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Review

Cedar virus biology and its applications as a surrogate for highly pathogenic henipaviruses

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ABSTRACT

Nipah Virus (NiV) and Hendra Virus (HeV), are the prototype species of the genus *Henipavirus* and are highly pathogenic agents capable of causing fatal diseases in both animals and humans. Both NiV and HeV are classified as biosafety level-4 (BSL-4) restricted pathogens and remain the only henipaviruses within the genus known to cause systemic, severe respiratory and encephalitic henipaviral disease, and represent substantial transboundary threats. There are no approved prophylactic or therapeutic treatments for human henipavirus infections, and the World Health Organization acknowledges them as priority pathogens needing urgent research. The discovery of Cedar virus (CedV), the only recognized non-pathogenic henipavirus, has provided a number of unique opportunities to study henipavirus and host interactions and also facilitate countermeasure development research at lower BSL-2 containment. This review will highlight the unique aspects of CedV biology and how it has been exploited as a model for developing therapeutic strategies against more virulent henipavirus species.

1. Introduction

The order *Mononegavirales* comprises the nonsegmented, negative-sense, single-stranded RNA (ssRNA) viruses (Broder & Wong, 2016) from families such as the Filoviridae [example Zaire ebolavirus], Rhabdoviridae [example Rabies virus] and Paramyxoviridae [example measles virus, mumps virus, Newcastle disease virus, human parainfluenza virus, and Sendai virus] (Latiff et al., 2004; Meng et al., 2014). Within the Paramyxoviridae family is the subfamily *Orthoparamyxovirinae*, which comprises the *Henipavirus* genus. The first recognized henipaviruses, Hendra virus (HeV) and Nipah virus (NiV), are highly pathogenic zoonotic agents that emerged in the mid and late 1990s in Australia and Malaysia respectively, and together are the prototype members of this genus (Eaton et al., 2006). The henipaviruses as a group have since received increased attention in recent years due to the discovery of several new henipaviruses, some with known or suggested zoonotic potential, together with the continued spillovers of HeV in Australia and NiV in South and Southeast Asia and their potential to cause severe and often deadly diseases in humans and animals (Kummer & Kranz, 2022; Li et al., 2023; Quarleri et al., 2022; Roman et al., 2022). Three other species of fruit bat-borne henipaviruses, distinguished based on the nucleic acid sequence, include Cedar virus (CedV), Ghana virus (GhV), and Angavokely virus (AngV) (Fig. 1) (Drexler et al., 2012; Madera et al., 2022; Marsh et al., 2012). The previously classified rodent-/shrew-borne henipaviruses, proposed as a new genus *Parahenipavirus* according to the International Committee on Taxonomy of Viruses (ICTV), include Mòjiāng virus (MojV), Langya virus (LayV), Gamak virus (GAKV), Daeryong virus (DARV), Melian virus (MeliV), and Denwin virus (DewV) (Fig. 1) (Lee et al., 2021; Vanmechelen et al., 2022; Wu et al., 2014; Zhang et al., 2022).

The rodent-borne henipaviruses, MojV was suspected to infect humans and shrew-borne LayV was associated with nonfatal febrile illnesses in humans and also infection of domestic animals (Wu et al., 2014; Zhang et al., 2022). However, HeV and NiV remain the only members associated with henipaviral disease, a systemic infection with severe respiratory and/or neurological pathogenesis, and infection transmitted via exposure to human or animal secretions or respiratory droplets (Luby & Broder, 2023; Halpin and Paul, 2015). Since 1994, outbreaks of HeV, which appears restricted to Australia, has occurred in horses 67 times resulting in 7 cases of human infections with 4 fatalities along with many

