Hendra and Nipah viruses: different and dangerous

Bryan T. Eaton, Christopher C. Broder‡, Deborah Middleton* and Lin-Fa Wang**

Abstract | Hendra virus and Nipah virus are highly pathogenic paramyxoviruses that have recently emerged from flying foxes to cause serious disease outbreaks in humans and livestock in Australia, Malaysia, Singapore and Bangladesh. Their unique genetic constitution, high virulence and wide host range set them apart from other paramyxoviruses. These features led to their classification into the new genus Henipavirus within the family *Paramyxoviridae* and to their designation as Biosafety Level 4 pathogens. This review provides an overview of henipaviruses and the types of infection they cause, and describes how studies on the structure and function of henipavirus proteins expressed from cloned genes have provided insights into the unique biological properties of these emerging human pathogens.

1934 (REF. 1) 2005 (REFS 2,3). *Pteropus* [4] (FIG. 1).

1998, [5,6] *Pteropus* *Pteropus hypomelanus*[7]. *Rousettus* [8]. *Sturnira* [9].

The genus Henipavirus

Paramyxovirinae *Pneumovirinae* (BOX 1).

Paramyxovirinae (FIG. 2a,b), [10] (FIG. 2c).

3′ 5′ [11,12], [12,13]. 18,234 18,246 2,700 (15%) [12,13]. [14] [15] 300 >700 3′ [10 12] (FIG. 2c).

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doi:10.1038/nrmicro1323

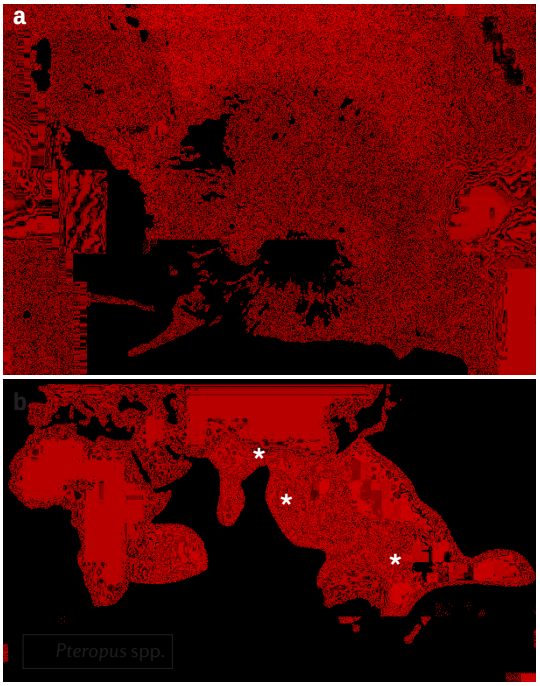


Figure 1 | **F. i g f , hei di ib j a d he ca j f di ea e b eak ca ed b He d a i a d Ni ah i . a** | *Pteropus poliocephalus* is an Australian flying fox and member of the family Pteropodidae, one of 18 bat families in the order Chiroptera. There are four *Pteropus* species in Australia[4]. **b** | Sixty-five *Pteropus* species are distributed from Madagascar through the Indian subcontinent to south-eastern Asia and Australia and as far east as the Cook Islands[4]. Some *Pteropus* species are among the largest of all bats, weighing as much as 1.2 kg and displaying a wing span of up to 1.7 m. *Pteropus* species are unique because they lack the complex neural and behavioural mechanisms required for echolocation that characterize the vast majority of bat species. Instead, they have large eyes and they navigate visually, feeding mainly on fruit and flowers, which they locate by smell. The sites of disease outbreaks caused by henipaviruses are indicated. Map modified with permission from REF. 4 © (2002) University of New South Wales Press.

( ), 100 200 [13,16].

p zoonotic 2004, 75% [17].

p j [18 23]. w

[24,25]. w

l l ( ) [26,27].

Biosafety Level 4 (4)

4 l

j

j

j

l (TIMELINE)

Henipavirus infections

l (BOX 2).

l [20,22,28 30].

