M outbreak, human cases were linked to horse slaughtering and horse meat consumption or exposure to other human patients, indicating both horse-to-human and human-to-human transmission (55) (**Figure 1**). The NiV-B outbreak in Kerala had a very high rate of human-to-human transmission (22 of 23 cases) at three different hospital locations (51).

Naturally acquired NiV infections were also recorded in cats, dogs, and horses in the initial Malaysian outbreak (**Figure 1**), and serological evidence of natural NiV infection in dogs was linked to outbreak farms (11, 61, 78). In the Philippines, both dogs and cats were linked to NiV-M infection, with cats dying after eating horse meat and dogs having NiV-neutralizing antibodies (55) (**Figure 1**). In Australia, a dog was found to be seropositive for HeV and later euthanized but showed no signs of disease, and a second HeV-positive dog was identified in 2013 following exposure to blood from an infected horse (79) (**Figure 1**). Dogs are susceptible to experimental HeV infection and shed virus but show little evidence of clinical illness (80).

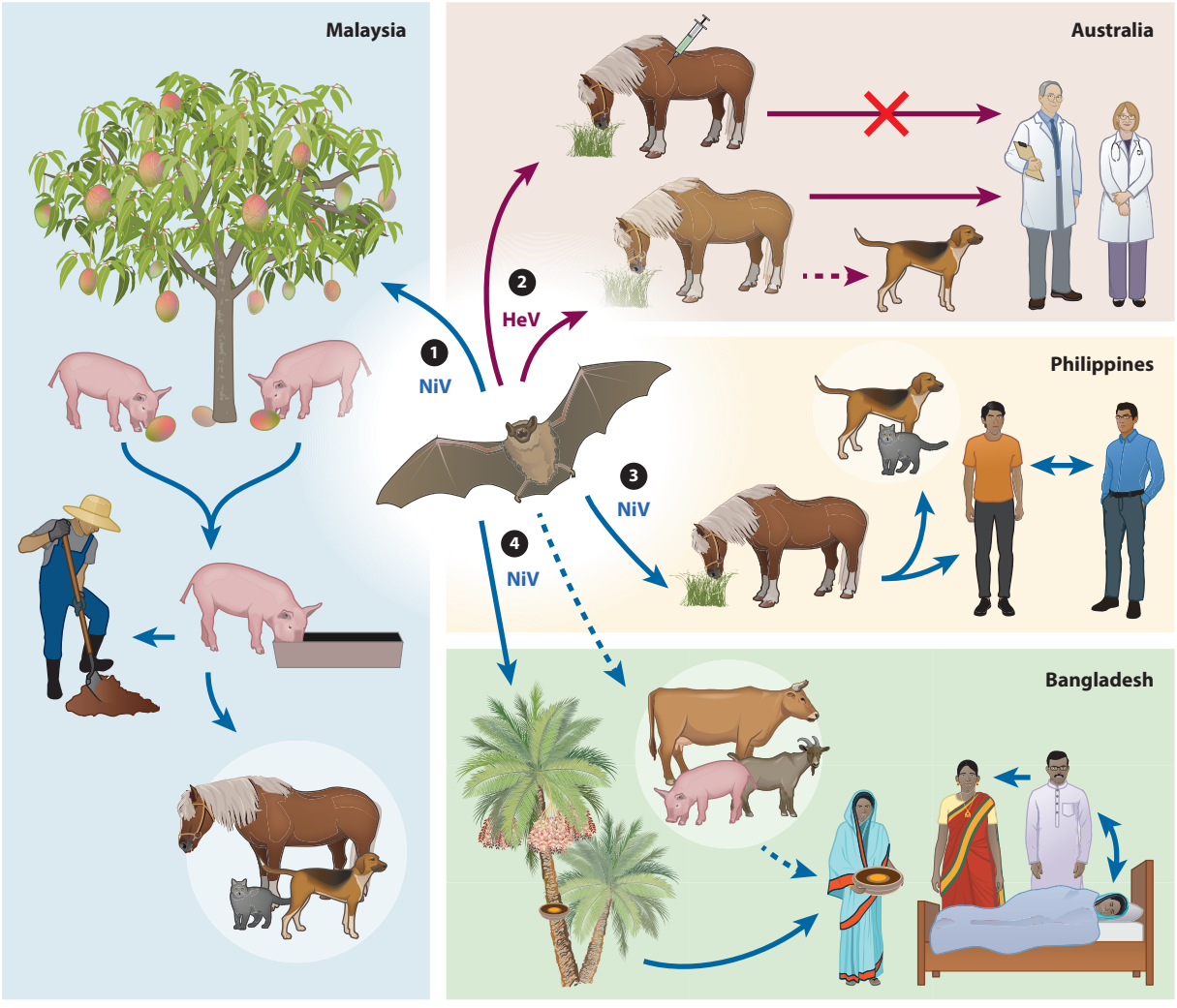

Figure 1

Nipah virus (NiV) and Hendra virus (HeV) modes of transmission in different countries. The transmission routes of NiV in Malaysia (*left*), Philippines (*middle right*), Bangladesh (*bottom right*), and HeV (*top right*) are depicted. Solid lines represent transmission that has been observed and documented, and dashed lines represent suspected transmission in natural conditions. Fruit bats are the natural reservoirs of NiV and HeV. (①) Pigs are infected by consuming partially eaten or contaminated fruit from infected bats (urine, saliva) and transmit NiV to other pigs, pig farmers, or other animals (dogs, cats, and horses) through close or direct contact. (②) Horses can be infected from grazing in contaminated pastures and transmit HeV to humans and on occasion domestic dogs through close contact. A One Health vaccine approach was developed for vaccination of horses in Australia with the dual purpose of saving horses from lethal HeV infection and preventing HeV transmission from horses to humans. (③) NiV is transmitted to humans through close contact with infected horses. NiV transmission to humans, cats, and dogs appears to have occurred following close contact with or consumption of infected horse meat. Human-to-human NiV transmission can occur through close contact. (④) Bat-to-human NiV transmission occurs through consumption of contaminated date palm sap. Human-to-human transmission can occur through close contact with infected patients. Humans may also become infected through contact with infected animals. Figure adapted with permission from Reference 171.