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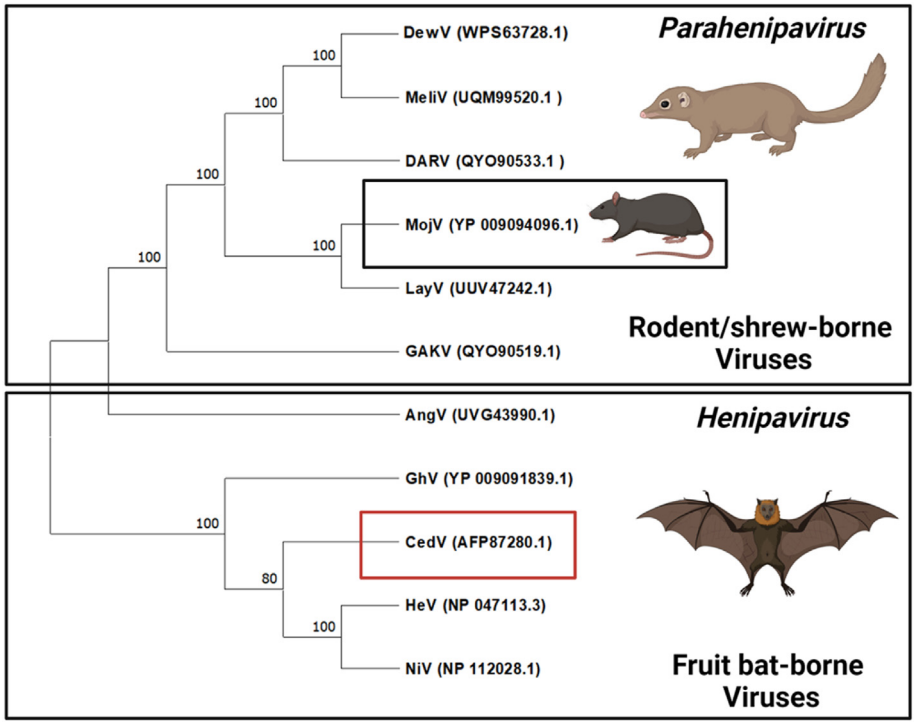

Fig. 1. Phylogenetic relationships of the 11 characterized henipaviruses based on amino acid sequences corresponding to putative L-proteins. Amino acid sequences were aligned using ClustalW and the phylogenetic tree was generated using the Neighbor-Joining method. The percentage of replicate trees where the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. GenBank accession numbers are indicated in parentheses. Evolutionary analyses were conducted using MEGA11. The proposed reclassification of a novel '*Parahenipavirus*' genus is indicated in boxes.

exposures and risk of infection (Playford et al., 2020; Queensland Government, 2023). Whereas NiV outbreaks have been seen in several countries since its first recognition in Malaysia, including Bangladesh, India, and the Philippines (Luby & Broder, 2023). While the total number of confirmed cases in these outbreaks has been less than 1000, the fatality rate for NiV infections can reach as high as 100%, depending on the specific circumstances and limitations of healthcare systems where these outbreaks occur (Enchery & Horvat, 2017). The geographical distribution of various *Pteropus* fruit bat species is extensive, contributing to the potential spread of the virus (Berge & Torheim, 2019; Enchery & Horvat, 2017), spanning across the Indo-Pacific territories in Southeast Asia and the western Pacific regions, this area encompasses approximately half of the global population (WHO, 2023a).

Both HeV and NiV have a unique broad species tropism that spans six mammalian orders posing significant transboundary threats to livestock and people and are classified as Biosafety level-4 (BSL-4) agents (Amaya & Broder, 2020). NiV and henipaviral disease (NiV and HeV) has been recognized by the World Health Organization (WHO) as an epidemic threat requiring urgent research and countermeasure development and is included in the WHO R&D Blueprint list of priority pathogens with epidemic potential (Sweileh, 2017; WHO, 2023b). During an outbreak, the use of widely accessible, highly sensitive rapid diagnostic tests (Koczula & Andrea, 2016), combined with effective preventive and non-pharmaceutical interventions can reduce the number of infections. In the specific case for HeV, a subunit vaccine called Equivac® HeV has been available for use in horses in Australia since 2012 for the prevention of HeV infection and thus reducing infection exposure risk to humans, a One-Health vaccine approach (Middleton et al., 2014). Nevertheless, there are no approved vaccines or effective antivirals against NiV or HeV infection for human use, and the development and global accessibility of vaccines, antivirals, or other medical interventions, particularly for populations at the highest risk of henipavirus infection, remains an urgent and unmet need (Roman et al., 2022).

CedV, the third member of the *Henipavirus* genus, was first isolated in 2009 from the pooled urine samples collected from flying fox (pteropid bats) population colonies in Queensland, Australia (Marsh et al., 2012), during a routine screening for novel henipaviruses. CedV caused distinct syncytial cytopathic effects in *Pteropus alecto* primary kidney cell monolayers, a common feature observed in various viral families, including emerging viruses and the Paramyxoviridae family (Chang & Dutch, 2012; Marsh et al., 2012). Phylogenetic analysis revealed that CedV is most closely related to HeV and NiV among all currently known viruses in this genus (Caruso & Edwards, 2023; Clayton et al., 2013; Marsh et al., 2012).

A significant challenge in researching and developing treatments for highly pathogenic viruses like NiV and HeV is the requirement for BSL-4 containment facilities. The isolation of CedV the only known non-pathogenic henipavirus species (Marsh et al., 2012) provided an opportunity to subsequently generate recombinant CedV (rCedV) and allow the study of an authentic henipavirus in BSL-2 facilities (Amaya et al., 2021; Laing et al., 2018, 2019).

