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研究報告の概要

新しく確認された脳炎惹起性の人獣共通感染ウイルス３種（ヘンドラウイルス、オーストラリアコウモリリッサウイルス、ニパウイルス）について、その出現、生態、病原性、生物学的特徴を概観している。これらウイルスは過去 10 年間に Pteropus 属のフルーツコウモリから広まり、ヒト疾患の流行をもたらした。3 件のヘンドラウイルス大発生はすべてウマの感染に関連しており、ウマからヒトへの伝播が 2 度認められている。一般的に、ウマにおけるヘンドラウイルス感染症は肺水腫やうっ血を惹起させ、非化膿性脳炎も伴う。またヘンドラウイルスがネコからウマへ伝播した 1 例にも言及している。オーストラリアコウモリリッサウイルスに感染したヒト 2 例について記述しており、1 例は 5 週間前に Yellow-bellied Sheathtail Bat（Saccolaimus flaviventris、サシオコウモリの仲間）（1 頭）に引っかかれ、さらには咬まれた可能性もある患者に発症し、他の 1 例はフルーツコウモリ（1 頭）に咬まれた後、27 か月間の潜伏期間をおいて発症した患者である。1 例目の患者は四肢の進行性の衰弱および意識低下を来たし、その後びまん性の脳炎による大脳障害で死亡した。2 例目の患者は発熱、嘔吐、食欲減退、疼痛、喉の痛み、人工呼吸を要する麻痺に至る筋痙攣を来した。この女性患者は発症から 17 日後に死亡した。

報告企業の意見	今後の対応
新たに発見された人獣共通感染ウイルスであり、本剤の原材料であるヒト血液の採血時には、現時点では同ウイルス感染に対し特別な対応を行っていないが、本剤の供血国とは異なる国での発現のため、本剤に対する影響はないと考えている。また、本剤はその製造工程でウイルスに対する不活化処理を実施しているため、万が一混入があったとしても、感染の危険性は少ないと考えている。	今後も情報の収集に努める。

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使用上の注意記載状況
重要な基本的注意：
・患者への説明：本剤の使用にあたっては疾病の治療における本剤の必要性とともに、本剤の製造に際して感染症伝播を防止するための安全対策が講じられているが、血液を原料としていることに由来する感染症伝播のリスクを完全に排除することができないことを、患者に対して説明し、理解を得るよう努めること。
・ウイルス不活化処理を行っているが、肝炎ウイルス等の感染の可能性を完全に否定することはできないので、観察を十分行うこと
・血液分画製剤の現在の製造工程では、ヒトパルボウイルスＢ１９等のウイルスを完全に不活化・除去することが困難であるため、本剤の使用によりその感染の可能性を否定できないので、使用後の経過を十分に観察すること。

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Emerging encephalitogenic viruses: lyssaviruses and henipaviruses transmitted by frugivorous bats

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Summary. Three newly recognized encephalitogenic zoonotic viruses spread from fruit bats of the genus *Pteropus* (order *Chiroptera*, suborder *Megachiroptera*) have been recognised over the past decade. These are: Hendra virus, formerly named equine morbillivirus, which was responsible for an outbreak of disease in horses and humans in Brisbane, Australia, in 1994; Australian bat lyssavirus, the cause of a severe acute encephalitis, in 1996; and Nipah virus, the cause of a major outbreak of encephalitis and pulmonary disease in domestic pigs and people in peninsula Malaysia in 1999. Hendra and Nipah viruses have been shown to be the first two members of a new genus, *Henipavirus*, in the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, whereas Australian bat lyssavirus is closely related antigenically to classical rabies virus in the genus *Lyssavirus*, family *Rhabdoviridae*, although it can be distinguished on genetic grounds. Hendra and Nipah viruses have neurological and pneumonic tropisms. The first humans and equids with Hendra virus infections died from acute respiratory disease, whereas the second human patient died from an encephalitis. With Nipah virus, the predominant clinical syndrome in humans was encephalitic rather than respiratory, whereas in pigs, the infection was characterised by acute fever with respiratory involvement with or without neurological signs. Two human infections with Australian bat lyssavirus have been reported, the clinical signs of which were consistent with classical rabies infection and included a diffuse, non-suppurative encephalitis. Many important questions remain to be answered regarding modes of transmission, pathogenesis, and geographic range of these viruses.

Introduction

Three novel emergent viruses that can cause encephalitis or meningo-encephalomyelitis have been described over the past decade from frugivorous bats (flying foxes) in the genus *Pteropus* from Australasia and South-East Asia [45]. Two of

these, Hendra and Nipah viruses, are members of a new genus, *Henipavirus*, in the family *Paramyxoviridae*, and the third, Australian bat lyssavirus, is a recently recognized member of the genus *Lyssavirus* in the family *Rhabdoviridae*. Additionally, two non-encephalitogenic viruses have also been isolated from pteropid bats, Menangle and Tioman viruses, which are members of the genus *Rubulavirus* in the family *Paramyxoviridae*. This paper describes the emergence and biological characteristics of these viruses, and addresses some of the questions that remain to be answered about their ecology and pathogenesis.

Henipaviruses

Hendra virus

The first of the novel bat-borne viruses to be recognized was Hendra virus, formerly known as equine morbillivirus, which was the cause of a 1994 outbreak of acute respiratory disease in two humans and 21 thoroughbred race horses in Hendra, a suburb of Brisbane, Australia [52, 61]. In that outbreak, one human, the racehorse trainer, and 14 horses died, and a further 7 horses with subclinical infection were later euthanased. A number of recent reviews have described the isolation, ecology and molecular biology of Hendra virus [13, 16, 29, 34, 43, 66, 68]. The clinical features and human and equine autopsy findings of the original outbreak were consistent with interstitial pneumonia [51, 52, 61]. A virus was isolated from equine lung tissue and from human kidney tissue taken at autopsy, and was initially named equine morbillivirus on the basis of its structure and a weak one-way cross reaction with rinderpest virus.

