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Henipaviruses: epidemiology, ecology, disease, and the development of vaccines and therapeutics

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SUMMARY Henipaviruses were first identified 30 years ago and have since been associated with over 30 outbreaks of disease in humans. Highly pathogenic henipaviruses include Hendra virus (HeV) and Nipah virus (NiV), classified as biosafety level 4 pathogens. In addition, NiV has been listed as a priority pathogen by the World Health Organization (WHO), the Coalition for Epidemic Preparedness Innovations (CEPI), and the UK Vaccines Research and Development Network (UKVN). Here, we re-examine epidemiological, ecological, clinical, and pathobiological studies of HeV and NiV to provide a comprehensive guide of the current knowledge and application to identify and evaluate countermeasures. We also discuss therapeutic and vaccine development efforts. Furthermore, with case identification, prevention, and treatment in mind, we highlight limitations in research and recognize gaps necessitating additional studies.

KEYWORDS henipavirus, Hendra virus, Nipah virus, epidemiology, clinical disease, animal model, vaccine, antiviral, therapeutic

INTRODUCTION

Henipaviruses (HNVs) are negative-sense, single-stranded RNA viruses in the family *Paramyxoviridae*, order *Mononegavirales* (1). The viral particle has six structural proteins and three accessory proteins (Fig. 1A). Like all paramyxoviruses, HNVs have enveloped, pleomorphic but mostly spherical virions with a ribonucleoprotein (RNP) core containing an RNA genome protected by a helical nucleocapsid protein (N), the polymerase-associated phosphoprotein (P), and the large protein (L), which includes RNA-directed RNA polymerase, capping, and cap methylation activities. The accessory proteins encoded within the P gene (V, W, and C) are translated via mRNA editing and an alternative start codon. The viral envelope contains two glycoproteins: the fusion glycoprotein (F) and the attachment glycoprotein (G). The genome arrangement is 3′-N-P/V/W/C- M- F- G- L-5′ (2, 3). HNVs have a larger genome than most members of the *Paramyxoviridae* family due to a longer open reading frame encoding P and comparatively longer 3′ untranslated regions (UTR) that flank each gene except L (4).

Viruses belonging to the five species of the genus *Henipavirus* are distinguished based on the amino acid sequences of their L protein and include Angavokely virus (AngV; *Henipavirus angavokelyense*), Cedar virus (CedV; *Henipavirus cedarense*), Ghana virus (GhV; *Henipavirus ghanaense*), Hendra virus (HeV; *Henipavirus hendraense*), and Nipah virus (NiV; *Henipavirus nipahense*) (Table 1) (2, 5). The most recent 2023 ICTV master species list, created in March 2024, introduced a new *Parahenipavirus* genus, encompassing 11 species (Table 2). The new genus was developed to separate the distinct rodent-/shrew-borne and bat-borne clades within the genus *Henipavirus*. The *Parahenipavirus* genus includes the previously recognized henipavirus Mòjiāng virus (MojV; *Parahenipavirus mojiangense*), and Langya virus (LayV; *Parahenipavirus langyaense*), which was reported in 2022 as a novel henipavirus species (6) but was not recognized by ICTV as such prior to the current classification (Fig. 1B).

Pathogenic HNVs include two species, NiV and HeV, both of which can cause severe and often fatal neurological and respiratory diseases. HeV and NiV are classified as biosafety level 4 (BSL-4) zoonotic pathogens and are considered overlap select agents, as they can cause severe disease in both humans and animals. Notably, these agents have been recognized by the WHO as top priority pathogens with the potential to result in severe future outbreaks.

In this article, we present current knowledge of NiV and HeV and touch on other HNVs and henipa-like viruses identified in the absence of associated clinical disease or without known infectious isolates. We present epidemiology, ecology, and pathogenesis data, and discuss how current knowledge can aid in developing medical countermeasures. We also highlight critical gaps and suggest prioritization for preparedness and response efforts.

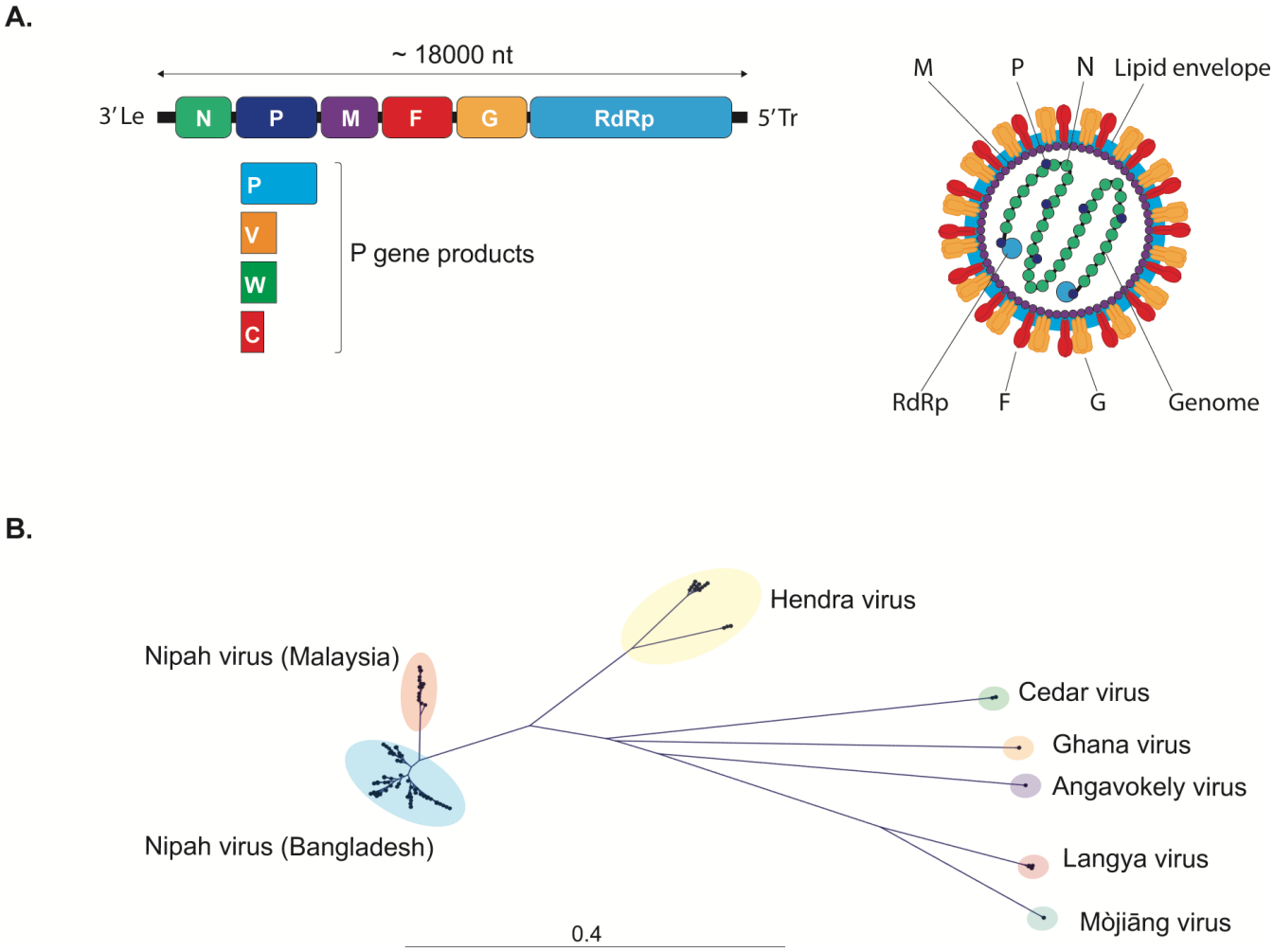

FIG 1 Henipavirus structure and phylogeny. (A) Henipavirus genomes are approximately 18,000 nucleotides in length and contain six genes: N, nucleocapsid protein; P, phosphoprotein; M, matrix protein; F, fusion glycoprotein; G, attachment glycoprotein; and RdRp, RNA-dependent RNA-polymerase. The P gene encodes three additional proteins, V, W, and C, translated via mRNA editing and an alternative start codon. Genes are separated by short intergenic regions, meaning each gene is preceded and followed by conserved transcriptional control signals involved in the initiation and termination of viral mRNA synthesis, respectively. At the genome termini, a 3′ leader sequence (3′Le) contains promoters for both transcription initiation as well as for the synthesis of the positive-sense replicative intermediate antigenome, and a 5′ trailer sequence (5′Tr) promotes the synthesis of new negative-sense genomes from the antigenome. Henipavirus virions are pleomorphic and consist of a viral lipid envelope containing multiple copies of the F and G, surrounding a single-stranded negative-sense RNA genome encapsidated by N and associated with P and RdRp. (B) Neighbor-joining phylogenies of full-length henipavirus genome nucleotide sequences showing relationships between all the genus *Henipavirus* species, as well as the previously recognized species Langya virus and Mòjiāng virus.

HISTORY AND EPIDEMIOLOGY

Hendra virus

HeV (originally called equine morbillivirus) was first recognized in September 1994 after a disease cluster of unknown etiology involving 21 racehorses and 2 humans was reported in Hendra (a suburb of Brisbane), Australia (12, 13) (Fig. 2). This outbreak resulted in the deaths of 14 horses and one human, who succumbed after 6 days of interstitial pneumonia. Subsequent analysis showed that the first case had occurred earlier, in March 1994, after an individual originally infected that month suffered a fatal relapsing encephalitis in April 1995. Epidemiological studies showed that, 13 months before his death, he had assisted a veterinarian in performing a post-mortem examination on two horses later shown to have died from HeV in MacKay, Australia (14, 15). Between 2004 and 2023 (excluding 2005), at least one equine case has been reported annually (16). To

TABLE 1 Summary of species of the genus *Henipavirus*

Species	Virus name	Year	Location first identified	Reservoir	Human disease reported?
Henipavirus angavokelyense	Angavokely virus[a]	2022	Angavokely Cave, Madagascar	*Eidolon dupreanum* bats	No
Henipavirus cedarense	Cedar virus	2009	Cedar Grove, Australia	*Pteropus* spp. bats	No
Henipavirus ghanaense	Ghana virus[a]	2008	Kumasi, Ghana	*Eidolon* spp. bats	No
Henipavirus hendraense	Hendra virus	1994	Brisbane, Australia	*Pteropus* spp. bats	Yes
Henipavirus nipahense	Nipah virus	1999	Malaysia and Singapore	*Pteropus* spp. bats	Yes

[a]Indicates that no infectious isolates have been reported; identification is based on sequence data alone.

date, there have been a total of 109 equine cases (89 confirmed, 20 suspected; all fatal or euthanized) and 7 human cases, of which 4 were lethal (57% case fatality rate [CFR]). In the initial search for the natural HeV reservoir, a range of animals, including horses, cats, cattle, chickens, dogs, and goats, were serologically evaluated for HeV infection, but all were negative (17). Because of the genetic similarity between the 1994 and 1995 HeV strains, they were hypothesized to have come from a common source, and given the geographical separation (~500 miles/800 km) between the two sites, the host was likely capable of migrating this distance. With these details, *Pteropus* spp. fruit bats were identified as the most likely host species. Subsequent sampling revealed the presence of HeV-neutralizing antibodies (18) and infectious virus (19) in these animals. Transmission to horses is thought to occur via close contact with *Pteropus* bats or their excreta and/or birthing material (19, 20), with transmission risk increasing during bat breeding seasons and during times of nutritional stress (21). In retrospective studies involving stored samples from horses and other assorted potential viral reservoirs (including rodents, marsupials, reptiles, and birds), no evidence of HeV prior to 1994 was detected (22). No human cases of HeV have been reported since 2009 (Table 3), and all equine and human cases remain restricted to Australia. In October 2021, Hendra virus genotype 2 (HeV-g2)

TABLE 2 Summary of species of the genus *Parahenipavirus*[a]

Species	Virus name	Location first identified	Detection method	GenBank accession no.	Ref.
Parahenipavirus chodsigoae	Wufeng Chodsigoa smithii henipavirus 1	China	S	OM030316	–
Parahenipavirus crocidurae	Wufeng Crocidura attenuata henipavirus 1	China	S	OM030317	–
Parahenipavirus daeryongense	Daeryong virus	Republic of Korea	S	MZ574409	(7)
Parahenipavirus gamakense	Gamak virus	Republic of Korea	S, VI	MZ574407	(7)
Parahenipavirus jingmenense	Jingmen Crocidura shantungensis henipavirus 1	China	S	OM030314	–
Parahenipavirus langyaense	Langya virus	China	VI	OM101125	(6)
Parahenipavirus meliandouense	Melian virus	Guinea	S	OK623353	(8)
Parahenipavirus mojiangense	Mòjiāng virus	China	S	KF278639	(9)
Parahenipavirus soricis	Ninorex virus	Belgium	S	OQ438286	(10)
Parahenipavirus wenzhouense	Wenzhou Apodemus agrarius henipavirus 1	China	S	MZ328275	–
Parahenipavirus winnikense	Denwin virus	Belgium	S	OK623354	(8)

[a]Additional detection and host information in Table 5, and reviewed in references (8, 11). –, no further details published; S, genome sequencing; VI, virus isolation.

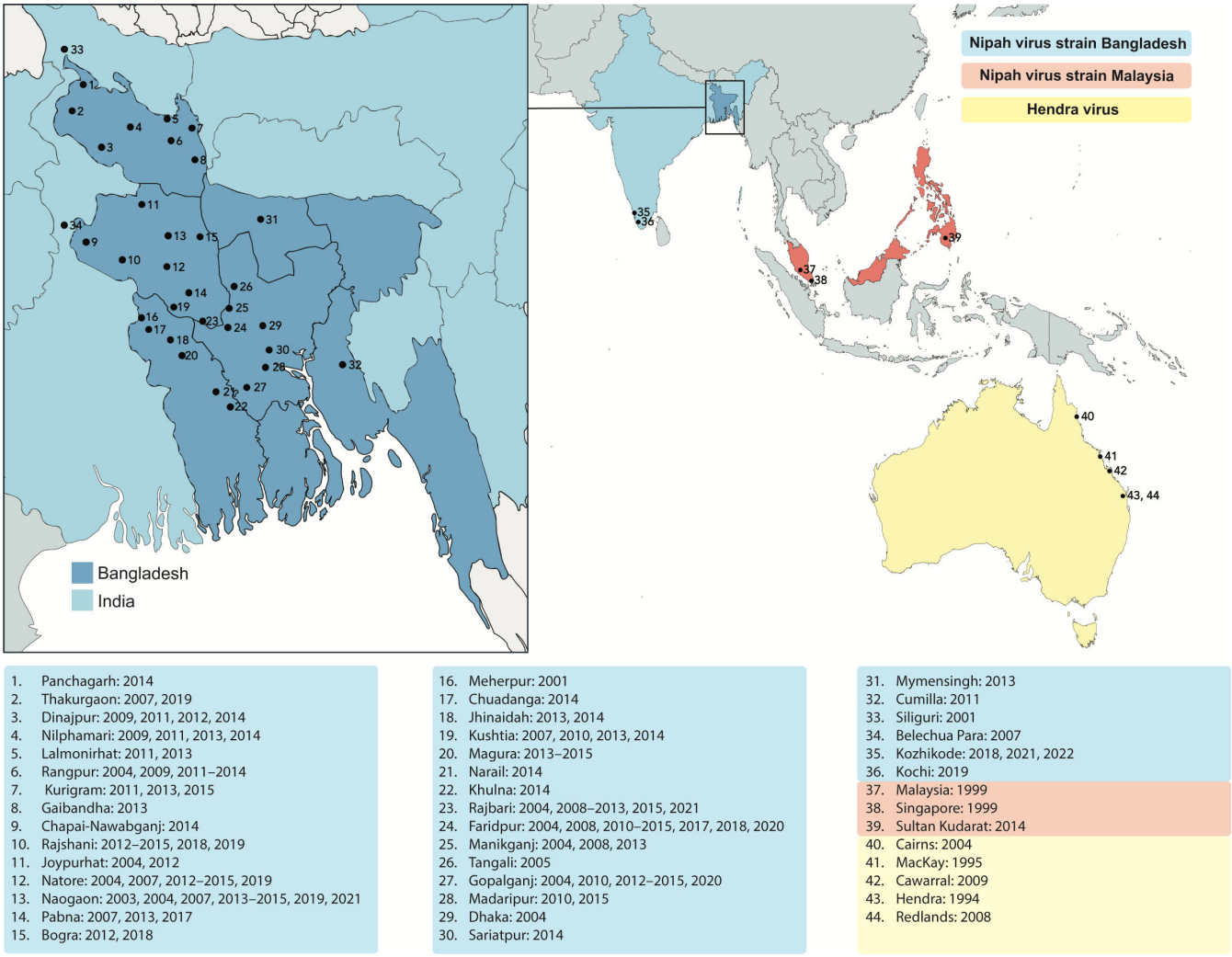

FIG 2 Reported henipavirus outbreaks in different countries from 1994 to 2022. Summary of geographic locations and year of report for henipavirus outbreaks involving human cases for Hendra virus (yellow—Australia), Nipah virus strain Malaysia (red—Malaysia, Singapore, and the Philippines), and Nipah virus strain Bangladesh (blue—India and Bangladesh).

was first identified in an equine case during a veterinary priority disease investigation in a horse near Newcastle, New South Wales, Australia (23). To date, no human cases of HeV-g2 have been recognized.

