Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER 28

Paramyxoviruses in Bats

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Bats are an extraordinary group of mammals; not only are they the only mammals capable of sustained flight, but they are also found in almost all habitats, having a worldwide distribution, except for the highest mountains and extreme polar regions. Bats also occupy a diverse array of ecologic niches and contribute significantly to mammalian diversity, with more than 1000 species.[39]

Bats are known to host six paramyxoviruses: Nipah virus, Hendra virus, Menangle virus, Tioman virus, bat parainfluenza virus, and Mapuera virus. At least three of these are able to infect humans and domestic animals. Ever-increasing human encroachment on natural habitats, combined with the ability of some bats to adapt to anthropogenic environmental changes, has led to increased contact between bats and domestic animals and humans. This may be a key reason for the repeated emergence of several paramyxoviruses from bats in recent years. These viruses have been able to jump the species barrier, and in the case of Nipah virus in Bangladesh, then spread from person to person.

DESCRIPTION OF PARAMYXOVIRUSES

There are two subfamilies and six genera within the family Paramyxoviridae. The genera *Morbillivirus*, *Respiovirus*, *Rubulavirus,* and *Henipavirus* make up the subfamily Paramyxovirinae, and the genera *Pneumovirus* and *Metapneumovirus* constitute the subfamily Pneumovirinae.[56] Each of the six genera contains highly contagious human and animal pathogens.[62]

Paramyxoviruses have been found predominantly in mammals and birds, and most have a narrow host range in nature, but display a broad host cell range in culture.[26] Transmission is generally horizontal, mainly through airborne routes, and no vectors are known.[26] Primary replication is usually in the respiratory tract. Infection is generally cytolytic, but persistent infections often occur.[56]

Paramyxoviruses are pleomorphic and 150 to 300 nm in diameter.[18] Virions are made up of a lipoprotein envelope and a nucleocapsid that surrounds a single strand of linear, negative-sense ribonucleic acid (RNA).[13] Virion proteins common to all genera include three nucleocapsid-associated proteins: a nucleocapsid protein (N or NP), a phosphoprotein (P), and a large putative polymerase protein (L); and three membrane-associated proteins: an unglycosylated envelope protein (M) and two glycosylated envelope proteins, comprising a fusion protein (F) and an attachment protein (G or H or HN).[56] The attachment and fusion proteins are of primary importance in inducing virus-neutralizing antibodies and immunity against reinfection.[56,57] Antibodies to other viral proteins are also produced and some, nucleocapsid proteins in particular, are known to play a role as antigens for cytotoxic T cells.[37]

BATS AS VIRAL HOSTS

Historically, a wide range of viral infections, including flaviviruses, alphaviruses, rhabdoviruses, arenaviruses, reoviruses, and paramyxoviruses, have been identified in bats.[60] More recently, a number of emerging zoonotic viruses have been detected in bats.[32] These include Hantaan virus, isolated from the common serotine bat *(Eptesicus serotinus)* and the horseshoe bat *(Rhinolophus ferrumepuinum)* in Korea; Rift Valley fever virus, isolated from the bats *Micropteropus pusillus* and *Hipposideros albae* in the Republic of Guinea; a strain of yellow fever isolated from an *Epomophorus* Old World fruit bat in Ethiopia; and serologic evidence of Venezuelan equine encephalitis, St. Louis encephalitis, and eastern equine encephalitis viruses in bats in Guatemala.[32] Although bat-variant rabies has long been recognized in the United States, the prevalence of human rabies cases attributed to that variant has increased in recent years.[46] Most recently, strong evidence shows that horseshoe bats (*Rhinolophus* spp.) may be the source of the severe acute respiratory syndrome (SARS) coronavirus.[40,42]

Fig 28-1 Administration of oral rehydration solution to greater flying fox *(Pteropus neohibernicus)* after general anesthesia for application of satellite collar in Papua New Guinea. (See Color Plate 28-1.) *(Courtesy Andrew C. Breed.)*

Also, Old World fruit bats of the genera *Hypsignathus*, *Epomops,* and *Myonycteris* may be natural hosts of Ebola virus, as found in Gabon and Republic of Congo.[41]

Bats may travel hundreds of kilometers (or miles) in a matter of days. Besides having significant implications for disease spread, this also suggests that populations of pathogens carried by bats are likely to be relatively homogenous across wide geographic areas.[9] Studies of Old World fruit bats using satellite telemetry have shown that individuals can travel more than 2000 km in 1 year and traverse significant bodies of open sea, such as the Torres Strait between Australia and New Guinea and the Strait of Malacca between peninsular Malaysia and Sumatra (www.henipavirus.com)[10,61] (Figure 28-1). These long-distance movements may transmit pathogens over great distances and enable exchange between bat populations on different land masses.

