

REVIEW



## Bats as a continuing source of emerging infections in humans

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### SUMMARY

Amongst the 60 viral species reported to be associated with bats, 59 are RNA viruses, which are potentially important in the generation of emerging and re-emerging infections in humans. The prime examples of these are the lyssaviruses and *Henipavirus*. The transmission of Nipah, Hendra and perhaps SARS coronavirus and Ebola virus to humans may involve intermediate amplification hosts such as pigs, horses, civets and primates, respectively. Understanding of the natural reservoir or introductory host, the amplifying host, the epidemic centre and at-risk human populations are crucial in the control of emerging zoonosis. The association between the bat coronaviruses and certain lyssaviruses with particular bat species implies co-evolution between specific viruses and bat hosts. Cross-infection between the huge number of bat species may generate new viruses which are able to jump the trans-mammalian species barrier more efficiently. The currently known viruses that have been found in bats are reviewed and the risks of transmission to humans are highlighted. Certain families of bats including the *Pteropodidae*, *Molossidae*, *Phyllostomidae*, and *Vespertilionidae* are most frequently associated with known human pathogens. A systematic survey of bats is warranted to better understand the ecology of these viruses. Copyright © 2006 John Wiley & Sons, Ltd.

Supplementary electronic material for this paper is available in Wiley InterScience at <http://www.interscience.wiley.com/jpages/10529276/suppmat>

Received: 24 May 2006; Revised: 8 August 2006; Accepted: 29 August 2006

### INTRODUCTION

Zoonoses are diseases and infections which are naturally transmitted between vertebrate animals and man [1]. Zoonotic pathogens are currently considered to be the major sources of emerging and re-emerging infections in humans and RNA viruses account for the overwhelming majority of emerging pathogens [2]. At the time of writing, the most important non-mammalian vertebrates associated with emerging infections in humans are the birds. The avian species have an enormous population: the estimated world poultry population in agriculture (chickens, ducks, geese and turkeys) in 2005 was over 18 billion [3]. Poultry birds often

have large populations reared in relatively restricted environments. Wild birds have a tendency to gather in flocks and many species fly or migrate over long distances. All these are important attributes which allow them to harbour and disseminate infectious agents over vast geographical areas. Their impacts on public health and economy are best exemplified by the West Nile virus encephalitis in North America and the global influenza A H5N1 outbreaks. The latter carries major economic losses in agriculture and the potential to cause a pandemic associated with significant morbidity and mortality in humans.

Infectious agents exist in commensal, mutual or parasitic relationships with their harbouring animal hosts. The relationship is often restricted to a limited number of host species owing to genetic adaptations that developed during co-evolution. This gives rise to the concept of species barrier in infectious diseases. However, spillage of infectious agents between different animal species does

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#### Abbreviations used

SARS, severe acute respiratory syndrome.

occur sporadically. This may result from accidental or deliberate intrusion by one animal species into the ecological niche of another, resulting in the direct exposure to the infectious agents from the original harbouring species or acquisition from the environment. Severe diseases often occur in the new animal hosts in the absence of genetic adaptations of the infectious agent to these new hosts. These new animals may become 'dead end' hosts when the infectious agent fails to cause further transmission under natural conditions (e.g. Japanese encephalitis in humans), or they may contribute to further transmissions resulting in an epidemic (e.g. SARS). The transmission dynamics of some important bat-associated viruses are summarised in Table 1. Understanding the amplifying host, the routes of transmission, the type of susceptible human hosts, and the epicentres for zoonotic and human transmissions is crucial in the control of these infections.

The relative probability of an infectious agent jumping from one animal species to another is often assumed to be related to the phylogenetic relatedness of the host species. However, experience from zoonoses acquired from birds shows that we should focus not just on those animals which are phylogenetically close to humans (such as the primates and rodents), but also on those which are more distantly related but have attributes that enable them to harbour and spread novel microbes. Hunting of non-human primates for bushmeat has been practised in tropical Africa for aeons. Such exposures have resulted in transmission of pathogens like Ebola virus, simian foamy viruses, simian immunodeficiency virus, and human immunodeficiency viruses to humans [4–6]. Rodents are not only the largest order of all mammals but also often exist in large numbers in urban and rural habitats. They are important sources of zoonotic pathogens including Sin Nombre and other hantaviruses. Bats are the only mammals possessing true flying ability. In recent years bats have increasingly been recognised to be potential reservoirs for various emerging infections. The bats and their associated viral pathogens will be discussed in this article.

#### BAT BIOLOGY, ECOLOGY AND HUMAN INFECTIOUS DISEASES

The bats represent the second largest order within the mammals. There are currently over 4600

known species of mammals (Class Mammalia) [7]. The largest order within Mammalia consists of the rodents (Order Rodentia) with over 2000 species, and there are over 930 species of bats (Order Chiroptera), or about 20% of all mammalian species. There are two suborders within Chiroptera, the Megachiroptera and Microchiroptera. *Pteropodidae* (flying foxes) is the only family within Megachiroptera (166 species) and they mainly feed on plant materials. There are 16 families within Microchiroptera (over 760 species) with diverse biology and ecology (Table 2) [7,8].

Bats are found in all continents except the polar regions and a few oceanic islands. The nature of their diet is equally diverse, varying from plants, insects, animals and, unique among mammals, blood. The diversity of bat species and some of their unique biological and ecological features allow them to become the hosts for a large number of medically important infectious agents. Most bats are nocturnal animals and seek shelter in roosts during the day. A few species are diurnal. The roosting environment ranges from natural to man-made structures and they can be temporary or permanent (Figure 1). Natural roosts can be found in caves, rock crevices, nests of birds or ants and termites, cavities in trees, or exposed on tree branches and trunks [9]. Man-made habitats include mines, tombs, buildings, bridges and so on. The occupation of man-made habitats could bring the bats into closer association with humans and their companion animals or livestock. The significance of this is seen in the transmission of some pathogens, such as bat rabies viruses, from peridomestic bats to humans and livestock. The number of bats in each colony varies greatly from less than 10 to over 200 000 individuals [9]. The large number of individuals, together with the social habits of the bats, such as mutual grooming and biting during courtship and mating, facilitates the transmission of infectious agents between them through direct contact, aerosols or arthropod vectors.

The dietary habits of bats can broadly be divided into insectivorous, frugivorous, carnivorous, omnivorous and sanguivorous [10]. Predatory bats could potentially acquire infectious agents from other animal species, such as birds and insects. Sanguivory in bats is limited to only three species of vampire bats, *Desmodus rotundus*, *Diphylla ecaudata*, and *Diaemus youngi*, all belonging to the

Table 1. Transmission dynamics of selected bat-associated viruses

Virus	Natural reservoir host	Amplification host(s)	Populations at-risk of zoonotic infection	Epicentre for animal-to-human transmission	Epicentre for human-to-human transmission
Coronaviruses related to SARS-CoV	Bats <sup>a</sup>	Palm civets and possibly other animals in wet markets	Restaurant or market workers handling wild animals (SARS-CoV)	Wet markets (SARS-CoV)	Health care facilities (SARS-CoV)
Ebola virus	Bats <sup>a</sup>	Non-human primates	Biologists handling primates, bushmeat hunters	Forests	Health care facilities, families
Hendra virus	Bats	Horses	Close contacts with horses	Horse farms	Nil
Menangle virus	Bats	Pigs	Pig farmers	Pig farms	Nil
Nipah virus	Bats	Pigs	Pig farmers, abattoir workers	Pig farm, abattoirs	Health care facilities (possible), families (possible)
Rabies viruses	Bats, carnivores	Nil (direct bat-to-human transmission); occasionally spillover to terrestrial mammals; rarely through organ transplantation	Biologist and other personnel handling bats; residents in endemic areas (e.g. Latin America)	Rural residents; occasionally in urban environment	Nil; rarely through organ transplantation
European and Australian bat Lyssaviruses	Bats	Nil (direct bat-to-human transmission)	Biologist and other personnel handling bats	Sporadic cases only	Nil

<sup>a</sup>Transmission from bats to human has been postulated for these viruses but has not been proven.

Table 2. Summary of bat families (7,8)