Nipah virus

The first recognized outbreak of NiV occurred in Malaysia from 1998 to 1999, with patients initially presenting with fever and neurological symptoms that progressed to acute encephalitic disease in some cases. The disease was initially thought to be caused by the Japanese encephalitis virus, but the epidemiology was inconsistent with this pathogen. In March 1999, NiV was identified as the etiologic agent (26, 27); the virus was named after Kampung Sungai Nipah (Nipah River Village), where the first isolates were obtained (28, 29). In total, the Malaysia outbreak caused 283 symptomatic cases resulting in 109 deaths (39% CFR) (27). By March 1999, the outbreak spread to nearby Singapore through the transport of infected pigs, resulting in 11 symptomatic human cases and one death (9% CFR) (30, 31). In both outbreaks, close contact with swine or their contaminated excreta was shown to be the major risk factor. Although the pigs themselves only demonstrated mild respiratory illness, the intensive farming

TABLE 3 Summary of Hendra virus human cases[a]

Year	Country	Division/district/region	Total cases	Fatal cases	CFR, %	Reference
1994	Australia	Brisbane	2	1	50	(12, 13)
1995	Australia	Mackay	1	1	100	(14, 15, 17)
2004	Australia	Cairns	1	0	0	(24)
2008	Australia	Redlands	2	1	50	(25)
2009	Australia	Cawarral	1	1	100%	(25)

[a]CFR, case fatality rate.

techniques likely aided the mechanical transmission of NiV between the pigs, amplifying the outbreak (32, 33). As with the earlier HeV outbreak, epidemiological studies eventually identified *Pteropus* spp. fruit bats as the virus reservoir, with transmission to pigs likely occurring via the pigs' foraging fruit dropped by feeding or roosting bats into the piggeries (32).

The strain of NiV associated with the 1998–1999 outbreak in Malaysia and Singapore was subsequently classified as strain NiV-Malaysia (34). Outside of this initial outbreak, the strain has not been associated with any further human infections except a cluster of cases in the Philippines in 2014 (35), in which 17 individuals were infected, with nine deaths (CFR 53%). Epidemiological data suggested that human infections predominantly resulted from direct contact with infected horses (via contaminated body fluids, during slaughter, or by consumption of horse meat from infected animals), although clinical and epidemiological evidence pointed to direct human-to-human transmission in at least five cases (35). Although the source of the infection in horses was not identified, it was presumed to be *Pteropus* spp. bats, which are common to the area and were reported as being present during the outbreak.

All subsequent NiV outbreaks have been associated with the second, genetically similar but distinct strain, referred to as NiV-Bangladesh. Since 2001, almost annual NiV-Bangladesh outbreaks have occurred in Bangladesh or India, resulting in hundreds of human infections (Table 4). The first outbreak in India was reported in Siliguri Town, West Bengal, in 2001 and is the largest to date, with a total of 66 cases and 49 deaths (74% CFR). Subsequently, between 2007 and 2023, five separate outbreaks were reported in India (a total of 31 cases, 78% CFR). The first two outbreaks occurred in the state of West Bengal, which borders Bangladesh. However, in the last 6 years, all NiV outbreaks have occurred in Kerala State on the western coast of India, approximately 1,200 miles (2,000 km) from West Bengal. In Bangladesh, since the first reported outbreak in 2001, a total of 343 NiV cases have been reported, with 244 deaths (71% CFR) (36–38). Most cases have been reported from four administrative divisions: Dhaka, Khulna, Rajshani, and Rangpur. As with other NiV outbreaks, epidemiological data suggest *Pteropus* spp. bats in these regions as the host species (39). In Bangladesh especially, the harvesting and consumption of date palm sap (DPS) is a known transmission risk factor, with outbreaks closely correlated to the DPS harvesting season (November to March) (37, 39).

Other HNVs and parahenipaviruses

Apart from HeV and NiV, no other HNV or parahenipavirus has been associated with human disease except LayV and MojV. LayV was first identified in 2018 from a throat swab during a sentinel surveillance program of febrile patients with a history of close contact with animals (6). Further investigation identified 35 additional cases of acute LayV infections occurring in 2018–2021 in the same geographic region of China, namely Shandong and Henan provinces. Epidemiological surveys suggest that shrews may be the natural reservoir (6). To date, none of the human LayV cases have been fatal, and evidence suggests that human-to-human transmission is unlikely. No cases of LayV have been detected to date outside China. Sequence analysis showed that LayV was most closely related to MojV, which was linked to a small cluster of human fatal pneumonia cases in South China (9). While LayV was first considered as an emerging HNV, it was never formally classified as such and has notable ecological and virological differences

TABLE 4 Summary of Nipah virus human cases

Year	Country	Division/district	Total cases[a]	Fatal cases	CFR, %
1998	Malaysia	Kinta, Negeri Sembilan, Selangor	265	105	40
1999	Singapore	N/A	11	1	9
2001	India	Siliguri	66[b]	45	68
2001	Bangladesh	Khulna	13	9	69
2003	Bangladesh	Rajshahi	12	8	67
2004	Bangladesh	Dhaka, Rajshahi, Rangpur	67	50	75
2005	Bangladesh	Dhaka	12	11	92
2007	Bangladesh	Khulna, Rajshahi, Rangpur	18	9	50
2007	India	Nadia	5	5	100
2008	Bangladesh	Dhaka	11	7	64
2009	Bangladesh	Dhaka, Rangpur	4	1	25
2010	Bangladesh	Dhaka, Khulna	18	16	89
2011	Bangladesh	Chattogram, Dhaka, Rangpur	43	37	86
2012	Bangladesh	Dhaka, Rajshahi, Rangpur	17	12	71
2013	Bangladesh	Dhaka, Khulna, Mymensingh, Rajshahi, Rangpur	31	25	81
2014	Bangladesh	Dhaka, Khulna, Rajshahi, Rangpur	37	16	43
2014	Philippines	Sultan Kudarat	17	9	53
2015	Bangladesh	Dhaka, Khulna, Rajshahi, Rangpur	15	11	73
2017	Bangladesh	Dhaka, Rajshahi	3	2	67
2018	Bangladesh	Dhaka, Rajshahi	4	2	50
2018	India	Kerala	23[b]	21	91
2019	India	Kerala	1	0	0
2019	Bangladesh	Rajshahi, Rangpur	8	7	88
2020	Bangladesh	Barishal, Dhaka	7	5	71
2021	India	Kozhikode, Kerala	1	1	100
2021	Bangladesh	Dhaka, Rajshahi	2	0	0
2022	Bangladesh	Dhaka, Rajshahi	3	2	67
2023	India	Kozhikode, Kerala	6	2	33
2023	Bangladesh	Dhaka, Rajshahi	14	10	72
2024	Bangladesh	Dhaka	4	4	100

[a]Confirmed and probable cases; CFR, case fatality rate; NR, not reported.
[b]Values obtained from WHO reports (https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON490).

from the other pathogenic HNVs, including different natural reservoirs and unique, yet unknown, cellular receptors (40).

Case-associated transmission

There is no evidence to date of HeV transmission directly between humans. The large number of cases in the 1994 outbreak is thought to reflect inadvertent human-assisted transmission associated with the index case's treatment of both sick and healthy horses. Although only a small minority of NiV-infected patients transmit the virus, more than half of identified cases may result from person-to-person transmission (41). In addition to direct transmission, NiV can be transmitted via fomites. Patient-associated contamination of nearby hospital surfaces, including towels, bed sheets, and bed rails, has been reported (42). Furthermore, experimental studies indicate that NiV is quite stable in blood in closed tubes at room temperature, but decay is observed when samples are exposed to air or increased temperatures (43). Comparable pathology is observed in human cases with HeV and NiV infection (44), indicating that the absence of documented person-to-person transmission of HeV may reflect differences in the epidemiology of the viruses and does not necessarily indicate that transmission of HeV between people would not be possible. However, other contributors may include differences in receptor

TABLE 5 Geographical identification and proposed reservoirs for parahenipaviruses and henipa-like viruses[a]

Primary site of detection	Parahenipavirus/henipa-like virus	Year[b]	Proposed reservoir (isolate spp.)	Isolate	Reference or GenBank no.
Korea	Daeryong virus	2021	Shrew (*Crocidura shantungensis*)	–	(7)
Korea	Gamak virus	2021	Shrew (*Crocidura lasiura*)	+	(7)
China	Langya virus	2022	Shrew	+	(6)
China	Jingmen *C. shantungensis* henipavirus 1	2021	Shrew	–	OM030314.1
China	Jingmen *C. shantungensis* henipavirus 2	2021	Shrew	–	OM030315.1
China	Wufeng *Chodsigoa smithii* henipavirus 1	2021	Shrew	–	OM030316.1
China	Wufeng *Crocidura attenuata* henipavirus 1	2021	Shrew	–	OM030317.1
China	Wenzhou *Apodemus agrarius* henipavirus 1	2021	Striped field mouse	–	MZ328275.1
Belgium	Denwin virus	2022	Shrew	–	(8)
Guinea	Melian virus	2022	Shrew (*Crocidura grandiceps*)	–	(8)
Brazil	Peixe-Boi virus	2022	Opossum (*Marmosa demerarae*)	–	(98)

[a]Data from Caruso and Edwards (11). –, indicates that no infectious isolates have been reported; dentification based on sequence data alone. +, indicates isolation of infectious virus has been reported.
[b]Year of first report or GenBank submission.

engagement, as NiV G engages the alternative receptor ephrin B3 (EFNB3) with higher affinity than HeV G (45).

Person-to-person transmission was rare during the Malaysian NiV outbreak (46), although the infectious virus was isolated from mucosal specimens (throat and nasal swabs), urine, and cerebrospinal fluid (47, 48). Person-to-person transmission was more clearly documented in later outbreaks and has been associated with case-patient activities and care (49, 50). Studies of all NiV cases identified during outbreak investigations in Bangladesh from April 2001 through April 2014 determined that 82 of the 248 (33%) cases were caused by person-to-person transmission, corresponding to a reproductive (R) number of 0.33. However, patient characteristics affected this estimate; the reproductive number increased with age and clinical presentation, especially in patients ≥45 years of age and those with respiratory symptoms, such as difficulty breathing. Other factors that increased the risk of transmission included duration of exposure and contact with body fluids (51).

Person-to-person transmission of NiV-Bangladesh can be frequent and rapid; 9 of the 11 nosocomial cases in Kerala acquired the infection in a span of 2 days (52). In Siliguri, West Bengal (2001), 45 (75%) of the 60 cases were hospital workers who cared for the patients or who had exposure to a patient with NiV disease in a hospital (53). In Kerala (2018), all patients except for the index patient acquired the infection from one of three community hospitals, where they were exposed to other patients with NiV infection (52, 54). The wards, emergency room, and radiology room were all identified as sites of exposure.

Viral shedding investigated in serial samples obtained from survivors in Kerala indicated that the timing of virus detection in blood, oral swabs, and urine varied significantly among patients (55). In one patient, NiV RNA was detected in throat swabs and urine 6 days after disease onset; urine and throat swabs were later found to be negative on days 9 and 24, respectively. Another patient in the same study had a positive throat swab sample on day 2, negative blood and urine samples on day 5, a positive blood sample on day 7, and positive blood and urine samples on day 9. However, subsequent blood and urine samples collected on days 10, 11, and 40 were all negative. Notably, semen samples from a male survivor tested positive on days 16 and 26 after illness onset, becoming negative starting on day 42.

Outbreak response and control

The impact of isolation and barrier measures against NiV transmission has been demonstrated in several outbreaks. In 2001 in Siliguri, an outbreak ended 5 days after the introduction of barrier nursing in the hospitals (53). Similarly, in 2018 in Kerala State,

the outbreak ended soon after the introduction of barrier nursing and total isolation of patients (52).

HNV ECOLOGY AND SPILLOVER

Within 18 months of the first HNV outbreak, neutralizing antibodies to HeV were discovered in fruit bats of the genus *Pteropus* (commonly known as flying foxes). These animals forage nocturnally on fruit and floral resources and roost diurnally in colonies sometimes numbering in tens or hundreds of thousands. *Pteropus* spp. bats are now the recognized natural reservoir of both HeV and NiV and maintain the virus without any clinical signs of infection (56). Surveys in areas where HNV outbreaks have occurred have demonstrated evidence (molecular detection, serological evidence, or viral isolation) of NiV infection in *Pteropus* spp. in Malaysia (57–59), India (60–62), and Bangladesh (50, 63), and of HeV in Australia (18–20, 64–67). Similar evidence of NiV or NiV-like viruses has been reported in surveys of *Pteropus* spp. fruit bats in Cambodia (68–70), Thailand (71–73), Vietnam (74), Indonesia (75), and East Timor (76). NiV RNA has also been detected in an insectivorous bat, *Hipposideros larvatus* (73), and serological evidence of NiV infection has been seen in several other species of frugivorous and insectivorous bats, including *Rousettus leschenaultia* (59, 77). Pteropid bats are also considered reservoirs for CedV, which was originally isolated from fruit bats in Australia (78).

While the natural reservoirs of HeV and NiV have been identified, transmission pathways of HNVs from bats to humans remain poorly characterized. Transmission to humans, particularly of HeV and NiV-Malaysia, is often bridged by an intermediate amplifying animal host, of which horses and pigs are the best described, respectively. Transmission of NiV can occur indirectly via contact with other infected domestic animals (cattle, sheep, goats) that presumably also consume food contaminated with bat saliva or urine. Different from most paramyxoviruses, HNVs have a wide host range. In addition to bats, pigs, horses, and humans, serological evidence of NiV infection in cattle, goats, cats, and dogs has been reported (35, 46, 79, 80). The highly conserved ephrin B2 (EFNB2), part of a family of receptor tyrosine kinases that are critical for a variety of functions, including vascular, lymphatic, neuronal, and renal development, neurotransmission, synaptic plasticity, and tumor metastasis, was identified as the main functional receptor for both HeV and NiV. The widespread occurrence of this molecule in vertebrates, particularly in arterial (but not venous) endothelial cells, in the smooth muscle of the tunica media, and neurons explains the wide host range of HNVs and the systemic nature of the infections they cause (81–83).

In Malaysia, exposure to swine was identified as the primary risk factor for NiV infection. The outbreak is believed to have started around pig farms in Ipoh, Perak, and spread by the movement of sick pigs to a second epicenter south (~160 miles) to the state of Negri Sembilan (28). The outbreak subsequently spread to workers in an abattoir in Singapore, where pigs from Negri Sembilan were held and slaughtered (31, 84). The pigs were likely infected by *Pteropus* spp. bats feeding on fruit trees near the farms, either by direct exposure to bat urine or through eating dropped pieces of contaminated fruit (58). Experimental studies of virus stability in bat urine, in fruit juice, and on mango flesh indicated that NiV can be recovered for several days in conditions with neutral pH and moderate temperatures (22°C). However, virus survival in the environment appears highly sensitive to higher temperature (37°C) and extreme pH values (<3 or >12) (85).

In Bangladesh, NiV transmission from bats to people has occurred primarily when raw DPS was contaminated by bats and then consumed by humans, amplified by human-to-human spread (86–89). Studies in Bangladesh have demonstrated that interventions like covering DPS collection vessels can significantly reduce bat contact with DPS and reduce the risk of NiV transmission (90). Bat exposure was also implicated in spillover events in the 2018 Kerala cases: the index patient, who presented with a fever that progressed to typical encephalitis, had suspected exposure to bats (91). Subsequent cases were identified to have had contact with this patient directly or indirectly (92). However, spillover in recent outbreaks in India is less understood, with the primary route

of transmission from the bat reservoir to the initial human infection case unknown and no intermediate host established.