Population size and density are positively associated with the diversity of pathogens hosted by mammalian species.[2] A large population size, as seen for many colonial bats, supports pathogen reproduction by providing a constant supply of individuals susceptible to infection and thus allows persistence of the pathogen.[3]

Regular, but not constant, contact between individual bats from different subpopulations allows for partial connectivity between colonies of bats. A metapopulation may exist where a spatial mosaic involves a constellation of subpopulations of which, at any given time, some are susceptible, some infected, and some immune to a particular disease.[43] This is beneficial for genetic diversity and may permit pathogens, particularly viruses, to persist in a species with a total population that would otherwise be too small to maintain the disease.[8] This results in these species having considerable potential to act as vectors for, and disseminators of, viruses and other pathogens.

Some authors have proposed that bats are unique in their response to viral infection and are able to sustain viral infections without disease.[60] However, many other small mammals act as reservoirs for viruses without evidence of disease, and recent analysis of a database on all emerging infectious diseases of humans suggests that bats (which represent as much as a quarter of all mammalian species) do not harbor a disproportionate number of the known emerging zoonotic viruses.[71]

PARAMYXOVIRUSES OF CHIROPTERA

Hendra Virus

In September 1994, an outbreak of severe respiratory disease of horses occurred in the Brisbane suburb of Hendra in eastern Australia (Queensland)[48] (Figure 28-2). The index case was a pregnant Thoroughbred mare, and 16 other horses at two sites showed signs of loss of appetite, dyspnea, and copious frothy nasal discharge. Twelve of the affected horses died a few days after the onset of signs.[7] Two people who had close contact with the index case also became infected. One of them, a stable worker, developed flulike signs and recovered. The other person, a horse trainer, showed