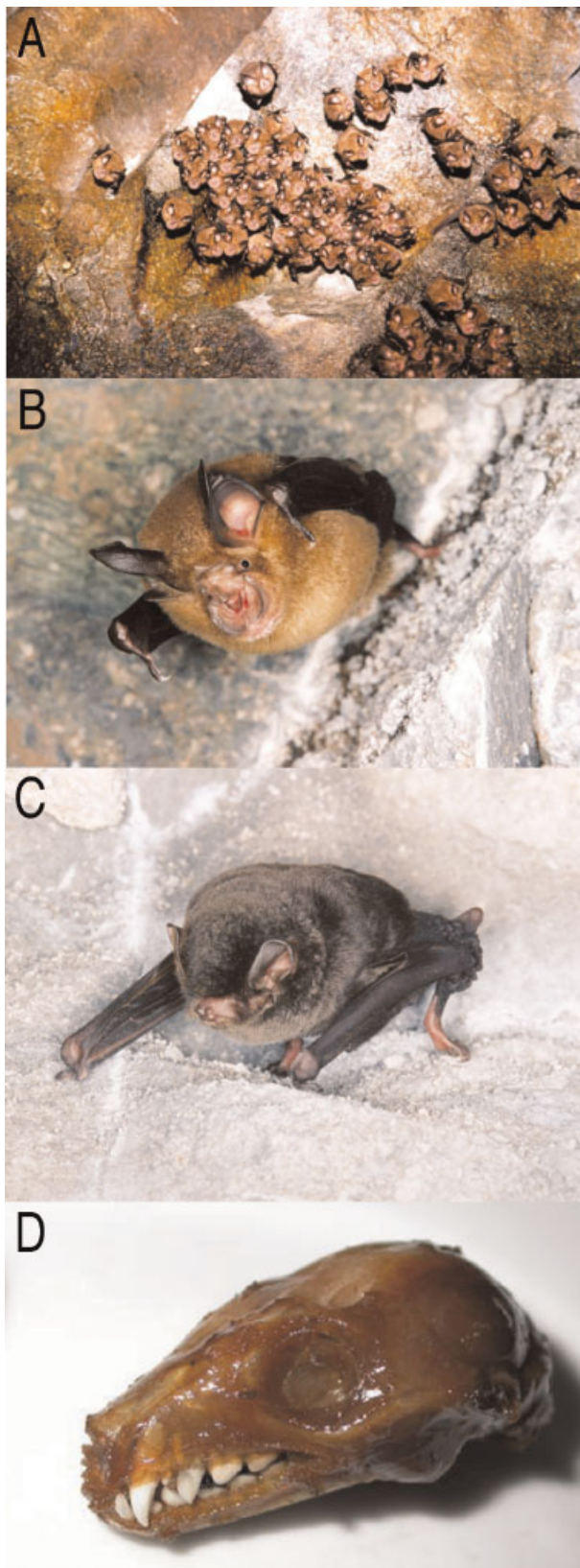
Family <sup>a</sup>	Common names	Major feeding habits	Main geographical distribution
<i>Pteropodidae</i> *	Flying foxes	Fruit, nectar, pollen, insects	Tropical Asia, Australia, Africa
<i>Craseonycteridae</i>	Bumblebee bats	Insects	Thailand
<i>Emballonuridae</i> *	Sheath-tailed bats	Insects	Worldwide, tropical
<i>Furipteridae</i>	Smoky bats	Insects	New World, tropical
<i>Megadermatidae</i>	False vampires	Arthropods, small vertebrates	Tropical Asia, Australia, Africa
<i>Molossidae</i> *	Free-tailed bats	Insects	Worldwide, tropical and subtropical
<i>Mormoopidae</i>	Mustached bats	Insects	New World, tropical
<i>Mystacinidae</i>	Short-tailed bats	Fruit, pollen, nectar, small vertebrates, carrion	New Zealand
<i>Myzopodidae</i>	Sucker-footed bats	Insects	Madagascar
<i>Natalidae</i>	Funnel-eared bats	Insects	New World, tropical
<i>Noctilionidae</i>	Bulldog bats, fisherman bats	Arthropods, fish	New World, tropical
<i>Nycteridae</i>	Slit-faced bats	Arthropods	Africa, Southeast Asia
<i>Phyllostomidae</i> *	New World leaf-nosed bats	Insects, fruit, pollen, nectar, small vertebrates, blood (only <i>Desmodus rotundus</i> , <i>Diaemus youngi</i> , and <i>Diphylla ecaudata</i> )	New World, tropical
<i>Rhinolophidae</i> *	Horseshoe bats and Old World leaf-nosed bats	Insects	Old World, tropical and temperate
<i>Rhinopomatidae</i>	Mouse-tailed bats	Insects	North Africa, India
<i>Thyropteridae</i>	Disc-winged bats	Insects	New World, tropical
<i>Vespertilionidae</i> *	Evening bats	Insects	Worldwide

<sup>a</sup>Families for which bat species carry known human pathogens are marked with an asterisk.

family *Phyllostomidae* and found only in Latin America. The vampire bats prey on birds and mammals and may also supplement their diet with insects. The diet of bats may not have direct relationships with the nature of pathogens they carry, as the same group of infectious agents could be carried by diverse bat species with different dietary habits. However, the search for foods may bring certain bats into close contacts with humans and other animals, thereby facilitating interspecies transmission of infectious agents. Such aberrant interactions often occur as a result of natural or man-made environmental perturbations and have been implicated in the transmission of the Nipah

and bat rabies viruses to humans and Ebola virus to primates.

Bats fly over a relatively large distance from their roosting sites in search for food. Microchiropterans commonly have a foraging distance of 10–15 km while some may fly over 80 km a night. Megachiropterans can travel up to 50 km [10]. Some species may utilise night roosts near feeding sites and distant from their day roosts. Despite their small sizes, bats have a relatively long life span and develop slowly. Many species live for over 10–20 years with some species living for over 30 years in the wild [10,11]. Host longevity facilitates the persistence of infectious agents in



bats and thereby increases the chance of spread through natural or accidental dispersal of the bats into new geographical areas.

When faced with winter and shortage of food supply, bats may either hibernate or migrate. The megachiropterans do not hibernate. Hibernation in microchiropterans involves substantial physiological changes during torpor, including an accumulation of brown adipose tissue. Hibernating bats can remain torpid continuously for up to 75 days, though many will have periodic arousal alternating with torpor periods lasting from 2 to 15 days [12]. Seasonal migration has been observed for bats living in both temperate and tropical environments, which demonstrate substantial seasonal changes in food supply and/or temperature. The maximal distance of migration of the studied bat species ranges from 200–300 km to almost 2000 km [13].

Of all the vertebrate hosts, bats are considered to be less important than ungulates, carnivores, rodents, non-mammals and primates in terms of the prevalence of zoonotic pathogens [2]. Nevertheless, bats do harbour a relatively large number of known or potential pathogens. Some of these appear to have co-evolved with specific bat hosts (e.g. lyssaviruses). Transmission of viruses carried by bats to humans can occur in a variety of ways, with direct contact through bites and scratches the most obvious example. Bats do not normally prey on humans. However, *Desmodus rotundus* has been known to feed on humans in Latin America when alternative animal hosts are scarce [14,15]. Bites sustained from other bat species are usually the result of accidental encounters rather than deliberate attack on humans. Lyssaviruses are typical examples of direct bat-to-human transmission of pathogens.

Another possible route of transmission involves inhalation of infectious particles by humans. These infective aerosols could arise from the secretions (e.g. saliva) or guano of the bats. This route of transmission may possibly occur in lyssavirus

Figure 1. (A) Typical roosts of *Rhinolophus sinicus*, a cave-dwelling bat that can be found in Hong Kong. (B) *Rhinolophus sinicus*, bats that carry SARS-like coronaviruses in Hong Kong. (C) *Miniopterus magnater*, bats that carry group 1 coronaviruses. (D) Bats are often consumed as delicacies in some parts of the world including China. A *Cynopterus sphinx* bat being served in a restaurant in southern China

infections. Arthropod vectors could serve as another mode of transmission of pathogens between bats and humans. Important categories of arthropods in this regard include mosquitoes, sand flies, and ectoparasites infesting bats such as bat ticks, bugs and bat flies. Insects, such as mosquitoes and sand flies may feed on both bats and humans as shown in entomological and virological studies [16,17]. There is currently no evidence to implicate an active role of these vectors in disease transmission, presumably because of the lack of efficient vector-pathogen combinations or host specificity of the vectors. Vectorborne transmission is therefore a route that requires further study.

The consumption of bat meat is practised in some parts of the world including China, Guam, and some parts of Asia where it is considered to have therapeutic effects against asthma (Figure 1). Adequately cooked bat meat is unlikely to pose any risk for transmission of infections. However, capture and slaughtering of bats could expose the handler to the bats' blood and body fluids or bites and scratches. In situations where live bats are kept in captivity in game markets, they may come into close contact with other animals, which are susceptible to viruses carried by bats. This is one of the postulated mechanisms linking the finding of SARS-coronavirus (SARS-CoV) in bats and palm civets in southern China. The bats may have transmitted a SARS-CoV-like virus to the civets, which in turn act as amplifying hosts before transmitting the virus to humans.

In some situations, transmission of bat pathogens to humans occurs via a secondary vertebrate host serving as an amplifying host. The outbreak of Nipah virus in Southeast Asia involved pigs as an efficient secondary host for multiplication of the virus and horses were involved in the transmission of Hendra virus to humans, while the palm civet is postulated to be the intermediate host for SARS-CoV. In an extreme example, another human host could be involved before such transmissions are clinically evident. This is exemplified by the transmission of the rabies virus to four organ transplant recipients from a donor who died of encephalitis of unknown aetiology who, in retrospect, was found to have been bitten by a bat [18].

The factors that promote the transmission of pathogens from bats to humans are incompletely

understood. One aspect is environmental changes, either as a result of natural or climatic alterations or conditions related to human activities. Examples include a change of feeding hosts of the vampire bat *Desmodus rotundus* from livestock to humans due to a decline in the livestock population [14]. Another example is the Nipah outbreak in Malaysia in which the loss of the normal forest habitats of the bats forced them to reside in areas close to livestock and human inhabitation [19,20]. The spread of the infectious disease within human communities was further augmented by trade in domestic animals, such as the transport of infected pigs in the spread of Nipah virus infection. Movement of bats over large geographical distances either as a result of natural migration, accidental or deliberate transport via artificial vehicles can occur and these may further facilitate the long-distance spread of potential pathogens [21].

#### SYSTEMATIC REVIEW OF VIRUSES ASSOCIATED WITH BATS

The following section summarises the important viruses that have been found to be associated with bats. Emphasis is put on viruses that have clinical or veterinary significance and those that are better characterised. Supplementary Table summarises the viruses together with their main geographical distribution and natural hosts. Table 3 summarises their potential to be transmitted to humans based on current clinical, virological and epidemiological information.

The detection of viruses in bats or other animals is problematic. Unequivocal evidence for natural infection can be obtained with a positive viral culture obtained from the animals. Another acceptable detection method is the use of direct immunofluorescent staining of bat tissues, most commonly used in the detection of lyssaviruses in the brains of bats. Nucleic acid amplification may offer similar confidence if the tests are performed competently without contamination. Unfortunately, many viruses are not readily cultivable *in vitro*. Consequently, a number of field studies employed serological tests to look for evidence of infection. While serology can offer valuable information, cross-reactivity between different members of the same family of viruses may be observed for some test formats (e.g. haemagglutination inhibition, complement fixation).