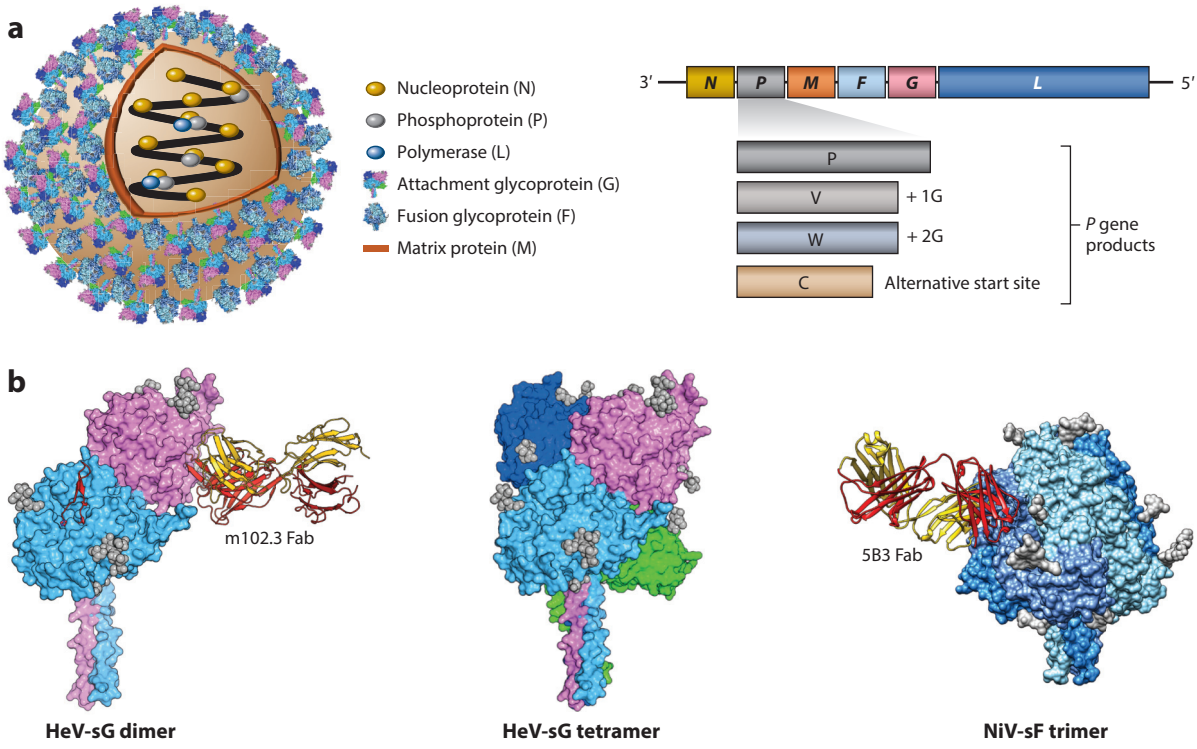

Figure 2

Henipavirus structure and genome organization and models of the G and F glycoprotein soluble ectodomains, Hendra virus (HeV-sG) and Nipah virus (NiV-sF), respectively, and their complexes with respective NiV and HeV cross-reactive neutralizing monoclonal antibodies m102.3 (anti-G) and 5B3 (anti-F). (*a*) Schematic representation of a henipavirus particle with the structural proteins depicted in different colors (*left*) and the henipavirus genome (*right*). HeV and NiV *P* genes encode 3 nonstructural proteins: The C protein is expressed from an alternative start site, and the V and W proteins are expressed following the addition of one or two G residues at the messenger RNA editing site, respectively (*right*). (*b*, *left*) HeV-sG shown as a dimer solvent-accessible surface view with one monomer (*cyan*) overlaid with the monoclonal antibody m102.3 CDR-H3 loop (*red*) at the receptor binding site, and the other monomer (*magenta*) in complex with m102.3 Fab, which has an identical heavy chain and a similar light chain, that was used in place of the m102.4 monoclonal antibody (mAb) in the structural solution of the complex (109). The HeV-sG consists of amino acids 76–604, and the structures of the two globular head domains of HeV-sG are derived from the crystal structure (103, 172). The stalk regions of each G monomer (residues 77–136) are modeled (173). The light chain of m102.3 Fab is colored in yellow, and the heavy chain is colored in red. (*b*, *middle*) The HeV-sG tetramer surface view is modeled with one dimer (*cyan* and *magenta*) in front and the other dimer (*blue* and *green*) in back. *N*-linked glycans are gray spheres. (*b*, *right*) Structural model of the NiV-sF trimer in complex with the 5B3 Fab derived from the cryo–electron microscopy structure (110). The NiV-sF consists of amino acid residues 1–494 with a FLAG tag (DYKDDDK) introduced between residues L104-V105 and a C-terminal GCN4 motif. Each monomer of NiV-sF is in a different shade of blue, 5B3 heavy chain is in red, and light chain is in gold. *N*-linked glycans are illustrated in gray.

Entry and Tropism of Nipah and Hendra Viruses

NiV and HeV are enveloped viruses containing an unsegmented, single-stranded, negative-sense RNA genome (2). **Figure 2*a*** is an illustration of the viral particle and the associated viral proteins. The genomes of HeV and NiV, and also CedV, GhV, and MojV, are considerably longer than the genomes of other paramyxoviruses, at greater than 18 kb. Henipavirus genomes encode 6 structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion glycoprotein (F), attachment glycoprotein (G), and the polymerase protein (L) (**Figure 2*a***). The N, P, and L proteins comprise the replication complex. The *P* gene undergoes RNA editing

to produce 2 additional nonstructural proteins, V and W, that are interferon (IFN) antagonists (81–84). The C protein is transcribed from a second open reading frame in the *P* gene (**Figure 2*a***). NiV has been central to understanding the V, W, P, and C protein roles in antagonizing the innate immune responses via a diverse set of mechanisms (85, 86). Recent in vivo studies with recombinant NiV variants have further defined the varying importance of these nonstructural proteins in pathogenesis, but only a lack of the V protein results in a nonlethal infection (87–89).

The henipavirus virion bears surface projections composed of the F and G glycoproteins that are anchored in the viral membrane and together mediate infection of host cells, and they are the major antigens of vaccine strategies (1) (**Figure 2*a***). The F glycoprotein facilitates membrane fusion between the virus and host cell. The G glycoprotein consists of a characteristic stalk with a globular head that engages entry receptors on host cells, leading to the fusion activation of F and virus infection. The native structure of G is a tetramer while F is a trimer, and together they are the key determinants of infection and tropism (90–92). Models of the soluble ectodomain of the HeV G (HeV-sG) as a dimer and tetramer and the soluble ectodomain of the NiV F (NiV-sF) as a trimer are shown in **Figure 2*b***. NiV and HeV utilize the host cell proteins ephrin-B2 and ephrin-B3 for entry (93–96). Ephrin-B2 and ephrin-B3 are members of a large family of ligands that bind to Eph receptors and are highly sequence conserved among mammals (97, 98). Ephrin-B2 expression is prominent in the vasculature of multiple organs, whereas ephrin-B3 is found predominantly in the nervous system (99–101). The ability of HeV and NiV to use these ephrins as receptors provided explanations of their broad host and tissue tropism (32, 33, 102). The NiV and HeV G head domain structures alone and in complex with ephrin-B2 and ephrin-B3 receptors have been determined (103–106). The structures of both the NiV and HeV F in their prefusion conformation have also been determined (107, 108). These studies have provided insights into understanding the virus entry receptors and host tropism features of the viruses on the molecular level and also facilitated further structural studies of henipavirus G and F glycoproteins in complexes with specific virus-neutralizing antibodies, providing valuable information that has aided vaccine design and choice (109, 110).