This review will focus on the biology of CedV and its significance as an important tool for henipavirus experimentation and countermeasure development for use at low biocontainment (BSL-2) as an effective surrogate for authentic NiV and HeV.

2. Virion, genome organization and proteins

The CedV genome comprises an enveloped, single-stranded negative-sense RNA molecule of ~18,162 nucleotides (nt), with 6 sequentially arranged genes encoding 7 proteins (Fig. 2A). The relative gene order is conserved similar to those of prototype viruses in the Paramyxoviridae family, with the N (nucleoprotein) gene positioned first, followed by the P (phosphoprotein), M (matrix), F (transmembrane-anchored fusion), G (glycoprotein), and L (polymerase) genes in a 3′–5′ orientation (Broder & Wong, 2016). The surface glycoproteins F and G are integral components of the viral envelope, facilitating viral attachment, fusion, and entry into host cells. Despite a similar genome size, CedV exhibits a slightly increased coding capacity (~87%) when compared to HeV and NiV (~82% for each) but lower than the average of other Paramyxoviruses (92%). The increased coding capacity of CedV is primarily due to larger protein sizes, particularly the L protein which is 257 amino acids larger than HeV and NiV. At 2501 amino acids, the CedV L protein stands as the largest among all known viruses within the order *Mononegavirales* (Fig. 2B) (Marsh et al., 2012).

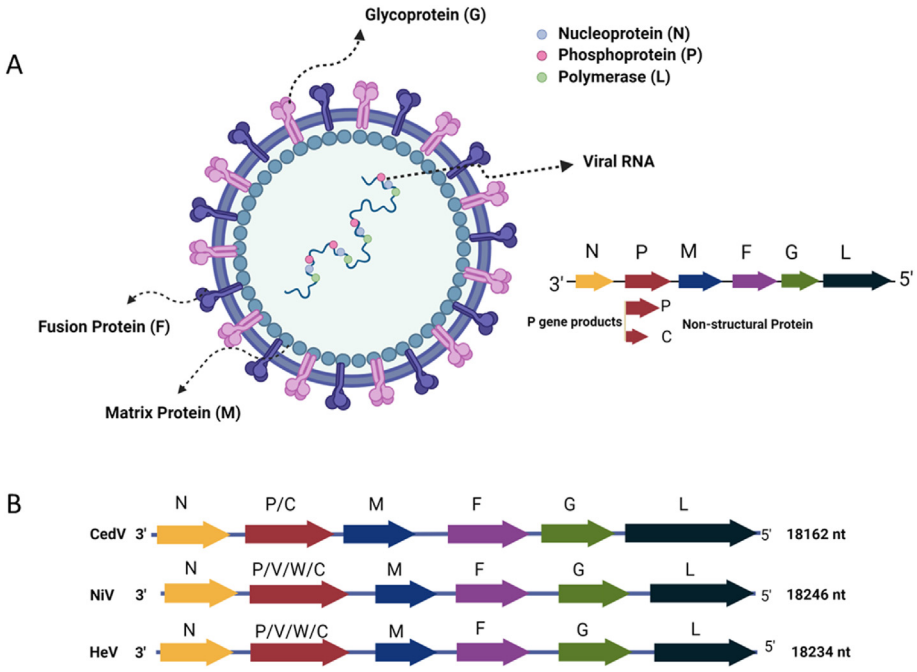

Fig. 2. Cedar virus (CedV) structure, viral genome organization and comparison of the genome sequence to those of HeV and NiV. (A) The N (nucleoprotein), P (phosphoprotein) and L (polymerase) proteins interact with the nonsegmented, single-stranded negative-sense viral RNA genome, forming the ribonucleoprotein (RNP) complex. The M (matrix) protein is involved in viral assembly, while the G (glycoprotein) and F (fusion) proteins are responsible for attachment to ephrin receptors and fusion, respectively. The C protein is produced from an alternative open reading framein the P gene. The six coded genes are flanked by a 3′ leader and a 5′ trailer region. **(B)** Comparison of genome organization to those of NiV and HeV. The HeV and NiV P gene encodes accessory proteins using an alternative start codon (C protein) or using mRNA editing (V and W proteins).

All members of the Paramyxoviridae family possess a unique P gene that undergoes RNA editing, resulting in the addition of non-template G residues, thereby generating 2 additional proteins, V and W, while the C protein is expressed from an alternate reading frame. These 3 nonstructural proteins are important for virulence and pathogenesis (Thomas et al., 1988; Yea et al., 2009). The products of the P gene can antagonize double-stranded RNA signaling and interferon (IFN) signaling (Bossart et al., 2013). However, a notable contrast between NiV and HeV in comparison to CedV lies in the absence of an RNA editing site and the coding capacity for the V and W proteins within the CedV P gene, potentially restricting in vitro virulence and pathogenesis (Marsh et al., 2012). The analysis of amino acid homology percentages across the genes of CedV revealed distinct patterns when compared to HeV and NiV (Table 1). Specifically, CedV N and M proteins exhibited the highest percentage identity with HeV and NiV. In contrast, CedV C protein demonstrated the lowest percentage identity among the analyzed genes. These differences in sequence identity provide insights into their evolutionary relationships and may impact viral replication and pathogenesis.