Table 3. Risk ranking of bat-associated viruses in causing human infections<sup>a</sup>

Bat families	<i>Not specified</i>	<i>Pteropodidae</i>	<i>Craseonycteridae</i>	<i>Emballonuridae</i>	<i>Furipteridae</i>	<i>Megadermatidae</i>	<i>Molossidae</i>	<i>Mormoopidae</i>	<i>Mystacinidae</i>	<i>Myzopodidae</i>	<i>Natalidae</i>	<i>Noctilionidae</i>	<i>Nycteridae</i>	<i>Phyllostomidae</i>	<i>Rhinolophidae</i>	<i>Rhinopomatidae</i>	<i>Thyropteridae</i>	<i>Vespertilionidae</i>
Tacaribe virus														D				
Kaeng Khoi virus							B											
Mojuí dos Campos virus	D																	
Bimiti virus, Catu virus, Guama virus, Manzanilla virus, Nepuyo virus, Oriboca virus							D	D			D			D				
Toscana virus																		C
Coronaviruses		C													C			C
Ebola virus		B																
Dengue virus		C												C				
Ilheus virus								C						C				
Japanese encephalitis virus															C			C
Kyasanur Forest disease virus		C													C			
Montana <i>Myotis</i> leukoencephalitis																		D
Rio Bravo virus							B											
St Louis encephalitis virus							C	C			C			C				C
Tamana bat virus																		
West Nile virus		C																C
Yellow fever virus							C	C						C				
Yokose virus																		D
Influenza viruses		C				C												C
Hendra virus		A																
Menangle virus		A																
Nipah virus		A																
Bat parainfluenza virus		D																
Mapuera virus														D				
Tioman virus		D																
Nelson Bay virus		D																
Pulau virus		D																
Betaretrovirus														D				
Mount Elgon bat virus															D			
Rabies virus		A					A							A				A
Lagos bat virus		D																
Duvenhage virus																		A

(Continues)

Table 3. (Continued)

Bat families	<i>Not specified</i>	<i>Pteropodidae</i>	<i>Craseonycteridae</i>	<i>Emballonuridae</i>	<i>Furipteridae</i>	<i>Megadermatidae</i>	<i>Molossidae</i>	<i>Mormoopidae</i>	<i>Mystacinidae</i>	<i>Myzopodidae</i>	<i>Natalidae</i>	<i>Noctilionidae</i>	<i>Nycteridae</i>	<i>Phyllostomidae</i>	<i>Rhinolophidae</i>	<i>Rhinopomatidae</i>	<i>Thyropteridae</i>	<i>Vespertilionidae</i>
Viruses																		
European bat lyssavirus 1		B					B								B			A
European bat lyssavirus 2																		A
Australian bat lyssavirus		A		A														
Aravan virus																		D
Irkut virus																		D
Khujand virus																		D
West Caucasian virus																		D
Other lyssaviruses in Southeast Asia		C		C			C								C			C
Mount Elgon virus															D			
Chikungunya virus																		C
Eastern equine encephalitis virus				C										C				
Mucambo virus							D							D				
Venezuelan equine encephalitis virus														C				
Western equine encephalitis virus														C				C

<sup>a</sup>Risk of bat-to-human transmission of the respective pathogens: A, documented direct bat-to-human transmission or through secondary hosts or definite epidemiological linkage; B, potential risk of transmission directly from bats or through secondary hosts or vectors because presence of known human pathogens documented and microbes present in relatively high frequency in bats; C, low risk of transmission directly from bats or through vectors because of low prevalence of pathogens in bats, inefficient vectorial capacity of bats or arthropod vectors, or insufficient epidemiological data to support a link; D, little risk of transmission because viruses not known to infect humans.

This is especially common in families such as *Flaviviridae*. Neutralising antibodies are generally considered to be the most specific for individual viruses. Newer studies frequently utilise RT-PCR and sequencing information to discriminate between viral orders, families and genera, irrespective of whether the viruses are cultivable or not.

### Arenaviridae

An arenavirus, Tacaribe virus, was found in a number of bat species in Trinidad by culture and

serology [22,23]. It has not been found to infect humans.

### Bunyaviridae

Bunyaviridae includes five genera, *Bunyavirus*, *Hantavirus*, *Nairovirus*, *Phlebovirus*, and *Tospovirus*. They constitute a huge family of viruses with a number of prominent human pathogens such as the hantaviruses (e.g. Hantaan, Seoul, and Sin Nombre viruses), Crimean-Congo haemorrhagic fever virus, and Rift Valley fever virus. The bunyaviruses have a global occurrence and many of



them are arthropod-borne and have reservoirs in vertebrates.

The Kaeng Khoi virus was initially isolated in 1969 from the wrinkle-lipped free-tailed bat (*Chaerephon plicata*) and Theobald's tomb bat (*Taphozous theobaldi*) in a cave in Thailand. Subsequent studies also found virological and serological evidence of infection in these two bats in Thailand [24]. The virus was recently isolated from *Chaerephon plicata* in Cambodia [25]. Interestingly, the virus was also isolated from bedbugs (*Stricticimex parvus* and *Cimex insuetus*) collected in the same bat caves, and these insects have been postulated to play a role in transmission of the virus between bats [24]. There is some serological evidence showing that Kaeng Khoi virus can infect humans entering bat caves to collect guano, and a seroprevalence of 29% among these workers has been described. Bedbug bites were incriminated to cause an influenza-like illness in the workers, which might be related to this viral infection.

The Toscana virus is endemic in Mediterranean countries where it is a major cause of viral meningitis and encephalitis [26]. It has been isolated from the brain of a bat (*Pipistrellus kuhli*) once but the sandflies—rather than vertebrates—are considered to be its natural reservoir host [27]. Other bunyaviruses that have been detected in bats but with uncertain clinical significance are listed in Supplementary Table.

### Coronaviridae

The coronaviruses have become the focus of research since the 2003 global epidemic of SARS caused by the SARS-CoV. Coronaviruses are known to have diverse animal hosts ranging from mammalian to avian species. Human coronavirus infections prior to the SARS outbreak were caused by human coronaviruses 229E and OC43 which commonly result in the common cold.

The first cases of SARS occurred in late 2002 in the southern Chinese province of Guangdong. The first confirmed case of SARS was a chef working in a local restaurant and he had had contacts with wild game animals prior to the onset of disease [28]. The emergence of a novel viral infection, together with the occupational history of contact with wild animals among four index patients in the Guangdong outbreak, prompted the search for an animal reservoir of SARS-CoV. The live

animal retail markets in the city of Shenzhen, Guangdong, was investigated and evidence of infection was found in the Himalayan palm civet (*Paguma larvata*) and to a lesser extent, Chinese ferret badgers (*Melogale moschata*) and raccoon dogs (*Nyctereutes procyonoides*) [29]. Further evidence for zoonotic transmission of SARS-CoV came from studies which showed that wild animal traders and slaughterers had a significantly higher seroprevalence (20 to 45%) against SARS-CoV than vegetable traders and control populations (0 to 5%) [30]. Review of seroprevalence studies showed that persons with an occupational history of contact with wild animals had a substantially higher prevalence of antibodies as compared to healthy blood donors, household contact or health care workers caring for SARS patients (12.99 to 16.69% vs. 0 to 2.92%, respectively) [31]. A recent study showed that contact with game animals could result in asymptomatic infection with the SARS-CoV but that the individual could still have detectable coronavirus antigenaemia and seroconversion [32]. Restrictions on the sale and consumption of game animals therefore became important control measures during the SARS epidemic in China. In contrast to other zoonotic pathogens that crossed the species barrier to humans, the SARS-CoV is notable in that it spreads readily from person-to-person and therefore outbreaks within health care facilities was a prominent feature during the SARS epidemic with a fifth of all global cases being health care workers [33]. It is also unique amongst coronaviruses in that a wide host range is observed, with the virus being able to infect (with or without the development of illness) humans, Chinese ferret badgers (*Melogale moschata*) and raccoon dogs (*Nyctereutes procyonoides*), cynomolgus macaques (*Macaca fascicularis*), African green monkeys (*Cercopithecus atheiops*, *Chlorocebus sabeus*), rhesus monkeys (*Macaca mulata*), ferrets (*Mustela furo*), domestic cats (*Felis domesticus*), mice, golden Syrian hamsters (*Mesocricetus auratus*), common marmosets (*Callithrix jacchus*), pigs, and chickens [34,35,36,37,38].

The sudden emergence of the SARS-CoV was initially linked to the palm civet and related animals which were found to carry the SARS-CoV asymptotically. The palm civet was consumed as a game animal in southern China and sold in the wet markets. Subsequent studies however

did not show a significant amount of SARS-CoV in wild or farmed palm civets. This suggested that the caged palm civets in the markets may not be a primary reservoir host of SARS-CoV in nature, but merely a secondary amplifying host that increases the viral burden and provides ample contact with humans, thereby facilitating animal to human transmission, a situation that is similar to the *Henipavirus* discussed below. The search for the primary reservoir host of SARS-CoV led to the finding of novel coronaviruses among bats in Hong Kong. One of these was detected in Chinese horseshoe bats (*Rhinolophus sinicus* (*R. rouxii*)) in Hong Kong which is currently named bat SARS-CoV (bat-SARS-CoV) [39]. The bat-SARS-CoV was initially found by RT-PCR in 39% of *R. sinicus* tested but seropositivity of bats ranged from 67 to 84% when the sera were tested by western blot and enzyme immunoassay respectively. It is postulated that the bats might serve as the natural reservoir of the SARS-CoV (or a related virus) and the palm civets acquired the virus from the bats before passing it on to humans.

