Henipaviruses: bat-borne paramyxoviruses

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Found on every continent except Antarctica, bats are one of the most abundant, diverse and geographically widespread vertebrates globally, making up approximately 20% of all known extant mammal species[1,2]. Noted for being the only mammal with the ability of powered flight, bats constitute the order Chiroptera (from the Ancient Greek meaning 'hand wing'), which is further divided into two suborders: Megachiroptera known as megabats or flying foxes, and Microchiroptera comprising of echolocating microbats[1,3].

Known for their important role in the pollination of flora, seed dispersal and insect population control, bats also play a key role in the spread and perseverance of many notable zoonotic viruses which cause severe disease and potentially fatal outcomes for humans, livestock and many other species[1,4]. In particular, megabats belonging to the genus *Pteropus*, are important natural reservoirs for many significant pathogenic viruses such as Hendra virus (HeV), Nipah virus (NiV), SARS Coronavirus, Australian bat Lyssavirus and Menangle virus[4–9].

The emergence of Henipaviruses

HeV was first observed in 1994 during a severe respiratory disease outbreak in Brisbane, Australia, in which 13 of 21 infected horses died within a two week period[10]. Additionally, two humans who frequently had extensive contact with the affected horses were confirmed as infected, with one later dying as a result[10,11]. Since the emergence of HeV, sporadic outbreaks have occurred within the equine population of Australia, which have resulted in four human fatalities, as well as the death or euthanasia of over 84 horses and two dogs[12,13] (Table 1). In late 2012 an HeV vaccine, based on the G glycoprotein, was released for use in horses[14]. Since this time, all identified cases of HeV have been in unvaccinated horses.

Following initial HeV outbreaks, the closely related NiV emerged in 1998 amongst the pig and human population in Peninsular Malaysia. During the outbreak, 105 people died from 265 cases of infection with febrile illness and encephalitis[15]. The disease was also later recorded in Singapore, transmitted via the importation of infected swine from the outbreak region of Malaysia. Over 1 million pigs were culled in a large-scale effort to eradicate the causative pathogen – an effort that has proven successful in this region[11,16]. Three years later, disease with hallmarks of febrile neurological symptoms resulted in the death of 9 people in a Bangladeshi village. An investigation into subsequent outbreaks showed a reaction of antibodies to NiV antigens[17], however, unlike the

Table 1. Occurrence of Hendra virus disease events recorded in Australia, 1994–2016.

Year	Number of HeV events	Confirmed cases in horses	Confirmed cases in humans	Fatality rate in humans %
1994	2	22	3	66
1999	1	1	–	–
2004	2	2	1	0
2006	2	2	–	–
2007	2	2	–	–
2008	2	8	2	50
2009	2	6	1	50
2010	1	1	–	–
2011	18	23	–	–
2012	8	10	–	–
2013	7	7	–	–
2014	1	1	–	–
2015	1	1	–	–
2016	1	1	–	–

Malaysian NiV outbreak, pigs were not the intermediate and amplifying host. Instead, human-to-human and bat-to-human transmission routes were involved in NiV outbreaks occurring within the Bangladesh region – events not previously observed of the henipaviruses[11]. Furthermore, the more recent Nipah outbreaks demonstrated a notably higher fatality ratio of 74%, compared to 38.5% for the Malaysia outbreak[15,18].

During 2014, severe illness among humans and horses in southern Philippines was attributed to henipavirus infections[19]. Seventeen cases met the case definition with 2 survivors, of these seven had participated in horse slaughtering and horse meat consumption. Horse-to-human and human-to-human transmission occurred.

Additionally, although recovery from HeV and NiV infection is possible, relapsed encephalitis has been shown to occur in 3–7% of individuals anywhere from months to years following recovery from acute infection[20].

Bats as hosts to the Henipaviruses

Following the 1994 Brisbane HeV outbreak, a large serological survey of horses showed no evidence of HeV infection outside the index property[21], suggesting horses were not commonly infected with these viruses. An extensive sampling exercise of native and introduced animal species was carried out with antibodies to HeV being detected in all four mainland Australian flying fox species: the Black flying fox (*Pteropus alecto*), Grey-headed flying fox (*P. poliocephalus*), Spectacled flying fox (*P. conspicilla- tus*) and the Little red flying fox (*P. scapulatus*)[9,22]. Further research has resulted in isolation of HeV directly from pteropid bats[23,24].

Following the 1998 Malaysian NiV outbreak, investigations of bat colonies across a large area of Peninsular Malaysia revealed evidence of NiV antibodies in the two pteropid species found in Malaysia: the Island flying fox (*P. hypomelanus*) and the Malayan flying fox (*P. vampyrus*)[25,26]. NiV was also isolated from urine and partially eaten fruit collected from a colony of *P. hypomelanus* on Tioman Island, off the coast of Peninsular Malaysia[27].

Following the emergence of NiV in India and Bangladesh in 2001, *P. giganteus*, a flying fox found across the Indian subcontinent, was shown to have antibodies to NiV[17]. To date, no NiV isolate has been reported from bats in either Bangladesh or India.

Although disease associated with HeV and NiV has only been observed in Australia and South and South-east Asia, serological and molecular evidence of Henipavirus have been reported from many different countries, including Africa[28–31] and Asia[32]. This includes 2 full genome sequences that have been obtained, one from a bat in Ghana (Kumasi virus)[33] and the other from a Chinese rat (Mòjiāng virus)[34].