3. Cellular tropism and viral entry

Ephrin (Eph) receptors are members of the largest family of receptor tyrosine kinases important for cell adhesion, vascular development, cell migration and tissue-border maintenance (Darling & Lamb, 2019; Janes et al., 2020; Tuzi & Gullick, 1994). There are two subclasses of Ephrins, Eph-As (Eph-A1-10) and Eph-Bs (Eph-B1-6) that bind to ephrin ligands which are similarly classified as ephrin-As (ephrin-A1-5) and ephrin-Bs (ephrin-B1-3) (Frisen, 1999; Gale et al., 1996; Smith et al., 1997). This Eph-ephrin interaction between neighboring cells initiates contact-dependent bidirectional signaling, shaping cellular behavior and development (Darling & Lamb, 2019; Lisabeth et al., 2013). Ephrin-As are tethered to the membrane through a glycosylphosphatidylinositol (GPI) moiety, whereas ephrin-Bs are anchored by a transmembrane domain and possess a cytoplasmic tail with a PDZ binding motif, reviewed in (Kania & Klein, 2016).

Like other henipaviruses such as HeV and NiV, the membrane-anchored envelope glycoproteins (G and F) of CedV are pivotal in facilitating virus attachment and subsequent entry into the host cell (Wang et al., 2001). CedV G has a β-propeller structure, harboring the receptor-binding site that is similar to other henipaviruses and it exhibits regions that are structurally conserved with ephrin-tropic henipaviruses, suggesting a preserved mode of receptor recognition. The CedV G receptor binding site, is uniquely distinct from other henipaviruses, as evidenced by the lack of neutralization by the HeV/NiV cross-neutralizing human monoclonal antibody m102.4 that competitively inhibits HeV/NiV G ephrin-B2/B3 interactions (Laing et al., 2019).

Similar to HeV and NiV, CedV utilizes ephrin-B2 as an entry receptor (Laing et al., 2018, 2019; Marsh et al., 2012), despite CedV G sharing only 30% sequence similarity with HeV and NiV G (Laing et al., 2019) but not ephrin-B3 (Fig. 3A and B) (Bonaparte et al., 2005; Negrete et al., 2005; Bossart et al., 2009; Laing et al., 2019; Lee et al., 2021; Negrete et al., 2006; Zeltina et al., 2016). Moreover, CedV utilizes as entry receptors additional human ephrins, ephrin-B1, -A2, -A5 and murine ephrin-A1 (Fig. 3A) (Laing et al., 2019). The usage of murine ephrin-A1 (Fig. 3A) but not the human ephrin-A1 suggests species-specificity (Laing et al., 2019). Ephrin-B1 exhibits a dynamic expression pattern during embryogenesis (Compagni et al., 2003) while ephrin-B2 expression is prominent in arteries, arterioles and capillaries in multiple organs and tissues (Gale et al., 2001). Both ephrin-B1 and ephrin-B2 demonstrate high efficiency as cellular receptors compared to ephrin-A2 and ephrin-A5; however, the in vivo significance of A-class ephrin receptor

Table 1
A comparison of the nucleotide and amino acid percentage identities between the CedV genes (NC_025351.1) and those of NiV (NC_002728.1) and HeV (NC_001906.3). The complete genome sequences were aligned using MAFFT algorithm within Geneious Prime (version 2023 2.1). The resulting alignment data was used to determine the percentage identity for nucleotide sequences. Whereas the amino acid homology percentages were obtained using the protein BLAST function on NCBI/UniProt databases.

CedV Genes	NiV	HeV	NiV	HeV
	Nucleotide % identities		Amino acid % identities	
N	49.23	50.33	61.49	62.01
P	40.70	37.17	36.25	36.65
C	57.21	58.12	27.27	28.48
M	47.55	47.67	61.85	61.60
F	42.40	43.55	43.41	42.76
G	48.41	48.19	32.14	30.75
L	52.55	52.31	56.79	57.23