Another new coronavirus was detected in the lesser bent-winged bats (*Miniopterus pusillus*), large bent-winged bats (*Miniopterus magnater*), and Japanese long-winged bats (*Miniopterus schreibersii*) [40]. This virus belongs to group 1 coronaviruses, which also includes the human pathogens human coronaviruses 229E and NL63. The virus was detected in 3 of 25 (12%) *Miniopterus pusillus* bats in a subsequent survey in Hong Kong which also revealed a great diversity of coronaviruses among the bat population [41]. Coronaviruses were detected by RT-PCR of the anal swabs in 37 of 309 bats (12%) with the bat-SARS-CoV being detected in 21 of 118 (17.8%) *R. sinicus*. Six other coronaviruses were also discovered in different bat species: bat-CoV-HKU2 from 2 of 118 *R. sinicus*; bat-CoV-HKU4 from 4 of 21 (19%) lesser bamboo bats (*Tylonycteris pachypus*); bat-CoV-HKU5 from 4 of 14 (28.6%) Japanese pipistrelle bats (*Pipistrellus abramus*); bat-CoV-HKU6 from 1 of 23 (4.3%) Rickett's big-footed bat (*Myotis ricketti*); bat-CoV-HKU7 from 1 of 51 (1.9%) *Miniopterus magnater*, and bat-CoV-HKU8 from 1 of 25 (4%) *Miniopterus pusillus*. Bat-CoV-HKU2, HKU6, HKU7, and HKU8 all belong to group 1 coronaviruses, while bat-CoV-HKU4, HKU5 belong to group 2 coronaviruses, the latter also includes human coronaviruses OC43, HKU1

and the SARS-CoV. The association of coronaviruses with bats is also confirmed in another study by Li *et al.* who documented the presence of SARS-like coronavirus infection of bats in Guangxi and Hubei, two provinces in southern and central China respectively [42]. Notably, with the exception of two positive samples which came from *Rousettus leschenaulti* (Family *Pteropodidae*), the majority of bats carrying SARS-like coronaviruses in China were *Rhinolophus* spp., members of which were also found to be infected with SARS coronavirus-like virus in Hong Kong. Figure 2 shows the phylogenetic relationship between the known human and bat-associated coronaviruses. These findings suggest that bats could represent a hitherto undiscovered reservoir of the coronaviruses, many of which might have important clinical or veterinary significance.

### Filoviridae

Filoviruses known to cause human infection belong of two genera: *Marburgvirus* and *Ebolavirus*, the latter containing four species, *viz* Sudan, Zaire, Reston and Ivory Coast. Both Marburg and Ebola viruses are endemic in Africa, except the Ebola Reston virus which was acquired from primates imported from the Philippines. Human infections due to filoviruses typically occur in outbreaks due to interpersonal and nosocomial transmission, and mortality rates in outbreaks reach a staggering 50–80%. The most recent outbreak of Marburg virus occurred in Angola in 2005, involving 374 cases and 329 deaths (88% case-fatality rate) [43]; the Ebola virus outbreak which occurred in 2005 in the Republic of Congo involved 12 cases and 9 deaths (75% case-fatality rate) [44].

Primary human infections in most cases resulted from contact with non-human primates which are generally sick or dead as a result of filovirus infection. The primates, however, are not believed to be important reservoirs of filoviruses in nature since they regularly die from the infections. Epidemiological and virological studies suggested that the regular outbreaks of filovirus infection in primates probably did not occur as a result of interspecies transmission between primates, but due to introduction to a hitherto unknown animal or environmental source [45]. The nature of such natural reservoirs remains elusive despite extensive surveys until a recent study which discovered the presence of Ebola virus (by serology and

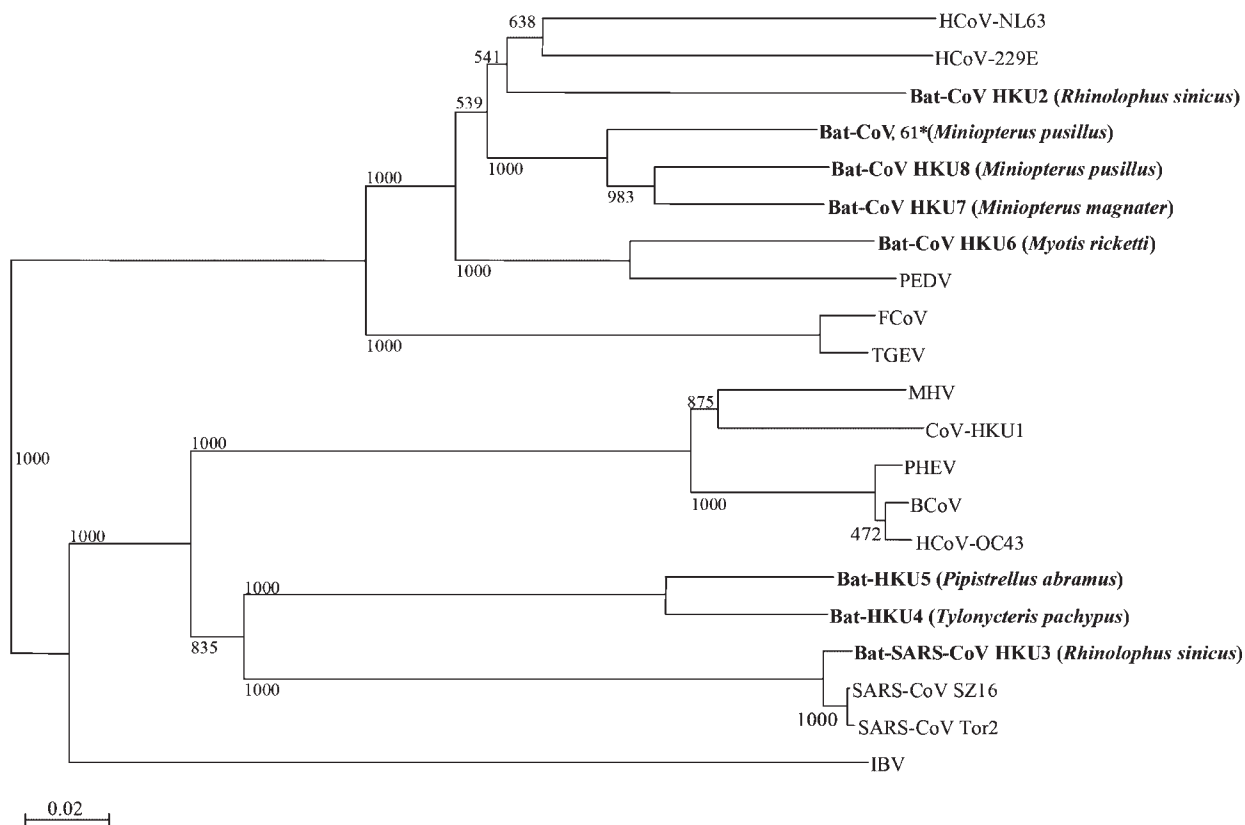


Figure 2. Phylogenetic tree of RNA-dependent RNA polymerase showing the relationship of the bat coronaviruses to other coronaviruses. The trees were inferred from amino acid sequence data (949 amino acid positions) by the neighbour-joining method. Numbers at nodes indicated levels of bootstrap support calculated from 1000 trees. The scale bar indicates the estimated number of substitutions per 50 amino acids. Coronaviruses found in bats are in bold and species of bats in which the corresponding coronaviruses are found are in brackets. HCoV-229E, human coronavirus 229E; PEDV, porcine epidemic diarrhoea virus; TGEV, porcine transmissible gastroenteritis virus; FCoV, feline coronavirus; HCoV-NL63, human coronavirus NL63; HCoV-OC43, human coronavirus OC43; MHV, murine hepatitis virus; BCoV, bovine coronavirus; PHEV, porcine haemagglutinating encephalomyelitis virus; CoV-HKU1, coronavirus HKU1; SARS-CoV, SARS coronavirus; bat-SARS-CoV, SARS coronavirus-like virus found in bats; IBV, infectious bronchitis virus

RT-PCR) in three species of bats captured in Gabon and the Republic of Congo. These include *Hypsignathus monstrosus* (4 of 17 animals, 23.5%), *Epomops fraqueti* (8 of 117 animals, 6.8%), and *Myonycteris torquata* (4 of 58 animals, 6.9%), all belonging to the *Pteropodidae* family of flying foxes [46]. If the bats are indeed the natural reservoir of the Ebola virus, two factors have been suggested to facilitate their transmission to primates [46]. The scarcity of food during the dry season is thought to affect the immune system of bats, thereby facilitating viral replication. At the same time, primates and bats could come into closer contact in search for food so cross species transmission of the virus from bats to primates could

become easier. These findings have important implications in future studies of the ecology and epidemiology of Ebola virus. The natural reservoir hosts of Marburg virus have not been identified, though bats would now be an obvious and important group of animals to investigate. Direct transmission of either of the filoviruses from bats to humans has not been confirmed. However, it is interesting to note that in some of the previous outbreaks of filoviruses in Africa not involving contact with primates, bats were sighted in caves or buildings which the index cases had visited or worked in [45]. With the present knowledge in the epidemiology of the filoviruses, it appears that direct bat-to-human transmission of

the viruses might occur, though it is a relatively inefficient route. The more important and efficient route of transmission could involve primates which develop illness and/or fatal infections, thereby serving as an amplifying host before transmission to humans, a theme which is shared by the ecology of Nipah virus and SARS-CoV.

### Flaviviridae

The flaviviruses encompass a diverse group of viruses with global distribution, important public health and clinical significance, and distinctive clinical syndromes. Many of the clinically important viruses are transmitted via arthropod vectors and have reservoirs in either arthropods or vertebrates. The most important clinical syndromes caused by flaviviruses include central nervous system infection (e.g. St. Louis encephalitis virus, Japanese encephalitis virus, West Nile virus, Murray Valley encephalitis virus, and tick-borne encephalitis virus), fever and rash (e.g. dengue viruses), and haemorrhagic fever (e.g. yellow fever virus, dengue viruses, Omsk haemorrhagic fever virus, and Kyasanur Forest disease virus). Bats are not generally considered to be important reservoirs of flaviviruses though a number of bats have been shown to be susceptible to viral infection either in experimental or natural settings.