Nipah Virus and Hendra Virus Infection in Humans and Animals

Human NiV and HeV infections are generally accepted to occur via the oronasal route, and the incubation periods for both have been estimated to be 1 to 2 weeks (31, 51, 111). Acute infection in people is a systemic infection likely via hematogenous spread of the virus from the respiratory system (112). In general, HeV and NiV disease onset is characterized by fever, myalgia, shortness of breath, and cough (38, 111). Human HeV infections have resulted in both fatal respiratory or encephalitic disease and also recovery from infection (31, 38, 42, 68). The predominant clinical feature in the NiV-M outbreak in Malaysia was encephalitis, but respiratory symptoms were also common with fever, cough, and headache (47, 111, 112). The clinical presentation of NiV-B infections in Bangladesh also includes severe respiratory disease. In the 2018 NiV-B outbreak in Kerala, 83% of cases presented with acute respiratory distress syndrome (ARDS) (51, 113). Central findings of human NiV and HeV infection are a widespread endothelial cell tropism and systemic vasculitis, with prominent parenchymal cell infection in most major organs with the brain and lung significantly affected (45, 112, 114). Human NiV and HeV infections can also take a protracted course following apparent recovery, and some patients can experience late-onset encephalitis or relapsed encephalitis can occur in patients who previously recovered (42, 115). Relapsed encephalitis caused by NiV appears to result from a recrudescence of virus replication in the central nervous system (CNS), with cases presenting from a few months to as long as 11 years later (116–118). Recrudescence of virus has important implications for vaccine development.

The development of animal models of NiV and HeV infection and pathogenesis has been a major focus since the late 1990s and an essential component of vaccine development and testing. Also, the approval process of countermeasures for NiV and HeV would fall under the animal rule requirement set forth by the US Food and Drug Administration (FDA) in 2002 as an alternative licensing pathway for countermeasures against highly pathogenic agents when human efficacy studies are not feasible or ethical (119, 120). Several animal models of NiV and HeV infection have emerged that well reflect the pathogenesis seen in infected people, which includes a systemic vasculitis with both respiratory and neurological diseases. Detailed reviews of NiV and HeV infections of a variety of mammalian species have recently been published (33, 121–123). It is generally accepted that the pathogenic processes of NiV and HeV infection in the hamster, ferret, and African green monkey (AGM) best reflect the pathogenesis observed in humans, whereas the most appropriate models for livestock are the horse and pig themselves.

VACCINATION

The attachment and fusion glycoproteins of paramyxoviruses such as measles, mumps, and parainfluenza viruses are the viral antigens to which virtually all neutralizing antibodies are directed (124–126). Likewise, immunization strategies for NiV and HeV have largely targeted their G and F glycoproteins.

Passive Immunization Strategies

Early passive immunization studies in the hamster model demonstrated that polyclonal antiserums or mouse monoclonal antibodies (mAbs) to NiV F or G could provide complete protection against NiV-M or HeV when administered before and immediately after virus infection (10, 127, 128). These studies demonstrated a major role of a viral glycoprotein-specific antibody in protection.

Recombinant human antibody technology was used to generate a potent cross-neutralizing mAb against NiV and HeV (m102.4) (129, 130). The m102.4 mAb epitope maps to the ephrin receptor binding site of G and blocks virus infection (see the left side of **Figure 2*b***), and it can neutralize NiV-M, NiV-B, and HeV (8, 109). The m102.4 mAb provided complete protection from NiV-M-mediated disease in ferrets as a single 50 mg dose administered 10 h post-challenge (8). In the AGM model, m102.4 administered as two 20 mg/kg doses, intravenously, at 10 h and again on day 3, on days 1 and 3 (days 1/3), or on days 3/5, after HeV challenge [4×10^5 50% tissue culture infectious dose ($TCID_{50}$)] by intratracheal (i.t.) administration, protected 100% of treated subjects (131). All treated subjects seroconverted against HeV F glycoprotein with a rise in antibody titer over time, indicating all animals had become infected with HeV and recovered, whereas untreated control subjects succumbed to HeV disease and failed to mount a protective immune response. No clinical signs were evident at any time in the early treatment groups; although neurological symptoms were observed in subjects in the late treatment group (days 3/5), all later recovered from infection. There was no HeV antigen or virus-specific histopathology detected in the lung or brain at the conclusion of the study in any treated subject, and infectious virus could not be recovered from any tissue. A similar study evaluated m102.4 against NiV-M disease in the AGM model at several time points following virus challenge (5×10^5 PFU), including a late cohort where treatment was initiated at the onset of clinical illness (day 5) (132). All subjects became infected after challenge, and all subjects that received m102.4 survived infection and all controls succumbed to disease. Subjects in the late day 5/7 treatment group exhibited disease, but all recovered. A comparative study in AGMs using NiV-M and NiV-B [5×10^5 PFU divided by i.t. and intranasal (i.n.) administration] revealed that NiV-B caused a more aggressive disease, with a

shortened time to death and higher virus loads in tissues and fluids (133). When m102.4 was tested in this model, all subjects in the days 1/3 and days 3/5 post-infection treatment groups survived NiV-B challenge, but subjects in the days 5/7 treatment group succumbed, indicating a shorter therapeutic window in treating NiV-B infection (133). Another well-characterized, humanized mouse mAb, 5B3 (h5B3.1), that is cross-reactive to the F glycoprotein of NiV and HeV and binds a prefusion conformation epitope on F, preventing membrane fusion, was recently tested (110, 134) (**Figure 2*b***). The h5B3.1 mAb was given to ferrets in 20 mg/kg doses by intraperitoneal (i.p.) injection, at 1 to several days post-challenge, with either NiV or HeV ($\sim 5 \times 10^3$ PFU) delivered i.n. (135). All subjects that received h5B3.1 after infection were protected from disease and had increasing neutralizing antibody titers, whereas all controls died. No pathology was observed, and no infectious virus could be isolated at the study endpoint. Altogether, these studies demonstrate that passive immunization with mAbs can provide therapeutic benefit and allow the infected host an extended period to mount a protective immune response. The findings from these experiments were also important because they suggest that vaccine approaches designed to induce adequate neutralizing antibody responses to NiV and HeV should be effective.

The m102.4 mAb producing cell line was provided to the Queensland Government, Australia, to produce the mAb for compassionate use in future cases of high-risk human HeV infection. To date, 14 individuals exposed to either HeV in Australia ($n = 13$) or NiV in the United States ($n = 1$) have been given high-dose m102.4 therapy (15–20 mg/kg) by emergency use protocols, and all have remained well. In Australia, m102.4 was used in a randomized, controlled phase I study in healthy adults (136). The study included four single and one repeat dosing groups, and the m102.4 mAb was found to be safe and well tolerated, with a half-life ranging between ~16.5 and 27 days, and no observed immunogenicity was reported. Two doses of 20 mg/kg (days 1/3) were as well tolerated as a single dose. This study's findings will aid in the design of future dosing regimens of mAbs for evaluating their ability to prevent and/or treat HeV and NiV human infections.