A second small outbreak came to light about 12 months later, although it actually pre-dated the Hendra outbreak by more than a month. Two horses died (one of acute respiratory distress and the other with rapid-onset of neurological symptoms) of unknown cause in August, 1994, on a farm near Mackay, about 1000 km north of Brisbane. The farmer became ill with a mild meningitic illness after assisting in the necropsy of the dead horses, but subsequently recovered. About 13 months later, he became ill and died from a severe encephalitis, which was shown to have been caused by Hendra virus [54]. Retrospective investigations showed that the horses had died of Hendra virus infection [30, 59] and that the farmer had also been infected at the same time, indicating that the virus had presumably remained latent and reactivated 13 months later [54]. These were the first indications that Hendra virus could cause neurological disease in humans and horses. A third incident of equine infection occurred in Cairns in January 1999, when a single infected horse was identified [15, 31].

In efforts to find the source of the virus, an extensive serological investigation was undertaken of wild and domestic animals, and over 5000 animals were tested [51, 59, 70, 79]. Only fruit bats (flying foxes) of the genus *Pteropus* in the suborder *Megachiroptera* were found to be seropositive [78, 79]. At least some of all four members of the genus found in Australia (the black flying fox, *Pteropus alecto*; the grey-headed flying fox, *P. poliocephalus*; the little red flying fox, *P. scapulatus*; and the spectacled flying fox, *P. conspicillatus*) were shown to be seropositive.

Indeed approximately 47% of flying foxes sampled over their full geographic range have been found to have antibodies to Hendra virus, although variation in prevalence was found between species [16]. Hendra virus isolated from the uterine fluid and from foetal tissues from a euthanased flying fox [23, 24] was found to be indistinguishable from the equine and human isolates.

Molecular studies have clearly shown that Hendra virus is a member of the sub-family *Paramyxovirinae* within the family *Paramyxoviridae*, but that it differs significantly from members of other genera in the sub-family in a number of molecular and biological properties [66, 68]. Thus, sequence studies have shown that although the gene arrangement is very similar to viruses in the genera *Respirovirus* and *Morbillivirus*, the genome is much larger (18.2 kb), due partly to a larger P gene and partly to longer untranslated regions at the 3′ ends of the six transcription units [19, 23, 67, 69, 80, 81]. It was therefore suggested that Hendra virus should be the type species of a new genus in the family and that the new genus should be called *Henipavirus* [67]. Biological and other properties also distinguish Hendra virus from other paramyxoviruses. It was shown to have an unusual double fringe morphology comprising 15 nm and 18 nm projections [33, 34], a wide *in vitro* host range, and a predilection for endothelial cells [29].

A number of major questions about the ecology of Hendra virus are still to be addressed. The most important questions relate to fruit bats, the reservoir hosts of the virus, and the mode of transmission of the virus from bats to horses, as the basis for the genesis of further epidemic activity, and thus of possible management strategies. There is also an apparent predilection of Hendra virus for pregnancy, which may also be an important epidemiological guide in providing an indication of seasons of potential transmission. Natural infection in fruit bats appears to be asymptomatic, and virus has been isolated from the kidney [16] and is present in uterine fluids of pregnant animals [24]. These findings are borne out by experimental infection; most bats seroconverted, but no gross lesions were observed at necropsy, although systemic vascular lesions were observed, and there was positive staining for virus in the endothelial cells [76] and in the placentas of two bats, with virus isolated from the foetus of one [74]. All three occurrences of Hendra virus activity have been associated with infection in horses, and in two instances, transmission from horses to humans. Interestingly, no evidence of direct transmission from fruit bats to humans has been found, despite the close relationships often over many years between bat carers and sick, injured or orphan fruit bats [61] nor with the exception of the three human cases, among people who had variable levels of exposure to infected horses (such as veterinarians with necropsy contact) [49]. Furthermore, no cases of Hendra virus infection have been found in archival human tissue specimens from autopsies of people who had died of pneumonia or acute lung infection of unknown aetiology (C Allen, LA Selvey, JS Mackenzie, unpublished results).

A characteristic pathology has emerged for Hendra virus, regardless of differences between hosts, which is primarily vasotropic and/or neurotropic, generating interstitial pneumonia or encephalitis, with syncytial cells in vascular tissues and

with a pronounced endothelial tropism. Investigations of experimental infections in a range of animal species provide some indication of modes of transmission. Thus horses, cats and guinea pigs have been investigated. Horses generally develop severe pulmonary oedema and congestion. A characteristic change was the appearance of endothelial syncytial cells in the capillaries and arterioles in lung, lymph node, spleen, brain, heart and kidney. Interestingly, experimental infections differ from field infections in that the latter often display a frothy nasal discharge, sometimes blood-tinged, which fills the airways, whereas this has not been observed in experimental infections. This discharge has been suspected as a means of horse-to-horse transmission during the outbreak in Mackay [16]. Non-suppurative encephalitis was also observed in some horses [28, 76]. Virus has been isolated from various horse tissues, and also from urine and in one case, from the oral cavity. No transmission was observed between infected bats and in-contact horses and bats, or between infected horses and in-contact horses or cats [76]. The disease in experimentally-infected cats resembled that in horses and was characterised by generalised vascular disease with the most severe effects in the lung [27, 73]. Encephalitis has not been observed in cats [29]. Virus was isolated from various tissues and from blood and urine of infected cats. Transmission was observed from an infected cat to a horse [76]. Guinea pigs also developed a generalised vascular disease, but there was little pulmonary oedema, and congestion and oedema were seen more commonly in the gastrointestinal tract [27]. Encephalitis was seen in some guinea pigs after inoculation with high doses of virus [75], and thus they may provide a model system for exploring an encephalitic tropism. Virus can also be found in lesions in the bladder by immunostaining, and virus detected in the urine [75]. Infection of pregnant guinea pigs resulted in vascular disease in the placenta and virus infection of the foetuses [74].