The ardeid birds are the major reservoir of Japanese encephalitis virus while the domestic pig is the most important amplifying host responsible for human infections and outbreaks. Birds are likewise the key reservoirs of St. Louis encephalitis and West Nile viruses. However, West Nile virus was isolated from bats more than 30 years ago in India [47]. Recent surveillance studies in the USA also showed occasional infection of bats by the West Nile virus [48,49,50]. In a series of experiments by Sulkin *et al.*, bats (*Tadarida brasiliensis mexicana*, *Myotis lucifugus lucifugus*, *Eptesicus fuscus fuscus*) were shown to be susceptible to infection by Japanese encephalitis and St. Louis encephalitis viruses by either the subcutaneous and intracerebral route [51]. The susceptibility of bats to infection and the level of viral replication varied with different viral strains and the species of bats tested. Of particular interest is that bats developed relatively long periods of viraemia (upto 30 days) after infection and developed no clinicopathological sequelae following inoculation, even though there was significant viral multiplication in the brain fol-

lowing intracerebral inoculation. Importantly, infections due to these neurotropic viruses produced significant viral loads not only in the blood but also in the interscapular brown fat, in contrast to brains and kidneys which were less frequently found positive for viruses. Sulkin *et al.* also demonstrated that infection at low temperatures (from 8°C to 24°C) was associated with prolonged viraemia of over 100 days and persistence of viruses in the brown fat [52]. Transplacental infection of Japanese encephalitis virus also occurred readily in *Tadarida brasiliensis mexicana*, again without any clinicopathological sequelae to the fetuses [53]. Similar experiments also showed that the big brown bat (*Eptesicus fuscus*) can maintain St. Louis encephalitis during hibernation [54]. Isolation of Japanese encephalitis virus in naturally infected bats in Japan and Taiwan, and serological evidence of St. Louis encephalitis infection in Ohio and Trinidad have been reported [16,23,54,55]. These data suggested that bats could easily serve as a suitable host for the persistence of these viruses in nature, perhaps with special significance to overwintering of viruses.

Similarly, natural infection of bats by dengue virus was suggested by a serological study in Costa Rica and Ecuador, and more convincingly by positive RT-PCR of the brains of *Rousettus leschenaulti* bats in southern China [56,57]. Kyasanur Forest disease is a flaviviral haemorrhagic fever mainly seen in western India. The virus is mainly transmitted between rodents and insectivores via ixodid ticks. Isolation of the virus from bats (*Rhinolophus rouxii* and *Cynopterus sphinx*) had been reported on two occasions from India. The role of bats in the ecology of these medically important flaviviruses is unknown [58,59]. Antibody against the yellow fever virus was described in a bat in one study, though primates are still believed to be the most important reservoir of sylvatic yellow fever [23]. A virus named Montana Myotis leukoencephalitis (MML) virus was initially isolated in the 1960s from *Myotis lucifugus* [60]. Subsequent genome analysis placed it into the family *Flaviviridae* [61]. No arthropod vectors have been identified for this virus and the virus can be transmitted directly to mice from bat bites. The Rio Bravo virus was isolated from the salivary glands of Brazilian free-tailed bats (*Tadarida brasiliensis*) in the USA and Mexico and also from *Molossus ater* in Trinidad [62,63]. It has no known arthropod vectors and is

believed to be transmitted amongst bats by direct contact or aerosols. Human infections by the Rio Bravo virus, including cases of laboratory-acquired infections (resulting in meningitis, orchitis or oophoritis), have been documented [64]. Other flaviviruses that have been described in bats include the Ilheus virus, Tamana bat virus, and Yokose virus [23,63,65]. The Ilheus virus causes an acute febrile illness in humans in Latin America and cases of encephalitis have been reported but without mortalities. The main reservoir hosts are birds and it is transmitted by mosquitoes.

It is evident that bats can be infected by numerous flaviviruses in nature. It is also biologically plausible that they have the potential to serve as reservoir hosts in certain situations, such as overwintering of viruses. However, as pointed out by Scott, evidence of viral infection in a vertebrate host does not equate to a significant role for them in the natural ecology of the viruses [66]. Virological profiles of infected animals, density of vectors, feeding habits of vectors and their vectorial capacity are all essential factors to be considered in this regard.

### Orthomyxoviridae

Isolation of influenza virus A/H3N2 from *Nyctalus noctula* bats in Kazakhstan was reported in the 1970s in the Russian literature [67]. Subsequently, serological evidence of influenza A/H2N2 and A/H3N2 infections of various bat species was found in India [68]. Although bats are likely to be susceptible to influenza virus infection, current epidemiological studies do not consider them a significant reservoir of the viruses in nature.

### Paramyxoviridae

Paramyxoviruses are common pathogens of humans. Important human pathogens include *Respirovirus* (human parainfluenza viruses 1 and 3), *Rubulavirus* (human parainfluenza viruses 2 and 4, mumps virus), *Morbillivirus* (measles virus), and *Metapneumovirus*. The first finding of a paramyxovirus in bats was described in 1966 [69,70]. A parainfluenza virus type 2 was isolated from the frugivorous bat *Rousettus leschenaulti* in India. The Mapuera virus was the second paramyxovirus isolated from the yellow-shouldered bat (*Sturnira lilium*) in Brazil in 1979 [71]. Both have not been found to cause human infections. The significance of paramyxoviruses in bats, however, became

evident in the 1990s with the occurrence of two outbreaks in humans and animals. The new genus *Henipavirus* was proposed to encompass two of the three zoonotic paramyxoviruses that are pathogenic to humans, namely Hendra and Nipah viruses. Whether the Indian type 2 parainfluenza virus is related to the newly described paramyxoviruses is unknown.

In 1994, an outbreak of fatal hyperacute respiratory illness occurred in horses in Queensland, Australia. Two human contacts of the index horse were infected and developed an influenza-like illness. One of them died of pneumonitis, respiratory failure, and renal failure and a virus was isolated from both the fatal patient and the horses [72]. The virus was initially called equine morbillivirus and later named Hendra virus. In the 1995 outbreak in Queensland, another patient died of encephalitis after Hendra virus infection [73]. Following the initial outbreaks of Hendra virus infection, a serological survey in Australia involving 5264 sera from 46 terrestrial animal species failed to show any evidence of infection by this new virus. However, seropositivity was detected in the spectacled fruit bat (*Pteropus conspicillatus*), black fruit bat (*P. alecto*), little red (*P. scapulatus*), and grey-headed bat (*P. poliocephalus*) [74]. Subsequently, Hendra virus was isolated from the viscera and fetuses of *P. poliocephalus* and *P. alecto* [75]. Retrospective analysis of bat sera showed that the virus was present in Australian flying foxes as early as 1982 and in some surveys, seroprevalence as high as 47% had been documented [76]. As is the case for many other bat-associated infections, Hendra virus does not cause disease or pathology in bats, and transplacental transmission to the fetus is possible. The route of transmission from flying foxes to horses has not been directly documented, though exposure to infected urine has been considered possible [76]. In addition to horses, cats and guinea pigs are also susceptible to Hendra virus infection and these animals can shed the virus in urine [77]. Humans acquired the infection solely from horses to date and not from flying foxes. The route of transmission to humans probably occur through contact with the respiratory secretions of the infected horses.

From September 1998 to June 1999, an outbreak of encephalitis occurred amongst pig-farmers in Malaysia. The aetiology of the disease is a novel paramyxovirus name Nipah virus isolated from

the cerebrospinal fluid of the patients [78,79]. There were 265 patients involved in the outbreak, with a case fatality rate of 39.6% and 15% of the survivors had residual neurological deficits [79,80]. The outbreak in humans was preceded by sudden and massive deaths of the pigs due to acute respiratory distress syndrome and encephalitis, and close contacts with pigs was confirmed to be the major risk factor for acquisition of the infection in a case-control study [80]. The presence of the virus in the respiratory secretions and urine of infected pigs suggested that contact with these secretions is the likely route of transmission. About 8% of the patients did not have a contact history with sick pigs; dogs were suspected to be another possible vector for transmission as they can also be infected by Nipah virus. Culling of over 1 million pigs was needed to terminate the outbreak. Singapore was concurrently hit by an outbreak of Nipah encephalitis in March 1999 which affected 11 abattoir workers who handled pigs imported from Malaysia [81]. Contact with live pigs was again confirmed to be a major risk factor for infection [82]. No interpersonal or nosocomial transmission of Nipah virus infection was noted in these two outbreaks [83].

A search for the reservoir hosts of Nipah virus found that in addition to pigs, wild boars (*Sus scrofa*), domestic dogs (*Canis lupus*) and rodents (*Rattus rattus*) could be secondarily infected. But the main natural reservoir of the virus appears to be flying foxes. Serological studies showed that, in Malaysia, a number of bat species are seropositive, with the majority of the animals belonging to the Megachiroptera [84]. The highest seroprevalence was found in *Pteropus hypomelanus* (31% seropositive), followed by *Pteropus vampyrus* (17%), *Eonycteris spelaea* (5%), *Cynopterus brachyotis* (4%), and the Microchiropteran *Scotophilus kuhlii* (3%). Subsequently, Nipah virus was cultured from the urine of the flying fox *P. hypomelanus* [85]. It was postulated that the combination of anthropogenic deforestation and drought caused by the El Niño Southern Oscillation drove the frugivorous flying foxes from their natural habitats into orchard plantations. The close proximity between the bats and pigs thus created, resulted in the cross-species infection of the pigs and subsequently humans [86].

There was no epidemiological evidence to suggest that Nipah virus was transmitted from flying foxes to humans directly and no person-to-person

transmission of the infection has been confirmed in the outbreaks in Malaysia and Singapore. Nevertheless, the presence of the virus in the respiratory secretions and urine of infected humans has been documented, suggesting that the risk of interpersonal spread may not be zero [87]. Although the number of cases that has been studied was small, it appeared that the presence of Nipah virus in respiratory secretions and urine is confined to the first week of illness before the appearance of IgM antibodies.