Active Immunization Strategies

A variety of immunization strategies have been developed to prevent NiV and HeV infection including several live-recombinant virus vectors, protein subunit, and virus-like particle (VLP) approaches, and all target the virus attachment and entry steps of infection by employing the G and/or F glycoprotein antigens. Here we summarize these various vaccination countermeasure approaches to NiV and HeV infection (**Tables 1** and **2**).

Poxvirus vectored. Poxviruses have a long history as a platform for the expression of heterologous genes to study protein function and serve as vaccine candidates as a live-attenuated viral vaccine platform capable of inducing both cell-mediated and humoral immune responses (137). The F and G glycoproteins of NiV and HeV were functionally characterized using recombinant vaccinia viruses in the early 2000s (138, 139). The first NiV vaccine tested used a highly attenuated vaccinia virus strain (NYVAC) encoding either the F or G glycoproteins from NiV-M (127). Hamsters were vaccinated by subcutaneous (s.c.) injection in a prime-boost strategy with NYVAC-NiV-F or NYVAC-NiV-G, individually and in combination, and then 3 months later challenged i.p. with NiV-M. Vaccination yielded complete protection from NiV-M with no detection of viral RNA, and control subjects succumbed 7–10 days after challenge (127) (**Table 1**). Another poxvirus-based approach was examined as a vaccine for pigs using canarypox (ALVAC) vaccine vectors encoding either NiV-M F or G glycoprotein (140). A prime-boost strategy with ALVAC-NiV-F or ALVAC-NiV-G vectors was tested alone or in combination in pigs. The animals were then challenged 28 days later with NiV-M via i.n. administration. All vaccinated animals survived NiV-M challenge

Table 1 Virus vectored vaccine strategies for NiV and HeV

Approach	Name(s)	Animal model	Vaccination dose, route, schedule	Adjuvant	Challenge	Challenge dose, route, schedule	Survival	Correlate(s) of immunity/ protection	Reference
Poxvirus	NYVAC-NiV-F and/or -G	Hamster	2 doses at 1×10^7 PFU, s.c., 1 month apart	None	NiV-M	1×10^3 PFU, i.p., 3 months later	100%	NAb response, viral RNA	127
	ALVAC-NiV-F and/or -G	Pigs	2 doses at 1×10^8 PFU, i.m., 2 weeks apart	None	NiV-M	2.5×10^5 PFU, i.n., 28 days later	100%	NAb response, viral RNA, infectious virus, viral shedding, cytokine production	140
	ALVAC-HeV-F and/or -G	Hamster	2 doses at 7.4 or 5.4 log10 $CCID_{50}$, s.c., 3 weeks apart	None	HeV	1×10^3 LD_{50}, i.p., 21 days later	89% and 63%	NAb response, viral RNA, viral antigen, viral shedding	141
		Ponies	2 doses at 6 log10 $CCID_{50}$, i.m., 3 weeks apart	None	NT	NA	NA	High NAb titers	
	MVA-NiV-sG and/or MVA-NiV-G	IFNAR−/− mice	1 or 2 doses at 1×10^8 PFU, i.m., 3 weeks apart	None	NT	NA	NA	High serum IgG titers, NiV-G-specific CD8 and CD4 T cells	142

(*Continued*)

Table 1 (*Continued*)

Approach	Name(s)	Animal model	Vaccination dose, route, schedule	Adjuvant	Challenge	Challenge dose, route, schedule	Survival	Correlate(s) of immunity/ protection	Reference
VSV	VSV-NiV-F and/or -G	Mice	5×10^3 PFU, i.n. or i.m.	None	NT	NA	NA	High NAb titers	144
		Hamster	1×10^6 infectious particles, i.m.		NiV-M	1×10^5 $TCID_{50}$, i.p., 32 days later	100%	NAb response, viral RNA, viral antigen	145
	VSV-NiV-B F and/or G	Ferret	1×10^7 PFU, i.m.	None	NiV-M	5×10^3 PFU, i.n., 28 days later	100%	Serum IgG response, viral RNA, viral antigen	146
		AGM			NiV-B	5×10^5 PFU, i.t. and i.n., 28 days later		NAb response, viral RNA, viral antigen	147
	VSV-ZEBOV-GP-NiV F, G, or N	Hamster	1×10^5 PFU, i.p.	None	NiV-M	1×10^3 LD_{50}, i.p., 28 days later	100%	NAb response, viral RNA, infectious virus	148
		AGM	1×10^7 PFU, i.m.			1×10^5 $TCID_{50}$, i.t., 29 days later		NAb response, viral RNA, infectious virus, viral shedding	149
	VSV-HeV-G	Mice	1×10^5 PFU, i.m.	None	NT	NA	NA	Serum IgG, NAb response	150
AAV	AAV8 NiV.G	Mice	2×10^{10} genome particles, i.m. or 1×10^{10} genome particles, i.d.	None	NT	NA	NA	Serum IgG, NAb response	151
		Hamster	6×10^{11} genome particles, i.m.		NiV-M	1×10^4 PFU, i.p., 5 weeks later	100%	Serum IgG, NAb response, viral RNA, viral antigen	
					HeV		50%		

(*Continued*)

Table 1 (*Continued*)

Approach	Name(s)	Animal model	Vaccination dose, route, schedule	Adjuvant	Challenge	Challenge dose, route, schedule	Survival	Correlate(s) of immunity/ protection	Reference
Adenovirus	ChAdOx1 NiV-B G	Hamster	2 doses at 1×10^8 IU, i.m., 28 days apart	None	NiV-B	5.3×10^5 $TCID_{50}$, i.p., 28 days later	100%	NAb response, viral RNA, infectious virus, virus shedding	153
			1×10^8 IU, i.m.		NiV-M	6.8×10^4 $TCID_{50}$, i.p., 28 days later	100%		
					HeV	6×10^3 $TCID_{50}$, i.p., 28 days later	33%		
Measles virus	rMV-Ed-G or rMV-HL-G	Hamster	2 doses at 2×10^4 $TCID_{50}$, i.p., 3 weeks apart	None	NiV-M	1×10^3 $TCID_{50}$, i.p., 1 week later	100%	Serum IgG response	NA
	rMV-Ed-G	AGM	2 doses at 1×10^5 $TCID_{50}$, s.c., 4 weeks apart			1×10^5 $TCID_{50}$, i.p., 1 week later		Serum IgG response, viral RNA	154
Inactivated RABV	RABV-HeV-G	Mice	3 doses at 10 µg, i.m., 2 weeks apart	None	NT	NA	NA	High NAb titers, serum IgG response	150
	RABV-NiV-B G		2 doses at 10 µg, i.m., 4 weeks apart						155
RABV	RABV-NiV-F and/or -G	Mice	$1 \times 10^{6.5}$ FFU, oral	None	NT	NA	NA	Serum IgG, NAb response	156

All NiV glycoprotein vaccines employ the NiV-M strain unless otherwise indicated.