The importance of pregnancy in providing a potential time of increased risk of transmission has not been adequately considered in management plans. Thus the virus was first isolated from fruit bat uterine fluid and subsequently from aborted foetuses; the index or initial horse in each of the outbreaks was pregnant; the season of each outbreak corresponded to the birthing period of the local fruit bat population [16]; the virus has been shown experimentally to infect the placenta in fruit bats and guinea pigs, and to invade the foetus. As pregnancy leads to immunosuppression, it may be that the virus is reactivated in pregnant bats, excreted in the urine, and transmitted to other bats during mutual grooming during which they use urine in the grooming process. Transmission from bats to horses must be a rare event; it may be due to infected urine contaminating pasture, or possibly through placental material and afterbirth dropping to the ground from 'birthing trees' in fields where horses graze.

Are other wild or domestic animal species at risk? No information is yet available about the susceptibility of sheep, goats, deer or pigs, all of which are potentially at risk of exposure. Similarly, there is no information on the susceptibility of indigenous species. Information of this nature is important in formulating management plans.

Nipah virus

Nipah virus first came to light as the causative agent of a major outbreak of disease in pigs and humans in Peninsula Malaysia between September 1998 and April 1999, which resulted in 265 human cases with 106 fatalities, and the eventual culling of about 1.1 million pigs [11, 12]. The emergence, ecology, clinical course, molecular biology and pathogenesis of Nipah virus have been reviewed recently [5, 16, 29, 34, 38, 64, 68, 77], and so will only be described briefly here. The outbreak began as sporadic cases of encephalitis among pig farmers in the Ipoh area in the state of Perak in late 1998. The outbreak was initially ascribed to Japanese encephalitis virus (JEV), and wide scale JEV immunisation was therefore undertaken. However, as pigs were also dying, which is not a characteristic of JEV infection, and cases of encephalitis were occurring in JEV-immunised individuals, it became apparent that the cause of the outbreak had to be another pathogen. The epidemic then spread through movement of infected animals to a second epicentre, about 160 miles south, in the state of Negri Sembilan in an intensive pig farming area, where the outbreak increased significantly in late February 1999, reaching a peak in late March and early April. The outbreak was contained by the slaughter of more than 1.1 million pigs. Almost all cases were in people in close contact with pigs, especially those associated with pig farming or involved in the transport or slaughter of pigs. There were 106 deaths among 265 reported cases of encephalitis, a mortality rate of nearly 40% [55]. The outbreak later spread to abattoir workers in Singapore, where pigs from Negri Sembilan had been sent for slaughter [56, 63]. A virus was first isolated from brain tissue of a fatal human case and found to be a novel agent closely related to Hendra virus, and thus the second member of the *Henipavirus* genus [11, 12]. The virus was called Nipah virus after Kampung Sungai Nipah (Nipah River Village) from where this first virus isolate had come [12, 39].

Most patients presented with a severe rapidly progressive encephalitis syndrome, although some also had significant pulmonary symptoms [18, 39, 56]. The main presenting features were fever, headache, myalgia, dizziness, drowsiness and vomiting [11, 18]. About half of the patients had a reduced level of consciousness and prominent brain-stem dysfunction. Indeed death was probably due to severe brain-stem involvement [18]. There was a significant association between the presence of virus in the cerebral spinal fluid and mortality [9]. The key pathological findings included systemic endothelial infection and cell damage accompanied by vasculitis with syncytia in affected vessels [29]. In a follow up 24 months after the outbreak, 7.5% of patients who survived the acute encephalitis had recurrent neurological disease or relapsed encephalitis, 3.4% of patients who initially had non-encephalitic disease or asymptomatic infections subsequently developed late onset encephalitis after an average interval of about 8.4 months, and 4 of the 22 relapsed or late onset encephalitis patients (18%) died, suggesting that both conditions were due to complications caused by persistent Nipah virus infection of the central nervous system [65]. There was no evidence of human-to-human transmission, even between close family contacts. Nevertheless, secretion of Nipah virus was reported from the upper respiratory tract and urine of some patients [7].

Epidemiological evidence suggested that the primary means of spread between farms and between regions was the movement of pigs. The primary mode of transmission on pig farms was believed to be via the respiratory route, which was subsequently confirmed by laboratory evidence [50]. Retrospective investigations suggest that Nipah virus has been responsible for disease in pigs in Peninsular Malaysia since late 1996, but was not recognised as a new syndrome because the clinical signs were not markedly different from those of several endemic diseases, and because morbidity and mortality were not remarkable [16]. The clinical disease in pigs could result in lesions in both the brain and lungs of individual pigs or in either organ independently. Importantly, the lesions were those of hyperplasia of the columnar epithelium, peribronchiolar and peribronchial infiltration of lymphocytes, and exudation of live and dead cells to the bronchial and bronchiolar lumens. Also, there were numerous alveolar macrophages, and often neutrophils, in a proteinaceous oedema fluid in the alveoli, bronchioles and bronchi [29]. Syncytial cells were seen in the small blood and lymphatic vessels, and possibly as multinucleated macrophages in the alveolar spaces. Thus, unlike Hendra or Nipah virus infection of humans, the infection in pigs targeted epithelial cells rather than endothelial cells. Of other species, a serological survey showed that many dogs from infected pig farms had antibodies to Nipah virus, but only two cases of disease in canids were detected, one in a moribund animal and the other in a dead animal. The former had clinical symptoms resembling those of canine distemper, histologically characterized as severe pulmonary oedema and interstitial pneumonia with numerous alveolar macrophages [29]. A single infected cat was found to have a generalised vasculitis, and experimentally, infection was similar to that observed in cats with Hendra virus, except that there was virus antigen in the respiratory epithelium and virus shedding in the oropharynx [50].

As with Hendra virus, a number of questions must be addressed, the most important of which relates to the transmissibility of the virus from pigs. Thus the most significant difference observed between Nipah virus infection of pigs and possibly cats compared to either Hendra or Nipah virus infections of most other species, was the epithelial tropism, resulting in virus excretion in the respiratory tract. This undoubtedly explains the contagious nature of the pig infection both to other pigs and to humans, and why there has been little evidence of transmission from or between other species in which the infection was largely endothelial [29]. Exceptions to this are the vascular transmission of Hendra virus through the placenta to the foetus in bats and guinea pigs, and the excretion of the two viruses in urine in bats and cats, and occasionally in horses infected with Hendra virus.