[31].

j [22].

l [22].

l [32]. 25% [30,33].

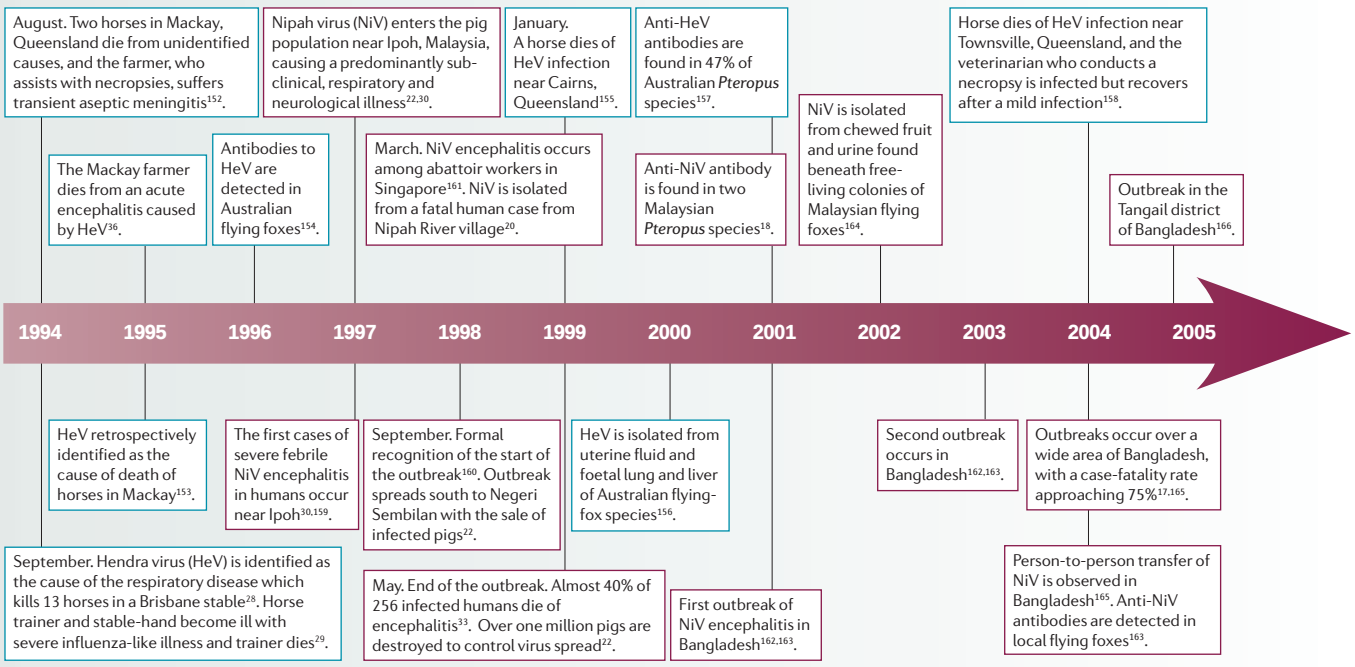
Zoonotic
A zoonotic infection is an infection of animals that can be transmitted to humans.

Biosafety Level 4
(BSL4). BSL4 is the highest safety rating for laboratories, used for handling agents that pose a high risk of life-threatening disease and for which there is no vaccine or therapy. Other BSL4 agents include Ebola virus and Marburg virus.