Abbreviations: AAV, adeno-associated virus; AGM, African green monkey; $CCID_{50}$, 50% cell culture infectious dose; ChAdOx1, chimpanzee adenovirus Oxford 1; F, fusion glycoprotein; FFU, focus forming units; G, attachment glycoprotein; HeV, Hendra virus; i.d., intradermal; i.m., intramuscular; i.n., intranasal; i.p., intraperitoneal; i.t., intratracheal; IFNAR, interferon receptor; IgG, immunoglobulin G; IU, infectious unit; LD_{50}, 50% lethal dose; MVA, modified vaccinia virus Ankara; NA, not applicable; NAb, neutralizing antibody; NiV, Nipah virus; NiV-B, Nipah virus Bangladesh; NiV-M, Nipah virus Malaysia; NT, not tested; PFU, plaque forming unit; RABV, rabies virus; rMV-Ed, recombinant measles virus Edmonston; rMV-HL, recombinant measles virus HL; s.c., subcutaneous; sF, F glycoprotein soluble ectodomain; sG, G glycoprotein soluble ectodomain; $TCID_{50}$, 50% tissue culture infectious dose; VSV, vesicular stomatitis virus; ZEBOV-GP, *Zaire ebolavirus* glycoprotein.

Table 2 VLP, subunit, and nucleic acid–based vaccine strategies for NiV and HeV

Approach	Name	Animal model	Immunization dose, route, schedule	Adjuvant	Challenge	Challenge dose, route, schedule	Survival	Immune correlate(s) of survival	Reference
VLPs	VLPs-NiV M/F/G	Mice	2 doses at 1.75, 3.5, 7, or 14 μg and 6 μg, s.c., 2 weeks apart	None	NT	NA	NA	High NAb titers	159
		Hamster	1 dose or 3 doses at 30 μg, i.m., 3 weeks apart	Alhydrogel/MPLA or Alhydrogel/CpG	NiV-M	1.6×10^4 PFU (3-dose trial) or 3.3×10^4 PFU (1-dose trial), i.p., 28 days later	100%	NAb response, viral RNA	160
Subunit vaccines	NiV-sG	Cat	3 doses at 100 μg, s.c., 2 weeks apart	CSIRO triple adjuvant	NiV-M	5×10^2 $TCID_{50}$, s.c., 2 months later	100%	NAb response, viral antigen, viral genome	14
	HeV-sG	Cat	3 doses at 100 μg, s.c., 2 weeks apart	CSIRO triple adjuvant	NiV-M	5×10^2 $TCID_{50}$, s.c., 2 months later	100%	NAb response, viral antigen, viral genome	14
			2 doses at 50, 25, or 5 μg, i.m., 3 weeks apart	CpG/Alhydrogel		5×10^4 $TCID_{50}$, o.n., 2 weeks later	100%	Serum IgG, NAb response, viral RNA, viral shedding, infectious virus	162
		Ferret	2 doses at 100, 20, or 4 μg, i.m., 20 days apart	CpG	HeV	5×10^3 $TCID_{50}$, o.n., 3 weeks later	100%	NAb response, viral RNA, infectious virus	163
				CpG/Alhydrogel	NiV-B	5×10^4 $TCID_{50}$, 20 days later or 12 months later	100%	viral RNA, viral antigen, infectious virus	164

(*Continued*)

Table 2 (*Continued*)

Approach	Name	Animal model	Immunization dose, route, schedule	Adjuvant	Challenge	Challenge dose, route, schedule	Survival	Immune correlate(s) of survival	Reference
		AGM	2 doses at 100, 50, or 10 μg, i.m., 3 weeks apart	CpG/Alhydrogel	NiV-M	1×10^5 $TCID_{50}$, i.t., 3 weeks later	100%	Serum IgG, NAb response, viral RNA, viral antigen, infectious virus	165
			2 doses at 100 μg, i.m., 3 weeks apart	Alhydrogel or CpG/Alhydrogel	HeV	5×10^5 PFU, i.t., 21 days later	100%	NAb response, viral RNA	166
		Horse	2 doses at 100 or 50 μg, i.m., 3 weeks apart	Zoetis	HeV	2×10^6 $TCID_{50}$, o.n., 28 or 194 days later	100%	NAb response, viral RNA, viral antigen, infectious virus	167
		Pig	2 doses of 2 mL preformulation, i.m., 3 weeks apart	Zoetis	HeV	5×10^5 PFU, i.n., 35 days later	Partial	NAb response, viral RNA, infectious virus, viral shedding	168
					NiV-M		0%		
Nucleic acid–based vaccine	HeV-sG mRNA LNP	Hamster	10 or 30 μg, i.m.	None	NiV-M	1×10^5 $TCID_{50}$, i.p., 30 days later	30% or 70%	Serum IgG, NAb response	169

All NiV glycoprotein vaccines employ the NiV-M strain.

Abbreviations: AGM, African green monkey; CSIRO, Commonwealth Scientific and Industrial Research Organisation; F, fusion glycoprotein; G, attachment glycoprotein; HeV, Hendra virus; i.m., intramuscular; i.n., intranasal; i.p., intraperitoneal; i.t., intratracheal; IgG, immunoglobulin G; LNP, lipid nanoparticle; M, matrix protein; MPLA, monophosphoryl lipid A; mRNA, messenger RNA; NA, not applicable; NAb, neutralizing antibody; NiV, Nipah virus; NiV-B, Nipah virus Bangladesh; NiV-M, Nipah virus Malaysia; NT, not tested; o.n., oronasal; PFU, plaque forming unit; s.c., subcutaneous; sG, G glycoprotein soluble ectodomain; $TCID_{50}$, 50% tissue culture infectious dose; VLP, virus-like particle.

as determined by the lack of NiV RNA and infectious virus from nasal washes, pharyngeal swabs, and a variety of sampled organs (140).

ALVAC-vectored vaccines encoding HeV F or G glycoprotein for potential use in horses were also examined (141). ALVAC-HeV-F or ALVAC-HeV-G vectors were combined and first used to vaccinate hamsters at a high or low dose of each vector, by s.c. injection, and then challenged with HeV by i.p. administration. Vaccination did not result in complete protection, with 8 out of 9 subjects in the high-dose group and 5 out of 8 subjects in the low-dose group surviving challenge. No signs of disease were noted, and viral antigen or viral RNA could not be detected in survivors. Nine ponies vaccinated using the same prime-boost regimen were able to develop high cross-neutralizing antibody titers to HeV and NiV-M at day 28 after vaccination. Although ponies were not challenged, most animals yielded titers of at least 1:32 and were considered likely protective (141).