As is obvious for two viruses in the same genus, Nipah and Hendra viruses share many of the same properties, although with small and subtle differences, including morphological similarities [34], genomic sequence and organisational similarities [26, 68], and ecological similarities [16].

Questions still remain concerning the genesis of outbreaks, and the transmission from the wildlife reservoir to pigs. The close genetic and antigenic similarities between Nipah and Hendra viruses has led to the suggestion that bats, particularly pteropid bats (flying foxes), might also be the wildlife hosts of Nipah virus. This

was confirmed initially by a seroepidemiological study of bats, which found seropositive individuals among four species of fruit bats (*P. hypomelanus*, the island flying fox; *P. vampyrus*, the Malaysian flying fox; *Cynopterus brachyotis*, the lesser dog-faced fruit bat; and *Eonycteris spelaea*, the cave bat) and one species of insectivorous bat (*Scotophilus kuhli*, the house bat) [35]. Subsequently Nipah virus was isolated from urine samples collected from island fruit bats on Tioman Island and from partially eaten fruit [8]. The mechanism by which the virus passed from bats to pigs to initiate the outbreak still remains to be determined, but it is of major importance with respect to formulating management plans [44] (Field, Mackenzie and Daszak, this volume).

Are there similar or related viruses waiting to emerge elsewhere? There are about 60 species within the genus *Pteropus*, with a distribution that extends from the west Indian Ocean islands of Mauritius, Madagascar, Comoro, and Pembe, along the sub-Himalayan region of Pakistan and India, through southeast Asia, Philippines, Indonesia, New Guinea, southwest Pacific Islands as far east as the Cook Islands, and Australia, excluding Tasmania. They are not found on mainland Africa, Europe, Asia, North America or South America [13]. With the exception of the populations in the western Indian Ocean, the various pteropid species occur as a series of overlapping populations, and thus it might be suspected that other related henipaviruses would occur throughout the range [13, 16, 43]. Indeed, bats have been reported with antibodies to Hendra-like or Nipah-like viruses in Papua New Guinea [16] and Cambodia [53], and suspected outbreaks of disease associated with a Nipah-like virus have been recently reported twice from Bangladesh [58] (RF Breiman, ICDDR,B Centre for Health and Population Research, Dhaka, Bangladesh, personal communication) and Uttar Pradesh [37] and North Bengal, India. Interestingly, the outbreaks in Bangladesh and India had no apparent association with an animal source, although it is probable that epidemiological investigations may have been inadequate to demonstrate this, and in India may have involved human-to-human transmission. The major observations and findings from the latter outbreak still remain to be shared with the international community, and given its importance to our understanding of the biology, geographic range and risk factors associated with this novel group of viruses, it is hoped that this information will be published in the near future. Perhaps some of the most interesting future results might come from investigations of the pteropid bat populations of the western Indian Ocean, which are believed to have been separated from other pteropid species for many thousands of years; they might provide a valuable insight into the evolution of this new genus in the family *Paramyxoviridae*.

Two additional paramyxoviruses have been described recently from pteropid bats, Menangle and Tioman viruses [10, 57]. Menangle was the first of the two to be recognised, and shown to be the cause of an outbreak of reproductive disease and congenital malformations in a large piggery in New South Wales in 1997 [57]. There was a decrease in the farrowing percentages and a decline in the number of live piglets per litter. Mummified foetuses, stillborn piglets with arthrogryposis, craniofacial deformities and degeneration of the brain and spinal cord, were observed along with occasional abortions.[40, 57]. Menangle virus

also appeared able to infect humans, with two piggery workers becoming ill with an influenza-like illness and rash [4, 57]. Sero-epidemiological studies indicated that the virus came from pteropid bats, with neutralising antibodies found in sera sampled from animals at two colonies in the vicinity of the piggery [36, 57]. There was no evidence of infection in cattle, sheep, birds, rodents or feral cats, nor in a dog at the affected piggery [36]. Over 96% of the pigs in the affected piggery were found to have neutralising antibodies to the virus, and 88% of pigs at two other piggeries which had received animals from the affected piggery were also found to be sero-positive for the virus [36]. No serological cross-reactivity was detected between Menangle virus and other known paramyxoviruses, Molecular studies, however, showed that Menangle virus was a new member of the genus *Rubulavirus* [3], with only limited amino acid sequence homology to other members of the genus. A second virus, Tioman virus, was isolated in 1999 from urine collected from island flying foxes on Tioman Island, and shown to be closely related to Menangle virus serologically and genetically [6, 10]. It has not been associated with disease in animals or humans.

Australian bat lyssavirus

Australian bat lyssavirus (ABLV) was first reported in July 1996 from a black flying fox (*P. alecto*) from Ballina in northern New South Wales; the bat had displayed abnormal behaviour and had been euthanased [17]. The virus subsequently was shown to be closely related to classical rabies virus antigenically but distinct from it genetically [20]. Subsequent studies demonstrated that ABLV occurred in all four Australian species of flying-fox (black flying-fox, grey-headed flying-fox, little red flying-fox, and spectacled flying-fox) throughout their Australian range, and in at least one species of insectivorous bat in the suborder *Microchiroptera*, the yellow-bellied sheath-tailed bat (*Saccolaimus flaviventris*) [21, 32, 46] (HE Field, unpublished observations). The prevalence of ABLV in these species, and its possible occurrence in other microchiropterans, are not yet known. However, the prevalence in 366 sick, injured, or orphaned bats in southern Queensland, or in bats submitted for testing after biting or scratching individuals, or for other public health reasons, was 6–9% [46, 72]. The virus was found to be antigenically similar to classical rabies virus and therefore a member of Lyssavirus serotype 1, but was distinguishable on genetic sequence and was therefore ascribed to a new genotype 7 [20]. Results from the U.S. Centers for Disease Control and Prevention have indicated that rabies vaccine may elicit a protective immune response to ABLV [32, 41].