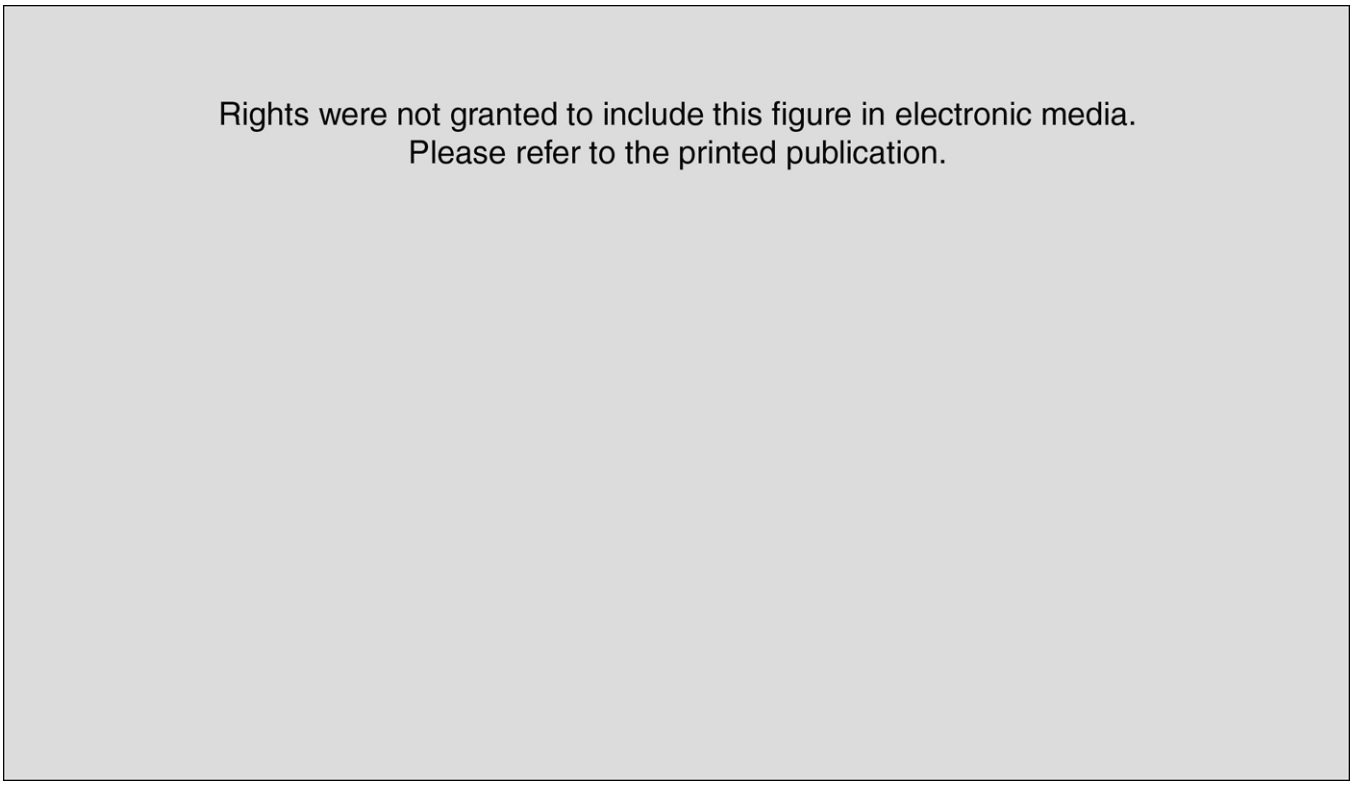

Fig 28-2 Timeline indicating emergence of henipaviruses. *(Eaton BT, Broder CC, Middleton D, Wang L: Hendra and Nipah viruses: different and dangerous,* Nat Rev Microbiol *4:23-35, 2006, Macmillan Magazines.)*

rapid development of respiratory illness and died 11 days later.[58]

It was thought that the pattern of disease in the horses reflected a point source of infection, and that all other cases were a direct result of transmission from the pregnant mare.[7] A range of pathogens and toxins were investigated and excluded from the diagnosis. A novel virus was cultured from the lungs of five of the affected horses and from the kidneys of the fatal human case.[49] The virus showed characteristics suggesting it belonged to the family Paramyxoviridae, although there was minimal cross-reactivity between the virus and a range of antisera to other paramyxoviruses. The virus showed 50% homology of the partial M protein gene sequence of several morbilliviruses and thus was initially called equine morbillivirus (EMV).[48] The name was subsequently changed to Hendra virus (HeV) when it became apparent that horses were not the natural host for the virus and that it did not belong in the genus *Morbillivirus.*

Surveillance of wildlife species identified flying foxes (genus *Pteropus*, family Pteropodidae) as the likely natural host of the virus. The infection was found to be widespread in four of the flying fox species found on mainland Australia: the black flying fox *(Pteropus alecto),* gray-headed flying fox *(P. poliocephalus),* little red flying fox *(P. scapulatus),* and spectacled flying fox *(P. conspicillatus).*[22,29] Sampling of 46 species of ground-dwelling mammals revealed no evidence of HeV exposure.[69] Studies of seroprevalence of HeV antibodies in flying foxes in Australia indicate a prevalence of approximately 50%[25] (Figures 28-3 through 28-5).

Since the first outbreak, further outbreaks of HeV have occurred in Queensland, including Mackay in 1994, involving fatal infections of both horses and a human; Cairns in 1999, involving a single horse; and Cairns and Townsville in 2004, involving both horses and a veterinarian.[23]

Hendra virus infection of terrestrial mammals, including humans, results in a systemic vasculitis with significant pathology of the lung and central nervous system (CNS).[34,66,70] Viral antigen is detected in vascular endothelium and frequently recovered from nasopharangeal swabs, urine, and internal organs, including lung and brain.[19,34] Experimental HeV infection of flying foxes, however, appears to cause only a sporadic subclinical vasculitis, even at infective doses lethal to horses.[68,69] Viral antigen is detected in the tunica media rather than endothelial cells, which may help explain why flying foxes appear to be spared from clinical disease.[20] Experimental infection of flying foxes has also shown placental transfer of the virus to a fetus.[34]

Fig 28-3 Anesthesia of wild-caught, spectacled flying fox *(Pteropus conspicillatus),* using isoflurane and oxygen, for Hendra virus surveillance in North Queensland, Australia. (See Color Plate 28-3.) *(Courtesy Jack Shield.)*

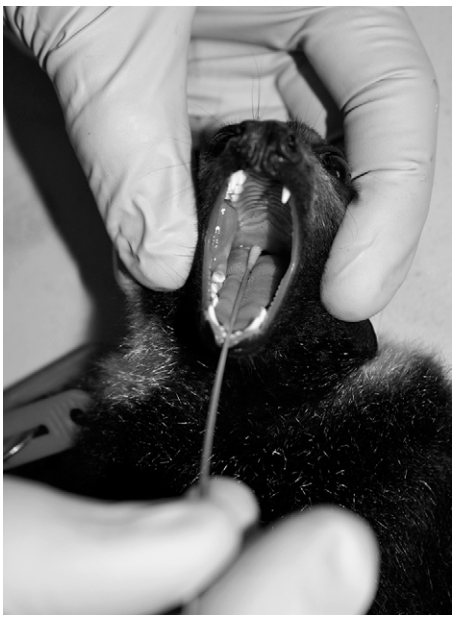

Fig 28-4 Collection of oral swab from anesthetized spectacled flying fox *(Pteropus conspicillatus)* for Hendra virus antigen detection. (See Color Plate 28-4.) *(Courtesy Jack Shield.)*