Molecular insights into henipavirus biology

The henipavirus G protein. *Paramyxoviridae*, *in vitro*

Timeline | **Emergence of henipaviruses**

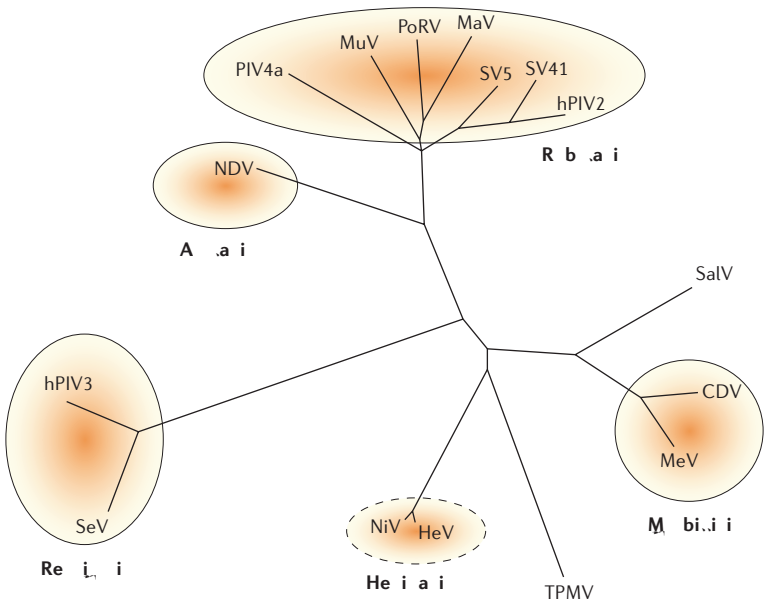


The emergence of Hendra virus and Nipah virus is detailed in boxes outlined in turquoise and purple, respectively.

Box 1 | Classification of henipaviruses

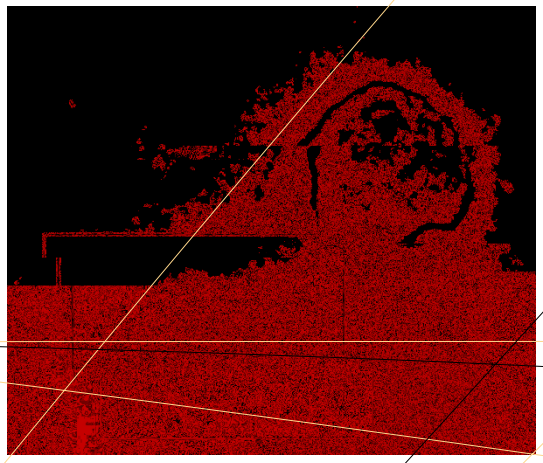
Viruses in the family *Paramyxoviridae* are classified in two subfamilies, *Paramyxovirinae* and *Pneumovirinae*. The latter subfamily contains two genera, Pneumovirus and Metapneumovirus. The number of genera in the *Paramyxovirinae* was increased in 2002 from three (Respirovirus, Morbillivirus and Rubulavirus) to five by the addition of two new genera, Avulavirus and Henipavirus[133]. The Avulavirus genus contains avian paramyxoviruses that were previously classified in the Rubulavirus genus, and the Henipavirus genus was created to accommodate Hendra virus and Nipah virus.

The phylogenetic tree shown here is based on an alignment of the deduced amino-acid sequence of the N gene of selected *Paramyxovirinae* subfamily members using the Neighbour-Joining method (see the genome organization of henipaviruses in FIG. 2). Viruses are grouped according to genus and abbreviated as follows. Morbillivirus genus: MeV (measles virus), CDV (canine distemper virus); Henipavirus genus: HeV (Hendra virus), NiV (Nipah virus); Respirovirus genus: SeV (Sendai virus), hPIV3 (human parainfluenza virus 3); Avulavirus genus: NDV (Newcastle disease virus); Rubulavirus genus: hPIV2 (human parainfluenza virus 2), MaV (Mapuera virus), MuV (mumps virus), PIV4a (parainfluenza virus 4a), PoRV (porcine rubulavirus), SV5 (simian parainfluenza virus 5), SV41 (simian parainfluenza virus 41); and unclassified viruses SalV (Salem virus) and TPMV (Tupaia paramyxovirus).



Type II membrane glycoproteins
Transmembrane glycoproteins with a cytoplasmic N terminus.