Although the natural reservoirs of other HNVs and parahenipaviruses remain less characterized, the discovery of these viruses outside the geographic distribution of *Pteropus* spp. bats indicates that reservoirs for emerging HNVs likely include additional animal species. LayV transmission is thought to occur directly from animals to humans; symptomatic cases were predominately farmers and factory workers, with no human-to-human transmission reported (6, 93). A serosurvey conducted in 25 species of wild small animals and several domestic animals reported that LayV RNA was predominantly detected in *Crocidura* spp. shrews (71 of 262; 27%), although small numbers of goats and dogs were also seropositive (6). The highest positivity rate was in *Crocidura lasiura* shrews commonly found in Northeast Asia; of the 121 tested *C. lasiura* shrews, 52.1% were positive for LayV. The virus was also found in 20% of *Crocidura shantungensis* shrew samples. Notably, *C. lasiura* and *C. shantungensis* are the same shrew species in which Gamak virus and Daeryong virus were detected, respectively (11). These data suggest the involvement of multiple hosts, with shrews potentially being the natural reservoir of LayV and other parahenipaviruses. Other novel henipa-like viruses have also been linked to bats, rodents, and other mammals (11, 77, 94–97) (Table 5). For example, a rodent host has been suggested for MojV, associated with three fatal cases of severe pneumonia reported in June 2012 in Mòjiāng Hani Autonomous County, Yunnan Province, China, in persons who had been working in an abandoned mine. Ecological testing in the cave associated with the initial disease report identified *Rattus flavipectus* rats as a potential reservoir for the virus (3 of 9 rat rectal swabs positive); all swabs taken from 20 *Rhinolophus ferrumequinum* bats and 5 *Crocidura dracula* musk shrews were negative (9).

PATHOGENESIS AND CLINICAL DISEASE

Cell targets, virus entry, and replication

HNV targets multiple tissues and organ systems, particularly epithelial, endothelial, smooth muscle, and neuronal cells (99). Infection and replication in epithelial surfaces contribute to viral shedding and transmission, whereas vascular endothelial cell infection results in systemic vasculitis and severe disease-associated endothelial damage both peripherally and in the central nervous system (CNS). Within the CNS, NiV and HeV show a marked predilection toward direct infection of neurons (99, 100). HNV virions adhere to host cells via the near-ubiquitously expressed EFNB2 (82, 83). Cell culture studies generating pure populations of human artery and vein cells highlighted that pathogenic HNV viruses preferentially infected arteries, consistent with receptor preference: arterial endothelial cells express higher levels of EFNB2 (101). Ephrin B3 (EFNB3), a related molecule predominantly expressed in brain tissue, serves as a functional alternative receptor for NiV (45). In addition to EFNB2, the less pathogenic CedV also utilizes other B-class (EFNB1) and A-class (EFNA1, EFNA5) ephrin receptors (102). On the other hand, the recently emerged LayV does not use either EFNB2 or EFNB3 (103, 104).

For cell entry, engagement of EFNB2/B3 by the G attachment glycoprotein induces a multi-step conformational change in the F glycoprotein, resulting in the fusion of the viral envelope and cellular membrane (105), effectively releasing the RNP into the cytoplasm. Once RNP is released into the cell, the encapsidated genome serves as the template for mRNA synthesis. The P and L proteins serve as the polymerase complex, which initiates transcription at the promoter region in the 3′ leader (106–109). Individual genes are regulated by corresponding transcriptional start and stop sites. Synthesis of mRNA poly-A tails occurs through a process in which the polymerase pauses at the gene transcriptional stop signal, crosses the intergenic region, and reinitiates transcription at the next gene start signal. This process can cause a subset of polymerase complexes to disengage from the template. As the only promoter is found at the 3′ leader, polymerase complexes can only reinitiate transcription at the 3′ terminus, resulting in a transcription gradient, with genes nearer to the 3′ terminus transcribed more frequently than genes closer to the 5′ terminus of the genome (1, 110).

In addition to transcriptase activity, the polymerase complex provides replicase activity; as viral mRNA translation occurs, the relative availability of N protein is believed to signal a switch from transcription to replication activity (111), with observable non-homogeneous ordered structures of N protein detected in infected cells (112–114). For genomic replication, the polymerase complex similarly enters the 3′ genomic promoter but ignores the mRNA transcriptional signals at gene junctions, allowing transcription of a full-length, positive-sense antigenome. A promoter in the 3′ non-coding region of the antigenome provides the location for the synthesis of new, negative-sense genomic RNA that can serve as additional templates for transcription (112).

Although glycoproteins are synthesized in the endoplasmic reticulum and typically mature through the Golgi network to the cell membrane, HNV F protein processing and maturation occur in the endosomes (115, 116). Cytoplasmic tails of the F and G glycoproteins interact with the M protein, which drives viral particle assembly and budding through interactions with other virus-specific and host-specific factors (117–124). Viral particle assembly requires termination of genome replication and proper encapsidation of new genomes. In addition, regions of the cell membrane must be prepared to accept budding nucleocapsids. Polymerase complexes stay associated with packaged nucleocapsids and are responsible for the next round of infection (125).

Immune evasion

Like many viruses, pathogenic HNVs have well-recognized immune antagonists. HNV interferon (IFN) antagonism is mediated by P gene products: C, V, and W proteins. V and W proteins are encoded by co-transcriptional mRNA editing during P gene transcription, and the C protein is translated from the P/V/W gene transcripts via an alternate open reading frame. Although the C, V, and W proteins are historically considered accessory proteins, all are present in infected cells and purified virions. In infected cells, W is detected in the nucleus, while P, V, and C are found in the cytoplasm (126). Immune antagonism and modulation are achieved by a variety of mechanisms. P, V, and W interact with a number of host proteins but were first characterized for their ability to block type 1 IFN signaling by binding STAT1 via their shared N-terminal region (127–131). By way of its unique C-terminus, the V protein alters MDA5 helicase folding dynamics, and also interacts with the RIG-I/TRIM25 regulatory complex to inhibit RIG-I signaling (132, 133). The unique C-terminus of W interacts with importin alpha-3 and the 7 isoforms of 14-3-3 proteins and interferes with both virus- and TLR-3-mediated IRF3 activation pathways (134, 135). The C protein can suppress innate immunity both by modulating viral replication to reduce the formation of aberrant pathogen-associated molecular patterns (e.g., defective interfering genomes) and by suppressing inflammatory cytokines (136, 137). The matrix protein (M) and N protein also have IFN antagonism activities. M, which is involved in viral assembly and budding, can also inhibit IFN through interactions with TRIM6 (138), whereas N can interfere with STAT complex formation, thereby reducing STAT nuclear accumulation and thus decreasing IFN-stimulated gene activation (139). The ability to efficiently modulate immune responses is associated with virulence. Several *in vivo* studies utilizing recombinant P gene mutant NiVs confirmed that V is the most important virulence factor (140–143). Also, the P gene of non-pathogenic CedV does not undergo RNA editing and thus does not produce V or W proteins (78). Studies of CedV-P targeting of STAT1 and STAT2 indicate that CedV does not effectively inhibit IFN/STAT signaling compared to pathogenic HeV. This correlates with the relatively reduced capacity of CedV to inhibit mRNA synthesis of IFN-inducible gene MxA (144).

Clinical presentation

The clinical manifestations of HeV and NiV in humans appear similar; overall, the infection can result in a rapidly progressing severe illness affecting the CNS and respiratory system. The CFR of HeV is 57% (4 of 7 cases). Following an incubation period of 7–16 days, HeV patients develop an influenza-like illness that can progress to acute

meningitis and/or encephalitis with tonic-clonic seizures and recurrent focal and motor seizures; the symptoms can rapidly devolve into coma (14). Of the four fatal human cases, three succumbed to severe neurological disease (encephalitis). The remaining patient exhibited severe respiratory distress, multiorgan failure, and arterial thrombosis, with chest radiographs showing bilateral alveolar and interstitial infiltration (12).

As HeV cases remain limited, most clinical data are derived from clinical investigations of NiV disease (Table 6). NiV infection typically presents as acute respiratory distress syndrome (ARDS), encephalitis, and/or myocarditis. The clinical disease can be exclusively respiratory or neurological (44) or may exhibit aspects of more than one common phenotype. In the initial outbreak, NiV infection was recognized in patients with encephalitic signs. In subsequent outbreaks, acute and severe respiratory signs were also associated with infection. In Malaysia, the CFR was 40%. Most patients presented with severe acute encephalitic syndrome, but a subset also had significant pulmonary signs (29, 31, 100). Case reports indicate a high prevalence of fever, and the majority (70%) of patients complained of drowsiness, headache, and disorientation or confusion. The most frequent clinical sign among patients was reduced consciousness (145). In fatal disease, intracerebral hemorrhage has been reported (145). In Singapore, the most common presenting symptoms were fever, headache, and drowsiness. Eight patients presented with signs of encephalitis (decreased level of consciousness or focal neurological signs). Three patients presented with atypical pneumonia, but one later developed hallucinations, and evidence of encephalitis was seen upon cerebrospinal fluid (CSF) examination (31). Eleven patients were confirmed to have acute NiV infection based on

TABLE 6 Henipavirus case reports and clinical investigations[a]

Year[b]	Virus	No.[c]	Focus	Reference
1994	HeV	2	Hendra, Australia: initial case reports of HeV; autopsy of first recognized lethal case	(13)
1995	HeV	1	Relapse case; clinical report and autopsy	(14)
2004	HeV	1	Clinical report, survivor; initial case report and follow-up 2 years later	(24)
2004, 2008	HeV	2	Specimens from acute-phase infection and convalescent phase for serology (blood) and qRT-PCR (nasopharyngeal aspirate, blood, urine, and CSF) from two of three known survivors	(146)
2008	HeV	2	Clinical report, virologic and serologic findings, human public health investigations and responses	(147)
1998–1999	NiV-Malaysia	32	Malaysia/Singapore: fatal cases, clinical and autopsy findings (29 full autopsies, 3 limited to brain)	(145)
1999	NiV-Malaysia	3	Malaysia: all pig farmers; two patients with relapsed and one patient with late-onset encephalitis up to 53 months after initial infection	(148)
1999	NiV-Malaysia	18	Kuala Lumpur Hospital, Malaysia: clinical features; case investigations	(149)
1999	NiV-Malaysia	94	Malaysia: clinical features	(100)
2001–2004	NiV-Bangladesh	92	Bangladesh: four outbreaks, clinical features	(150)
2007	NiV-Bangladesh	7	Thakurgaon District, Bangladesh: person-to-person transmission	(151)
2007	NiV-Bangladesh	8	Kushtia District, Bangladesh: case cluster; identifying risk factors for infection in Bangladesh	(152)
2007	NiV-Bangladesh	5	West Bengal, India: diagnostics; sequencing; person-to-person transmission	(153)
2008	NiV-Bangladesh	10	Bangladesh: investigation of cluster of patients with encephalitis, etiology and risk factors for disease; link to DPS[d]	(86)
2010	NiV-Bangladesh	16	Bangladesh: investigation of cluster and sporadic cases; contact with corpse of probable case implicated in disease transmission	(154)
2010–2014	NiV-Bangladesh	14	Bangladesh: investigation of three case clusters from hospital-based surveillance implicating fermented DPS[d] (tari) as a potential pathway for NiV transmission	(88)
2013–2014	NiV-Bangladesh	16	Faridpur, Rajshahi, and Rangpur, Bangladesh: patient contamination of surfaces; person-to-person transmission	(42)
2018	NiV-Bangladesh	2	Kerala, India: longitudinal characteristics of cell-mediated and humoral immunity during acute and convalescent phases	(155)
2018	NiV-Bangladesh	12	Kerala, India: clinical manifestations and outcome	(52)
2018	NiV-Bangladesh	23	Kerala, India: clinical and epidemiological profiles	(54)

[a]Summary is intended to provide a comprehensive but not exhaustive list of clinical reports to date.
[b]Year of cases or outbreak.
[c]Number of confirmed and/or probable cases included in report.
[d]DPS, date palm sap.

raised IgM in serum. NiV was identified by RT-PCR in the CSF and tissues of the patient who died.

In subsequent outbreaks, a higher incidence of respiratory disease has been reported. In Bangladesh, similar clinical signs and symptoms were seen: fever, altered mental status, headaches, vomiting, and convulsions, with the addition of respiratory indicators (cough, respiratory difficulty, and clinical and radiographic features of respiratory distress syndrome) (150). Chest radiographs showed diffuse bilateral opacities covering most lung fields, consistent with ARDS (150).

The median incubation period in NiV outbreaks in India has been reported as 9.5 days (range: 4–14 days), with a mean duration of 6.4 days (54). This is consistent with a study of clinical features during four outbreaks identified from 2001 to 2004, in which the median incubation period of patients with known exposure to another infected patient was 9 days (range: 6–11 days), with death occurring a median of 6 days (range: 2–36 days) after the onset of illness (150).

A study of 12 patients treated at the emergency medicine department of the Government Medical College, Kozhikode, during the 2018 outbreak in Kerala, India, reported a high frequency of non-specific clinical signs and symptoms: fever (100%), headache (75%), and vomiting (58.3%). Respiratory tract-associated changes were also frequent in these patients and included cough, breathlessness, hypoxia, chest infiltrates seen on X-ray, and the need for ventilatory support in 11 of 12 patients (91.7%). Other notable findings included cardiac abnormalities such as bradycardia and intractable hypotension necessitating the administration of inotropes to control cardiac contraction. Thrombocytopenia was observed in 6 of 11 patients (55%). An additional study summarized all 23 recognized cases in the Kerala outbreak. A comparable clinical spectrum was described, emphasizing the predominant occurrence of respiratory signs (shortness of breath in 73.6% of the patients) (54).

Although some reports note an absence of asymptomatic infection, such as a study that investigated two outbreaks of encephalitis in Meherpur and Naogaon, Bangladesh (50), subclinical or asymptomatic NiV infection has been recognized in outbreaks of both NiV-Malaysia and NiV-Bangladesh. Asymptomatic infection was seen in 8–11% of humans associated with infected pig farms in Malaysia and Singapore (30, 33). In addition, asymptomatic infection was detected in humans on farms where infected pigs were not reported; one study found 10 of 166 (6%) individuals who tested positive for NiV antibodies as part of a case-control study in Malaysia (33). In a serosurvey of 1,469 people potentially exposed to NiV following the 1999 outbreak in Singapore, 22 cases were identified; 10 of these (45%) had no history of compatible pulmonary or neurological disease (156). Subclinical infections have also been reported among contacts of patients in India. A serosurvey of 155 healthcare workers and 124 household and community members with close contact with 18 lab-confirmed patients reported two subclinically infected persons who developed anti-NiV IgM and IgG and one who developed only IgM (157). These individuals were two family members of a laboratory-confirmed patient and a healthcare worker in the emergency medicine department. A study using magnetic resonance imaging (MRI) identified lesions characteristic of NiV infection in the brain of an asymptomatically infected nurse who worked in the intensive care unit caring for NiV patients during the Malaysian outbreak (158). Further studies indicate that up to 16% of asymptomatic persons may have lesions detectable on brain MRI scans (159). Brain MRI was thus recommended to identify subclinical brain lesions in asymptomatic, seropositive patients; however, it is not clear how predictive these lesions are for the occurrence of late-onset encephalitis (160).

Clinical signs associated with closely related parahenipaviruses are less well described. Like NiV and HeV, LayV affects multiple systems. In all, 26 of the 35 patients identified with acute LayV infection in the Shandong and Henan provinces of China (6) were found to have LayV infection alone (i.e., no other pathogens detected). In this subset, all patients presented with fever; around half (46–54%) presented with fatigue, cough, anorexia, and/or myalgia, and a third or more (35–38%) presented with nausea,

headaches, and/or vomiting. Clinical pathology included thrombocytopenia, leukopenia, impaired liver function, and, less frequently, impaired kidney function.

Encephalitis

In both HeV and NiV infection, viral pathogenesis includes vasculitis-induced microinfarction and neuronal infection causing severe tissue damage in the CNS (161, 162), with neuronal infection implicated as more critical than microinfarction in acute NiV encephalitis (163). HNV acute encephalitis is associated with a high CFR (pooled CFR, 61%) (164). Lesions visualized on MRI associated with NiV encephalitis include the presence of numerous discrete lesions (2–7 mm) disseminated in the subcortical and deep white matter of the cerebral hemisphere (165).

Knowledge of mechanisms for viral entry into the CNS and of viral targets in the CNS is limited and derived predominantly from animal model studies. Based on studies in hamsters, entry into the CNS is proposed to occur via neurons extending from the olfactory epithelium through the cribriform plate into the olfactory bulb, a mechanism that resulted in viral presence before the presentation of neurological signs (166). The vascular endothelium in the brain was reported as the initial target of infection in experimentally infected African green monkeys (AGMs; *Chlorocebus aethiops*) (167). Mechanisms and localization of viral persistence in the CNS were also reported in these studies; in AGMs that survived infection, encephalitis affected the entire brain, with the majority of virus detected in the neurons and microglia of the brainstem, cerebral cortex, and cerebellum (167).