Nipah Virus

Nipah virus (NiV) was first described in March 1999 in the investigation of an outbreak of disease in pigs and humans in Malaysia (see Figure 28-2). In the course of the outbreak, 265 humans were infected, 105 fatally.[15] Infected pigs were identified as the primary source of human infection, and over 1 million pigs were culled to control the outbreak. Wildlife surveillance identified the Malayan flying fox *(Pteropus vampyrus)* and island flying fox *(Pteropus hypomelanus)* as probable natural hosts of NiV.[38]

Subsequent studies have also found serologic evidence of NiV infection in the Malayan flying fox, island flying fox, and Lyle's flying fox *(Pteropus lylei)* in Thailand, in Lyle's flying fox in Cambodia, and in the Indian flying fox *(Pteropus giganteus)* in Bangladesh.[35,51,63] NiV has strong serologic and sequence similarities to HeV and is the second member of the genus *Henipavirus*.[64]

Subsequent to NiV's emergence in Malaysia, five outbreaks of NiV-associated disease in humans were described in Bangladesh between April 2001 and February 2005.[4-6,35] As of 11 February 2005, a total of 122 cases had been recognized by the Bangladesh Directorate of Health Services, at least 78 (64%) of which were fatal. A number of the characteristics of the Bangladesh outbreaks were similar to the outbreak in Malaysia: delayed recognition, a primary presentation of humans with fever and CNS signs, and a high case-fatality rate. In marked contrast to the Malaysian outbreak, however, infection in humans was not associated with disease in pigs, and evidence indicated horizontal human transmission. Further, the pattern of the Bangladesh outbreaks suggests a sporadic, geographically scattered introduction of infection to humans. Nucleotide sequence data also support a different epidemiology in Bangladesh. Data obtained from human cases in Malaysia suggest a single source of human

Fig 28-5 Collection of piece of wing membrane from anesthetized spectacled flying fox *(Pteropus conspicillatus)* for molecular genetic studies. This technique is used to elucidate population structure of flying fox species for henipavirus epidemiologic studies. (See Color Plate 28-5.) *(Courtesy Jack Shield.)*

infection from the porcine amplifying host.[1,11,15] Data from Bangladesh cases formed a cluster clearly distinct from the Malaysian sequences, but differed from each other by approximately 0.8%, suggesting possibly multiple introductions of virus into humans.[30]

The pathologic effects of NiV in terrestrial mammals are similar to those of HeV, with infection resulting in a systemic vasculitis and significant pathology of the lung and CNS.[34,66] In contrast to HeV, however, viral antigen is often found in bronchial and alveolar epithelium. NiV has not been associated with clinical disease in flying foxes.[20]

Menangle Virus

In August 1997, Menangle virus was isolated from stillborn piglets at a swine farm in Menangle, New South Wales, Australia.[54] Many of the piglets had craniofacial and spinal deformities and degeneration of the brain and spinal cord. Additionally, the pig herd experienced a reduced pregnancy rate, increased abortion rate, decreased litter sizes, and increased proportion of stillborn and mummified piglets. Infection of humans also occurred; two swine farm workers developed an influenza-like illness and high-titer antibody responses to Menangle virus.[12]

Menangle virus was classified as a member of the Paramyxoviridae based on electron microscopy of the virus grown in cell culture.[67] Data on nucleotide and deduced amino acid sequences from the viral genome showed the closest relationship to members of the *Rubulavirus* genus, including mumps and simian parainfluenza type 5.[67] These preliminary genome sequence data suggested that Menangle virus was a new member of the genus *Rubulavirus* within the family Paramyxoviridae.

A colony of *Pteropus poliocephalus* was known to roost near the affected piggery in Menangle. Antibodies to Menangle virus were found in flying foxes from the colony,[54] and electron microscopy (EM) revealed virus-like particles in the feces of flying foxes from a nearby colony.[55]

Tioman Virus

During the search for the natural host for NiV, a novel paramyxovirus was isolated from a number of pooled urine samples of island flying foxes *(P. hypomelanus)* from Tioman Island off the eastern coast of the Malay peninsula.[17] Electron microscopy of virus-infected cells revealed spherical, enveloped virus particles compatible in structure with viruses of the family Paramyxoviridae.[16] The virus showed serologic reaction to antibodies to Menangle virus, but not to a number of other paramyxoviruses investigated.[16] Molecular characterization of the nucleocapsid (N) protein gene of the new virus and Menangle virus showed them to be approximately 70% homologous at the nucleotide level and approximately 85% homologous at the amino acid level.[44] Analysis of the full-length genome

indicated the virus to be a member of the genus *Rubulavirus* within the family Paramyxoviridae, and it was named Tioman virus.[17]

The potential for Tioman virus to cause disease in humans, flying foxes, or other animals is unknown.[44]