More recently, a modified vaccinia virus Ankara (MVA) vector encoding NiV-M G glycoprotein and a soluble version of G (NiV-sG) were examined in interferon receptor α and β (IFNAR−/−) knockout mice (142) (**Table 1**). IFNAR−/− mice were immunized once with MVA-NiV-G or MVA-NiV-sG or prime-boosted. IFNAR−/− mice developed high serum immunoglobulin G (IgG) titers to NiV-G and also generated NiV-G-specific CD8 and CD4 T cells following vaccination. MVA-NiV-sG vaccination induced rapid and significantly higher amounts of NiV-G epitope-specific CD8 T cells compared with the MVA-NiV-G candidate vaccine, suggesting superior immunogenicity. Together, these immunization studies with poxvirus vectors highlight that both T cell and B cell responses play a role in an adaptive immune response to NiV and HeV. However, detailed studies on the adaptive immune responses in animal experiments with henipaviruses have been limited. Future studies evaluating the role of NiV-specific T cells will be important because two human survivors of NiV-B infection in the 2018 outbreak in Kerala showed marked elevation of activated $CD8^+$ T cells, which coincided with virus clearance (143).

Vesicular stomatitis virus vectored. Recombinant vesicular stomatitis virus (rVSV) vectors as a vaccine platform suitable for single immunization strategies to potentially meet emergency use requirements have been tested by several groups (**Table 1**). A method of using two defective VSVΔG vectors each expressing only the NiV G or F glycoprotein was devised using VSV G glycoprotein complementation that can generate replication-defective VSV vectors that could elicit NiV-neutralizing antibodies (144). Using this technique, researchers tested rVSV vaccines expressing either NiV-M F or G glycoproteins (VSV-ΔG-NiVG, VSV-ΔG-NiVF) in hamsters by intramuscular (i.m.) vaccination (145). Hamsters were then challenged 32 days later with NiV-M by i.p. administration. All vaccinated animals survived lethal infection with no clinical signs of disease. No viral RNA or viral antigen could be detected in the sampled tissues when compared with controls, and there was a lack of an anamnestic immune response in vaccinated subjects following challenge, suggesting the induction of sterilizing immunity.

Another study used rVSV-ΔG vectors expressing NiV-B F or G glycoprotein and also tested them as single-injection vaccinations in NiV-M-challenged ferrets (146). Ferrets were vaccinated i.m. with rVSV-NiV-B F or rVSV-NiV-B G complemented with VSV G or a mix of both vectors, rVSV-NiV-B F/G, that was generated as a complementing pair in the absence of VSV G and then challenged at 28 days with NiV-M by i.n. administration. All vaccinated ferrets were completely protected against NiV-M challenge. Although viral RNA was detected in blood at day 6 post-challenge in 2 of 5 animals in each group, those levels were 100 times lower than in the unvaccinated controls, and by day 21 no viral RNA was detected (146). In a second study, rVSV-NiV-B F and rVSV-NiV-B G were assessed separately and in combination in AGMs (147). Cohorts were

vaccinated with the rVSVs by i.m. injection and challenged 28 days later with NiV-B divided between the i.t. and i.n. routes (147). Complete protection was recorded from NiV-B disease with no gross pathology and no detectable NiV antigen in lung or spleen tissues. Viral RNA was detected in nasal and oral swabs of the vaccinated groups, but no viral RNA could be detected in blood samples.

Replication-competent rVSV-NiV-M F or G vectors, generated by the retention of the envelope glycoprotein from *Zaire ebolavirus* (ZEBOV-GP), which allowed virus stocks to be propagated (rVSV-ZEBOV-GP-NiVF, rVSV-ZEBOV-GP-NiVG, and rVSV-ZEBOV-GP-NiVN), have also been tested (148). These rVSVs were used to immunize hamsters by i.p. administration and were challenged 28 days later with NiV-M. All subjects vaccinated with either the NiV F or G glycoprotein encoding rVSV vectors were completely protected with no clinical disease or pathology, whereas those vaccinated with the NiV N protein were only partially protected (2 of 6 animals) with no clinical signs of disease and the other subjects succumbed to infection. The protective efficacy of the rVSV-ZEBOV-GP-NiVG was also tested in AGMs, where vaccinated subjects were challenged with NiV-M by i.t. administration 29 days later (149). All vaccinated subjects were protected from lethal challenge and showed no signs of clinical disease, no viral RNA was detected in the blood or oral and nasal swabs, and no infectious virus could be recovered. Another study using a rVSV vector expressing a codon-optimized HeV *G* gene together with an inactivated counterpart was evaluated in mice for humoral immune responses only as a comparator to a recombinant rabies virus vaccine encoding HeV G as a HeV vaccine candidate (150). Here, the live rVSV vectors induced greater levels of HeV G-specific antibodies and higher levels of HeV-neutralizing antibodies than did the recombinant rabies virus vectors (see the section titled Rabies Virus Vectored).

Adeno-associated virus and adenovirus vectored. Adeno-associated virus (AAV) vectors as a vaccine platform against infectious diseases, particularly viral pathogens, have been explored. AAV is a small, single-stranded DNA virus in the family *Parvoviridae*. Immunization of hamsters with an AAV vector expressing NiV-M G glycoprotein (AAV8 NiV.G) by i.m. injection demonstrated complete protection from a challenge of NiV-M by i.p. administration, and no signs of clinical disease were recorded (151) (**Table 1**). Neutralizing antibodies to NiV were induced, no viral RNA or viral antigen was detected in any of the sampled tissues, and there was only a moderate anamnestic response observed in a single subject, suggestive of potential sterilizing immunity. However, in a cross-protection study, AAV8 NiV.G protected only 50% of hamsters challenged with HeV.

Chimpanzee adenoviral (ChAd) vectors circumvent issues of the preexisting immunity observed with human adenovirus vectors (152). Adenoviruses are double-stranded DNA viruses in the family *Adenoviridae*. An engineered replication-deficient ChAd vector, Oxford 1 (ChAdOx1), was tested as a NiV/HeV vaccine (153). Here, ChAdOx1 encoding NiV-B (ChAdOx1 NiV-B) G glycoprotein was used to vaccinate hamsters by i.m. injection, either as a single dose or as a prime-boost protocol. Hamsters were challenged by i.p. administration with NiV-B 42 days following the booster or the single vaccination. Neutralizing antibodies were detectable, and all vaccinated hamsters were protected against lethal disease with no lung pathology, suggesting that a single dose of ChAdOx1 NiV-B was sufficient to completely protect against NiV-B. No viral RNA in the lung tissue and no viral shedding in oropharyngeal swabs could be detected, and no infectious virus could be isolated. A second cohort using a single dose of ChAdOx1 NiV-B to vaccinate hamsters was trialed, and these animals were challenged 28 days later with NiV-M or HeV. All vaccinated animals were protected from lethal NiV-M challenge, but 4 out of 6 hamsters succumbed to HeV disease between days 5 and 7 post-challenge. Neither virus shedding in oropharyngeal swabs nor

infectious virus was detected in the lung or brain tissues of NiV-M-challenged vaccinated hamsters. In contrast, infectious virus was detected in the lung tissues of 75% of the HeV-challenged vaccinated animals. The lower cross-protection observation using NiV G vaccination followed by HeV challenge was not unexpected, as it was previously shown that when the G glycoprotein (as a recombinant soluble subunit immunogen) of either HeV or NiV was used to vaccinate cats, both could completely protect against lethal NiV-M challenge, and that the HeV-sG elicited greater heterologous neutralizing antibody responses in comparison to NiV-sG (14).