The first recognised human infection with ABLV was in November 1996 in a patient who had been scratched and possibly bitten 5 weeks earlier by a yellow-bellied sheathtail bat [1, 32, 60]. The patient developed progressive weakness in all limbs and eventually a depressed conscious state, and required ventilation. An electroencephalogram was consistent with a diffuse encephalitis. The patient exhibited progressive evidence of cerebral damage, and died [1]. A second case occurred in December 1998 after a 27-month incubation period following a bite

from a flying fox [25]. After a history of fever, vomiting, anorexia, pain about the left shoulder girdle and a sore throat, the patient's condition deteriorated with muscle spasms, and then paralysis requiring ventilation, and she died 17 days after onset. Thus both cases died following an encephalitis similar to that described for classical rabies.

The complete sequence of the ABLV isolate from the second human infection has been reported and, at 11,918 nucleotides, shown to be the smallest lyssavirus yet sequenced [71]. Further sequences have been reported for various parts of the genome [20–22], and a comparison of the sequence data from various isolates has shown that the isolates can be separated into two clades, based on the host of origin. Thus flying fox isolates can be distinguished from yellow-bellied sheathtail bat isolates, with nucleotide sequence divergence of up to about 20% and amino acid sequence divergence of 4–12% [21, 22, 71]. These analyses also provided additional evidence to distinguish ABLV from rabies on genetic grounds [22].

The disease caused by ABLV has been described in a captive juvenile black flying fox which was probably infected by its mother after birth [14], and between a wild black flying fox and a captive grey-headed flying fox housed in a captive colony of 23 animals [71]. The incubation period for the former was 6–9 weeks, and for the latter, about 4 weeks. In both cases, the disease began initially as an abnormal pattern of behaviour. The juvenile animal then exhibited dysphagia, and both animals exhibited progressive neurological symptoms to euthanasia or death. Experimental intramuscular infection of flying foxes with either ABLV or bat-associated rabies virus also has given some insight into the progression and outcomes of infection. Three of ten ABLV-infected animals and two of four rabies-infected animals developed abnormal clinical signs 15–24 days post-infection, and all five were found to have histological lesions and antigen in the central nervous system. None of the five animals developed anti-lyssavirus antibodies, although five of the seven ABLV-inoculated survivors and one of two rabies-inoculated survivors had seroconverted by 3 months after infection [48].

Murine studies have indicated that rabies vaccine offers protection against infection with ABLV [32, 41, 42], a finding that has had major positive public health implications. Pre-exposure vaccination with rabies vaccine is recommended for high-risk occupations such as wildlife workers and veterinarians. Post-exposure treatment with combined vaccine and rabies immune globulin is offered to anyone exposed to bats through bites or scratches unless ABLV infection can be ruled out in the bat. These management issues are discussed in an accompanying manuscript (Field, Mackenzie and Daszak, this volume).

The major questions still to be fully addressed are the incidence of ABLV in wild populations of flying foxes and yellow-bellied sheathtail bats, the possibility of transmission and adaptation of ABLV to terrestrial species, and the geographic range of ABLV outside Australia. Despite retrospective and progressive case investigation and passive surveillance, no evidence of ABLV infection has been found in domestic vertebrate species [47] or targeted wild mammalian species (HE Field, unpublished data). However, limited susceptibility studies to date have revealed that dogs and cats experimentally infected with ABLV do seroconvert

and, in some cases, show transient clinical signs of disease (KA McColl, CSIRO Australian Animal Health Laboratory, Geelong, personal communication). As with henipaviruses in pteropid bats, it would be expected that similar viruses might occur elsewhere in the geographic range of other members of this bat genus, but there has been no evidence of ABLV-like viruses in either Malaysia or Papua New Guinea. However, neutralising antibodies to an ABLV-like virus have been reported from bats collected in the Philippines [2].

Conclusions

Some important questions have yet to be addressed about the ecology, pathogenesis, and host range of the two henipaviruses and ABLV, and which undoubtedly will have significant implications for management practices if we are to minimise the risk of future outbreaks. Some of this information would not be difficult to generate, but could be essential to our understanding of transmission cycles and of species susceptibilities. Of particular importance is the knowledge of the domestic animal host ranges of the two henipaviruses, and of the potential for terrestrial transmission of ABLV. In addition, a better understanding of tissue tropisms and its genetic basis is crucial to determining potential contagiousness of the henipaviruses and the possibility of selection for increased transmissibility.

The emergence of three novel bat-borne viruses in Australia and Malaysia over the past 8 years and their associations with encephalitic and pneumonic diseases demonstrates the importance of wildlife as a source of disease emergence, and the need for continued vigilance through international surveillance programmes for the emergence of related viruses throughout the range of the pteropid bats. From recent experiences in Bangladesh and India, it appears that there might not always be an obvious zoonotic association with novel henipaviruses, and human-to-human transmission might be a major feature, which was not seen in either of the well-documented outbreaks described here. Thus, it is possible that the biological properties of presently unrecognized henipaviruses may show a number of differences from those described above.

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References

1. Allworth A, Murray K, Morgan J (1996) A case of encephalitis due to a lyssavirus recently identified in fruit bats. Commun Dis Intell 20: 504
2. Arguin PM, Murray-Lillibridge K, Miranda MEG, Smith JS, Calaor AB, Rupprecht CE (2002) Serologic evidence of Lyssavirus infection among bats, the Philippines. Emerg Infect Dis 8: 258–262
3. Bowden TR, Westenberg M, Wang LF, Eaton BT, Boyle DB (2001) Molecular characterization of Menangle virus, a novel paramyxovirus which infects pigs, fruit bats, and humans. Virology 283: 358–373