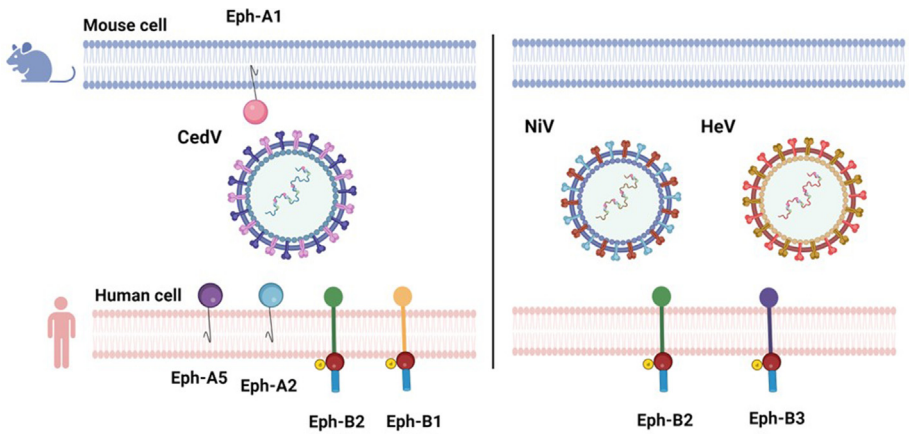

Fig. 3. CedV entry into host cells. (A) CedV can enter mouse cells through ephrin-A1 (upper left panel) and human cells through multiple ephrin receptors, including ephrins-B1, -B2, -A2, and -A5 (lower left panel). (B) NiV and HeV use ephrins-B2 and -B3 as entry receptors to infect host cells (lower right panel).

utilization remains uncertain, as their evidence is currently overshadowed by the dominant interaction with high-affinity ephrin-B1 and -B2 receptors (Laing et al., 2019). Structural studies revealed that the CedV G receptor binding site has only the first 3 receptor binding pockets, unlike HeV, NiV and GhV G proteins that each have 4 pockets each (Laing et al., 2019). The loss of the fourth pocket in CedV G is attributed to 2 amino acid changes: $W504_{HeV}/W514_{GhV}$ to $Y525_{CedV}$ and $L305_{HeV}/Y321_{GhV}$ to $D328_{CedV}$ (Laing et al., 2019). These modifications allow the accommodation of amino acids with larger side chains in the third pocket, thus providing a rationale for both CedV broad receptor tropism and the inability to use human ephrin-B3 or other ephrin-As (Laing et al., 2019). CedV broad receptor tropism highlights the diversity within the known henipavirus receptor repertoire, suggesting that only modest structural changes may be required to modulate receptor specificities within this group of lethal human pathogens (Laing et al., 2019).

4. Non-pathogenic nature of Cedar virus

The pathogenicity of HeV and NiV is attributed to the V and W proteins produced from the addition of nontemplate G nucleotides in the P gene sequence (Ciancanelli et al., 2009; Kulkarni et al., 2009; Lieu et al., 2015; Marsh et al., 2012; Schountz et al., 2019; Shaw, 2009; Shaw et al., 2004; Uchida et al., 2018). These virally encoded proteins typically function by inhibiting type I IFN production and/or IFN/STAT signaling, effectively countering the antiviral response (Randall & Goodbourn, 2008; Versteeg & García-Sastre, 2010). Additionally, studies have shown that NiV and HeV infections can suppress IFN-α and IFN-β gene expression in human cell lines, suggesting that complex cell-specific responses can occur during henipavirus infections (Virtue et al., 2011). Evasion of the host's primary antiviral response, particularly the IFN/STAT signaling pathway is essential to limit the antiviral response and initiate viral replication.

In contrast, CedV appears to be non-pathogenic in several animal models, including ferrets, guinea pigs, Syrian hamsters and Type I IFN Receptor Knock-out (IFNAR-KO) mice (Huaman et al., 2024; Marsh et al., 2012; Schountz et al., 2019). This phenotype is likely attributed to the lack of RNA editing ability and therefore production of the V and W proteins, resulting in the inability to counteract the IFN response (Marsh et al., 2012). Comparative studies have shown that CedV P targeting of STAT1 or STAT2 is compromised compared to HeV–P/V/W. While HeV P retained the capacity to bind to STAT1/2 and potentially inhibit IFN-α-induced STAT1 nuclear localization, CedV P showed compromised interference with STAT1/2 subcellular localization and *MxA* antiviral gene expression (Lieu et al., 2015). The compromised interaction of CedV with STAT1 and STAT2 could be attributed to the absence of several key glycine residues in CedV P that are highly conserved in the STAT binding domain of HeV/NiV P (Lieu et al., 2015). Additionally, CedV may encode other factors such as the C protein, which could inhibit IFN/STAT signaling.