Fc region
The region of an antibody that is responsible for binding to antibody receptors (FcR) on cells and the C1q component of complement.



c

Clathrin
A structural protein that polymerizes into polyhedral lattices to form a membrane coat around vesicles involved in membrane transport in both the endocytic and biosynthetic pathways.

in vivo [73,74]. [75]. [11]. [75,76]. *in vitro* [11,38,75]. *trans*- [76]. [77,78]. 45- clathrin- [79]. (REFS 77,78).

[79] [76 78]. [80]. [81].

The henipavirus P gene products. [82 85]. -α -β. -γ, -α/β (FIG. 3) -β -α [84,86]. ( ) [87] [88]. (FIG. 4), [82 84].

Box 2 | **Henipavirus infection in flying foxes**

Despite the high prevalence of antibodies to henipaviruses, particularly in Australian pteropids, neither Hendra virus (HeV) nor Nipah virus (NiV) has been associated with any naturally occurring disease of flying foxes. The subclinical nature of HeV infection of pteropids has been confirmed by experimental infection of several species of Australian flying foxes[134,135].

A comparison of the pathology observed in henipavirus-infected chiropteran and terrestrial mammals provides some insights into the different clinical outcomes of infection. The predominant lesion in natural and experimental henipavirus infection of terrestrial animals, including humans, is systemic vasculitis, which affects smaller vessels in many organs, with clinical symptoms arising predominantly from infection of the lung and/or the central nervous system[21,136,137]. Viral antigen is detected in syncytial cells in vascular endothelium and, in the case of NiV infection, in bronchial and alveolar epithelium. Henipaviruses are readily recovered from nasopharyngeal secretions, urine and internal organs including lung and brain[21,138]. By contrast, infection of flying foxes with doses of HeV consistently shown to be lethal in horses generated only sporadic vasculitis in the lung, spleen, meninges, kidney and gastrointestinal tract, and only in a proportion of infected bats[134,135]. Viral antigen is detected in the tunica media rather than endothelial cells. In infected pregnant flying foxes, antigen is observed in similar locations and in the placenta[135].

Two observations might explain the lack of systemic disease in flying foxes. First, the presence of antigen in the tunica media rather than endothelial cells indicates that the latter might be spared from infection, therefore reducing the clinical effects associated with vasculitis. Second, the striking reduction in the level of antigen in flying foxes compared to horses and cats indicates that factors not found in terrestrial mammals that limit the ability of HeV to replicate could be at play in flying foxes. Indeed, after experimental infection of flying foxes with HeV, only half the animals show a rise in antibody titre, which is often low and sometimes of short duration (<3 weeks). Despite rigorous sampling regimes, virus has been isolated only infrequently, and where isolation was successful, positive sources included urine and the foetus, heart, placenta, kidney and spleen of two pregnant bats[134,135].