Bat Parainfluenza Virus

The first recorded isolation of a paramyxovirus from a bat was described in 1971 by Pavri et al.[53] The virus was isolated from a suspension of pooled organs from an Old World fruit bat, *Rousettus leschenaulti* (family Pteropodidae), captured as part of ongoing investigations into rabies outbreaks in the district near Poona, India. Hemagglutination inhibition, complement fixation, neutralization tests, and growth characteristics revealed that this virus represented a new parainfluenza strain that was related to but distinct from simian virus 41 (SV41), placing it in the parainfluenza type 2 group.[33] Serosurveys revealed specific neutralization of the bat virus by serum specimens from 7% of 70 *R. leschenaulti* samples tested. Bat parainfluenza antibodies were also demonstrated in 10% of 200 human serum samples tested.[53] It is not known whether the observed antibody reactions in humans were caused by interspecies transmission of the virus or a serologic cross-reaction.[53]

Mapuera Virus

Mapuera virus was isolated from a little yellow-shouldered bat *(Sturnira lilium),* a New World leaf-nosed bat (family Phyllostomidae), from Brazil in 1979. It was tentatively classified as a member of the family Paramyxoviridae on the basis of its morphology and its ability to hemagglutinate guinea pig erythrocytes.[72]

The molecular biology of Mapuera virus has been studied at both the protein and the nucleic acid levels.[31] Seven virus-encoded proteins were detected in infected Vero cells. Based on the similarity of N-protein sequences, results indicate that Mapuera virus should be placed within the genus *Rubulavirus,* which includes mumps virus, simian virus 5 (SV5), and Menangle virus.[56]

DIAGNOSTIC TESTS

To date, diagnostic test development has been most successful for Hendra and Nipah viruses. Four diagnostic tests—virus isolation, EM, immunohistochemistry, and polymerase chain reaction (PCR) and sequencing—have been described for the detection of virus or viral antigen of these two viruses. Two diagnostic tests for the detection of antiviral antibodies are serum neutralization (SN) and enzyme-linked immunosorbent assay (ELISA).[19] Because Hendra and Nipah viruses are classified internationally as biosecurity (biosafety) level 4 (BSL4) agents, tests necessarily involving live virus (i.e., virus isolation and SN tests) should only be carried out under physical containment level 4 (PC4) conditions.

Virus Isolation

Hendra and Nipah viruses grow well in Vero cells from a range of tissue specimens, including brain, lung, kidney, and spleen.[19] Cytopathic effect usually develops within 3 days, and virus isolates may be specifically identified by immunostaining, neutralization with specific antiserum, PCR, and EM.

Immunohistochemistry

Immunohistochemistry (IHC) may detect viral antigen in a range of tissues. Because IHC uses formalin-fixed tissues, the technique is useful for retrospective investigations on archived materials, and the biosafety constraints of viral isolation and SN tests do not apply. The availability of a range of polyclonal and monoclonal antisera allows that test sensitivity and specificity to be tailored to testing objectives.

Electron Microscopy

Negative-contrast EM and immuno-EM have provided rapid and valuable information on virus structure and antigenic reactivity during primary virus isolation.[36]

Polymerase Chain Reaction and Sequencing

Diagnostic PCR assays for HeV and NiV are in routine use at the Australian Animal Health Laboratory (Geelong) and the U.S. Centers for Disease Control and Prevention (Atlanta). The ability to select primer sets for particular genes allows test sensitivity and specificity to be tailored to testing objectives. The technique may be used as a primary diagnostic tool to

detect viral sequences in fresh or formalin-fixed tissue and as an adjunct to virus isolation to characterize virus isolates rapidly.[22]

Serum Neutralization Tests

The SN test is regarded as the "reference standard" serologic test for Hendra and Nipah viruses. Sera are incubated with live virus in microtiter plates to which Vero cells are added, and cultures are read at 3 days.[19] The use of live virus means that SN tests should only be performed in a PC4 facility.

Enzyme-Linked Immunosorbent Assay

The ELISA tests provide a rapid, inexpensive, and safe means of conducting serologic investigations. Indirect ELISAs have been developed for the detection of anti-Nipah and anti-Hendra immunoglobulin G (IgG), and a capture ELISA has been developed for detection of anti-Nipah IgM.[19] Currently available ELISA tests still need to combine excellent sensitivity and specificity with respect to SN results. Further improvement of ELISAs or other serologic tests is required for future epidemiologic studies of HeV and NiV in bats. Recent advances in the development of multiplexed microsphere assays show particular promise in this area.