Another factor contributing to the pathogenicity of both HeV and NiV, is their efficient binding to ephrin-B3, facilitating replication and leading to severe respiratory and neurological symptoms (Bonaparte et al., 2005; Negrete et al., 2006; Xu et al., 2012). Ephrin-B3 is primarily expressed in the central nervous system, particularly in the brain and spinal cord and is crucial for neural development and axon guidance and is also found in various peripheral tissues and developing embryos (Kullander et al., 2001). Furthermore, compared to NiV, CedV exhibits lower fusogenic activity, which may serve as an additional factor contributing to its non-pathogenicity (Yu et al., 2021). Syncytia formation (multi-nucleated giant cells) is the hallmark of henipaviral and paramyxoviral infection and appears to be directly correlated with the pathogenesis of HeV and NiV, as demonstrated by histopathological examination of NiV and HeV disease animal models (Li et al., 2010; Torres-Velez et al., 2008). However, further studies are required to demonstrate the quantitative impact of immune response versus fusogenic impacts on CedV pathogenicity (Yu et al., 2021). Although, the pathogenic impacts of other novel henipaviruses like GhV and MojV remain uncertain (Pernet et al., 2014; Wu et al., 2014), these viruses also appear to be less fusogenic compared to HeV and NiV (Rissanen et al., 2017). Future studies using CedV as a tool may determine whether in vitro fusogenicity could serve as an indicator to assess the pathogenic potential of henipaviruses. It is evident that the absence of V and W protein expression, coupled with the inability to engage with ephrin-B3 receptors and lower fusogenic activity are contributing factors to the non-pathogenicity of CedV. These aspects provide an opportunity to use CedV as an invaluable model for understanding the determinants of henipavirus pathogenicity (Laing et al., 2018; Marsh et al., 2012).

5. Immune responses to Cedar virus

The expression of several innate immune genes was examined in hamster primary endothelial cells infected with CedV or NiV. It was found that genes involved in the type I IFN response, such as IFNA7, DDX58, STAT1, STAT2, CCL5, CXCL10, ISG20, IRF7 and IIGP1 were significantly upregulated in CedV-infected cells at 48 h post-infection. In contrast, the expression of IFNA7 and IIGP1 was significantly suppressed during NiV infection (Schountz et al., 2019). In another study, the responses of *P. alecto* kidney cells (PaKi) and human (HeLa) cells to infection with HeV and CedV showed significantly different transcriptomic profiles for both viruses. CedV triggered a strong response in PaKi cells, indicated by a large number of differentially expressed genes

(DEGs) at 24 h post-infection. In contrast, HeV-infected HeLa cells showed relatively fewer DEGs. Immune-related proteins and pathways, such as pattern recognition receptors, IFNs, cytokines, apoptosis, NF-κB signaling and JAK–STAT signaling, were upregulated in both cell types following CedV infection (Chen et al., 2020). IFN responses upon infection to CedV and HeV in HeLa cells revealed comparable IFN-α induction but significantly higher IFN-β production in CedV and recombinant CedV (rCedV) infected cells (Marsh et al., 2012). In vitro replication kinetics of CedV and NiV in BHK-21 cells deficient in type I IFN (Nagai et al., 1981), both CedV and NiV showed similar viral kinetics. However, in type I IFN-competent hamster primary cells, CedV replication was reduced by up to 4 logs compared to NiV (Schountz et al., 2019). Analysis of antibody responses to rCedV-Luc in wild-type and IFN-KO mice showed significantly elevated levels of total IgG, IgG1, IgG2b, and IgG3 compared to controls. Furthermore, IFN-KO mice had significantly higher levels of rCedV-Luc neutralizing antibodies compared to controls. Some wild-type mice showed similar levels, but their neutralizing activity was lower and not significantly different from controls (Huaman et al., 2024). Overall, these studies suggest that differences in the P gene coding strategy between CedV and other henipaviruses may contribute to variations in host immune responses and pathogenesis.

6. Animal models of Cedar virus

Several well-established models of henipaviral infection have been developed and include ferrets, Syrian hamsters, guinea pigs, mice and non-human primates that exhibit varying responses to henipaviral infections (Bossart et al., 2009; Geisbert et al., 2010; Pallister et al., 2009; Rockx et al., 2010; Westbury et al., 1995; Williamson et al., 2000; Wong et al., 2003). The development of several animal models specifically for HeV and NiV pathogenesis and infection have been extensively reviewed in (Geisbert et al., 2012; Rockx, 2014; Dhondt & Horvat, 2013; Pigeaud et al., 2023; Wit & Vincent, 2015).

Animal models of CedV infection are developing and include ferrets, guinea pigs, hamsters and mice (Table 2). Each of these will be discussed below.