In 2001 and 2003, outbreaks of a febrile neurological disease occurred in Bangladesh. Retrospective studies showed that the outbreak was again caused by Nipah virus infection [88,89]. The Bangladeshi outbreaks are notable in two aspects. Firstly, pig populations were very small in Bangladesh and there were no reports of massive animal deaths in the vicinity of the affected villages. Direct bat-to-human transmission through accidental exposures to bats or bat secretions was suspected. Secondly, there were familial clusters of cases suggesting the possibility of interpersonal spread through contact with the patients' secretions. Nipah virus antibodies were found in two flying foxes (*Pteropus giganteus*) in Bangladesh. A similar outbreak of encephalitis occurred in the northeastern part of India in 2001 and retrospective studies again confirmed Nipah virus as the cause of encephalitis [90]. Animal studies were not performed in the Indian outbreak but nosocomial spread of the infection was observed.

In Cambodia, *Pteropus lylei* has a 11.5–12.3% seroprevalence for Nipah virus but only 0.26% of the urine samples were culture positive, suggesting a transient infection of the flying foxes by the virus [91,92]. In Thailand, seroprevalence among *Pteropus hypomelanus*, *P. lylei*, *P. vampyrus*, and *Hipposideros larvatus* ranged from 1.3% to 15.4%, and the virus was detectable by RT-PCR in *P. lylei* (saliva and urine) and *H. larvatus* (saliva) bats [93]. Recently, serological evidence of infection by *Henipavirus* was also reported among *Pteropus vampyrus* in Indonesia [94]. Phylogenetic analysis of the nucleocapsid gene sequence suggested that there are three lineages of Nipah virus, with Nipah–Bangladesh and Nipah–India being closer to each other than Nipah–Malaysia and Nipah–Cambodia [88,90].

From April to September in 1997, sows in New South Wales, Australia, were found to have

decreased pregnancy rate and litter size. There was an increase in mummified, stillborn, and deformed piglets. The affected stillborn piglets were found to have severe degeneration of the central nervous system and some had myocarditis. Subsequently a new virus—later named the Menangle virus—was isolated from the infected piglets [95]. Among 251 persons with contact with the pigs in the piggeries, 2 were seropositive for the virus [96]. Both of the patients developed a febrile illness with headache, malaise, myalgia, and skin rashes with no long term sequelae. The flying foxes *Pteropus poliocephalus* and *P. alecto* were seropositive for the Menangle virus [95]. The route of transmission of menangle virus from flying foxes to pigs, amongst pigs, and from pigs to human is unknown. The complete genome of the Menangle was reported which confirmed its position in the family *Paramyxoviridae* [97].

As a result of enhanced surveillance for bat-associated viruses in Southeast Asia, the Tioman virus was discovered. Tioman virus is a novel paramyxovirus isolated from the urine of the flying fox *Pteropus hypomelanus* in Malaysia and the genome of the virus has been published. It was placed under the genus *Rubulavirus* by genomic analysis [98,99]. Human infections due to Tioman virus have not been described.

### Reoviridae

Two reoviruses have been isolated from bats. They include the Nelson Bay virus and Pulau virus [100,101]. The transmissibility and pathogenicity of these viruses to humans are unknown.

### Retroviridae

By searching through the mouse (*Mus musculus*) and rat (*Rattus norvegicus*) genomes, genetic elements belonging to betaretroviruses were discovered in these rodents. In the same study, sequences similar to the *env* gene of an endogenous retrovirus CpERV- $\beta$ 5\_AC138156 was found in the genome of the Seba's short-tail bat (*Carrollia perspicillata*) [102].

### Rhabdoviridae

The Rhabdoviridae contains six genera, *Vesiculovirus*, *Lyssavirus*, *Ephemerovirus*, *Novirhabdovirus*, *Cytorhabdovirus*, *Nucleorhabdovirus*, the last two being plant viruses. Vesicular stomatitis (*Vesiculo-*

*virus*) occurs in horses, cattle, and pigs in the Western Hemisphere. It produces a disease in livestock similar to foot-and-mouth disease. Seroprevalence amongst humans can be high in enzootic areas but human infection is generally mild or asymptomatic. Bats have been implicated, but not proven, as a possible carrier for vesicular stomatitis virus [103]. In contrast, the Chandipura virus, another *Vesiculovirus*, causes a much more severe encephalitis, sometimes in large outbreaks, in India [104]. Sandflies were believed to be the vector of Chandipura virus but it has not been found in association with bats. Mount Elgon bat virus was another rhabdovirus isolated from *Rhinolophus hildebrandtii eloquens* and described in 1969 [105]. It has not been associated with human infections.

The lyssaviruses are arguably the most important of all bat-associated viruses. The *Lyssavirus* species include rabies virus, (genotype 1), Lagos bat virus (genotype 2), Mokola virus (genotype 3), Duvenhage virus (genotype 4), European bat lyssaviruses 1 and 2 (genotypes 5 and 6, respectively), Australian bat lyssavirus (genotype 7), and four newly described genotypes found in Eurasia, Aravan (isolated in 1991), Khujand (isolated in 2001), Irkut (isolated in 2002), and West Caucasian bat viruses (isolated in 2002) [106,107,108]. These four Eurasian genotypes and the Lagos bat virus have not been shown to cause human infections to date [109]. There had only been one reported case of human infections caused by the Duvenhage virus to date which occurred in South Africa in 1970 [110,111]. *Miniopterus schreibersii* was considered to be the host associated with the infection and the virus was isolated once from a *Nycteris thebaica* bat [111].

The lyssaviruses differ from each other not only in their genotypes (based on nucleoprotein gene sequences), but also in their pathogenicity in animals and cross-neutralisation by antibodies. Based on the transmembrane glycoprotein sequence, these seven lyssavirus genotypes were further divided into two phylogroups [112]. Phylogroup I consists of genotypes 1, 4, 5, 6 and 7 while phylogroup II consists of genotypes 2 and 3. Phylogenetic analyses of the four Eurasian lyssaviruses using the glycoprotein, nucleocapsid and phosphoprotein gene sequences has also been published [113]. Aravan, Irkut, Khujand and West Caucasian bat viruses are considered to be separate genotypes according to nucleoprotein gene

sequences. Aravan, Irkut and Khujand viruses are related to each other and to genotypes 4, 5 and 6 viruses, and these six viruses are believed to represent a phylogroup of Old World bat lyssaviruses. The West Caucasian bat virus is the most divergent of all known lyssaviruses.

All the above lyssaviruses have been isolated from bats except Mokola virus which is mainly isolated from cats and occasionally from rodents and shrews. For the bat-associated lyssaviruses, only rabies virus is also associated with other terrestrial animals (especially carnivores); all the others have bats as the sole natural reservoir hosts. Rabies virus has a worldwide distribution while the other lyssaviruses are relatively restricted geographically. A large number of animals are susceptible to infection by the classical rabies virus. However, only mammals of the orders Chiroptera and Carnivora transmit the virus efficiently in nature. In countries which are free from canine rabies, bats are the most important source for human rabies. The evolution of rabies virus has been studied in detail. Phylogenetic studies showed that the different genotypes of lyssaviruses are clearly distinct from each other and that, within genotype 1, distinct lineages with respect to the reservoir host range can be recognised [114]. The chiropteran rabies viruses are likely to be older than the carnivoran viruses, and the current rabies viruses amongst carnivores probably arose as a result of two cross-species spillover events from bats to carnivores. One of the events resulted in the North American raccoon and skunk rabies virus lineage while the other resulted in the carnivore rabies lineage in the rest of the world. Cross-species transmission of rabies virus still occurs today but all these incidents are the result of bat-to-terrestrial animals spillover not the reverse [115]. Phylogenetic division of bat rabies viruses was clearly shown to be associated with clustering of specific bat species in two studies, suggesting that some rabies viruses co-segregate with their bat hosts [116,117]. In Canada, for example colonial and non-migratory *Myotis* bats are associated with rabies virus clades that are distinct from those associated with solitary, tree-dwelling and migratory *Lasiurus* bats [117].

The vast majority of human rabies in the world is acquired from canine sources. In some countries, however, bats carrying variants of the genotype 1 rabies virus are equally, if not more important,

causes of human infections. From 1958 to 2000, bat rabies accounted for 32 of the 35 indigenous cases of rabies in the USA [118]. In 26 of the patients, there was no history of bat bites. Nineteen of these 26 'cryptic' rabies were associated with two species, *Lasionycteris noctivagans* and *Pipistrellus subflavus*. Similarly in Latin America, bat rabies is as important as canine rabies in causing disease in humans and livestock. In Brazil, analysis showed that canine- and bat-related rabies viruses reside in distinct groups, reinforcing the hypothesis that different rabies virus strains are preferentially related to different mammalian hosts [119,120].

Bat rabies viruses are associated with a large number of bat species, both frugivorous, insectivorous, and sanguivorous. In the Latin America, *Desmodus rotundus*, *Artibeus lituratus*, *Artibeus planirostris*, *Tadarida brasiliensis*, *Nyctinomops laticaudatus*, *Eumops auripendulus*, *Eptesicus furinalis*, *Lasiurus borealis*, *Molossus* spp. are often encountered [119,120,121,122]. In North America, *Eptesicus fuscus*, is one of the commonest rabid bat species, followed by *Myotis* spp. (e.g. *M. lucifugus*), *Lasionycteris noctivagans*, *Lasiurus* spp. (e.g. *L. cinereus*, *L. borealis*, *L. intermedius*, *L. seminolus*), *Tadarida brasiliensis* and *Pipistrellus subflavus* [117,118,123,124]. *Pteropus* bats have been found to be infected by rabies virus in India [125]. Rabid bats may become sick, though most of the animals will remain healthy and asymptomatic. Transmission of rabies virus among bats likely occurs orally and through biting. Although aerosolisation of bat rabies virus in saliva or excreta has been postulated as a possible means of transmission, humans are mainly infected via percutaneous or mucosal contacts with infected bats.