Pathological features of human infection

To date, there are two publications reporting human autopsy results each for HeV (13, 14) and NiV (145, 163). The initial autopsy report describes the findings of the first recognized fatal case of HeV infection (13); the most significant findings were in the lung and were consistent with viral disease. Both lungs were congested, hemorrhagic, and filled with serous fluid. Lung histology revealed focal necrotizing alveolitis with many giant cells, some syncytial formation, and viral inclusions. In addition, pulmonary autopsy findings reflected a history of heavy smoking (focal centrilobular emphysema, features of chronic bronchitis, and a small adenocarcinoma arising from a terminal bronchiole). Other tissue findings included mild non-specific chronic myocarditis, coronary atherosclerosis, areas of inflammation with necrosis scattered throughout the kidneys, and pulmonary embolism. The second available HeV autopsy report focused on a single fatal relapse case (14) and is discussed below regarding relapse and late-onset disease.

All NiV autopsy data are from patients in Malaysia and Singapore infected with NiV-Malaysia. Detailed descriptions of gross pathology and histopathological lesions are found in a report examining 32 such fatal cases and are summarized below (145). In the CNS, gross lesions were non-specific, difficult to identify, and observed in a small subset of brains; these lesions included small, discrete, occasionally hemorrhagic necrotic lesions, and herniation. In the CNS and spinal cord, the main histopathological findings were vasculitis, thrombosis, parenchymal necrosis, and viral inclusions. In the brain, in both the gray and white matter, plaques with various degrees of necrosis were reported and associated with inflammatory cellular infiltrate (neutrophils, macrophages, lymphocytes, and reactive microglia). Outside the CNS, the most notable lesions were seen in the lung, spleen, and kidney. Lesions were less frequently observed in the heart, mesentery, adrenal gland, and pancreas, and no significant pathology was reported in the liver, skeletal muscle, or other tissues examined. Microcystic degeneration was also observed, most commonly near necrotic plaques. Incidence of necrosis was reported from most frequent to least frequent in the brain, lung, spleen, kidney, and heart. Vasculitis was similarly reported from most to least frequently in the brain, lung, heart, and kidney; vasculitis was absent in the spleen. Correspondingly, in fatal cases, viral antigen was most frequently detected in the brain, followed by the lung and kidney, then the heart and spleen.

A more recent report examining brain tissues (cerebrum, brainstem, and cerebellum) from 15 autopsies described three types of parenchymal lesions with the following features: (i) neuroglial immunohistochemistry (IHC) positivity for viral antigens and minimal or no necrosis; (ii) neuroglial immunopositivity and necrosis; and (iii) necrosis with no viral antigen (163). Several important observations and corresponding conclusions were reported in these studies. Most viral antigen-positive or RNA-positive cells were neurons, and findings indicated that neuronal infection was more important than microinfarction as a mechanism of pathogenesis in acute encephalitis. In addition, as cerebral glial immunopositivity was rare, the authors also concluded that microinfarction played a more important role in white matter injury.

Relapse and late-onset disease

Both HeV and NiV (100) can cause relapsing encephalitis and other pathology, with similar pathogenesis (44). HNV relapse was first reported in a case of HeV (14). This patient experienced a brief encephalitic disease that resolved but was followed by relapse 13 months after exposure; relapse was characterized by a variety of non-specific signs including sore throat, headache, drowsiness, vomiting, neck stiffness, mood changes, and low back pain. The patient's other CNS signs included tonic-clonic, focal, or generalized motor seizures, which progressed to depressed consciousness; the patient later succumbed to the disease. Necropsy revealed leptomeningitis with lymphocyte and plasma cell infiltration. Discrete foci of necrosis were limited to the neocortex, basal ganglia, brainstem, and cerebellum; foci were not observed in the subcortical white matter. Occasional multinucleate endothelial cells were seen in the brain, liver, spleen, and lungs. IHC of brain tissue was positive for viral antigen, but the virus could not be isolated.

Several cases of relapsing and late-onset disease were also recognized during the initial NiV outbreak. In Malaysia, relapse encephalitis was reported in 7.5% of those who recovered from acute encephalitis; moreover, late-onset encephalitis was seen in 3.4% of those with initial non-encephalitic or asymptomatic infection (162). The mean interval between the initial illness and the onset of relapse signs was 8.4 months. Subsequent case reports increased the prevalence of NiV relapse to around 9% (148). Both fatal cases and survivors of relapse disease have been reported. One study, for example, described a necropsy performed on a 26-year-old male who recovered from initial illness but later died from relapse disease a few months later (145). Interestingly, the CNS pathology in this relapse encephalitis case was different from that seen in the acute cases examined during the outbreak; the relapse case had more extensive viral inclusions, larger parenchymal lesions that were associated with more severe histopathological findings (neuronal loss, gliosis, macrophage abundance). In addition, vasculitis or typical necrotic plaques were not seen, and perivascular cuffing was not a prominent feature (145).

Other patients survived relapse disease. For example, a 23-year-old male pig farmer was diagnosed in February 1999 with acute NiV encephalitis, given general supportive care, recovered uneventfully, and was discharged after 9 days. The patient presented after two episodes of generalized tonic-clonic seizures in late June 2002, 40 months after acute disease (148). Similarly, a 43-year-old female pig farmer was first admitted in April 1999 with fever, anorexia, myalgia, and generalized weakness that progressed to severe neurological disease. She fully recovered and was discharged after 3 weeks. In September 2003, she presented after a single generalized tonic-clonic seizure. Abnormalities were noted on electroencephalogram and brain MRI, including those consistent with previous acute NiV encephalitis. No additional neurological events were reported in this patient.

Late-onset NiV encephalitis has been reported up to 53 months after initial infection (148). A 51-year-old pig farmer presented in late March 1999 after a 1-week history of fever, headache, sore throat, anorexia, nausea, vomiting, arthralgia, dizziness, and drowsiness with fever but no additional signs or abnormalities; he was diagnosed with non-encephalitic NiV infection and given a 1-week course of oral ribavirin. The patient

was asymptomatic until September 2003, when he presented with fever, headache, and weakness, followed by seizure activity. In addition, areas of consolidation were detected on chest radiographs. Late-onset NiV encephalitis with secondary aspiration pneumonia was diagnosed, and he was treated with ribavirin, phenytoin and carbamazepine, ceftriaxone, and metronidazole prior to recovery, with only mild neurological signs remaining (148).

Sequelae

Severe chronic sequelae occur in many survivors, presenting primarily as neurological signs (168). In HeV survivors, residual neurological symptoms including ataxia have been observed (147). One study of pig farmers in Malaysia reported that 15% of cases developed persistent neurologic deficits (100). A later study followed 9 patients with NiV encephalitis over the course of 24 months; all but one developed clinical signs associated with the encephalitis. Neurological sequelae defined as depression developed either immediately after recovering from encephalitis or around a year after the outbreak. Other sequelae included personality changes, chronic fatigue syndrome, and marked deficits in attention, verbal, and/or visual memory. Verbal memory was more impaired than visual memory in these patients. Interestingly, no correlation was found between the total number of brain lesions and psychiatric or cognitive impairment (169).

Immunological response to infection

Limited clinical specimen availability has restricted studies to monitoring and evaluating the immune response to HNV infection in humans. The kinetics of the antibody response in human infection are still being characterized, and, due to the spectrum of clinical phenotypes, may vary based on disease course and clinical presentation. Several studies show that IgM and IgG antibodies are detected in the serum during the first week after illness onset; IgM antibodies persist from 3 to up to 7 months post-illness onset, while IgG antibodies have been shown to persist for many years in survivors (145, 155, 170, 171).

IgM positivity can be observed as soon as the first day after the appearance of symptoms in 50% of patients and 100% of patients after 3 days; IgG can be detected 2 days after symptom onset in 31% of patients, with 100% positivity reached by day 17 (171). Another small study in India detected antibodies in three symptomatic and two asymptomatic contact cases when contact cases of NiV-positive patients were tested regularly for several months (172). In symptomatic patients, IgM was detectable 5–27 days after disease onset, while IgG persisted for more than 1 year.

Interestingly, comparable IgM and IgG immune responses against NiV infection were observed in the presence and absence of clinical symptoms; however, the severity and duration of illness may affect antibody kinetics. In fatal cases from Malaysia and Singapore, NiV IgM antibodies were detected more often than IgG in serum and CSF. In patients with a longer duration of illness (6–10 days), IgM antibodies were found in serum samples of 94% of patients and CSF of 64%, while IgG antibodies were found in 12% of serum samples and 9% of CSF samples. IgM antibodies were detectable in serum before CSF (145). In two of three patients with total illness of ≥25 days (25–34 days), IgM and IgG were both detected in the CSF, whereas detection in serum was variable (145).

Characterization of adaptive immune responses is also limited. Responses were characterized in the two survivors of the 2018 Kerala outbreak, indicating the prominence of a CD8 T-cell response (155). In both cases, elevated total B lymphocytes, activated B cells, and plasmablast counts were observed. Interestingly, while the absolute number of T lymphocytes was within normal limits during the period of illness evaluated, a marked elevation of activated CD8 T cells was observed, and clearance of NiV RNA from blood coincided with peak levels of activated CD8 T cells.

DIAGNOSTICS

Acute patient diagnostics

Early clinical presentations in NiV infections can closely resemble other febrile diseases, so early diagnosis is vital for both the appropriate treatment of the individual and for controlling any potential outbreaks (173). First-line testing primarily relies on either molecular and/or serological approaches, with other methodologies, such as viral isolation, used for confirmatory studies (Table 7).

Detection of viral RNA (vRNA) in patient samples is the most common method for diagnosing acute HNV infection. Numerous nucleic acid amplification tests (NAATs) have been reported, but because many of them were developed internally within individual institutions, there is significant variation in assay design, reagent type, assay methodology, and instrumentation. Most of these assays are designed to target the N gene due to the abundance of N mRNA transcribed during infection; however, assays have also been developed to detect the L gene or multiple targets, including the M and P genes (assays summarized in Table 8). Currently, variations of reverse-transcriptase quantitative PCR (RT-qPCR) assays are the most frequently used method for diagnosing acute HNV infection given their high sensitivity, specificity, and relatively brief processing time (174). Development of an isothermal (65°C) reverse-transcription loop-mediated isothermal amplification (RT-LAMP) method targeting the N gene for the rapid detection of NiV has been reported and demonstrated high sensitivity, supporting future use of LAMP-based approaches (175).

RT-qPCR can identify NiV infection in the early stages of disease, with vRNA detectable in blood (55), serum, oropharyngeal swabs (42, 54, 55, 86), urine (54, 55, 153), and

TABLE 7 Summary of henipavirus diagnostic approaches[b]

HNV test type	Process time	Advantages for HNV detection	Disadvantages for HNV detection
Lateral flow device-based[a]	<1 hour	Rapid; low cost; adaptable to point-of-care field application	Decreased sensitivity
Loop-mediated isothermal amplification (LAMP)	<1 hour	Isothermal reaction; rapid; low cost; lyophilized reagents can be used	Challenging to design primers with adequate specificity
RT-PCR/ nested RT-PCR	Hours	Specific; high sensitivity; adaptable for minor sequence heterogeneity; low cost	Qualitative only; limited dynamic range; low throughput; can be prone to cross-contamination resulting in false positives
SYBR Green RT-PCR	Hours	Quantitative; high sensitivity; adaptable for minor sequence heterogeneity; low cost; melt curves can differentiate between strains	High cost of equipment; training required to interpret results; prone to false positives due to non-specific primer annealing/primer dimers
TaqMan RT-qPCR	Hours	Quantitative; high specificity; high sensitivity; adaptable for minor sequence heterogeneity; high throughput	Higher cost of equipment; training required to interpret results
Multiplex TaqMan RT-qPCR (e.g., TaqMan array cards [TACs])	Hours	Screen multiple pathogens simultaneously; medium throughput; less sample required	Limited utility for intermittent small outbreaks; cost-prohibitive; custom pre-developed panels (closed system); large footprint equipment
IgM ELISA (MAC)	Hours	Consistent early detection of infection	Requires inactivated viral antigen or purified recombinant antigen; not automated
IgG ELISA	Hours	Specific utility for surveillance; adaptable for recombinant	Requires inactivated viral antigen or purified recombinant antigen; not automated
Luminex bead-based	3–6 hours	High throughput	Requires advanced technology/expertise
Pseudotyped virus neutralization	1–3 days	No BSL-4 requirement; higher throughput; quantitative	Variable or lower sensitivity
Virus neutralization	1–7 days	Specific and sensitive	Requires BSL-4
Virus isolation	7–14 days	Specific	Requires BSL-4; sensitivity dependent on sample quality

[a]Antigen or immunoassay.
[b]HNV, henipavirus; MAC, IgM antibody capture enzyme-linked immunosorbent assay.

TABLE 8 Summary of published henipavirus molecular diagnostic assays[a]

Assay type	Viral target	Assay target	Sample types evaluated in assay development	Reference
RT-PCR	NiV	N	Blood; respiratory secretions; urine; CSF	(27)
	NiV	N, M	Blood; urine	(53)
Nested/hemi-nested RT-PCR	HeV	F	Tissue samples; viral supernatant	(176)
	HeV	F	Tissue samples	(177)
	NiV	N	Urine	(178)
	NiV	L	Tissue samples; viral supernatant	(179)
	Pan-HNV	L	Viral supernatant	(180)
SYBR Green RT-qPCR	HeV, NiV	N	Viral supernatant	(181)
	NiV	N	Tissue samples; viral supernatant	(179)
TaqMan	HeV	M	Tissue samples; viral supernatant	(176)
RT-qPCR	NiV	N	Blood; respiratory secretions; urine; CSF	(182–184)
	NiV	N, P, M	Tissue samples; viral supernatant	(179)
	NiV	F to G IGR	Viral supernatant	(185)
Multiplex TAC	NiV	N	Serum; viral supernatant	(183, 186)
	NiV	N	CSF; viral supernatant	(183, 187)
RT-LAMP	NiV	N	Blood; respiratory secretions; urine; fecal swab	(175)
RT-RPA with LFD	HeV	N	Viral supernatant	(188)
	NiV	N	Viral supernatant	(189)

[a]IGR, intergenic region; LAMP, loop-mediated isothermal amplification; RPA, recombinase polymerase amplification; TAC, TaqMan array card.

CSF (153). In general, oropharyngeal swabs are one of the most sensitive sample matrices and are thus the most common sample type obtained for diagnostic evaluation (86, 190). Importantly, blood samples may not reliably serve as the preferred sample type for diagnostics, depending on exposure history, due to the transient nature of viremia associated with HNV infection (54, 55). In addition, in areas with limited access to care, patients most often present later in the course of infection, when the presence of viremia is unreliable. In addition, the timing of testing may depend on the characteristics of the outbreak. For example, in Kerala, most of the patients tested positive for NiV only later in the illness (54), suggesting that RT-qPCR-based assays may not always detect cases early after infection.

Diagnostic serological tests for HNV can be used to detect either viral antigens or the patient's antibody response to infection. Like with NAATs, most assays have been developed internally within individual institutions. HNV antigen-specific IgM enzyme-linked immunosorbent assays (ELISAs) are typically used in conjunction with RT-qPCR to confirm active infection. Serum IgM titers peak around 9 days post-symptom onset and can remain detectable for up to 3–7 months (171). ELISAs for IgG are generally used for epidemiological analysis rather than acute diagnosis because these titers peak later in infection but can persist for months (173). Again, due to its relative abundance and immunodominance, N protein is the most commonly used antigen in HNV ELISAs, although G, P, and M protein-based assays have also been described (27, 191–200). The homology between HNV N and G proteins often allows the use of these ELISAs for pan-henipavirus detection, and, in conjunction with the more heterologous P protein, allows differentiation between NiV and HeV infection.

Well-characterized and accessible rapid point-of-care tests for serology and antigen detection are currently unavailable and represent a notable gap in NiV diagnostics. However, the development of several point-of-care targeted assays has been recently reported, providing proof of principle that this technology can be applied to advance a simple and rapid diagnostic assay for HNV. A rapid lateral flow strip test for NiV detection was developed using EFNB2 as the capture ligand combined with a NiV mAb (F20NiV-65) as the detector (201). A competitive ELISA using baculovirus-expressed recombinant NiV

G antigen and a NiV G-specific monoclonal antibody detected NiV and/or HeV antibodies from different animal species (192). Three additional rapid lateral flow detection assays were developed based on reverse transcription recombinase-based isothermal amplification targeting the NiV N gene: two assays use recombinase polymerase amplification and one uses recombinase-aided amplification isothermal technology. All three were shown to be sensitive and did not detect other viruses that cause similar febrile symptoms (189).

Diagnostics for surveillance, research, and countermeasure evaluation

Neutralization assays are predominantly used as confirmatory assays or for research and vaccine evaluation. Considering its high specificity, serum neutralization testing is currently considered the reference standard for confirmatory NiV diagnosis by the World Organization for Animal Health (202). While cross-reaction of HeV-immune sera against NiV was key to the eventual definition of the henipavirus genus, cross-neutralization does not occur as widely. Antibodies generated against F and G glycoproteins of HeV and NiV have demonstrable cross-neutralizing activity (203–209); however, antibodies against CedV were shown to cross-react with, but not cross-neutralize, HeV and NiV (78).