When ferrets and guinea pigs were exposed to CedV orally or intraperitoneally they remained clinically healthy, with detectable neutralizing antibodies between 10 and 21 days post-infection. Ferrets euthanized earlier, however, exhibited reactive lymphoid tissue hyperplasia, with CedV antigen detected in bronchial lymph nodes, indicating viral replication. Viral RNA was found in various lymphoid tissues, suggesting transient replication in the upper and lower respiratory tracts, although the viral genome was not recovered from nasal washes, oral swabs, pharynx, or lung tissues, and the virus was not isolated from all PCR-positive tissues (Marsh et al., 2012).

Hamsters inoculated with CedV via intraperitoneal (IP) or intranasal (IN) routes did not exhibit signs of disease during the 28-day study period and gross examination of organs was unremarkable. The lungs and spleens were found to have vRNA through day 14, except for day 8 IP-inoculated hamsters. No CedV was detected by day 28 for either inoculation route. Neutralizing antibodies appeared in most hamsters by day 8, with all IN-inoculated hamsters having antibodies by day 28, while only two IP-inoculated hamsters showed neutralizing antibodies at lower titers. This suggests that CedV is non-pathogenic in hamsters compared to NiV, which causes a fatal disease in Syrian hamsters that resembles human disease (Schountz et al., 2019).

BALB/c mice exposed orally to CedV showed no clinical signs of disease and neutralizing antibodies could not be detected by day 21 post-infection (Marsh et al., 2012). This is not surprising considering that immunocompetent mice are not a uniformly lethal model for henipavirus infection and do not produce clinical henipavirus disease, reviewed in (Pigeaud et al., 2023). It was observed that mice lacking the type I interferon receptor (IFN–KO mice) could be used to support the replication of various human viruses that do not typically replicate in wild-type mice. The IFN-KO mouse could potentially be a useful BSL-2 lethal henipavirus disease model considering the low cost of housing mice, reagent availability and the ease of handling (Pigeaud et al., 2023). As such, it was demonstrated that HeV and NiV could efficiently replicate and cause disease and death in IFN-KO mice (Dhondt et al., 2013). Recent studies utilizing rCedV-Luc in IFN-KO mice demonstrated semi-quantitative tracking of replication kinetics through whole-animal bioluminescence imaging. Robust replication of rCedV-Luc was observed for about a week in IFN-KO mice, compared to the minimal replication in WT mice. PCR-based quantification confirmed a strong correlation between bioluminescence and viral genome numbers. Although histopathological and serological analysis indicated infection, no clinical signs were evident in either WT or IFN-KO animals. IFN-KO mice generated significant levels of anti-CedV G IgG, effectively neutralizing the virus in vitro. Taken together, an IFN-KO rCedV mouse model could potentially be a useful BSL-2 tool for studying henipavirus replication dynamics and immune responses, and for initial validation of henipavirus-directed therapeutics (Huaman et al., 2024).

Table 2
Animal models of CedV.

Animal Model	Route of Inoculation	Viral Replication	Clinical Symptoms/ pathology	Reference
Ferrets	Oronasal	Transient replication in lymphoid tissues, CedV antigen in bronchial lymph nodes	Clinically healthy, reactive lymphoid tissue hyperplasia	Marsh et al. (2012)
Guinea Pigs	Intraperitoneal	Neutralizing antibody was detected in serum between 10 and 21 dpi	Clinically healthy	Marsh et al. (2012)
Hamsters	Intraperitoneal, Intranasal	vRNA in lungs and spleen through day 14 (except day 8 IP-inoculated), no detection by day 28	No signs of disease, unremarkable gross examination of organs	Schountz et al. (2019)
BALB/c Mice	Oronasal	No neutralizing antibodies detected, minimal replication	No clinical signs	Marsh et al. (2012)
Female B6 albino and male IFNAR-KO mice	Intraperitoneal	Robust replication for about a week in IFNAR-KO mice and minimal in wild-type mice	No clinical signs	Huaman et al. (2024)

7. Cedar virus for henipavirus antiviral development

NiV and HeV are classified as BSL-4 restricted pathogens, posing a substantial public health threat due to their epidemic or pandemic potential (Mehand et al., 2018; Sweileh, 2017). The WHO has prioritized henipaviral diseases and listed them among pathogens with epidemic potential (WHO, 2023b). Currently, there are no approved vaccines or antivirals for henipavirus infection in humans, highlighting the urgent need for research and development efforts. The only licensed subunit vaccine for henipavirus infection is Equivac® HeV (Zoetis), available in Australia since 2012 for use in horses for the control of HeV infection (Middleton et al., 2014). Following the completion of the priming schedule and annual boosters, this vaccine consistently elicits a robust neutralizing antibody response (Halpin et al., 2021). Although a human monoclonal antibody m102.4 has been demonstrated to be well tolerated and safe in phase 1 trials in Australia (Playford et al., 2020), developing

vaccines and therapeutics against henipaviruses for use in people remains a high priority.