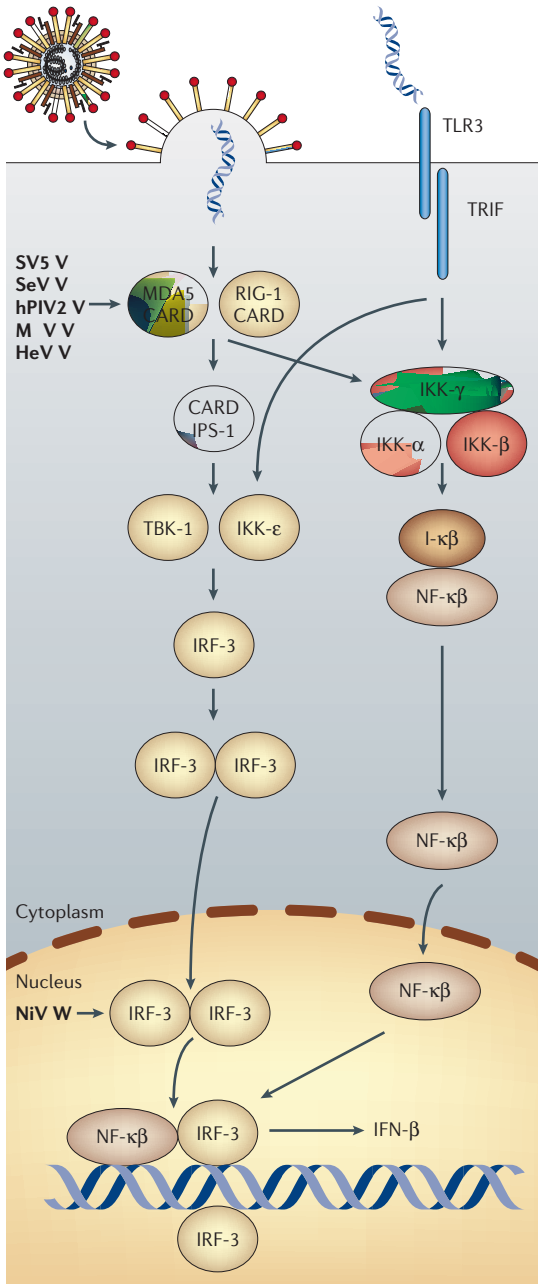
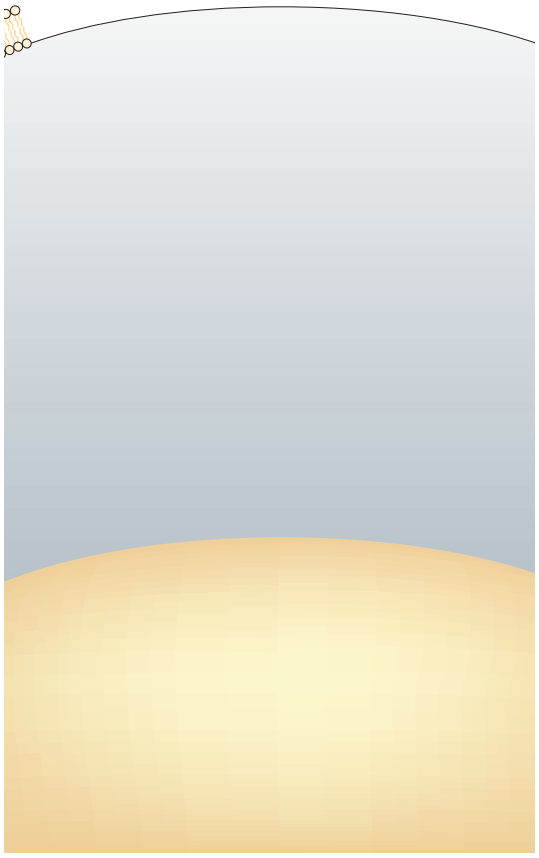


Figure 3 | **I e fe (IFN) i d c j d b.e- a ded (d)RNA ig a..i g.** The innate immune system depends on the ability of cells to detect the presence of unique, pathogen-specific molecules. The molecule considered most likely to be seen as foreign by virus-infected cells and activate the innate immune system is dsRNA, generated as a result of virus infection[87]. Several cellular sensors detect the dsRNA signal and respond by activating pre-existing transcription factors such as IFN-regulatory factor 3 (IRF-3) and the general transcription factor nuclear factor (NF)-κB[83,97,144–146]. Activated IRF-3 and NF-κB are redistributed to the nucleus, where they cooperate with other transcriptional activators to induce transcription of the interferon (IFN)-α/other 0 0 8.5 ron (IFN)-

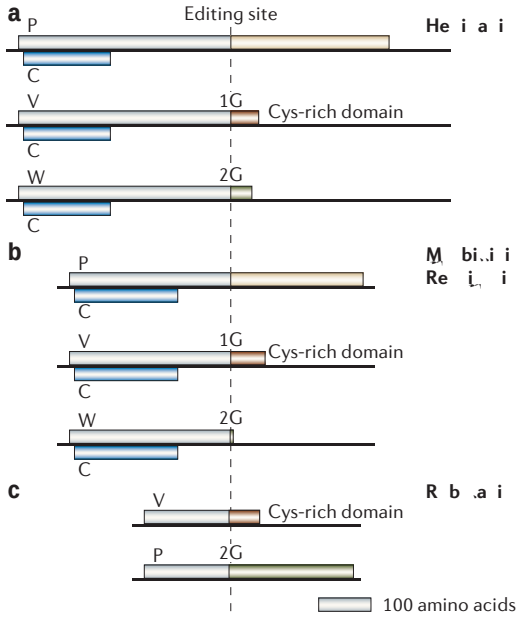
l [83,85,89,90]. l p 1 2 *Pneumovirinae* l [91,92], *Paramyxovirinae*. [93 96] [97 99]. l w