4. Chant K, Chan R, Smith M, Dwyer DE, Kirkland P (1998) Probable human infection with a newly described virus in the family *Paramyxoviridae*. The NSW Expert Group. Emerg Infect Dis 4: 273–275
5. Chua KB (2003) Nipah virus outbreak in Malaysia. J Clin Microbiol 26: 265–275
6. Chua KB, Wang LF, Lam SK, Eaton BT (2002) Full length genome sequence of Tioman virus, a novel paramyxovirus in the genus *Rubulavirus* isolated from fruit bats in Malaysia. Arch Virol 147: 1323–1348
7. Chua KB, Lam SK, Goh KJ, Hooi PS, Ksiazek TG, Kamaeulzaman A, Olson J, Tan CT (2001) The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia. J Infect 42: 40–43
8. Chua KB, Koh CK, Hooi PS, Wee KF, Khong JH, Chua BH, Chan YP, Lim ME, Lam SK (2002) Isolation of Nipah virus from Malaysian Island flying-foxes. Microbes Infect 4: 145–151
9. Chua KB, Lam SK, Tan CT, Hooi PS, Goh KJ, Chew NK, Tan KS, Kamarulzaman A, Wong KT (2000) High mortality in Nipah encephalitis is associated with presence of virus in cerebrospinal fluid. Ann Neurol 48: 802–805
10. Chua KB, Wang LF, Lam SK, Crameri G, Yu M, Wise T, Boyle D, Hyatt AD, Eaton BT (2001) Tioman virus, a novel paramyxovirus isolated from fruit bats in Malaysia. Virology 283: 215–229
11. Chua KB, Goh KJ, Wong KT, Kamarulzaman A, Tan PS, Ksiazek TG, Zaki SR, Paul G, Lam SK, Tan CT (1999) Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 354: 1256–1259
12. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Shieh WJ, Goldsmith CS, Gubler DJ, Roehrig JT, Eaton B, Gould AR, Olson J, Field H, Daniels P, Ling AE, Peters CJ, Anderson LJ, Mahy BWJ (2000) Nipah virus: a recently emergent deadly paramyxovirus. Science 288: 1432–1435
13. Field HE, Mackenzie JS, Hall LS (2001) Emerging zoonotic paramyxoviruses – the role of pteropid bats. In: Dodet B, Viscari M (eds) Emergence and control of zoonotic ortho- and paramyxovirus diseases. John Libby Eurotext, Montrouge, France, pp 205–209
14. Field H, McCall B, Barrett J (1999) Australian bat lyssavirus infection in a captive juvenile black flying fox. Emerg Infect Dis 5: 438–440
15. Field HE, Barratt PC, Hughes RJ, Shield J, Sullivan ND (2000) A fatal case of Hendra virus infection in a horse in north Queensland: clinical and epidemiological features. Aust Vet J 78: 279–280
16. Field H, Young P, Yob JM, Mills J, Hall L, Mackenzie J (2001) The natural history of Hendra and Nipah viruses. Microbes Infect 3: 307–314
17. Fraser GC, Hooper PT, Lunt RA, Gould AR, Gleeson LJ, Hyatt AD, Russell GM, Kattenbelt JA (1996) Encephalitiss caused by a lyssavirus in fruit bats in Australia. Emerg Infect Dis 2: 327–331
18. Goh KJ, Tan TC, Chew NK, Tan PS, Kamarulzaman A, Sarji SA, Wong KT, Abdullah BJJ, Chua KB, Lam SK (2000) Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med 342: 1229–1235
19. Gould AR (1996) Comparison of the deduced matrix and fusion protein sequences of equine morbillivirus with cognate genes of the *Paramyxoviridae*. Virus Res 43: 17–31
20. Gould AR, Hyatt AD, Lunt R, Kattenbelt A, Hengstberger S, Blacksell SD (1998). Characterisation of a novel lyssavirus isolated from pteropid bats in Australia. Virus Res 54: 165–187
21. Gould AR, Kattenbelt JA, Gumley SG, Lunt RA (2002) Characterisation of an Australian bat lyssavirus variant isolated from an insectivorous bat. Virus Res 89: 1–28

22. Guyatt KJ, Twin J, Davis P, Holmes EC, Smith GA, Smith IL, Mackenzie JS, Young PL (2003) A molecular epidemiological study of Australian bat lyssavirus. J Gen Virol 84: 485–496
23. Halpin K (2000) Genetic studies of Hendra virus and other novel paramyxoviruses. PhD Thesis, University of Queensland
24. Halpin K, Young PL, Field HE, Mackenzie JS (2000) Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. J Gen Virol 81: 1927–1932
25. Hanna JN, Carney IK, Smith GA, Tannenberg EG, Deverill JE, Botha JA, Serafin IL, Harrower BJ, Fitzpatrick PF, Searle JW (2000) Australian bat lyssavirus infection: a second human case, with a long incubation period. Med J Aust 172: 597–599
26. Harcourt BH, Tamin A, Ksiazek TG, Rollin PE, Anderson LJ, Bellini WJ, Rota PA (2000) Molecular characterisation of Nipah virus, a newly emergent paramyxovirus. Virology 271: 334–349
27. Hooper PT, Westbury HA, Russell GM (1997) The lesions of experimental equine morbillivirus disease in cats and guinea pigs. Vet Pathol 34: 323–329
28. Hooper PT, Ketterer PJ, Hyatt AD, Russell GM (1997) Lesions of experimental equine morbillivirus pneumonia in horses. Vet Pathol 34: 312–322
29. Hooper P, Zaki S, Daniels P, Middleton D (2001) Comparative pathology of the diseases caused by Hendra and Nipah viruses. Microbes Infect 3: 315–322
30. Hooper PT, Gould AR, Russell GM, Kattenbelt JA, Mitchell G (1996) The retrospective diagnosis of a second outbreak of equine morbillivirus infection. Aust Vet J 74: 244–245
31. Hooper PT, Gould AR, Hyatt AD, Braun MA, Kattenbelt JA, Hengstberger SG, Westbury HA (2000) Identification and molecular characterisation of Hendra virus in a horse in Queensland. Aust Vet J 78: 281–282
32. Hooper P, Lunt R, Gould A, Samaratunga H, Hyatt AD, Gleeson LF, Rodwell BJ, Rupprecht CE, Smith JS, Murray PK (1997) A new lyssavirus – the first endemic rabies-related virus recognized in Australia. Bull Inst Pasteur 95: 209–218
33. Hyatt AD, Selleck PW (1996) Ultrastructure of equine morbillivirus. Virus Res 43: 1–15
34. Hyatt AD, Zaki SR, Goldsmith CS, Wise TG, Hengstberger SA (2001) Ultrastructure of Hendra virus and Nipah virus within cultured cells and host animals. Microbes Infect 3: 297–306
35. Johara MY, Field H, Rashdi AM, Morrissy C, van der Heide B, Rota P, bin Adzhar A, White J, Daniels P, Jamaluddin A, Ksiazek T (2001) Nipah virus infection in bats (Order Chiroptera) in Peninsular Malaysia. Emerg Infect Dis 7: 439–441
36. Kirkland PD, Love RJ, Philbey AW, Ross AD, Davis RJ, Hart KG (2001) Epidemiology and control of Menangle virus in pigs. Aust Vet J 79: 199–206
37. Kumar S (2003) Inadequate research facilities fail to tackle mystery disease. Brit Med J 326: 12
38. Lam SK, Chua KB (2002) Nipah virus encephalitis outbreak in Malaysia. Clin Infect Dis 34 Suppl 2: S48–S51
39. Lee KE, Umapathi T, Tan CB, Tjia HT, Chua TS, Oh HM, Fock KM, Kurup A, Das A, Tan AK, Lee WL (1999) The neurological manifestations of Nipah virus encephalitis, a novel paramyxovirus. Ann Neurol 46: 428–432
40. Love RJ, Philbey AW, Kirkland PD, Ross AD, Davis RJ, Morrissey C, Daniels PW (2001) Reproductive disease and congenital malformations caused by Menangle virus in pigs. Aust Vet J 79: 192–198
41. Lyssavirus Expert Group (1996) Prevention of human lyssavirus infection. Commun Dis Intell 20: 505–507
42. Lyssavirus Expert Group (1996). Update on bat lyssavirus. Commun Dis Intell 20: 535