The WHO is actively working on developing a target product profile for potential NiV therapeutics. The standard care for individuals infected with henipaviruses primarily involves intensive supportive therapy to manage severe respiratory and neurological complications (Roman et al., 2022). However, the development of substantive guidance protocols for both pre- and post-exposure prophylaxis against henipavirus encephalitis necessitates further evidence (Tan et al., 2002). It is worth noting that the potential therapeutic options against henipavirus encephalitis, predominantly consist of compounds with broad-spectrum antiviral activity. These compounds were not specifically developed as medical countermeasures against henipaviruses but rather target a wide range of RNA and DNA viruses. Ribavirin, a drug originally intended for hepatitis C, is currently the only known treatment with some evidence of effectiveness during a human outbreak of NiV in Malaysia from September 1998 to June 1999 (Chong et al., 2001). This treatment empirically reduced mortality by 36%. However, the reliability of this evidence is constrained due to the study's open-label design, the inclusion of data from control patients who either declined treatment or received standard care before Ribavirin was an option, and the simultaneous provision of intensive supportive care to patients (Banerjee et al., 2019). This underscores the need for more dedicated research and development efforts to identify and develop therapeutics specifically tailored to combat henipavirus infections.

A significant challenge in research and drug discovery for highly pathogenic henipaviruses, such as HeV and NiV is the stringent requirements of BSL-4 containment. However, the close phylogenetic relatedness of CedV to HeV and NiV allows for its utilization as a suitable surrogate for (pan-)henipavirus antiviral discovery. Several of CedV proteins show high amino acid identity to those of HeV and NiV (Table 1), with highly conserved domains particularly in the N, M and L proteins (Marsh et al., 2012). Although the L polymerase protein is 257-aa larger in comparison to HeV and NiV, it still shares a high amino acid identity to NiV and HeV L protein. Indeed, CedV L is ~56% aa identical overall to NiV-Bangladesh (NiV–B) and HeV L protein; similarities amongst important L domains are: catalytic domain ~59.5%; the mRNA capping domain ~64.7%; methyltransferase domain ~50.5% (NiV–B) and ~52% (HeV); C-terminal domain ~51% (NiV–B) and ~52% (HeV); and the 2-O-ribose methyltransferase domain ~60.8% (NiV–B) and ~61.7% (HeV). This overall high degree of protein identity shared between CedV, HeV and NiV suggests that other antiviral agents, in particular inhibitors of L polymerase, could be identified and potentially offer a pan-henipavirus countermeasure. The development of therapeutics targeting RNA-dependent RNA polymerase, as demonstrated in the discovery of treatments for other RNA viruses such as SARS-CoV-2 has been instrumental (Chen et al., 2022; Tian et al., 2021; Uppal et al., 2022).

Recombinant CedV generated using a reverse genetics approach, including those that express reporter proteins like green fluorescent protein (rCedV-GFP) or firefly luciferase (rCedV-Luc), as well as the G and F proteins from more pathogenic henipaviruses such as HeV and NiV–B provides an excellent platform to study henipavirus biology under BSL-2 conditions (Amaya et al., 2023; Amaya et al., 2021; Doyle et al., 2021; Laing et al., 2018). These recombinant viruses have been instrumental in advancing basic research and the development of potential antiviral compounds and monoclonal antibodies. For example, the utilization of rCedV-Luc in high-throughput screening assays to identify small molecule inhibitors of rCedV replication (Amaya et al., 2021). In this study, several compounds having robust antiviral activity against rCedV-Luc were validated in vitro using infectious HeV and NiV–B in BSL-4 (Amaya et al., 2021). Among these compounds, four out of eight that effectively inhibited rCedV-Luc replication were found to similarly inhibit the replication of authentic NiV–B. Furthermore, two out of these four compounds validated against NiV–B, exhibited antiviral activity against HeV, while demonstrating minimal toxicity (Amaya et al., 2021). In summary, the rCedV-based platform proves to be a strong tool for screening potential antiviral compounds against henipaviruses.

8. Closing remarks

In this review, we have presented an overview of the potential of CedV as a promising tool for research against highly pathogenic henipaviruses under BSL-2 containment. This unique perspective opens new doors for future investigations into antiviral treatments and the possibility of discovering a pan-henipaviral drug target. The ongoing work on CedV provides a valuable opportunity to further understand henipaviral biology and gain insights that may ultimately contribute to our understanding of and ability to combat these deadly pathogens.

CRediT authorship contribution statement

Ahmad Jawad Sabir: Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Lijun Rong:** Writing – review & editing, Supervision, Conceptualization. **Christopher C. Broder:** Writing – review & editing. **Moushimi Amaya:** Writing – review & editing.

Declaration of Competing interest

We have no conflicts of interest to disclose.

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