A major limitation of neutralization assays with authentic viruses is the requirement for BSL-4 facilities. Pseudovirion-based neutralization assays, such as those based on Moloney murine leukemia virus, vesicular stomatitis virus (VSV), and human immunodeficiency virus (HIV) (210–218), are an alternative approach for detecting antibodies against viral surface glycoproteins that preclude the need for BSL-4 facilities. In general, studies comparing live viruses to pseudotype systems indicate a good correlation in results (211, 212, 217), though under some circumstances, pseudotype system-derived data may vary. For example, a study comparing plaque reduction neutralization test (PRNT) data to those obtained with replication-deficient luciferase-based VSV particles pseudotyped with NiV F and G (pVSV-NiV-F/G) found that the results were similar except when detecting low levels of antibodies. When anti-NiV antibody levels were relatively low, PRNT showed higher sensitivity as it more consistently detected neutralizing activity (211). Another study indicated that while a green fluorescent protein (GFP)-based VSV pseudotype generally correlated with PRNT, the neutralizing titers of the pseudotype were consistently higher than those from PRNT (212). Interestingly, the successful deployment of pseudovirions as a surveillance tool identified seroprevalence among those living in proximity to deforestation as well as those who butchered bat meat (219).

Other technologies applied to surveillance and research include virus isolation and genetic sequencing. Virus isolation is also used for confirmatory diagnosis and downstream phenotypic and genomic characterization of the isolate. NiV can be isolated from throat swabs (47, 62, 86), nasal swabs (47), urine (47, 150), and CSF (48, 150). Cytopathic effect usually occurs within 7 days, depending on the number of infectious virions present in the patient sample. Finally, high-throughput sequencing can be used as a metagenomics approach to study viral evolution and phylogenetics and to investigate transmission or new introductions (220).

MODELING ECOLOGICAL DYNAMICS IN ANIMALS

Animal models of HNV infection can be applied for two purposes: modeling maintenance and transmission in nature and modeling human disease. HNVs can infect a wide range of animal species. The natural host reservoirs of HNVs, predominantly bats, appear to carry the virus asymptomatically (221, 222). In other susceptible species such as pigs, horses, cats, and dogs, the disease can range from mild to severe, with severe disease reported in both horses and young pigs (223). While severe clinical signs are typically limited in animals, several animal models have been developed that more closely mimic disease progression seen in humans, both respiratory and neurological (further reviewed in references (224–228). Here and in the subsequent section, we summarize experimental infection studies in bats, livestock, and other species used to investigate

viral ecology. In the following section, we will review the development and use of rodent and non-human primate (NHP) models of disease.

Bats

Early experimental infection studies of HNVs in fruit bats investigated transplacental infection as a possible mode of transmission, with four gray-headed fruit bats (*Pteropus poliocephalus*) inoculated with HeV. While no overt clinical disease was observed in the bats, subclinical infection and associated pathology were indicated by viral isolation, seroconversion, vascular lesions, and positive immunostaining (56). Positive immunostaining in two placentas, along with isolation of the virus from one of the associated fetuses, supported transplacental transmission. In a separate study, HeV infection of Australian black flying foxes (*Pteropus alecto*) again demonstrated no overt clinical disease, with viral antigen detected but confined to the lungs in these animals (229).

In a 2008 report, *P. poliocephalus* were inoculated subcutaneously with an isolate of NiV-Malaysia derived from a fatally infected human (222). As with HeV, infection was subclinical but the animals seroconverted, transient presence of the virus within selected viscera was noted, histopathological tissue changes were observed, and episodic viral excretion in urine was detected. Later studies (2011) reported inoculation of *Pteropus* spp. bats from Malaysia and Australia with NiV and HeV, respectively (64). Again, no disease was seen, and, despite intensive sampling, no NiV was recovered; HeV was re-isolated from only one bat. Conclusions from these initial experiments suggested that opportunities for transmission may be limited and the probability of a spillover event low. More recent studies in the Egyptian fruit bat (EFB; *Rosettus aegyptiacus*) were carried out to evaluate the potential of this species to serve as a NiV disease model (230). However, NiV did not efficiently replicate in EFBs *in vivo*; no viral replication or shedding was detected, and no seroconversion against NiV glycoprotein occurred, suggesting that some species of bats may be refractory to infection.

Livestock and domestic species

Experimental challenge studies in larger species like pigs and horses are currently limited to two research facilities: the Australian Animal Health Laboratory (Geelong, Victoria, Australia) and the Canadian Science Centre for Human and Animal Health (Winnipeg, Manitoba) (225). Natural HeV infection in horses is highly variable and shows a broad range of signs. In general, it is characterized by rapid progression, with an increase in body temperature around 6 days post-challenge accompanied by an elevated heart rate. Within 9 days post-challenge, respiratory signs (respiratory distress, increased breathing rate, and nasal discharge at death) and neurological signs (restlessness, weight shifting between legs, irritability) indicate HeV infection in horses (231). Three horses were experimentally infected with HeV by the oronasal route to monitor potential routes of shedding, which resulted in systemic disease. In two of the three animals, HeV RNA was detected continually in nasal swabs as early as 2 days post-exposure, highlighting that the systemic spread of the virus may be preceded by local viral replication in the nasal cavity or nasopharynx (232).

In response to the discovery of HeV, other species in addition to horses have been experimentally infected to investigate their putative roles in natural transmission and utility as animal models of disease. Experimental HeV infection studies in cats resulted primarily in respiratory disease but did not consistently recapitulate neurological disease (233). Cats given the virus orally, intraperitoneally, or subcutaneously developed the disease, as did one in-contact cat. Gross pathology was seen in all affected cats and was consistent with respiratory disease (hydrothorax; dark, heavy, wet, congested, and/or hemorrhagic lungs with froth sometimes found in the respiratory passages). Histologically, lesions in the lungs of the cats indicated severe interstitial pneumonia along with clearly defined vascular lesions. Vascular lesions accompanied by parenchymal degeneration were also seen in the gastrointestinal and lymphoid organs. Vascular damage was associated with HeV antigen and syncytia in small blood vessels in the

lungs and other organs (234). In addition, *in utero* transmission of NiV in cats has been reported, with evidence of high levels of viral replication in many tissues of a pregnant adult cat and in fetal tissues, suggesting potential for both vertical and horizontal transmission (235). Experimental inoculation in other domestic and peri-domestic species (mice, rats, rabbits, dogs, and chickens; subcutaneous inoculation) failed to elicit clinical disease (236). No remarkable gross or histological lesions were observed in mice, rats, rabbits, chickens, or dogs 21 days after inoculation, and no virus was isolatable. Significant concentrations of specific neutralizing or fluorescent antibodies were not detected in the mice or chickens but were detected in 3 of 4 rats, 1 of 2 dogs, and both rabbits (236).

Based on the well-recognized epidemiological link between NiV and porcine farmers, several experimental infection studies in pigs have been performed, demonstrating a predominance of mild to subclinical HNV infection with strain- and age-associated outcomes. Experimental HeV infection in pigs indicated susceptibility to infection and their potential role as intermediate hosts in transmission to humans. Pigs developed fever and depression; a subset developed respiratory signs, sometimes followed by mild neurological signs. Virus was detected in all infected pigs 2–5 days post-infection in oral, nasal, and rectal swabs, and 3–5 days post-infection in ocular swabs. vRNA was mainly distributed in tissues from respiratory and lymphoid systems at an early stage of infection, and the presence of the virus was confirmed by virus isolation (237).

Like with HeV, experimental infection in pigs with NiV-Malaysia results in self-limiting respiratory disease, with rare involvement of the CNS. Studies of 5-week-old piglets infected intranasally, orally, and ocularly with NiV-Malaysia and euthanized 3–8 days post-infection reported neurological signs in 2 of 11 animals, while the rest remained clinically healthy (238). The virus was detected in the respiratory system, lymphoreticular system, and nervous system of both sick and subclinical animals. Data suggested that virus entry to the CNS was via cranial nerves and by crossing the blood-brain barrier after initial virus replication in the upper respiratory tract (238).

In contrast to experimental infection with NiV-Malaysia, pigs experimentally infected with NiV-Bangladesh do not display overt clinical signs of disease (223). The absence of clinical disease in pigs oronasally infected with the Bangladesh strain is based on a single study to date. In these animals, viremia was undetectable throughout the study, and only low titers of neutralizing antibodies were measured around a month post-infection. The virus was, however, detected in oral, nasal, and rectal excretions, and viral dissemination from the upper respiratory tract to the brain, lungs, and associated lymphatic tissues was observed.

ANIMAL MODELS OF DISEASE

NHPs, ferrets, cats, hamsters, guinea pigs, and, most recently, mice have all been used to investigate disease; some of these animal models are now frequently used as critical systems to evaluate countermeasures. Early after the discovery of HeV, cats were observed to develop clinical disease following experimental infection (239) and later shown to develop the similar disease when infected with NiV (233). While cats were the first model used to test vaccine efficacy (207, 240), currently used animals are predominantly hamsters and African green monkeys (AGMs), with other models (ferrets and mice) in active development. Importantly, all these models recapitulate, to varying levels, both the respiratory and neurological features reported in human infection. Here we provide background on HNV-associated disease in animal models, emphasizing that these are models of a complex clinical disease in humans. While some species, such as hamsters, have been studied more frequently, continued model development and natural history studies for all species detailed below are needed to support robust conclusions regarding outcomes associated with viral dose, strain, and administration route, and to provide robust tools for evaluating pathogenesis and medical countermeasures. Overall, interpretations of data and comparisons between

HNV animal model studies must carefully consider sample size, number of independent investigations, experimental design (e.g., infection route), and endpoint criteria.

Non-human primates

The AGM (*Chlorocebus aethiops*) is the primary NHP species used to model human HNV disease and evaluate medical countermeasures. The AGM model of disease was reported first for HeV (241), followed by studies on NiV-Malaysia (242). Intratracheal inoculation of AGMs with HeV produced a uniformly lethal infection within 7–9 days, and the observed clinical signs and pathology displayed largely as ARDS, consistent with HeV-mediated disease in humans. Animals developed extensive diffuse interstitial infiltrates only 1–2 days prior to death, and severe lung lesions covering up to 90% of the tissue were observed at necropsy. Histopathological changes were primarily found in the lung and included alveolar hemorrhage, pulmonary edema, inflammation, and syncytial vascular endothelium, as described in human cases of both HeV and NiV. Similarly, initial investigations of NiV in AGMs confirmed their susceptibility to disease; infection caused severe systemic infection and high lethality that recapitulated many key aspects of human disease, including severe respiratory pathology and generalized vasculitis (242). Detailed clinical analyses have been performed in the NiV model, characterizing lethality, clinical parameters, and other observations, as well as virological indices and pathological findings (243). In contrast to HeV, NiV infection in AGMs is not uniformly lethal. Circumstances resulting in a protracted disease course or survival (i.e., due to type of exposure or intervention studies) appear to support a high propensity for neurological involvement in the model. Viral RNA and neurological lesions may be detectable in surviving animals even in the absence of clinical signs. Histopathological evidence of relapse encephalitis, which was not apparent clinically, was reported in AGM survivors euthanized 32 days post-infection (243). NiV genomic RNA was detected only in the brains of both survivors but not in any other organs evaluated (colon, eyes, liver, lungs, lymph nodes, spleen, stomach, and testes) (167).

More recent model development efforts have focused on further refinement of the AGM model, characterizing clinical disease associated with inoculation routes, specifically comparing intratracheal/intrabronchial and aerosol exposure and further defining variation associated with aerosol particle size to determine appropriate models for the spectrum of clinical phenotypes in human disease. Disease presentation in AGMs following aerosol exposure to NiV can vary based on estimated inhaled dose, aerodynamic particle size, and deposition site (244). Intratracheal and small-particle (1–3 µm) aerosol infection of AGMs with NiV-Malaysia leads to severe respiratory disease without neurological indications (245). Aerosol exposure to intermediate-size (6–8 µm) virus particles, to mimic potential human exposure by facilitating virus deposition in the upper respiratory tract, results in pulmonary parenchymal disease (i.e., consolidations, ground-glass opacities, and reactive adenopathy) but also induces neurological disease indicated by MRI lesions in the brain of a subset of animals in the absence of neurological signs (246). Neurological abnormalities were also observed in the disease following large-particle (10–14 µm) aerosol exposure to NiV (247). Efforts to modify aerosol exposure and characterize associated neurological signs in NHP studies are critical to the development of a model that can also recapitulate the incidence and spectrum of neurological disease reported in human infection.

Experimental infection studies have been performed in other NHP species, demonstrating species-associated differences in outcome. Squirrel monkeys (*Saimiri sciureus*) infected intranasally or intravenously with NiV-Malaysia developed clinical signs. The disease course was more acute in intravenously infected monkeys (2–3 days) and was lethal in three of four animals in which the disease was allowed to proceed; the disease was associated with acute neurologic and respiratory illness (248). The illness lasted longer in intranasally infected animals (7 days), and clinical signs were milder and seen only in two of four animals before recovery after 3–7 days. More recently, the common marmoset (*Callithrix jacchus*) was evaluated for susceptibility to NiV-Bangladesh infection

(249). All four intratracheally/intranasally inoculated marmosets succumbed to infection within 8–11 days post-challenge. Among these, three animals primarily developed respiratory disease, while one animal recapitulated neurologic clinical manifestations and cardiomyopathy seen upon gross pathology investigations. Interestingly, the gene ontology of the brainstem transcriptome from the lone animal that displayed neurological signs indicated enrichment in the expression of genes related to myelination and oligodendrocyte function.

By contrast, only mild or asymptomatic infections were reported in cynomolgus macaques (*Macaca fascicularis*) intratracheally and intranasally inoculated with HeV, NiV-Malaysia, or NiV-Bangladesh (250). Although HeV and NiV replicated with similar kinetics as in AGMs, NiV or HeV antigen was not detected in any of the tissues analyzed at the endpoint (day 28) except in one NiV-Bangladesh-infected animal, which exhibited a focal cluster of neurons with cytoplasmic immunolabeling within the brainstem. In addition, histopathological and IHC analyses of formalin-fixed tissues were unremarkable. Notably, analyses of cytokine/chemokine profiles and targeted transcriptome profiling indicated that differences in cell-mediated and humoral immunity could be delineated by clinical outcomes, suggesting that characteristics and quality of the immune response to infection (e.g., which cells are activated/recruited, the magnitude of the inflammatory response, and/or Th1 vs Th2 skewing) may be more important than early control of viral replication (250).

Ferrets

Initial studies on HNV disease in ferrets (*Mustela putorius furo*) were reported in 2009 (251, 252). HNV infection in ferrets is typically performed via oronasal or intranasal inoculation. Ferrets are very sensitive to HeV infection; regardless of route, doses as low as 10 $TCID_{50}$ result in 75–100% lethality (251, 253). The disease is characterized by fever (developing 4–7 days post-infection), depression, lack of grooming, hunched posture, respiratory signs (labored breathing), and/or neurological signs (ataxia, paralysis, generalized tremors, myoclonus, head tilt, seizures), with animals succumbing around a week post-infection (253). Although similar clinical signs and disease course are observed in ferrets infected with NiV, severe disease manifestations are seen less frequently than with HeV: 10 $TCID_{50}$ of NiV results in 25% lethality (254).

As with other animal models, HNV infection leads to widespread viral dissemination and replication. The presence of replicating HNV and/or viral antigen has been reported in syncytial cells of small blood vessels and highly vascular tissues, including the lung, kidney, spleen, and brain. Low-level virus shedding has been detected via both pharyngeal and rectal swabs (225). Recapitulating features of human disease, systemic vasculitis involving the pulmonary system and CNS has been reported in both HeV-infected and NiV-infected ferrets (251, 253).

Hamsters

Multiple studies have investigated HNV clinical course and pathogenesis in hamsters and have characterized clinical analytes (255) and serology (256) of these animals. Hamster studies have also employed reverse-genetics approaches to generate recombinant virus-expressing reporter proteins (e.g., luciferase or *Zoanthus* spp. green fluorescent protein [ZsGreen1]) for use in advanced imaging to examine infectivity, pathogenicity, and real-time viral dissemination, and to correlate clinical signs with anatomical localization of fluorescence at the study end points (257–260).