43. Mackenzie JS, Field HE (2001) Hendra virus: a new zoonotic Paramyxovirus from fruit bats in Australia. In: Dodet B, Viscari M (eds) Emergence and control of zoonotic ortho- and paramyxovirus diseases. John Libby Eurotext, Montrouge, France, pp 177–184
44. Mackenzie JS, Field HE, Guyatt KJ (2003) Managing emerging diseases borne by fruit bats (flying foxes), with particular reference to henipaviruses and Australian bat lyssavirus. J Appl Microbiol 94 Suppl 1: 59–69
45. Mackenzie JS, Chua KB, Daniels PW, Eaton BT, Field HE, Hall RA, Halpin K, Johansen CA, Kirkland PD, Lam SK, McMinn P, Nisbet DJ, Paru R, Pyke AT, Ritchie SA, Siba P, Smith DW, Smith GA, van den Hurk AF, Wang LF, Williams DT (2001) Emerging viral diseases of South-East Asia and the Western Pacific: a brief review. Emerg Infect Dis 7 (Suppl 3): 497–504
46. McCall BJ, Epstein JH, Neill AS, Heel K, Field H, Barrett J, Smith GA, Selvey LA, Rodwell B, Lunt R (2000) Potential exposure to Australian bat lyssavirus, Queensland, 1996–1999. Emerg Infect Dis 6: 259–264
47. McColl KA, Tordo N, Aguilar Setién A (2000) Bat lyssavirus infections. Rev Sci Tech Off Int Epizoot 19: 177–196
48. McColl KA, Chamberlain T, Lunt RA, Newberry KM, Middleton D, Westbury HA (2002) Pathogenesis studies with Australian bat lyssavirus in grey-headed flying foxes (Pteropus poliocephalus). Aust Vet J 80: 636–641
49. McCormack JG, Allworth AM, Selvey LA, Selleck PW (1999) Transmissibility from horses to humans of a novel paramyxovirus, equine morbillivirus (EMV). J Infect 38: 22–23
50. Middleton DJ, Westbury HA, Morrissy CJ, van der Heide BM, Russell GM, Braun MA, Hyatt AD (2002) Experimental Nipah virus infection in pigs and cats. J Comp Path 126: 124–136
51. Murray K, Rogers R, Selvey L, Selleck P, Hyatt A, Gould A, Gleeson L, Hooper P, Westbury H (1995) A novel morbillivirus pneumonia of horses and its transmission to humans. Emerg Infect Dis 1: 31–33
52. Murray K, Selleck P, Hooper P, Hyatt A, Gould A, Gleeson L, Westbury H, Hiley L, Selvey L, Rodwell B (1995) A morbillivirus that caused fatal disease in horses and humans. Science 268: 94–97
53. Olson JG, Rupprecht C, Rollin P, An US, Niezgoda M, Clemins T, Watson J, Ksiazek T (2002) Antibodies to Nipah-like virus in bats (Pteropus lylei), Cambodia. Emerg Infect Dis 8: 987–988
54. O'Sullivan JD, Allworth AM, Paterson DL, Snow TM, Boots R, Gleeson LJ, Gould AR, Hyatt AD, Bradfield J (1997) Fatal encephalitis due to novel paramyxovirus transmitted from horses. Lancet 349: 93–95
55. Parashar UD, Sunn LM, Ong F, Mounts AW, Arif MT, Ksiazek TG, Kamaluddin MA, Mustafa AN, Kaur H, Ding LM, Othman G, Radzi HM, Kitsutani PT, Stockton PC, Arokiasamy J, Gary HE, Anderson LJ (2000) Case-contol study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998–1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 181: 1755–1759
56. Paton NI, Leo YS, Zaki SR, Auchus AP, Lee KE, Ling AE, Chew SK, Ang B, Rollin PE, Umapathi T, Sng I, Lee CC, Lim E, Ksiazek TG (1999) Outbreak of Nipah virus infection among abattoir workers in Singapore. Lancet 354: 1253–1256
57. Philbey AW, Kirkland PD, Ross AD, Davis RJ, Gleeson AB, Love RJ, Daniels PW, Gould AR, Hyatt AD (1998) An apparently new virus (family *Paramyxoviridae*) infections for pigs, humans, and fruit bats. Emerg Infect Dis 4: 269–271
58. ProMED (2002) Bangladesh: A Nipah-like virus linked to neurological disease outbreak in 2001. <http://www.promedmail.org> Archive number 20020830.5187