There is evidence that less pathogenic Henipaviruses may also be circulating in Australia, exemplified by the newly identified CedPV virus[35]. This was observed to share a similar genome size and organisation with HeV and NiV, showed cross-reactivity with henipavirus antigens, and also used the same host cell receptor for infection. Despite these similarities, disease was not observed with CedPV infection in various animal models in which a lethal disease results from HeV or NiV challenge. This is thought to be due to differences in its phosphoprotein gene in which it lacks RNA editing sequences and a highly conserved V open reading frame[35], whose protein product is responsible for modulating the host innate immune response[32].

The molecular biology of Henipaviruses

HeV, NiV and CedPV belong to the genus *Henipavirus* (order *Mononegavirales*, family *Paramyxoviridae*). HeV and NiV were the first observed zoonotic Paramyxoviruses resulting from spillover events of *Pteropid* bats[36]. High virulence, human susceptibility, broad host species range, and a lack of human vaccines and therapeutic treatment has resulted in HeV and NiV being restricted to biosafety level four (BSL4) containment – the only Paramyxoviruses to be restricted to this level[35,37,38]. The lack of a human vaccine and licensed therapeutics necessitates on-going research to understand these viruses and the virulence determinants.

Members of the *Paramyxoviridae* family are enveloped, nonsegmented negative-stranded RNA viruses which can cause a range of respiratory and systemic disease in both humans and animals. Paramyxovirus genomes share similar ultrastructural appearance, and organisation of genes contained within a genome which can range from 15–20 kilobases (kb), with henipaviruses having a genome size of 18.2 kb[39,40]. Henipavirus genomes encode six major proteins. Reading in order from 3' to 5' on the antigenome they are: nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), attachment glycoprotein (G), and RNA-dependent RNA polymerase (L)[39,41] (Figure 1).

Essential for the replication and transcription of paramyxoviruses, the genomic RNA is encapsidated by the N protein, the most abundant protein in the purified virion. The N protein associates with both the P and L proteins, forming the ribonucleoprotein (RNP) complex, and it is this complex that serves as a template for replication[42,43]. Following transcription and translation of viral proteins, the M protein mediates the association of the plasma membrane with the ribonucleoprotein and glycoproteins F and

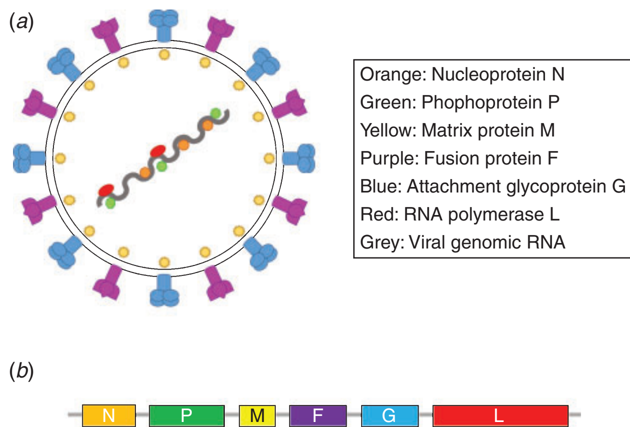

Figure 1. A simplified schematic of the henipavirus virion (*a*) and organisation of the henipavirus genome (*b*).

G. After assembly, the newly matured virion is then ready to bud and be released from the host cell[44].

The attachment glycoprotein (G glycoprotein/HN haemaglutinin-neuraminidase/H haemaglutinin; depending on the virus), is essential for the initiation of infection by recognising and attaching to membrane-bound host cell receptors. In the case of the henipaviruses, the G glycoprotein attaches to receptors ephrin B2 and B3[45–47]. The binding of the G protein to the ephrin host cell receptor facilitates the F protein-dependent fusion event, however the exact signalling cascade from the bound G glycoprotein to the fusion-competent F protein is not well understood[47]. Due to the enveloped nature of the paramyxoviruses, it is essential that fusion-competent F proteins generate membrane fusion between the virus envelope and the plasma membrane of the receptive host cell through a pathway of conformational changes in the viral envelope proteins[38].

Similar to other *Paramyxoviridae* genomes, the henipavirus genomes strictly follow the 'rule of six' which requires that the genome has a nucleotide length of a multiple of six (also known as a polyhexameric length). The importance of nucleotide numbers being a multiple of six is thought to be due to the nucleocapsid proteins interacting with precisely six nucleotides therefore forming a hexamer within the viral RNP complex[48,49].

Given the global zoonotic threat posed by these viruses, there is a great importance to further understand these viruses. However, given that these agents are amongst the most dangerous pathogens known, research methods have been developed to allow many of these agents to be handled outside of BSL4 containment. Reverse genetics has advanced the study of negative-sense RNA viruses, and is a commonly used tool for the *in vitro* study of negative-stranded RNA virus replication by allowing the manipulation of genes within the viral genome[50,51]. These systems rely on the transfection of 'helper' plasmids which each encode a gene required for viral replication and gene expression. The genes encoded on these expression vectors are present as cDNA, and are therefore not infectious[50,52,53].

Future perspectives

The infection of HeV and NiV in horses and pigs, respectively, demonstrate the potential threat of dangerous bat-borne zoonotic agents to livestock as the intermediate and amplifying host for transmission to humans. Factors contributing to the emergence of these viruses into the human population include deforestation, closer living proximities between humans and bats, and a greater yield demand and turnover of livestock farming practices. Considering the close human interaction with livestock within current farming practices globally, livestock interaction with the bat population is of high importance for both intervention and countermeasure strategies to prevent incidences of human infection. In addition to surveillance of both bat populations and any circulating zoonotic agents they may harbour, further study of these viruses on a molecular biology and immunology scale is needed. Broadening our understanding of key factors such as bat immunity, virus transmission, host response and pathogenesis, could make a significant contribution to the development of vaccines and therapeutic treatments for these highly pathogenic bat-borne infections.

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