The first hamster model of NiV disease was reported by Wong et al. (261) in 2003. Syrian (golden) hamsters (*Mesocricetus auratus*) infected intranasally or intraperitoneally with NiV-Malaysia succumbed to infection within 9–29 days or 5–9 days, respectively (261). At the terminal stage of infection, virus and/or vRNA could be recovered from most organs (both vascular and extravascular tissues) and urine, but not from serum, consistent with intermittent or absent detection of virus in blood and sera of human cases. Lesions were reported in multiple tissues but were most severe and extensive

in the brain; vasculitis and multinucleated syncytia were detected in blood vessels. Subsequent studies have shown that NiV replication in hamsters is detectable early (≥8 hours post intranasal inoculation) in type I pneumocytes, bronchiolar respiratory epithelium, alveolar macrophages in the lung, and in respiratory and olfactory epithelial linings of nasal turbinates (262). In addition, associated with early infection of olfactory epithelium in nasal turbinates, visible NiV-infected neurons extending through the cribriform plate into the olfactory bulb have been reported, providing evidence for early axonal transport of NiV into the CNS in the absence of concurrent neurological signs (166). Viral spread appears to be rapid; within the first day of infection (at 16 hours), the virus disseminates to epithelial cells lining the larynx and trachea (262).

In 2009, HeV infection in Syrian hamsters was first reported; like with NiV, infection was widespread and involved both the respiratory and neuronal systems (263). While there is variation between reports, like ferrets, hamsters appear to be more sensitive to low-dose infection with HeV (263) than with NiV (261). Interestingly, replication patterns differ between NiV and HeV following intranasal infection; NiV initially replicates in the upper respiratory tract epithelium, whereas HeV initiates infection primarily in the interstitium (264). For both NiV and HeV, a dose-associated difference in clinical phenotype has been reported in hamsters; high-dose (10^5 $TCID_{50}$) intranasal infection with either virus results in acute respiratory distress, whereas low-dose (10^2 $TCID_{50}$) infection results in neurological signs and more systemic spread of the virus through involvement of the endothelium (264). However, subsequent studies report a broad spectrum of disease within experimental cohorts, even at relatively high doses, indicating that the relationship between inoculation dose and outcome is more complex (265).

Comparisons between NiV strains have been investigated in hamsters. In studies following respiratory exposure, NiV-Malaysia and NiV-Bangladesh both demonstrated similar endotheliotropism in small- and medium-caliber arteries and arterioles (but not in veins) in the lung, correlating with EFNB2 localization (266). Correspondingly, although a slower rate of spread was noted in hamsters infected with NiV-Bangladesh (262), the pattern of viral dissemination was similar for both strains, and they both resulted in comparable respiratory tract lesions (266). An isolate from Bangladesh was found to cause slightly more severe rhinitis and bronchointerstitial pneumonia 2 days after inoculation, but differences in lesion severity could no longer be detected 4 days after inoculation (266).

NiV-Malaysia has been suggested to result in more rapid and severe disease in hamsters: experimental intraperitoneal infection with NiV-Malaysia leads to accelerated viral replication, pathology, and death when compared to NiV-Bangladesh infection, and NiV-Malaysia infection was shown to activate host immune response genes at an earlier time point (267). These studies are relatively limited; more data are needed using various inoculation routes to support this conclusion and identify trends associated with experimental design.

Guinea pigs

HNV studies in guinea pigs are relatively limited and most studies to date have focused on HeV infection. Guinea pigs appear susceptible to disease after subcutaneous inoculation; however, signs may not be present in all animals. Early studies reported that a subset of guinea pigs infected subcutaneously with HeV succumbed to respiratory disease and exhibited gross lesions of pneumonia (236). In a later study, all guinea pigs inoculated subcutaneously with HeV developed disease; at necropsy, all guinea pigs were cyanosed and had congestion and edema in the gastrointestinal tract. Histologically, widespread vascular disease was observed in arteries and veins and in many organs, including the lung, kidney, spleen, lymph node, gastrointestinal tract, and skeletal and intercostal muscle, but no severe pulmonary edema was detected, unlike in horses and cats (234). Similarly, in a study of 18 pregnant guinea pigs inoculated with HeV mid-gestation, half (9 of 18) of the animals developed the disease as confirmed by

viral isolation, histopathology, and IHC. These studies demonstrated vertical transmission of HeV from the dam to fetus. In the placentas of five of the nine clinically affected guinea pigs, necrosis and strong positive immunostaining for HeV antigen were detected; one of these five animals aborted and HeV was isolated from its three fetuses, one of which also demonstrated positive immunostaining. In three other sick guinea pig dams, the virus was isolated from the fetuses, with positive immunostaining in two of them (56).

In 2001, a guinea pig model of HeV encephalitis was reported (268). Subcutaneous (but not intranasal or intradermal into the footpad) inoculation with HeV consistently produced disease in guinea pigs. Most (14 of 15; 93%) of the animals developed vascular disease with positive HeV IHC labeling in a range of tissues. Interestingly, the authors used immunolabeling to report, for the first time, the presence of lesions, including syncytial cells, in the transitional epithelium of the bladder. Virus isolation from urine rather than from nasal, oral, rectal, or conjunctival swabs was consistent with previous epidemiological work in horses, indicating a limited possibility of transmission. In addition, experimental infection produced microscopic lesions of encephalitis in 8 of 15 (53%) guinea pigs, with positive immunolabeling in blood vessels and neurons, especially in the medulla, cerebellum, and thalamus. The virus was recovered from six of the eight (75%) encephalitic brains. Severe vascular degeneration in the centers of these encephalitic lesions and positive immunolabeling in the choroid plexus of another animal indicated that the virus entered the brain following virus-induced vascular injury and choroid plexus invasion.

NiV studies in guinea pigs are more limited. The first report primarily focused on experimental infection of bats but included a control group of eight guinea pigs that were inoculated intraperitoneally with a human isolate of NiV-Malaysia. Three of eight (38%) guinea pigs developed clinical signs 7–9 days post-inoculation (222). Follow-up studies performing histopathologic and IHC characterization of NiV infection in the guinea pig reported that intraperitoneal inoculation with NiV is primarily associated with encephalitis, recapitulating disease with notable resemblance to that in humans but with reduced pulmonary involvement (269).

Mice

The utility of wild-type mice as disease models of HNV infection appears limited. Early reports in juvenile BALB/c and Swiss Brown mice inoculated with HeV and NiV, respectively, indicated resistance to infection in mice (236, 261). To date, only one study has reported clinical signs in HNV-infected wild-type mice; 1 of 5 juvenile (8-week-old) and 5 of 5 aged (12-month-old) C57BL/6 mice developed the clinical disease after intranasal but not subcutaneous exposure to HeV, with aged mice reliably developing the encephalitic disease (270). Similar results were seen with HeV in intranasally inoculated BALB/c mice; 0 of 5 juvenile and 3 of 5 aged mice presented with clinical signs. Here, mice developed transient lower respiratory tract infection without progressing to viremia and systemic vasculitis which is commonly seen in other animal models. Viremia and systemic spread were not an important feature of infection, and viral antigens and lesions in the brain were largely confined to neuroanatomical sites associated with the afferent olfactory pathway. However, there are no reports of clinical signs in wild-type immunocompetent mice inoculated with NiV; a comparable study to that which observed signs of HeV in young adult and aged BALB/c and C57BL/6 mice reported subclinical infection following intranasal exposure to NiV-Malaysia or NiV-Bangladesh (271). vRNA was detected in the lung tissue of mice at euthanasia (21 days post-inoculation) along with a non-neutralizing antibody response. Serial euthanasia studies indicated that presence of this vRNA reflected an earlier self-limiting and subclinical lower respiratory tract infection. No evidence of viremia or infection of other organs, including the brain, was observed.

The varied clinical outcomes in wild-type mice inoculated with HeV compared to NiV were similarly observed by Edwards et al. (272). Following intranasal inoculation of

3-week-old BALB/cArc mice with HeV or NiV-Bangladesh, neurological disease developed in 2 of 5 HeV-infected mice, whereas NiV infection was restricted to the lungs (272). The two HeV-infected mice with clinical disease were euthanized on day 10 post-infection due to depression, isolation, increased respiration, and erratic hypersensitivity to stimuli; one of the animals also displayed piloerection and hunched posture. All other mice remained clinically normal for the duration of the study and were euthanized at the study's end (21 days post-inoculation). Weight loss and temperature changes were not observed other than expected minor daily fluctuations. To investigate molecular determinants of clinical outcome, HeV proteins were substituted into recombinant NiV viruses, but none of the attempted combinations changed the NiV disease phenotype (272).

The factors which confer resistance to disease in immunocompetent mice are not fully understood. Rodent cells express the ephrin B2 receptor and are generally permissive to infection, but the efficiency of virus replication in mouse (and rat) cells in culture was found to be markedly lower than in human and hamster cells, suggesting that factors other than receptors may be involved in the control of virus replication (273). A key breakthrough in mouse models was a report indicating that resistance to lethal virus infection in wild-type mice was elicited by type I IFN signaling (274). Mice lacking the type I IFN receptor (IFNAR-KO) were susceptible to both HeV and NiV. Intraperitoneally infected mice developed fatal encephalitis, with pathology and IHC features like those seen in humans. vRNA was found in the majority of analyzed organs, and sub-lethally infected animals developed virus-specific neutralizing antibodies. In addition, IFNAR-KO mice have been shown to support replication and transient infection with non-pathogenic CedV, whereas wild-type mice did not (275).

Beyond disease model development, mice have been used to investigate various aspects of infection. IFNAR, RIG-I-like receptor-associated mitochondrial antiviral signaling protein (MAVS), MyD88, and TLR-3 receptor (TLR3) knockout mice, and mice crossed to bear several deletions (including MyD88/TRIF, MyD88/MAVS, and MyD88/TRIF/MAVS knockouts) have been utilized to demonstrate the redundant but essential roles of both MAVS and MyD88 adaptors, but not TRIF, in the control of NiV infection in mice (276). A similar approach used C57BL/6 knockout strains (IFNAR, MyD88, and combinations of MyD88, TRIF, MAVS, and the cyclic guanosine monophosphate-adenosine monophosphate [cGAMP] synthase [cGAS]/stimulator of interferon genes [STING]) to support the role for STING in controlling NiV infection (277). A human lung xenograft mouse model has also been used to study the pathogenesis of NiV. NiV targeted the endothelium and respiratory epithelium in the human lung tissues, and infection resulted in the production of several cytokines and chemokines, including IL-6, IP-10, eotaxin, G-CSF, and GM-CSF (278).

Overall, mouse models of HNV infection may prove more useful than initially considered. Further model characterization and refinement as performed with the hamster model, with a focus on immunocompromised strains, could provide additional pre-clinical options with high utility due to low cost, reagent availability, ease of handling, and improved logistical considerations for BSL-4 studies, including housing and husbandry.

VACCINES

A HeV vaccine for horses (Equivac HeV) was licensed in Australia in 2012, 18 years after HeV was first identified (Fig. 3), representing the first commercialized vaccine against a BSL-4 agent (279, 280). Currently, no vaccines are approved for the prevention of NiV or HeV disease in humans. In June 2017, WHO released a Target Product Profile for NiV vaccines to inform vaccine development and provide a framework for evaluating the progress of vaccine candidates (Table 9) (281). Vaccine availability was prioritized for active immunization of at-risk persons (all age groups and populations at high risk of NiV disease) during an outbreak for the prevention of NiV disease, to be used in conjunction with other control measures to curtail or end an outbreak.

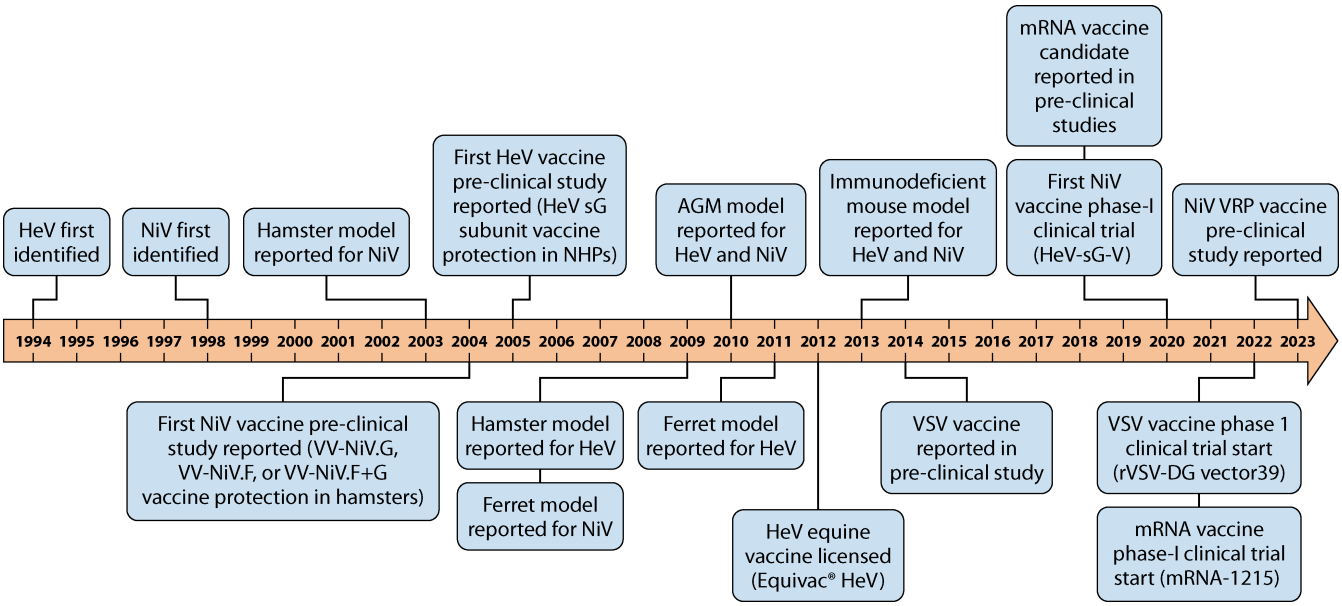

FIG 3 Timeline of henipavirus virus vaccine development and clinical trials.

The effectiveness of the vaccine depends on various factors, including the type of vector used for vaccination, the timing of the challenge after vaccination, and the route of vaccine administration. A critical aspect of vaccine development is defining the correlates of protection. Currently, no clear correlates of protection against NiV have been defined in either animal models or humans. Until recently, the prevalent assumption was that the presence of neutralizing antibodies is the gold standard correlate of protection for NiV vaccines. This assumption was largely based on the success of using neutralizing monoclonal antibodies (mAbs) as a therapeutic, and the survival of all vaccinated and challenged animals that had neutralizing antibodies at the time of challenge. However, recent studies point to additional mechanisms of protection. For example, vaccinated animals can survive virus challenges in the absence of detectable neutralizing antibodies. Even in animals that have neutralizing antibodies at times of challenge, the titers are often very low, raising the question of whether they truly contribute to protection (282–284). Immune responses to NiV can be multiple, and humoral and cellular immunity can contribute to protection. A longitudinal study of two survivors of the Kerala NiV outbreak has shown the induction of T-cell responses coinciding with viral clearance, which is followed by the development of strong and long-lasting neutralizing antibody responses (134).

TABLE 9 Summary of WHO target product profile for NiV vaccines (2017) for use as rapid deployment in outbreak settings

Characteristic	Preferred	Minimal
Efficacy in preventing disease	≥90%	≥70%
Onset of protection	<2 weeks after first dose	≤2 weeks after final dose
Dosing regimen	Single-dose series	Requires no more than two doses, with some level of protection conferred after initial dose
Durability	Lasting immunity for ≥1 year	Immunity for at least 6 months.
Stability	Shelf life of 5 years when stored at 2–8°C (long-term viability)	Shelf life of ≥ 12 months at −20°C, with demonstrated stability of ≥ 1 month at 2–8°C (suitability for field delivery in remote areas)

Currently, multiple NiV vaccine candidates are in various stages of development, with most still in pre-clinical phases and only a few progressing to clinical trials. These vaccine candidates use a range of vaccine platforms, all of which incorporate the viral glycoproteins F and/or G as the primary NiV antigens. NiV vaccine clinical trials are currently underway for four candidates, including (i) subunit sG HeV vaccine (ClinicalTrials.gov: NCT04199169) (ii); rVSV-DG vector39 (ISRCTN87634044 ClinicalTrials.gov: NCT05178901) (iii); mRNA (ClinicalTrials.gov: NCT05398796); and (iv) ChAdOx1 NipahB vaccine from the Oxford Vaccine Group. The University of Oxford is utilizing the ChAdOx1 platform, the same viral vector vaccine platform that was used for the Oxford/AstraZeneca COVID-19 vaccine (clinical trial; ISRCTN87634044) (Table 10).

Viral vector vaccines

Several recombinant viral vector vaccine platforms expressing the NiV F or G glycoprotein have been evaluated as vaccine candidates. The majority are VSV based; three types have been evaluated with varying levels of replication competency (replication-incompetent, single-cycle replication, or replication-competent); all conferred protection after a single dose.