European bat lyssaviruses 1 and 2 are found in a number of European countries including Denmark, Finland, France, Germany, Hungary, Lithuania, the Netherlands, Poland, the Russian Federation, Slovakia, Spain, Switzerland, Ukraine and the United Kingdom [126]. European bat lyssaviruses are generally restricted to bats. They only rarely infect other mammalian species including one stone marten (*Martes foina*), two sheep, and three human beings [127]. One of the three human infections was due to European bat lyssavirus 1 (1985, Ukraine) and two due to European bat lyssavirus 2 (1985, diagnosed in Finland but had exposures in Finland, Switzerland and Asia;



2002, Scotland) [126]. A fourth case occurred in 1977 in Ukraine and the isolate was believed to be European bat lyssavirus 1, but the strain was not genetically typed [128]. European bat lyssavirus 1 is the commoner of the two types and is mainly associated with *Eptesicus serotinus* [19,129,130,131]. Asymptomatic infection of a zoo bat *Rousettus aegyptiacus* has been reported [132]. The sole reservoirs of European bat lyssavirus 2 to date are the *Myotis* bats (*M. daubentonii* and *M. dasycneme*) [128,130,133,134].

Australian bat lyssavirus was first recovered from *Pteropus alecto* in New South Wales, Australia, and later also found in other bat species [135]. Two fatal human infections in Australia have been reported, both had sustained bat-related injuries prior to onset of disease [136,137]. Post-exposure prophylaxis with rabies vaccine and immunoglobulin were given in subsequent potential exposures to Australian bat lyssavirus and no human cases have ever been described [138,139]. In contrast to other bat-associated pathogens, Australian bat lyssavirus can cause encephalitis in infected bats [138,139]. Surveillance using immunofluorescent staining of bat brains showed that healthy flying foxes have a lower positive rate than sick and injured animals (<1% vs. 6.5%, respectively) [139].

Bat-associated lyssaviruses have also been increasingly recognised in Asian countries. In India, the flying fox *Pteropus poliocephalus* was found to be infected by rabies virus using direct immunofluorescent staining of brain tissue [125]. In the Philippines, no lyssaviruses were detected in the brains of 821 bats, but 9.5% of the bats in six species possessed neutralising antibodies against Australian bat lyssavirus, especially among *Miniopterus schreibersi* [140]. In Cambodia, neutralising antibodies were detected against a number of lyssaviruses, including rabies virus, European bat lyssavirus 1, Australian bat lyssavirus, and Lagos bat virus [141]. A similar picture was seen in Thailand in which serological evidence of infection by Aravan, Khujand, Irkit and Australian bat lyssavirus was found in 3.8% (all from *Pteropus lylei* and *Hipposideros armiger*) of the bat samples [142]. Neutralising antibodies against Aravan and Khujand viruses were detected in three *Pteropus giganteus* samples in Bangladesh [143]. In these latter four Asian surveys, no lyssaviruses were detected in the brains of the bats by direct immunofluorescence and/or

mouse inoculation. Although serological assays *per se* may be complicated by cross-reactions between different lyssaviruses, the studies do raise the possibility that these lyssavirus species may have a broader geographical distribution than previously described.

In addition to the natural route of zoonotic transmission of rabies virus to humans, organ transplantation has in recent years been recognised as an iatrogenic route of transmission. The first report of such transmission occurred in Texas in 2004 when four recipients received organs from a donor who died of subarachnoid haemorrhage. The organs transplanted included iliac artery, liver and two kidneys. All four recipients subsequently developed rabies encephalitis within 30 days of infection and all succumbed to the infection. In retrospect, the donor was found to have been bitten by a bat before his death [18]. In 2005, a similar incident occurred in Germany. A 26-year old woman visited India in October in 2004 and died in Germany in late 2004. Three recipients received lungs, combined kidney/pancreas and kidney, and all of them died of rabies. Subsequent examination of the donor's brain showed typical pathological features of rabies including Negri bodies and positive immunohistochemical staining [144]. The three recipients of liver and cornea remained asymptomatic as of February 2005. Such incidents highlight the importance of excluding rare and exotic diseases in potential organ donors and the novel iatrogenic routes of transmitting bat-associated pathogens.

The prognosis of human rabies after the onset of symptoms is extremely poor with almost 100% mortality. The only useful specific post-exposure management, apart from local wound cleansing, is the use of rabies vaccine and immunoglobulin (local infiltration and systemic injection). Although no useful antiviral agent is available to date, one recent report showed that a potent anti-excitotoxic therapy led to the survival of a patient. A 15-year old girl who sustained a laceration from a bat developed clinical rabies without post-exposure prophylaxis. A regimen of high doses of midazolam, barbiturates, diazepam and ketamine, together with ribavirin and amantadine, appeared to tide over the acute phase of infection before the development of immunity, and the patient eventually regained consciousness, albeit with some neurological deficits [145]. The currently available

rabies vaccines are extremely effective for pre- and post-exposure prophylaxis of rabies virus infections. Failures occur only in situations of delayed vaccination and multiple severe animal bites to the head. There are no controlled clinical trials on the efficacy of rabies vaccines or immunoglobulins towards other lyssaviruses. However, vaccines and immunoglobulin have been given to persons exposed to European and Australian bat lyssaviruses without failure so far. Animal studies of rabies vaccines against European bat lyssaviruses have yielded variable results depending on the vaccine strains used and the host species. In a recent study in mice, human diploid cell vaccine conferred cross-protection against both European and Australian bat lyssaviruses, thus providing some experimental evidence for the protective efficacy against these phylogroup I lyssaviruses [146]. Vaccine efficacy against Lagos bat virus is likely to be low. The efficacy of human rabies vaccines and immunoglobulin against the Eurasian genotypes appears to be unsatisfactory, and the degree of protection is related to the phylogenetic distance from the rabies virus [147].

### Togaviridae

The two genera of togaviruses contain several important human pathogens. Examples of *Alpha-virus* include Barmah Forest virus, Chikungunya virus, eastern equine encephalitis virus, O'nyong-nyong virus, Ross River virus, Semliki Forest virus, Sindbis virus, Venezuelan equine encephalitis virus, and western equine encephalitis virus. Eastern, western, and Venezuelan equine encephalitis viruses are primarily associated with encephalitis, while chikungunya, O'nyong-nyong, Ross River, sinbis, and Barmah Forest viruses commonly present as fever, rash and arthritis. Rubella virus is the only example of *Rubivirus* for which human is the only natural host.

All alphaviruses are transmitted by arthropods, especially mosquitoes. Evidence of infection by chikungunya, eastern and Venezuelan equine encephalitis viruses have been found in bats. Chikungunya virus causes recurrent and explosive outbreaks in many tropical countries. The most recent outbreak occurred in the southwest Indian Ocean involving La Réunion, Mauritius, Seychelles, Mayotte, Maldives and India. Since March 2005, there have been more than 3000 confirmed cases and an estimated infected population of

over 200 000 [148]. The urban cycle of chikungunya is maintained by human-to-human transmission via mosquitoes (mainly *Aedes aegypti* but also *Aedes albopictus*), while a rural cycle is maintained between non-human primates. Bats have occasionally been found to be infected by chikungunya virus, though the potential of the bat for further transmission requires confirmation [149]. The reservoir of eastern equine encephalitis virus is primarily birds, but other animals such as rodents, marsupials, and bats are often infected in Latin America. Serological evidence for bat infection by eastern equine encephalitis virus was documented in Trinidad and Guatemala [23,150]. Venezuelan equine encephalitis virus has a broad host range including rodents, bats, horses, sheep, dogs and birds, but the main sylvatic reservoir is believed to be rodents. Horses are efficient amplifying hosts and human infections and outbreaks mostly resulted from equine infections [151]. Several species of bats have serological evidence of infection by the Venezuelan equine encephalitis virus in Guatemala and positive viral cultures have been obtained from *Carollia perspicillata*, *Desmodus rotundus* and *Uroderma bilobatum* [152,153,154]. Bats might have been infected through mosquito bites, ingesting infected mosquitoes, or feeding on the blood of viraemic animals in the case of vampire bats. They may play a role in geographical dispersal of the virus and as an alternative reservoir for the virus [151,152]. The flying foxes (*Pteropus poliocephalus* and *Pteropus pscapulatus*) are considered to be unimportant reservoirs of Ross River virus in one study [155].

### IMPLICATIONS

To prevent transmission of bat-associated zoonoses to humans, the key is to gain a better understanding of the ecology of bats and the range of infectious agents associated with them. Surveillance of bat-associated viruses is based on passive surveillance in many countries of the world, in part because of the conservation status of bats. Passive surveillance usually targets towards sick, injured or dead animals, which may bias the results. To better understand the prevalence of old and new pathogens, active surveillance is obviously preferable.

The primary preventive measure against bat-associated infections is conservation of the natural habitats of bats. The intrusion of Nipah virus to the

pig population and subsequently to humans serves as the most recent example. In Latin America, outbreaks of human rabies from vampire bats almost always followed the destruction of the bats' habitats, forcing the animals to feed on humans and livestock. In situations where exposure to bats is unavoidable, measures to reduce contact between bats and humans may be considered, such as bat-proofing of houses in the endemic areas and the use of personal protective equipment for workers and scientists in frequent contact with bats. In Latin America, application of anticoagulants to bats and cattle have been utilised to control the vampire bat population. This approach, however, has not achieved much success and may have adverse effects on the ecology of bats or other fauna and flora. With the excellent results from the use of oral rabies vaccine to control rabies in wildlife in Europe and North America, oral vaccines against bat rabies were being tested in vampire bats with satisfactory experimental results [156,157]. Pre- and post-exposure prophylaxis using rabies vaccines appears to be effective against genotypes of lyssaviruses that are known to cause human infections despite the antigenic differences from classical rabies virus. Pre-exposure vaccination is especially important for persons with constant exposure to bats such as bat biologists. The value of vaccines against other bat-associated viruses remains unknown mainly because of the lack of effective vaccines (e.g. against *Henipavirus*) or the uncertain role of bats in the transmission of infections (e.g. Japanese encephalitis virus).