DISEASE ECOLOGY AND SPILLOVER MECHANISMS

The reasons for the emergence of these zoonotic bat-borne viruses in recent years are yet to be resolved. Although not yet established, it has been hypothesized that changes in bat ecology are driving disease emergence in these species.[25] Flying foxes are particularly vulnerable to habitat loss or modification resulting from the ephemeral nature of their food resources.[21] Land use change has resulted in population decline, population concentration during resource scarcity, distributional changes, and urbanization of flying fox populations throughout the Old World Tropics.[21,28,47] These processes could lead to disease emergence either by changes in viral dynamics or by increased contact with domestic animals and humans.

Hendra and Nipah viruses appear to be ancient viruses that co-evolved with and are well adapted to their natural flying fox hosts.[27,48] The emergence of these viruses in humans has required a bridge from the natural host to a susceptible "spillover" host. Such bridges typically result from changes to the agent, the host, or the environment. The close RNA sequence match among flying fox, livestock, and human isolates of Hendra and Nipah viruses suggests that emergence is more likely associated with ecologic changes that have promoted contact between bats and livestock, rather than with genetic change leading to increased virulence.[42]

Fig 28-6 Gray flying fox *(Pteropus griseus)*, East Timor. (See Color Plate 28-6.) *(Courtesy Andrew C. Breed.)*

Available data on many flying fox species suggest that populations in Australia and Southeast Asia are declining, with disruption occurring throughout their range (Figures 28-6 and 28-7). In Southeast Asia, anthropogenic activities (primarily habitat destruction and hunting) constitute the major threats. Deforestation, whether for agricultural land, commercial logging, or urban development, is widespread and results in loss or abandonment of roosting sites and loss of feeding habitats. This habitat loss caused by clearing is often exacerbated by tropical storms because the remnant forest may be particularly prone to high-wind damage. Hunting, whether for consumption, sport, or crop protection, at both a local and a commercial level, results in the abandonment of roost and feeding sites.[47] A scenario thus emerges of flying fox populations under stress with altered foraging and behavioral patterns, of niche expansion, and of closer proximity to humans. In Australia the geographic redistribution of roosting sites has been increasingly into urban areas in recent decades.[28]

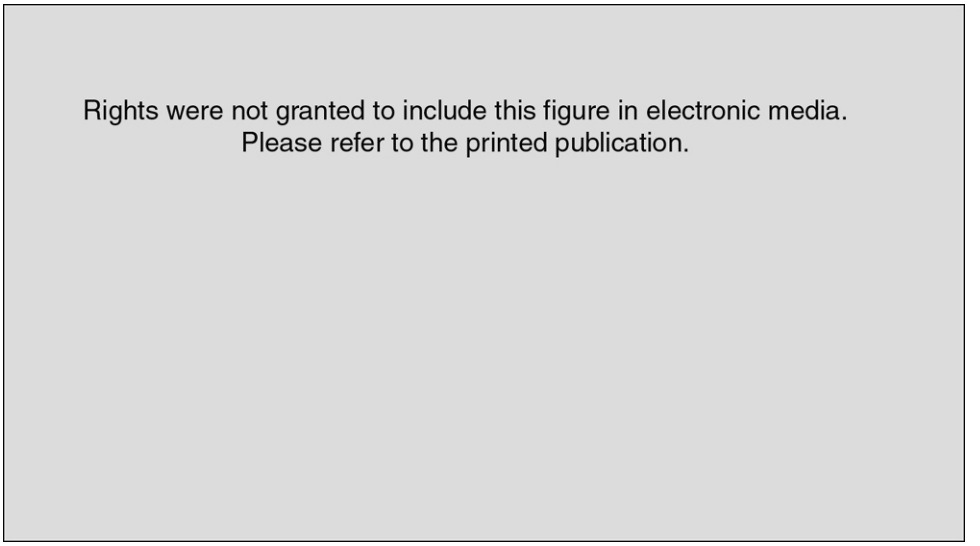

Fig 28-7 Global distribution of flying foxes (genus *Pteropus*). The sites of disease outbreaks caused by henipaviruses are indicated by asterisks. (See Color Plate 28-7.) *(Eaton BT, Broder CC, Middleton D, Wang L: Hendra and Nipah viruses: different and dangerous,* Nat Rev Microbiol *4:23-35, 2006, Macmillan Magazines.)*

RESERVOIR HOST MANAGEMENT STRATEGIES

The sporadic and apparently rare nature of HeV spillover events from flying foxes to horses, the low infectivity for horses (and thus limited economic impact), and the apparent absence of direct transmission from flying foxes to people have resulted in more emphasis on management strategies for horses than flying foxes. Quarantine of infected premises, movement controls on stock, and disinfection have so far proved effective.[7] Veterinarians involved in these disease investigations are advised to wear appropriate protective equipment and to use a limited necropsy approach, because horses have been the source of infection for all four human cases. Putative risk factors for infection in horses appear to be age (>8 years old), breed (Thoroughbred), housing (paddocked), season (late gestation or birthing season of local flying fox populations), and the presence of food trees favored by flying foxes in the index-case paddock.[23] A considerable research focus on the ecology of HeV has yet to define the route of virus excretion or any temporal pattern of infection in flying foxes. This information and knowledge of the actual mode of flying fox–to-horse transmission would facilitate a risk management approach to spillover infection in horses.