Replication-incompetent VSV pseudotypes in hamsters demonstrated that single-dose vaccination with VSV expressing either the NiV F or G glycoproteins protected hamsters from the lethal NiV challenge (285). VSV virions that express NiV F or G and are capable of a single round of replication have also demonstrated efficacy. A study of AGMs vaccinated with a single dose of recombinant VSV (rVSV) expressing NiV-Bangladesh strain glycoproteins (rVSV-ΔG-NiVBF or rVSV-ΔG-NiVBG) resulted in 100% efficacy against NiV-Bangladesh challenge; both vaccines induced strong neutralizing antibody responses (286). In addition, complete protection is seen in as little as a week in AGMs receiving rVSV-ΔG-NiVBG prior to NiV-Bangladesh challenge (282). Even when vaccinated animals were challenged on day 3, 67% of animals survived, despite no neutralizing antibodies detected at the time of challenge. Transcriptomic studies suggested activation of natural killer (NK) cells and cytotoxic T cells, indicating potential early protection mediated by non-neutralizing antibody mechanisms (282). Single-round replication rVSV-based vaccines can also confer long-lasting immunity against NiV disease. AGMs vaccinated with rVSV-ΔG-NiVBG 1 year before NiV challenge survived

TABLE 10 Nipah virus vaccines currently in pre-clinical and clinical trials[a]

Vaccine developer	Format	Clinical phase	Clinical trial status[b]	Funding
Auro Vaccines & PATH	HeV-sG-V recombinant subunit vaccine containing a portion of the HeV G glycoprotein	Phase 1 trial	Posted: 12/13/2019 Completed: 5/6/2022	CEPI
Moderna	mRNA-1215 mRNA vaccine encoding secreted prefusion-stabilized F component covalently linked to G monomer (pre-F/G)	Phase 1 trial	Start: 07/11/2022 Completion: 10/2024 (est.)	NIAID
Public Health Vaccines	rVSV-G recombinant VSV (rVSV) expressing NiV G protein	Phase 1 trial Phase 1 prime/boost trial	Start: 01/10/2022 Completed: 05/30/2023 Start: 02/2024 (est.) Completion: 12/2024 (est.)	CEPI
University of Tokyo	rMV-G recombinant measles virus (rMV) expressing NiV G protein	Pre-clinical	N/A	CEPI (discont.)
Janssen Vaccines & University of Oxford	ChAdOx1-NiV_B replication-deficient simian adenoviral vaccine vector expressing NiV G protein	Phase 1 trial	Recruiting start: 10/24/2023	CEPI
CDC, Viral Special Pathogens Branch	NiVΔF non-spreading viral replicon particle (VRP) lacking F gene	Pre-clinical	N/A	DARPA

[a]CEPI, Coalition for Epidemic Preparedness Innovations; DARPA, Defense Advanced Research Projects Agency; NIAID, National Institute of Allergy and Infectious Diseases, National Institutes of Health.
[b]N/A, not applicable (clinical trials not initiated to date).

with no clinical signs or evidence of NiV replication. The vaccine induced stable and robust humoral responses; all vaccinated animals exhibited low detectable neutralizing antibody levels and cellular responses (287).

Replication-competent VSV-NiVG or VSV-NiVF vaccine candidates have been developed that use the licensed VSV Ebola virus vaccine as a backbone, in which the VSV-G protein is replaced by Ebola virus glycoprotein and co-expresses NiV F or G (288–290). These vaccines induced total IgG and neutralizing antibodies and protected AGMs from lethal challenge. Vaccinated animals were protected even when neutralizing antibody titers were below the lower limit of detection, which means that either very low levels of neutralizing antibodies may be enough to provide protection or that total antibody level and perhaps cellular responses may be more important for protection than previously thought (283). A recombinant VSV that expresses NiV G protein is currently in phase 1 clinical trial supported by CEPI.

Other viral vector vaccines include those using vaccinia virus (291), poxvirus (292), canarypox (293), measles virus (294), Venezuelan equine encephalitis virus (295), rabies virus (296), Newcastle disease virus (297), adeno-associated virus (AAV) (298), and chimpanzee adenovirus (ChAd) (299). All the reported candidates (except for poxvirus, for which efficacy has not been reported to date) conferred complete protection against lethal challenge and/or elicited high titers of neutralizing antibodies. However, of these candidates, only the AAV- and ChAd-vectored vaccines conferred protection after a single dose. ChAdOx1 NiV vaccination has been also shown to protect against lethal NiV-Bangladesh infection in AGMs and induced almost sterilizing immunity. Neutralizing antibodies and specific T-cell responses were detected in all animals on the day of challenge (299). The vaccine is currently in phase 1 trial supported by CEPI.

Subunit vaccines

The first NiV vaccine to progress to clinical trials was a subunit vaccine candidate that used a purified, recombinant G glycoprotein from HeV, with the transmembrane domain removed to facilitate soluble G protein expression (sG). There is high sequence conservation between NiV and HeV G glycoproteins (83% amino acid homology) (4). Although vaccination with NiV G does not efficiently generate HeV cross-neutralizing antibodies (298), expression of HeV sG induces potent NiV cross-neutralizing antibodies (300). The efficacy of sG-based vaccines was first investigated in cat models of disease; the homologous efficacy of an sG vaccine against NiV was first evaluated (207), followed by heterologous protection against NiV when HeV sG was used (240). Although HeV-sG was first developed as a veterinary vaccine, the ability of HeV sG to protect against HeV (301) and NiV challenge warranted additional studies. Efficacy studies of HeV-sG vaccination for human use against NiV in AGM challenge studies indicated single-dose protection, with efficacy observed as early as 1 week post-immunization (302). These findings supported the potential use of the HeV sG subunit vaccine as an emergency countermeasure during NiV outbreaks. The vaccine is currently in phase one clinical trial supported by CEPI. In conjunction with sG, various adjuvant formulations have been tested, including aluminum +CpG (240, 303), CpG alone (304), and Quil A/DEAEdextran/Montanide (207). All formulations were 100% efficacious, eliciting neutralizing antibodies and providing complete protection to all vaccinated animals against lethal NiV challenge, with no signs of clinical disease.

mRNA vaccines

The mRNA vaccine platform has been widely pursued, particularly in recent times, due to its potential to allow rapid vaccine development against newly emerging pathogens. Pre-clinical data on mRNA vaccines for NiV were first reported in 2020; a single dose of an HeV soluble glycoprotein mRNA vaccine in liquid nanoparticles protected up to 70% of hamsters against a lethal intraperitoneal challenge with NiV-Malaysia (305). Immune responses were suboptimal, suggesting that improved protection may be conferred using a two-dose regimen. A recent mRNA NiV candidate vaccine was designed based

on a chimeric vaccine antigen that included both F and G proteins. Guided by protein structure studies, the F protein was stabilized in its pre-fusion trimeric conformation (pre-F) and covalently linked at the C-terminus to three G protein monomers. The resulting chimeric mRNA vaccine showed robust induction of both neutralizing antibody responses and cellular immune responses (306). This chimeric mRNA vaccine (mRNA-1215) is currently in phase 1 trials that began in July 2022 (307) supported by NIAID. In September 2023, Phylex BioSciences announced a NiV vaccine initiative with its nanoparticle mRNA vaccine technology (308). The Phylex BioSciences mRNA vaccine candidate encodes a nanoparticle displaying 60 copies of the antigen based upon the head domain of NiV G protein, applying the same technology used in its second-generation nanoparticle mRNA vaccine against the SARS-CoV-2 delta virus; this candidate has not begun clinical trials and no pre-clinical data have been reported to date.

Virus-like and replicon particle vaccines

A vaccine candidate comprised of an enveloped virus-like particle created by the co-expression of the NiV M, F, and G glycoproteins and adjuvanted in either aluminum hydroxide (Alhydrogel), monophosphoryl lipid A, or CpG has also been tested and was 100% protective in the hamster challenge model (309). The NiV replicon particle (VRP), NiVΔF, morphologically resembles authentic Nipah virions but encodes a genome lacking the F gene, which makes it replication-competent with regards to viral RNA transcription, genome replication, encapsidation, and budding, but incompetent in terms of cell-to-cell spread and to the generation of viable infectious particles (284). This vaccine approach combines the advantages of a live-attenuated virus platform expressing multiple viral antigens with a high safety profile due to the complete absence of the F gene. Using the hamster model and given intranasally as a single dose, NiVΔF confers protection against lethal disease even when given only 3 days before the challenge. Protection by this vaccine is not reliant on eliciting a neutralizing antibody response but appears to involve other antibody-mediated activities, including Fc effector functions (284).

Vaccine-targeting antigen-presenting cells

Recently, an approach targeting CD40 of antigen-presenting cells has been used to develop a vaccine that targets select epitopes of NiV-Bangladesh G, F, and N proteins. The CD40.NiV vaccine was shown to induce strong neutralizing immune responses towards NiV-Bangladesh, cross-neutralization against HeV, and protection in AGM pre-clinical models of disease (310).

TREATMENT AND THERAPEUTICS

Supportive care

Although a variety of treatments has been investigated pre-clinically and more are in development and screening, supportive care remains the mainstay of treatment for HNV infection and disease (311). Patients with respiratory distress receive non-invasive ventilation and intermittent positive pressure ventilation, as indicated. In certain outbreaks, most patients also received antibiotics to prevent secondary bacterial infection. Other treatments have included antiepileptics for patients with seizures and management of increased intracranial tension and brain edema with mannitol and dexamethasone. Patients in hypertensive crisis receive anti-hypertensives, and those in septic shock receive vasopressors and crystalloids (54).

Direct-acting and host-directed small molecule therapeutics

Although much work has been done to investigate direct-acting and host-directed small molecule therapeutics (summarized in Tables 11 and 12, respectively), no effective therapeutics for HNV infection are currently approved for use in humans. During the

TABLE 11 Overview of direct-acting small molecule therapeutics and their status[a]

HNV target	ID	Cell-based assay tested	*In vivo* model (% survival)	FDA/clinical trial status	Reference
L	Remdesivir (GS-5734)	LV, mG, RdRp	NHP (100% [NiV])	Approved for SARS-CoV-2, phase 3 discontinued for Ebola virus	(106, 314–317)
L	V2043, V2053, V2067, V2068	LV	NT	Pre-clinical	(318, 319)
L	Favipiravir (T-705)	LV	H (100% [NiV])	Approved for IAV, SARS-CoV-2	(320)
L	4′-Azidocytidine (Balapiravir)	LV, RdRp	NT	Phase 2 discontinued for dengue & HCV	(106, 321)
L	2′-Mono-/di-fluoro 4′-azidocytidine	LV	NT	Exploratory	(322)
L	Lumicitabine (ALS-8112)	LV	NT	Phase 2 discontinued for RSV	(323)
L	4′-FIU (EIDD-2749)	mG, LV	NT	Pre-clinical	(324)
L	Galidesivir (BCX4430)	LV	NT	Phase 1 completed for Marburg virus	(325)
L, N	si-RNA	mG, LV	NT	Exploratory	(326)
F	VIKI-PEG$_4$-chol, VIKI-PEG$_4$-Toco	LV, FUS	H (40–100% [NiV]); NHP (33% [NiV])	Pre-clinical	(327–329)
F & G (RBP)	GRFT, 3mG, Q-GRFT	LV, PSE, FUS	H (30–40% [NiV])	Phase 1 completed (for HIV, SARS-CoV-2)	(330)
Viral lipid membrane	LJ001, JL103	LV, PSE	NT	Pre-clinical	(331, 332)

[a]F, fusion protein; FUS, fusion assay; G, glycoprotein; RBP, receptor binding protein; H, hamster; L, viral polymerase; LV, live virus; mG, mini-genome; HCV, hepatitis C virus; NHP, non-human primate; IAV, influenza A virus; NT, not tested; RdRp, RNA-dependent RNA polymerase assay; RSV, respiratory syncytial virus; PSE, pseudotype entry assay.

1998–1999 NiV-Malaysia outbreak, the purine analog ribavirin was administered to 140 patients. There were 45 deaths (32%) in the ribavirin arm and 29 deaths (54%) in the control arm consisting of 52 untreated patients, suggesting a 36% reduction in mortality (312). However, the true impact of treatment is unclear, as ribavirin allocation was not randomized and the treated patients may have received better overall care (313). In the 2018 outbreak in Kerala, ribavirin was empirically tried on 6 patients, 2 of whom survived;

TABLE 12 Overview of host-directed small molecule therapeutics and their status[a]

Host target	Therapeutic	Cell-based assay tested	*In vivo* model (% survival)	FDA/clinical trial status	Ref.
Inosine monophosphate dehydrogenase	Ribavirin[b]	LV	H (0% [NiV & HeV]); NHP (0% [HeV])	Approved for HCV	(241, 333, 334)
Orotidine monophosphate decarboxylase	6-azauridine (Azaribine)	LV	H (0%)	Approval withdrawn for psoriasis	(333)
Inosine monophosphate dehydrogenase	EICAR	LV	NT	Pre-clinical	(333)
Immune activator	Rintatolimod (Ampligen, Poly(I)-poly(C$_{12}$U))	LV	H (80% [NiV])	Phase 1, 2a, two for various cancers, COVID-19 in cancer patients, long COVID; phase 3 for chronic fatigue syndrome/myalgic encephalomyelitis	(333, 335)
Immune activator	KIN1400, KIN1408	LV	NT	Exploratory	(336)
Kinase inhibitor (PDK-1, PAK)	OSU-03012 (AR-12)	LV	NT	Exploratory	(337)
Endosomal acidification	Chloroquine	LV	H (0% [NiV & HeV]); Fe (0% [NiV])	Approved for treatment of malaria, autoimmune diseases	(252, 334, 338)
Proteosome inhibitor	Bortezomib	LV, B	NT	Approved for multiple myeloma	(339)
Membrane fusion blocker	25-hydroxycholesterol (25HC)	LV, FUS	NT	Exploratory	(340)
Glycosaminoglycan	Heparin	LV	H (20% [NiV])	Pre-clinical	(341)
Cathepsin B/L Inhibitors	Cath I, Cath LIII, CA-074Me	FUS	NT	Exploratory	(342, 343)

[a]B, budding assay; F, fusion protein; Fe, ferret; FUS, fusion assay; H, hamster; HCV, hepatitis C virus; LV, live virus; NT, not tested.
[b]Ribavirin has also shown direct acting antiviral activity *in vitro* at high concentrations.

all 6 patients who did not receive treatment succumbed to the disease. However, no statistically significant decrease in CFR was seen in this cohort, precluding clinical support for ribavirin based on these data (54). Additional therapeutics administered during the Kerala outbreak included intravenous acyclovir (54).

Ribavirin, alongside other inhibitors of inosine monophosphate dehydrogenase and/or orotidine monophosphate decarboxylase (e.g., 6-aza-uridine), has shown *in vitro* efficacy against NiV replication. However, when these two small molecules were tested alongside the IFN inducer poly(I)-poly(C (12)U) for antiviral efficacy against a lethal NiV challenge in the Syrian hamster model, both ribavirin and 6-aza-uridine could only delay but not prevent NiV-induced mortality. Poly(I)-poly(C (12)U), on the other hand, protected 5 out of 6 animals receiving a daily dose of 3 mg/kg of body weight from the day of infection to 10 days post-infection (333, 336, 337, 339–343). A similar observation was shown in studies evaluating ribavirin against HeV AGM. Neither pre- nor post-exposure treatments protected against HeV challenge, but 24 hours pre-treatment and 12 hours post-treatment regimens significantly delayed the time to death. The delay in time to death was likely due to the reduction of viral titers in the respiratory tract; however, the inability of ribavirin to cross the blood-brain barrier rendered the brain susceptible to infection, culminating in the onset of neurological symptoms in the AGMs (241). Additional challenge studies in hamsters and ferrets also showed that ribavirin did not prevent death after NiV or HeV infection (252, 334, 338). While different classes of innate immune modulators and other host-directed antivirals have shown antiviral activity *in vitro* (336, 337, 339–343), they have either not yet been tested *in vivo* or have shown little or no efficacy.

Based on prior work demonstrating the antiviral activity of 4′-modified nucleosides against positive- and negative-stranded RNA viruses (344, 345), the repurposing potential of such compounds against HNVs was explored. 4′-Azidocytidine (R1479) is the parental nucleoside of the prodrug balapiravir, which was evaluated in clinical trials against the hepatitis C virus and dengue virus. R1479 exhibited single-digit micromolar EC_{50} potencies *in vitro* against HeV, NiV, and a number of other related paramyxoviruses (321). A follow-up study demonstrated enhanced *in vitro* antiviral activity of 2′-monofluoro- and 2′-di-fluoro-modified analogs of R1479 against NiV and HeV (322). The third and final study in this series investigated the *in vitro* activity of β-D-4′-chloromethyl-2′-deoxy-2′-fluorocytidine (ALS-8112), the parent nucleoside of lumicitabine (lumicitabine has been evaluated in phase 1 and 2 clinical trials to treat pediatric and adult respiratory syncytial virus infection). ALS-8112 demonstrated potent *in vitro* activity against NiV and HeV, further affirming the potential of 4′-modified nucleosides as antiviral therapeutics.