Measles virus vectored. Recombinant measles virus vectors based on the HL (rMV-HL) and Edmonston (rMV-Ed) measles virus strains have also been explored in which they encoded the NiV-M G glycoprotein (rMV-HL-G and rMV-Ed-G) (154) (**Table 1**). Hamsters were immunized twice by i.p. administration of rMV-HL-G or rMV-Ed-G. All vaccinated animals produced NiV G-specific antibody titers after the booster immunization. Animals were challenged 1 week after the second immunization with NiV-M by i.p. administration. All immunized hamsters exhibited no clinical symptoms and survived challenge. The study was extended to non-human primates (NHPs), where 2 AGMs were immunized twice by s.c. injection with rMV-Ed-G. Subjects were challenged 2 weeks after the second immunization with NiV-M by i.p. administration. Here, immunization completely protected the AGMs with no observed clinical disease and no detectable pathological changes, and no viral RNA could be detected in sampled tissues. Although this was a small study, the safety profile and success of the live-attenuated measles virus vaccine suggests that a recombinant platform encoding the NiV G glycoprotein as a NiV vaccine candidate is promising and should induce a balanced and long-lasting immune response against NiV.

Rabies virus vectored. A rabies virus (RABV) SAD B19 vaccine strain, BNSP333, expressing HeV or NiV G glycoproteins has been evaluated (150, 155). Recombinant BNSP333 encoding either the wild-type or a codon-optimized HeV *G* gene, together with their inactivated counterparts, was used in mice (150) (**Table 1**). Mice were immunized by i.m. injection with a single dose of the RABV-based vectors or with 3 doses of their inactivated versions. The inactivated RABV-based vectors induced higher and more rapid HeV G-specific antibody responses and higher neutralizing antibody titers than their live counterparts. The inactivated RABV-coHeV-G induced cross-neutralizing antibodies against NiV. A similar study used the BNSP333 vector expressing NiV-B G glycoprotein (RABV-NiV-BG) (**Table 1**) and elicited NiV G-specific neutralizing antibodies (155).

Recently, the recombinant RABV Evelyn-Rokitnicki-Abelseth (ERA) strain ($rERAG_{333E}$) expressing either NiV-M F or G glycoproteins was evaluated in mice and pigs (156) (**Table 1**). This vector, $rERAG_{333E}$, serves as an oral vaccine in dogs. Here, mice were orally immunized with RABV-NiV-F or RABV-NiV-G either individually or in combination. Pigs were also immunized in a similar manner but with 2 doses of each vector either alone or in combination. RABV-NiV-F and/or RABV-NiV-G immunization induced NiV F- and G-specific IgG antibody responses and neutralizing antibodies in both mice and pigs with the combination vaccine inducing higher titers. Although not suitable for human use, the live-attenuated $rERAG_{333E}$ vector is of interest as a potential veterinary vaccine for NiV because it is already approved for use in some animals and could be adapted for emergency use to protect against NiV infection in livestock, particularly swine.

Many of these virus-vectored vaccines for NiV are promising candidates because of their established safety profiles and ease of genetic modification. Several of these virus-vectored vaccines also require no adjuvants, and some are clearly efficacious as a single immunization strategy, suitable features for emergency use circumstances. In addition, several of these platforms are able to induce both cell-mediated and humoral immune responses, which may also be desirable but as yet

are not fully explored in the development of vaccines for NiV and HeV. Although animals immunized with viral vectors encoding the NiV G glycoprotein and challenged with the homologous virus were completely protected, cross-protection studies with some of these vaccines against a HeV challenge were less effective. For example, only 50% of AAV8 NiV.G-immunized hamsters or 33% of ChAdOx1 NiV-B G glycoprotein–immunized hamsters were protected from a lethal HeV challenge (151, 153). In addition, the ALVAC-HeV-F and ALVAC-HeV-G vaccination studies showed that these vectors did not provide 100% protection in hamsters challenged with HeV, perhaps due to either a suboptimal immunization dose or the immunization route (141).

Virus-like particles. VLPs have been explored as a vaccine platform because of the resemblance of their surface structure, dimensions, and compositions to authentic virus yet are of high safety because of the lack of viral genetic material. Earlier studies revealed that the M protein of NiV was capable of orchestrating the formation and budding of NiV VLPs when expressed in cells that appeared structurally similar to authentic NiV virions, and these VLPs could also incorporate other viral proteins such as the F and G glycoproteins (157, 158). VLPs composed of NiV M, F, and G were used to vaccinate mice s.c. at weeks 0, 2, and 4 and demonstrated they could induce high neutralizing antibody titers by day 35 (159) (**Table 2**). NiV VLPs were later used in NiV-M challenge studies either alone or in combination with adjuvant, monophosphoryl lipid A (MPLA) and Alhydrogel^{TM} (15 μg/50 μg) or CpG and Alhydrogel (40 μg/50 μg) (160). Hamsters were vaccinated i.m. either as a single dose or as a 3-dose regimen and then challenged via i.p. administration of NiV-M at 28 days or 58 days, respectively. In all cohorts, 100% of the vaccinated animals survived with no clinical signs of disease and no detection of viral RNA in any of the sampled tissues, regardless of the presence of adjuvant. VLPs are thus an alternative means, with inherent safety, of producing an inactivated whole virus vaccine from an otherwise highly pathogenic virus.

Subunit vaccine. The HeV-sG subunit vaccine has been extensively evaluated in several studies. Here, a brief summary of earlier reports is made, but the focus is on studies in NHPs and livestock. Recombinant HeV-sG and NiV-sG can elicit a potent neutralizing antibody response and were first tested as vaccine immunogens in the feline model (14, 161) (**Table 2**). Both HeV-sG and NiV-sG vaccination of cats completely protected against lethal NiV-M challenge, and HeV-sG elicited greater heterologous neutralizing titers than did NiV-sG, demonstrating that a single subunit vaccine may be effective against both NiV and HeV (14). Other studies using lower doses of HeV-sG (**Table 2**) demonstrated that a pre-challenge neutralizing titer of 1:32 could protect against NiV-M (162). Additional studies in ferrets showed that low doses of HeV-sG could protect against HeV and NiV-B (163, 164) (**Table 2**). Also, a longevity study showed that vaccinated ferrets challenged with NiV-B at 14 months post-immunization, with pre-challenge neutralizing titers of 1:16 to 1:128, were also protected (164).

The HeV-sG vaccine has been extensively evaluated in AGMs (**Table 2**). In a cross-protection study, 100 μg, 50 μg, or 10 μg doses of HeV-sG in combination with Alhydrogel and CpG were administered i.m. as a prime-boost, on days 0 and 21. Pre-challenge 50% neutralization titers ranged from 1:28 to 1:379. All subjects were challenged with NiV-M by i.t. administration on day 42. All vaccinated subjects were completely protected, displaying no clinical signs of disease, and no viral RNA could be detected in blood and tissues and no infectious virus was isolated (165). Similarly, HeV-sG vaccination HeV challenge in AGMs has also been performed. Using a prime-boost regimen, AGMs were vaccinated twice, 3 weeks apart, by i.m. injection with 100 μg HeV-sG with Alhydrogel or HeV-sG with Alhydrogel and CpG, and then challenged 3 weeks later with HeV by i.t. administration (166). All vaccinated animals were completely protected from clinical

disease, and no HeV RNA or viral antigen could be detected in swabs, blood, or tissues, and notably HeV-sG formulated in only Alhydrogel protected (166).