59. Rogers RJ, Douglas IC, Baldock FC, Glanville RJ, Seppanen KT, Gleeson LJ, Selleck PN, Dunn KJ (1996) Investigation of a second focus of equine morbillivirus infection in coastal Queensland. Aust Vet J 74: 243–244
60. Samaratunga H, Searle JW, Hudson N (1998) Non-rabies lyssavirus human encephalitis from fruit bats. Australian bat lyssavirus (peropid lyssavirus) infection. Neuropathol Appl Neurobiol 24: 331–335
61. Selvey L, Taylor R, Arklay A, Gerrard J (1996) Screening of bat carers for antibodies to equine morbillivirus. Comm Dis Intell 20: 477–478
62. Selvey LA, Wells RM, McCormack JG, Ansford AJ, Murray K, Rogers RJ, Lavercombe PS, Selleck P, Sheridan JW (1995) Infection of humans and horses by a newly described morbillivirus. Med J Aust 162: 642–645
63. Tambyah PA, Tan JH, Ong BK, Ho KH, Chan KP (2001) First case of Nipah virus in Singapore. Intern Med J 31: 132–133
64. Tan CT, Wong KT (2003) Nipah encephalitis outbreak in Malaysia. Ann Acad Med Singapore 32: 112–117
65. Tan CT, Goh KJ, Wong KT, Sarji SA, Chua KB, Chew NK, Murugasu P, Loh YL, Chong HT, Tan KS, Thayaparan T, Kumar S, Jusoh MR (2002). Relapsed and late-onset Nipah encephalitis. Ann Neurol 51: 703–708
66. Wang LF, Yu M, Eaton BT (2001) Molecular biology of Hendra virus. In: Dodet B, Viscari M (eds) Emergence and control of zoonotic ortho- and paramyxovirus diseases. John Libby Eurotext, Montrouge, France, pp 185–197
67. Wang LF, Yu M, Hansson E, Pritchard LI, Michalski WP, Eaton BP (2000) The exceptionally large genome of Hendra virus: support for creation of a new genus within the family *Paramyxoviridae*. J Virol 74: 9972–9979
68. Wang LF, Harcourt BH, Yu M, Tamin A, Rota PA, Bellini WJ, Eaton BT (2001) Molecular biology of Hendra and Nipah viruses. Microbes Infect 3: 279–287
69. Wang LF, Michalski WP, Yu M, Pritchard LI, Crameri G, Shiell B, Eaton BT (1998) A novel P/V/C gene in a new member of the *Paramyxoviridae* family, which causes lethal infection in humans, horses, and other animals. J Virol 72: 1482–1490
70. Ward MP, Black PF, Childs AJ, Baldock FC, Webster WR, Rodwell BJ, Brouwer SL (1996) Negative findings from serological studies of equine morbillivirus in the Queensland horse population. Aust Vet J 74: 241–242
71. Warrilow D, Smith IL, Harrower B, Smith GA (2002) Sequence analysis of an isolate from a fatal human infection of Australian bat lyssavirus. Virology 297: 109–110
72. Warrilow D, Harrower B, Smith IL, Field H, Taylor R, Walker C, Smith GA (2003) Public health surveillance for Australian bat lyssavirus in Queensland, Australia 2000–2001. Emerg Infect Dis 9: 262–264
73. Westbury HA, Hooper PT, Brouwer SL, Selleck PW (1996) Susceptibility of cats to equine morbillivirus. Aust Vet J 74: 132–134
74. Williamson M, Hooper P, Selleck P, Westbury H, Slocombe R (1999) The effect of Hendra virus on pregnancy in fruit bats and guinea pigs. J Comp Pathol 122: 201–207
75. Williamson M, Hooper P, Selleck P, Westbury H, Slocombe R (2001) Guinea pigs as an experimental model of Hendra virus encephalitis. J Comp Pathol 124: 273–279
76. Williamson M, Hooper P, Selleck P, Gleeson LJ, Daniels PW, Westbury HA, Murray PK (1998) Transmission studies of Hendra virus (equine morbillivirus) in fruit bats, horses and cats. Aust Vet J 76: 813–818
77. Wong KT, Shieh WJ, Kumar S, Norain K, Abdullah W, Guarner J, Goldsmith CS, Chua KB, Lam SK, Tan CT, Goh KJ, Chong HT, Jusoh R, Rollin PE, Ksiazek TG, Zaki SR; Nipah Virus Pathology Working Group (2002) Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. Am J Pathol 161: 2153–2167

78. Young P, Halpin K, Field H, Mackenzie J (1997). Finding the wildlife reservoir of equine morbillivirus. In: Asche V (ed) Recent advances in microbiology, Vol 5. Australian Society for Microbiology Inc, Melbourne, pp 1–12
79. Young PL, Halpin K, Selleck PW, Field H, Gravel JL, Kelly MA, Mackenzie JS (1996) Serologic evidence for the presence in pteropus bats of a paramyxovirus related to equine morbillivirus. Emerg Infect Dis 2: 239–240
80. Yu M, Hansson E, Langedijk JP, Eaton BP, Wang LF (1998) The attachment protein of Hendra virus has high structural similarity but limited primary sequence homology compared with viruses in the genus *Paramyxovirus*. Virology 251: 227–233
81. Yu M, Hansson E, Shiell B, Michalski W, Eaton BT, Wang LF (1998) Sequence analysis of the Hendra virus nucleoprotein gene: comparison with other members of the subfamily *Paramyxovirinae*. J Gen Virol 79: 1775–1780

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