w l (BOX 3). p l

Inhibition of dsRNA signalling. [93 95,100,101], (FIG. 3). l 5 (*MDA5*), j [102].



Box 3 | **The henipavirus P gene**



The paramyxovirus P gene encodes several proteins by means of internal translation-initiation sites, overlapping reading frames and an unusual transcription process in which one or more non-templated G nucleotides are inserted at a conserved editing site, resulting in a shift of reading frame during translation[127]. The figure shows a schematic representation of mRNAs transcribed from the P gene of henipaviruses compared with those of morbilliviruses, respiroviruses and rubulaviruses. In henipaviruses (**a**) and respiroviruses and morbilliviruses (**b**), the unedited P-gene transcript encodes the P protein, and the V protein is generated by a separate transcript containing a single G nucleotide inserted at the editing site. Insertion of two G residues generates a transcript encoding a protein usually called W. V and W proteins share their amino termini with the P protein. Compared with morbilliviruses and rubulaviruses, henipaviruses have an N-terminal 100–200-amino-acid extension that might have evolved to better equip the viruses to antagonize the cellular interferon response (see text). The P, V and W proteins have unique C-terminal domains. In the P protein, this region is essential for viral RNA synthesis and contains sites for binding to the N and L proteins in ribonucleoproteins. The C-terminal domain of the V protein is highly conserved among paramyxoviruses and contains seven perfectly conserved cysteine residues. The C-terminal domain of the W protein is frequently short because of the presence of a stop codon soon after the editing site, but in henipaviruses the W-specific domain is 43 amino acids in length, compared with 55 for the V-protein C-terminal domain[10]. The P genes of henipaviruses, morbilliviruses and most respiroviruses contain a second short discrete overlapping reading frame upstream of the editing site, which in P, V and W mRNAs encodes the C protein. The structure of the P gene differs in rubulaviruses (**c**), where the primary transcript encodes the V protein, and transcripts with two G nucleotides inserted at the editing site generate the P protein. Note the long 3′ untranslated region of the henipavirus P gene RNAs.

in vivo. *in vivo.* *in vitro*

Conclusions

(REFS 131,132).

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Acknowledgements
We thank A. Hyatt, F. Filippi and I. Pritchard for help with figures. B.T.E. and L.F.W. were supported in part by the Australian Biosecurity Cooperative Research Centre for emerging infectious diseases and the NIH/NSF 'Ecology of Infectious Diseases' award from the John E. Fogarty International Center. C.C.B. was supported by NIH grants to the Middle Atlantic Regional Center of Excellence for Biodefense and Emerging Infectious Disease.

Competing interests statement
The authors declare no competing financial interests.

DATABASES
The f ... i g e i hi a ic.e a e.i ked ... i e ... :
Entrez: http://www.ncbi.nlm.nih.gov/Entrez
canine distemper virus | HeV | hPIV2 | hPIV3 | *MDA5* | MeV | mumps virus | Newcastle disease virus | NiV | NS1 | NS2 | rabies virus | respiratory syncytial virus | SeV | SV5 | SV41 | Tioman virus | Tupaia virus | vaccinia virus
UniProtKB: http://us.expasy.org/uniprot
cathepsin L | CD64 | ephrin B2 | IFN-β | IFN-γ | IL-6 | IRF-3 | SLAM | STAT1 | STAT2 | STAT3 | TLR3 | TYK2

FURTHER INFORMATION
Bryan T. Eaton, Deborah Middleton and Lin-Fa Wang's homepage:
http://www.csiro.au/aahl
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