In marked contrast to HeV, the NiV outbreak in peninsular Malaysia in 1999 had an enormous economic and social impact.[50] Nipah virus was highly infectious for pigs, with all age and sex classes susceptible. The pattern of on-farm infection was consistent with respiratory transmission; between-farm spread was generally associated with the movement of pigs. Human infections were predominantly attributed to contact with live pigs; none was attributed to contact with bats.[15] Horizontal transmission was not a feature of infection in humans. Recommended host management strategies primarily targeted pig-to-pig transmission.[24]

Although strategies directed at the flying fox–pig interface are limited by the incomplete knowledge of the ecology of NiV, several simple on-farm measures may be taken to reduce the likelihood of spillover events. The removal of fruit orchards and other food trees favored by flying foxes from the immediate vicinity of pig farms greatly reduces the probability of flying fox–pig contact. Similarly, the wire screening of open-sided pig sheds is a simple and inexpensive strategy to prevent direct contact between flying foxes and pigs. Indirect contact (with flying fox urine or feces or partially eaten fruit) may be avoided by ensuring roof runoff does not enter pig pens.[14]

Henipavirus spillover to domestic animals may be effectively controlled by the methods previously mentioned, but events in Bangladesh warn against complacency in elimination of the zoonotic risk of henipaviruses using these methods alone.

A study has shown that an oral vaccine was capable of inducing a protective immune response to rabies in vampire bats after oral vaccine delivery, and therefore an oral vaccination approach may be plausible for other bat species.[59]

Other authors discuss the possibility of using an oral vaccine for henipaviruses in flying foxes in the future.[45] They observe that the presence of antibodies to Hendra and Nipah viruses in healthy flying foxes could warrant the inclusion of a biomarker in a vaccine to distinguish between vaccinated individuals

and naturally infected individuals. However, they also caution that various aspects of flying fox behavior require further study before development of an oral vaccine strategy.

Development of a vaccine for HeV or NiV to be used in wild flying fox populations is not likely to occur in the near future. However, a better understanding of flying fox behavior and ecology, henipavirus dynamics in flying foxes, and anthropogenic factors that facilitate spillover events will offer cost-effective and practical solutions for preventing future outbreaks.

CONCLUSION

The evident horizontal human transmission and the apparent absence of an intermediate domestic animal reservoir in the Bangladesh outbreaks of Nipah virus are disturbing epidemiologic features that highlight the potential for change in viral transmission dynamics and the urgent need for detailed study of bat paramyxoviral ecology and increased understanding of spillover mechanisms.[24] Also, given that four of the six paramyxoviruses known to naturally infect bats have been identified within the last 15 years, and that the vast majority of bat species have never been surveyed for evidence of paramyxoviral infection, there may well be other, currently unidentified paramyxoviruses in wild bat populations.

To understand fully the factors that drive disease emergence, we must attempt to understand these viruses and their hosts at a range of spatial scales. We currently know a considerable amount about the molecular biology of the viruses discussed,[65] little about the interaction between the viruses and their hosts,[68,69] and even less about the biology of the viruses at the level of the host population.[8,25]

Bats play vital roles in pollination, seed dispersal, and insect predation in the ecosystems where they occur[52]; they must be conserved to maintain ecologic health and biodiversity. The increasing anthropogenic encroachment on and change in these ecosystems will test our ability to assess and manage effectively the risk posed by the pathogens harbored by bats and other wildlife species.