The efficacy and inherent safety of the HeV-sG subunit led to its development as an equine vaccine to prevent HeV infection of horses and also reduce the risk of HeV transmission to people, as a One Health concept (**Figure 1**). HeV-sG, formulated in an approved equine adjuvant (Zoetis, Inc.), was evaluated in two efficacy studies; the first tested 50 μg and 100 μg doses of the same HeV-sG used in prior animal studies to vaccinate horses, and the second used 100 μg doses of HeV-sG produced in Chinese hamster ovary cells (Zoetis, Inc.). Two vaccinations were given by i.m. administration 3 weeks apart. All horses in these efficacy studies were challenged by oronasal inoculation with HeV (**Table 2**). Seven horses were challenged at 28 days and 3 horses were challenged at 194 days after the second immunization. All vaccinated horses remained clinically healthy following challenge; pre-challenge neutralization titers ranged from 1:128 to more than 4,096 in horses challenged 21 days after vaccination and only from 1:16 to 1:32 in horses challenged at 6 months. There was no gross or histologic evidence of infection in any of the vaccinated horses at study completion, and all tissues examined were negative for viral antigen, with no viral genome detected in any tissue. In 9 of 10 vaccinated horses, HeV nucleic acid was not detected in daily nasal, oral, or rectal swab samples or from blood, urine, or fecal samples collected before euthanasia, no recoverable virus was present, and no rise in antibody titer was detected in any vaccinated horse following challenge (167). The HeV-sG horse vaccine (Equivac® HeV) was launched by Zoetis, Inc., in November 2012 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 against a BSL-4 agent. All vaccinated horses are microchipped, and a database is maintained. Equivac HeV received full registration by the APVMA in 2015. To date, more than 765,000 doses of Equivac HeV vaccine have been administered to more than 179,000 unique horses, and laboratory-confirmed HeV infections in horses have since occurred only in unvaccinated animals.

Studies showed HeV-sG as a NiV vaccine in the pig model (which is a non-lethal challenge model) was much less effective in comparison to results observed in the cat, ferret, NHP, and horse, and HeV-sG was only partially protective against HeV challenge and unprotective against NiV-M in the pig (168). These experiments also indicated that both humoral and cellular immune responses were required for protection of swine against NiV and HeV. Here, pigs were immunized with HeV-sG in a proprietary adjuvant (Zoetis, Inc.), and subjects were challenged with HeV or NiV via i.n. administration (**Table 2**). HeV-sG-vaccinated pigs developed neutralizing titers ranging from 1:160 to 1:320 to HeV, but only partial protection was achieved with reduced viral RNA in tissues and no recoverable virus, and there was no reduction of viral shedding in nasal washes. These HeV-sG-vaccinated pigs did not develop neutralizing antibodies to NiV-M that were considered protective (low), nor did they have measurable activation of cellular immune memory. Only a comparative group of pigs that were first orally infected (vaccinated) with NiV (and recovered) were subsequently protected against an i.n. rechallenge with NiV. This group of pigs developed protective antibody levels and cell-mediated immune memory responses (168).

Single-dose lipid nanoparticle mRNA, HeV-sG vaccine. More recently, messenger RNA (mRNA)-based vaccines have emerged as an attractive vaccine strategy because of safety, efficacy, and rapid implementation features. In a recent study, the efficacy of an mRNA vaccine approach was assessed in a NiV-M animal challenge model (169). mRNA transcripts encoding HeV-sG were complexed with lipid nanoparticles (LNPs) to generate HeV-sG mRNA LNP. Two groups of 10 hamsters were vaccinated with a single dose of HeV-sG mRNA LNP at either 10 μg or 30 μg by i.m. injection. Subjects were challenged with NiV-M by i.p. administration 30 days

post-vaccination (**Table 2**). The HeV-sG mRNA LNP was only partially protective, with 3 hamsters from the low-dose group and 7 hamsters from the high-dose group surviving challenge. Of the surviving animals, signs of clinical disease were observed in 2 low-dose group and 6 high-dose group hamsters; however, disease symptoms were gone by study termination. NiV *N* gene RNA levels in the blood and a variety of tissues in surviving hamsters were lower compared with nonsurvivors, but NiV RNA copy levels were not different compared with controls. No anti-NiV IgG or virus-neutralizing activity was detected in vaccinated animals prior to challenge; however, all post-challenge survivors were positive for anti-NiV IgG antibodies, and all survivors (in both groups) had similar neutralizing titers ranging from 1:160 to 1:640. Euthanized animals had little to undetectable neutralizing activity, highlighting the correlation of this immune response to protection. Although promising, the partial efficacy of HeV-sG mRNA LNP observed in this study suggests that further optimization of vaccination route, addition of an adjuvant, and/or a prime-boost regimen is needed.

SUMMARY AND FUTURE PERSPECTIVES

The frequency of henipavirus outbreaks and human infections is a significant global health concern. A promising passive immunization strategy has been developed using a human mAb, m102.4, shown effective in the NHP challenge model, which has also been administered numerous times to people by compassionate use protocol and has successfully completed a phase I safety trial in Australia. In addition, the Equivac HeV vaccine is available, targeting the protection of horses and also people by breaking the chain of HeV transmission to people, and is an example of a One Health approach to counter an infectious disease threat. Over the past 15 years, nearly a dozen NiV and HeV vaccine approaches have been trialed in animal challenge models, and many show promise as effective human-use vaccines. Recently, the formation of the Coalition for Epidemic Preparedness Innovations (CEPI), a global partnership between public and private organizations, was undertaken with the goals of developing vaccines against emerging infectious diseases and offering equitable access to those vaccines (170). Indeed, without the support of CEPI, the prospects of having a NiV or HeV vaccine suitable for use in people, at a deployable stage in the event of a significant outbreak, would have remained academic. Research teams can now capitalize on the large body of basic and preclinical vaccine development data on a half-dozen important emerging viral threats including NiV and, with the support of CEPI, can develop vaccine candidates for clinical use and future licensure. Several of the NiV human vaccine candidates described in this review are now supported by CEPI.

DISCLOSURE STATEMENT

C.C.B. is a US federal employee and co-inventor on US and foreign patents pertaining to soluble forms of HeV and NiV G and F glycoproteins and monoclonal antibodies against HeV and NiV whose assignee is the United States as represented by the Henry M. Jackson Foundation for the Advancement of Military Medicine (Bethesda, Maryland). The soluble forms of the HeV and NiV G glycoproteins are licensed to Zoetis, Inc., and Aurobindo Pharma USA Inc. M.A. declares no competing interests.

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