Recently, orally bioavailable 4′-fluorouridine (4′-FlU; EIDD-2749) demonstrated broad-spectrum *in vitro* antiviral activity against respiratory syncytial virus (RSV), SARS-CoV-2, and multiple paramyxoviruses, including NiV and HeV (324, 346). This demonstrated *in vivo* efficacy of 4′-FlU against RSV, SARS-CoV-2, and influenza A viruses warrants further *in vitro* and *in vivo* efficacy studies of this compound against HNV (347).

Favipiravir (T-705), also known by its brand name Avigan, is a purine analog primarily used to treat severe non-seasonal influenza in Japan; it has garnered attention for its potential in treating other emerging zoonotic viral infections, including Ebola virus, Lassa virus, and SARS-CoV-2 (348). Favipiravir showed *in vitro* antiviral activity against NiV and HeV viruses and provided complete protection for hamsters when administered at the time of virus challenge either periorally twice daily for 14 days, or subcutaneously once daily for 13 days. The potential efficacy of post-exposure prophylactic favipiravir administration should be explored in future *in vivo* studies.

Remdesivir (RDV; GS-5734; Veklury), a broad-spectrum antiviral nucleotide prodrug that inhibits the RNA-dependent RNA polymerase (314, 318), was evaluated in AGMs when administered 24 hours post-inoculation with a lethal dose of NiV-Bangladesh. Treated animals developed mild respiratory symptoms, reduced appetite, and local virus replication, but no viremia was detected and all treated animals fully recovered (315). More recent studies support early initiation of RDV treatment. When administration

of treatment was delayed until 3 days post-infection in AGM challenge studies with NiV-Bangladesh, it conferred dose-dependent partial protection from severe disease but did not prevent clinical disease; surviving animals showed histologic lesions in the brain (316). Since the necessity for intravenous administration of RDV confines its utility to treatment in hospital settings (as observed for COVID-19 treatment), multiple investigators have sought to chemically optimize an orally available prodrug of the parent nucleoside GS-441524 (349, 350). *In vitro* studies evaluated orally available lipid-modified monophosphate prodrugs against HNVs and filoviruses, some of which showed equivalent and even improved antiviral activity over RDV in multiple primary-like cell types (318, 319). Most recently, an orally available prodrug of GS-441524 (Obeldesivir) was shown to prophylactically protect against Sudan ebolavirus in rhesus macaques (351). It remains to be seen whether obeldesivir can protect against NiV in the AGM model.

With the exception of HNV sequence-targeted small interfering RNA (326), other direct-acting non-nucleoside small molecule antivirals have targeted different aspects of viral entry. Griffithsin (GRFT), a high-mannose oligosaccharide dimeric binding lectin, has been shown to have *in vivo* broad-spectrum antiviral activity against SARS-CoV-1, HIV-1, hepatitis C virus, and Japanese encephalitis virus. GRFT and its synthetic trimeric tandemer (3mG) were evaluated against NiV; 3mG had comparatively greater potency than GRFT against NiV due to its enhanced ability to block NiV glycoprotein-induced syncytia formation. In studies evaluating *in vivo* prophylactic activity of an oxidation-resistant GRFT (Q-GRFT), the treatment showed significant protection against lethal NiV challenge in Syrian hamsters but did not prevent clinical disease (330). Paramyxovirus fusion (F) proteins share a common mechanism in which a conformational change is triggered via receptor engagement by their respective cognate receptor binding protein. A key step in inducing the fusion of viral and host membranes is the formation of the fusogenic 6-helix bundle. Peptides derived from the C-terminal heptad repeat regions (HRC) of paramyxovirus F proteins have been shown to interfere with their respective homotypic 6-helix bundle formation. Interestingly, the HRC of human parainfluenza 3 virus (hPIV-3) was shown to also inhibit NiV and HeV fusion (327). Sequence optimization and conjugation of the hPIV-3 HRC with a cholesterol moiety and a polyethylene glycol spacer (VIKI-PEG_4-chol) not only increased *in vitro* potency against NiV fusion by approximately 60-fold but was able to protect 40%–100% of lethally challenged Syrian hamsters (328). However, it only protected 33% of treated NHP (329). Finally, broad-spectrum membrane intercalating photosensitizing molecules have been shown to block cell fusion of many enveloped RNA viruses, including NiV and HeV (331). While certain classes of such molecules may have high *in vitro* potency, the prohibitive requirements for deep tissue penetration of light to consistently and systemically activate such compounds complicate the evaluation of *in vivo* efficacy.

Monoclonal antibodies

Exploring various epitopes of G- and F-specific antibodies and their characterization by crystallography have been key to the development of therapeutic antibodies. Neutralizing monoclonal antibodies (mAbs) specific for NiV-G or -F glycoproteins have been shown to protect hamsters, ferrets, and NHPs against lethal NiV infection (251, 263, 352, 353). The best-characterized and most clinically advanced mAb candidate to date is m102.4, a human mAb targeting the EFNB2/B3-binding site on NiV and HeV G glycoproteins (Table 13). Investigation of a near-identical analog m102.3 showed that the antibody occupies the same binding surfaces of HeV G typically accessed by EFNB2 (354).

m102.4 has been tested in NiV animal models for prophylactic and therapeutic use. Studies in ferrets evaluating m102.4 were the first successful post-exposure passive antibody therapy reported for NiV using a human monoclonal antibody. All ferrets that received m102.4 10 hours after a lethal oronasal NiV dose were protected from disease (251). Later studies in AGMs supported their efficacy against lethal NiV and HeV infection. Fourteen AGMs were challenged intratracheally with a lethal dose of HeV, and

TABLE 13 Overview of direct-acting antibody-based treatments for henipaviruses and their status[a]

HNV target	Antibody	Cell based assay tested	*In vivo* model (% survival)	FDA/clinical trial status	Reference
G (RBP)	m102.4	LV	Fe (100% [HeV]); NHP (100% [HeV, NiV])	Phase 1 completed	(251, 353–358)
G	HENV-26, HENV-32	LV	Fe (100%, 100% [NiV])	Pre-clinical	(359)
G	HENV-103, HENV-117, HENV 103 + 117	LV, PSE	H (40%, 40%, 100% [NiV])	Pre-clinical	(360)
G	Nip GIP 1.7, Nip 3B10	LV	H (75%, 100% [NiV])	Pre-clinical	(352)
G	NiV 41, NiV 41–6	LV, PSE	H (80–100% [NiV])	Pre-clinical	(361)
F	5B3, h5B3.1	LV, FUS	Fe (100% [NiV], 100% HeV])	Pre-clinical	(203, 208)
F	12B2, h12B2	LV, FUS, PSE	H (60% [NiV])	Pre-clinical	(204, 209)
F	1F5, h1F5	LV, FUS, PSE	H (100% [NiV]); NHP (100% [NiV])	Pre-clinical	(204, 209)
F	mAb92	LV, PSE	H (100% [NiV], 28% [HeV])	Pre-clinical	(362)
F	Nip GIP 35, Nip GIP 3	LV	HM (100%, 75% [NiV])	Pre-clinical	(352)

[a]F, fusion protein; Fe, ferret; FUS, fusion assay; G, glycoprotein; H, hamster; h, humanized; LV, live virus; NHP, non-human primate; NT, not tested; PSE, pseudotype entry assay.

12 subjects were infused twice with a 100 mg dose of m102.4 beginning 10, 24, or 72 hours after infection and again about 48 hours later. All animals that received m102.4 survived infection; animals in the 72-hour treatment group exhibited neurological signs of disease, but all started to recover by day 16 post-infection (355). Similarly, AGMs were challenged intratracheally with a lethal dose of NiV; treatment animals were infused twice with m102.4 (15 mg/kg) 1, 3, or 5 days after the viral challenge and again about 2 days later. All animals that received m102.4 survived infection, demonstrating successful treatment up to 5 days post-infection with NiV-Malaysia. Although half of the treated monkeys developed overt clinical signs (fever, respiratory, and neurological signs), all fully recovered (356). The window for successful treatment with m102.4 was only 3 days post-infection when AGMs were challenged with NiV-Bangladesh (353), suggesting that m102.4 may have utility as a post-exposure prophylactic or therapeutic in humans. In addition, m102.4 has been administered on an emergency basis as post-exposure prophylaxis to a handful of humans at high risk of exposure to NiV or HeV (279). None of the patients became ill, though whether the antibody prevented illness is unclear.

Aside from m102.4, additional monoclonal antibodies targeting the G glycoprotein (whether administered individually or as a cocktail) have shown post-exposure prophylactic protection in animal models (208, 359–361); whether these antibodies can improve upon the efficacy of m102.4 is unclear.

On the other hand, several studies evaluating the therapeutic potential of monoclonal antibodies targeting the F glycoprotein have demonstrated efficacy equivalent to or better than that of m102.4 in animal models (208, 209). Of note is h1F5, which outperformed m102.4 in a head-to-head comparison in which a single 25 mg/kg dose of either antibody was given to AGMs (six animals/treatment group) 5 days post-challenge with NiV-Bangladesh (the challenge was conducted via aerosolization). Whereas only 16% (1 of 6) AGMs were protected by m102.4, h1F5 not only provided complete protection from the lethal challenge but also markedly minimized clinical signs of illness; only three of six AGMs receiving h1F5 showed intermittent decreases in appetite throughout the study. Moreover, a lower dose of h1F5 (10 mg/kg) was still able to protect all three treated animals against lethal disease.

FUTURE OF VACCINE AND THERAPEUTIC DEVELOPMENT

Vaccine and therapeutic development for NiV has notably advanced in recent years. Currently, four vaccine candidates—HeV soluble glycoprotein vaccine (HeV-sG-V), rVSV-DG vector39 (PHV02), mRNA-1215, and ChAdOx1 NipahB—and one mAb (m102.4) have had a registered human clinical trial. All trials were phase 1, with dose-ranging trials taking place in the United States or Australia and enrolling healthy adults (363). However, a major limitation in the development of NiV vaccines and therapeutics is the practicality

of later-phase, large-scale vaccine efficacy trials in the field due to the relatively low number of annually reported NiV cases. This shortage of cases will complicate the potential evaluation of efficacy during phase 3 clinical trials. An assessment of the feasibility of NiV vaccine efficacy trials based on previous outbreaks in Bangladesh concluded that without a change in the epidemiology of NiV, ring vaccination or mass vaccination trials are unlikely to be completed within a reasonable time window (364). While the most advanced vaccine candidates have decidedly and understandably targeted the virus glycoprotein(s), characterizing the natural history of NiV disease in human patients as well as immune responses against multi-valent vaccines will likely provide key correlates of protection that also involve cell-mediated components of the immune response (155, 284). Given the sporadic and unpredictable nature of outbreaks, innovative strategies will be needed to circumvent the infeasibility of traditional phase 3 clinical trial regulatory pathways (365). Implementation of adaptive trial designs, early warning/disease surveillance systems, and the development of point-of-care rapid diagnostics can all contribute to minimizing logistical hurdles to vaccine/therapeutic administration and evaluation and should be considered wherever possible (189, 201, 366–368).

Licensure of vaccines and therapeutics may rely heavily on knowledge of regulatory pathways and the application of the animal rule, which allows for the extrapolation of vaccine efficacy from animal studies when human efficacy trials are not feasible. To address these challenges, extensive research is required to identify and clearly understand the correlates of protection during natural NiV infection. These studies will help in selecting the most promising vaccine candidates for licensure. In addition, the true burden of the disease remains unclear since the detection of cases occurs only at the advanced stages of illness when the patient presents at the hospital with acute encephalitis. We may be missing patients with milder disease or with respiratory symptoms. Increased efforts to support surveillance and monitoring of NiV outbreaks may be necessary to facilitate the evaluation of vaccine and therapeutic efficacy in real-world settings.

CONCLUSION

The identification of HNVs in association with severe lethal disease, as well as the recognition that transmission cycles involve complex interactions between wildlife and both agricultural and domestic species, has generated understandable concern. The pathophysiology of the disease is equally complex, involving many tissues and with the potential to develop into acute severe respiratory or neurological disease. Late-onset or recrudescent illness is also possible. Several key advancements have been made in the field since the initial discovery of HeV and NiV, including the identification of the natural virus reservoir and cellular receptor, the development of various diagnostic modalities, and the concurrent clinical advancement of promising therapeutic and vaccine candidates. However, gaps in knowledge remain, including the long-term consequences of infection (both asymptomatic and symptomatic), and how best to recapitulate and investigate the diverse clinical phenotypes manifested in human disease using pre-clinical models. The correlates of protection, critical to vaccine evaluation, are also unclear.

Efforts to accelerate the development of diagnostics, therapeutics, and vaccines were the focus of a development roadmap published by the WHO in 2019 (369). In 2024, an update to the roadmap was published detailing research priorities and identifying strategic goals and milestones focused on key achievements needed over the next 6 years for cross-cutting issues, diagnostics, therapeutics, and vaccines (370). As the field progresses to address known and new or emerging HNVs, the aim is to advance our understanding of disease and identify infection mitigation and treatment strategies. While monoclonal antibodies will likely contribute to any efficacious treatment regimen against HNVs, identifying and characterizing combinations of novel broad-spectrum, direct-acting, and host-directed small molecule therapeutics can potentially serve an

important complementary role in pandemic preparedness. The largest challenge to clinical advancement and approval of medical countermeasures will likely be limitations in late-stage clinical trials due to low case frequency. Relatedly, research and preparedness for these high-consequence, low-incidence viruses will always face challenges based on an exposure risk perceived as low compared to other public health threats. However, investment in efforts related to these agents is critical because increased incidence or changes, social or virological, facilitating more efficient spread could result in a devastating public health emergency requiring rapid deployment of diagnostics and countermeasures. Currently, this need is recognized by governmental agencies and other public, private, philanthropic, and civil society organizations aimed at preventing and treating emerging infectious diseases. Continued efforts and support in basic virological and clinical research, as well as investment and prioritization of vaccine and therapeutic development, will be key to future preparedness efforts.

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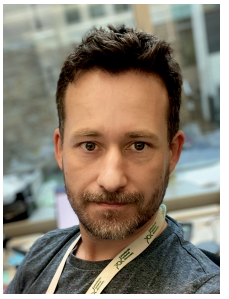
Michael K. Lo is a Microbiologist at the US Centers for Disease Control and Prevention (CDC) within the Viral Special Pathogens Branch. Michael earned his B.S. in Microbiology from the University of California, San Diego, received pre-doctoral training as an Emerging Infectious Disease Fellow at the Wadsworth Center before earning his Ph.D. in Immunology and Molecular Pathogenesis at Emory University, and completed his ASM postdoctoral training fellowship at the US Centers for Disease Control and Prevention (CDC) and has since then joined the Viral Special Pathogens Branch at CDC. The high pathogenicity and broad host species tropism of the Nipah virus piqued his interest and steered him toward characterizing the pathogenesis of the Nipah virus in various *in vitro* tissue models over the past 15 years. In the last 10 years, he has also developed and evaluated vaccines and therapeutics against Nipah and other lethal zoonotic viral pathogens requiring biosafety level-4 containment.



Stephen R. Welch earned his B.Sc. in Microbiology from the University of Plymouth, UK, his M.Sc. in Medical Virology from Liverpool John Moores University, UK, and his Ph.D. in Virology from the University of Glasgow, UK. He has worked with high-consequence biosafety level (BSL)-3 and BSL-4 pathogens for over 20 years, first as a Microbiologist within the Special Pathogens Research Unit at the current UK Health Security Agency (UKHSA) site at Porton Down, UK, before moving to the US Centers for Disease Control and Prevention (CDC) to work within the Viral Special Pathogens Branch. During this time, he has worked to evaluate medical countermeasures and develop suitable animal models for these pathogens, including the assessment of several Nipah vaccine platforms and antiviral candidates.

Christina F. Spiropoulou is the Deputy Branch Chief of the Viral Special Pathogens Branch (VSPB) at the US Centers for Disease Control and Prevention (CDC) and the Lead Scientist for the Molecular Pathogenesis and Therapeutics Team. For the past 30 years, Christina's research has primarily concentrated on high-consequence, low-incidence zoonotic RNA viruses, including Ebola, Lassa, Nipah, Crimean-Congo hemorrhagic fever, Rift Valley fever, and hantaviruses that cause fatal hemorrhagic fevers (VHFs) in humans. During her tenure at the CDC, she participated in the discovery of a number of emerging and reemerging viruses including the highly pathogenic New World hantaviruses and numerous VHF outbreaks. Currently, her team is focused on advancing prototype vaccine development and identifying antiviral targets and broad-spectrum therapeutics to combat VHFs.