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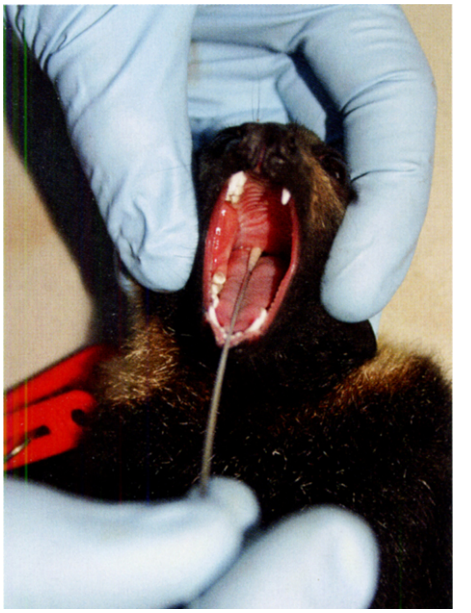
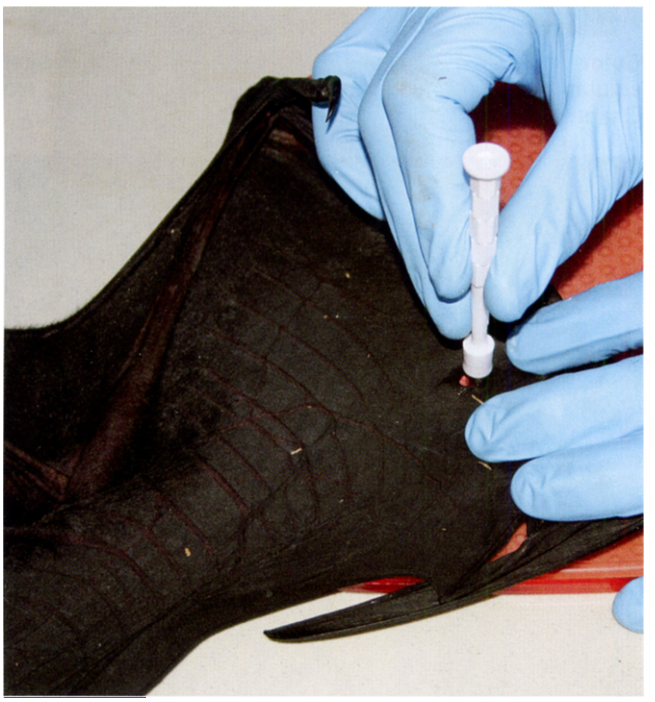
Color Plate 28-1 Administration of oral rehydration solution to greater flying fox *(Pteropus neohibernicus)* after general anesthesia for application of satellite collar in Papua New Guinea. (For text mention, see Chapter 28, p. 226.) *(Courtesy Andrew Breed.)*

Color Plate 28-3 Anesthesia of wild-caught, spectacled flying fox *(Pteropus conspicillatus),* using isoflurane and oxygen, for Hendra virus surveillance in North Queensland, Australia. (For text mention, see Chapter 28, p. 228.) *(Courtesy Dr. Jack Shield.)*

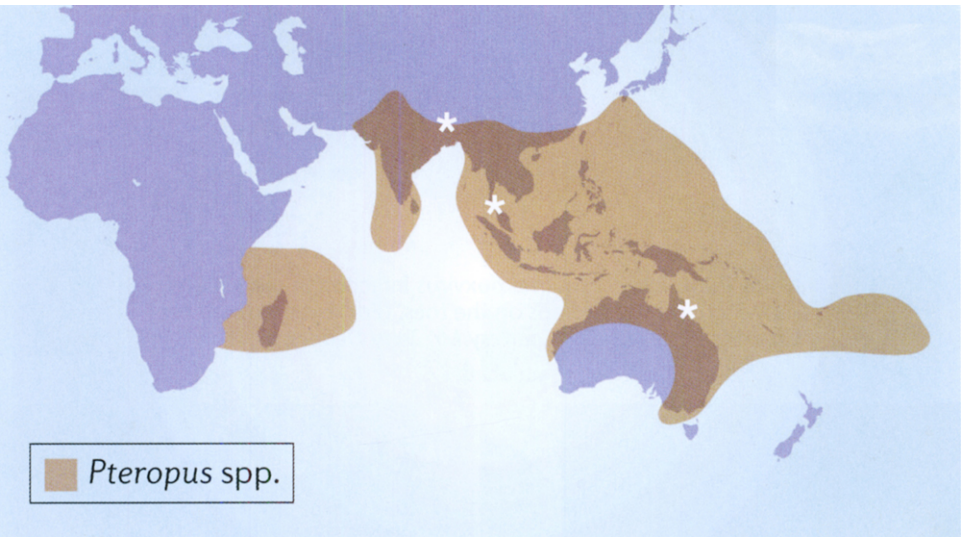


Color Plate 28-4 Collection of oral swab from anesthetized spectacled flying fox *(Pteropus conspicillatus)* for Hendra virus antigen detection. (For text mention, see Chapter 28, p. 228.) *(Courtesy Dr. Jack Shield.)*



Color Plate 28-5 Collection of piece of wing membrane from anesthetized spectacled flying fox *(Pteropus conspicillatus)* for molecular genetic studies. This technique is used to elucidate population structure of flying fox species for henipavirus epidemiologic studies. (For text mention, see Chapter 28, p. 229.) *(Courtesy Dr Jack Shield.)*

Color Plate 28-6 Gray flying fox *(Pteropus griseus)*, East Timor. (For text mention, see Chapter 28, p. 231.) *(Courtesy Andrew Breed.)*



Color Plate 28-7 Global distribution of flying foxes (genus *Pteropus*). The sites of disease outbreaks caused by henipaviruses are indicated by asterisks. (For text mention, see Chapter 28, p. 232.)