As depicted in the Supplementary Table, the known and potential viral pathogens are distributed in numerous families of bats. However, one thing that stands out from the table is that most of the important pathogens are often associated with four families: *Pteropodidae*, *Molossidae*, *Phyllostomidae* and *Vespertilionidae*. This could be due to a greater biodiversity of these species, better known biological features, or indeed, a co-evolution of the main pathogens with specific bat species. The understanding of the association between viruses and bat families could also be useful to predict the likelihood of transmission or dissemination to humans. For example, many of the flying foxes (*Pteropodidae*) are relatively large animals and, being strong flyers, they could reach relatively long distances from their roosting sites.

Being frugivorous bats, *Pteropus* spp. often encroach onto fruit plantations and hence may bring them into closer contacts with humans. The big brown bat (*Eptesicus fuscus*, *Vespertilionidae*), an insectivorous species, likewise is relatively close to humans in that it often roosts in buildings and man-made structures in cities or rural areas. The serotine bat (*Eptesicus serotinus*) is equally at home in buildings. The carriage of lyssaviruses by these bats is an obvious concern to man.

Although bats are currently not considered the most dominant group of mammals giving rise to emerging or re-emerging infections in humans, this could reflect a relative paucity of studies on this important group of mammals [2]. In the last decade, a number of new infections have caused outbreaks in different parts of the world. Some of these causative agents, such as the henipavirus and SARS coronavirus, have been found to have a reservoir in bats. Bats have also been proven to be a natural host for enigmatic viruses such as the Ebola virus and their significance in the natural ecology of filoviruses remains to be uncovered. It is interesting to note that many of the bat-associated pathogens do not cause any clinicopathological damage to the bats, and coupling with the longevity and migratory habits of the bats, make this animal a potentially important reservoir for emerging and re-emerging infections.

It can be seen from the above checklist of viruses that very often the geographical distribution and host range of many bat-associated infections broadens as more surveillance was performed in different parts of the world and when more bat species were studied. Examples include coronaviruses, *Henipavirus* and lyssaviruses. In lyssaviruses, for example surveys of South and Southeast Asian bats showed that Australian bat lyssavirus and the Eurasian lyssaviruses may indeed have a broader distribution than previously known. Many of the new viruses were discovered in bats following recent human or animal outbreaks of infectious diseases and enhanced surveillance in wild animals. A variety of coronaviruses were discovered in southern China after the SARS epidemic in diverse bat species. The outbreak of Nipah encephalitis in Malaysia and the association of the virus with flying foxes prompted enhanced surveillance of bats. This led to the discovery of the new Tioman and Pulau viruses. The finding of these new viruses is the

result of a systematic and targeted surveillance of bats, which underscores the importance of such an approach in the future study of zoonotic agents. It is therefore hardly surprising to predict that more viruses will be discovered in bats in the future. However, one should not forget that the mere isolation of a virus from bats does not necessarily implicate an active role of bats in the natural ecology of the microbe. Whether bats are incidental and dead-end hosts or actively support the maintenance of the viruses need to be investigated in more vigorous studies. Anthropogenic and natural changes in the environment due to deforestation, alteration of habitats of bats, alterations in animal diversity, and climatic events may shift the ecology of bats and expose humans to new pathogens. A long-term systematic surveillance of bats is essential to unravel the complex ecology between bats, humans, other animals, arthropod vectors and the environment. Such studies will benefit not only humans, but also conservation of wildlife and biodiversity.

#### ACKNOWLEDGEMENT

The authors are grateful to the generous support of the Providence Foundation Limited in memory of the late Dr Lui Hac Minh, and that of Mr Fred WC Chan and Ms Dorcas Suen on our research work on emerging infections. This work is partly supported by the Research Grant Council and the Research Fund for the Control of Infectious Diseases of the Health, Welfare and Food Bureau of the Hong Kong Special Administrative Region Government. The authors give special thanks to the Mammal Working Group, Agriculture, Fisheries and Conservation Department for their help in taking the photographs of Chinese horseshoe bats.

#### REFERENCES

- Palmer SR, Lord Soulsby, Simpson DIH. (eds). *Zoonoses: Biology, Clinical Practice, and Public Health Control*. Oxford University Press: Oxford, 1998.
- Woolhouse ME, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. *Emerg Infect Dis* 2005; **11**: 1842–1847.
- Food and Agriculture Organization of the United Nations. 2005. FAO Statistical Databases. <http://faostat.fao.org/faostat/form?collection=Production.Livestock.Stocks&Domain=Production&servlet=1&hasbulk=&version=ext&language=language=EN>. [13 April, 2006.]
- Kalish ML, Wolfe ND, Ndongmo CB, *et al.* Hunters exposed to simian immunodeficiency virus. *Emerg Infect Dis* 2005; **12**: 1928–1930.
- Wolfe ND, Daszak P, Kilpatrick AM, Burke DS. Bushmeat hunting, deforestation, and prediction of zoonotic disease emergence. *Emerg Infect Dis* 2005; **12**: 1822–1827.
- Wolfe ND, Switzer WM, Carr JK, *et al.* Naturally acquired simian retrovirus infections in central African hunters. *Lancet* 2004; **363**: 932–937.
- Integrated Taxonomic Information System. <http://itis.gbif.net>. [14 April, 2006.]
- Nowak RM. *Walker's Bats of the World*. Johns Hopkins University Press: Baltimore, 1994.
- Kunz TH, Lumsden LF. Ecology of cavity and foliage roosting bats. In *Bat Ecology*, Kunz TH, Fenton MB (eds). The University of Chicago Press: Chicago, 2003; 3–89.
- Neuweiler G. *The Biology of the Bats*. Oxford University Press: Oxford, 2000.
- Barclay RMR, Harder LD. Life histories of bats: life in the slow lane. In *Bat Ecology*, Kunz TH, Fenton MB (eds). The University of Chicago Press: Chicago, 2003; 209–253.
- Speakman JR, Thomas DW. Physiological ecology and energetics of bats. In *Bat Ecology*, Kunz TH, Fenton MB (eds). The University of Chicago Press: Chicago, 2003; 430–490.
- Fleming TH, Eby P. Ecology of bat migration. In *Bat Ecology*, Kunz TH, Fenton MB (eds). The University of Chicago Press: Chicago, 2003; 156–208.
- Gonçalves MA, Sá-Neto RJ, Brazil TK. Outbreak of aggressions and transmission of rabies in human beings by vampire bats in northeastern Brazil. *Rev Soc Bras Med Trop* 2002; **35**: 461–464.
- Schneider MC, Aron J, Santos-Burgoa C, Uieda W, Ruiz-Velazco S. Common vampire bat attacks on humans in a village of the Amazon region of Brazil. *Cad Saude Publica* 2001; **17**: 1531–1536.
- Cross JH, Lien JC, Huang WC, Lien SC, Chiu SF. Japanese encephalitis virus surveillance in Taiwan. II. Isolations from mosquitoes and bats in Taipei area 1969–1970. *Taiwan Yi Xue Hui Za Zhi* 1971; **70**: 681–686.
- Lampo M, Feliciangeli MD, Marquez LM, Bastidas C, Lau P. A possible role of bats as a blood source for the *Leishmania* vector *Lutzomyia longipalpis* (Diptera: Psychodidae). *Am J Trop Med Hyg* 2000; **62**: 718–719.
- Srinivasan A, Burton EC, Kuehnert MJ, *et al.* Transmission of rabies virus from an organ donor to four transplant recipients. *N Engl J Med* 2005; **352**: 1103–1111.
- Daszak P, Cunningham AA, Hyatt AD. Anthropogenic environmental change and the emergence of

- infectious diseases in wildlife. *Acta Trop* 2001; **78**: 103–116.
20. Weiss RA, McMichael AJ. Social and environmental risk factors in the emergence of infectious diseases. *Nat Med* 2004; **10**(12 Suppl): S70–76.
  21. Constantine DG. Geographic translocation of bats: known and potential problems. *Emerg Infect Dis* 2003; **9**: 17–21.
  22. Downs WG, Anderson CR, Spence L, Aitken THG, Greenhall AG. Tacaribe virus, a new agent isolated from *Artibeus* bats and mosquitoes in Trinidad, West Indies. *Am J Trop Med Hyg* 1963; **12**: 640–646.
  23. Price JL. Serological evidence of infection of Tacaribe virus and arboviruses in Trinidadian bats. *Am J Trop Med Hyg* 1978; **27**: 162–167.
  24. Williams JE, Imlarp S, Top FH Jr, Cavanaugh DC, Russell PK. Kaeng Khoi virus from naturally infected bedbugs (*Cimicidae*) and immature free-tailed bats. *Bull World Health Organ* 1976; **53**: 365–369.
  25. Osborne JC, Rupprecht CE, Olson JG, et al. Isolation of Kaeng Khoi virus from dead *Chaerephon plicata* bats in Cambodia. *J Gen Virol* 2003; **84**: 2685–2689.
  26. Charrel RN, Gallian P, Navarro-Mari JM, et al. Emergence of Toscana virus in Europe. *Emerg Infect Dis* 2005; **11**: 1657–1663.
  27. Verani P, Ciufolini MG, Caciolli S, et al. Ecology of viruses isolated from sand flies in Italy and characterized of a new *Phlebovirus* (Arabia virus). *Am J Trop Med Hyg* 1988; **38**: 433–439.
  28. Zhong NS, Zheng BJ, Li YM, et al. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. *Lancet* 2003; **362**: 1353–1358.
  29. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 2003; **302**: 276–278.
  30. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 2003; **302**: 276–278.
  31. Leung GM, Lim WW, Ho LM, et al. Seroprevalence of IgG antibodies to SARS-coronavirus in asymptomatic or subclinical population groups. *Epidemiol Infect* 2006; **134**: 211–221.
  32. Che XY, Di B, Zhao GP, et al. A patient with asymptomatic severe acute respiratory syndrome (SARS) and antigenemia from the 2003–2004 community outbreak of SARS in Guangzhou, China. *Clin Infect Dis* 2006; **43**: e1–5.
  33. World Health Organization. Consensus document on the epidemiology of severe acute respiratory syndrome (SARS): 2003. <http://www.who.int/csr/sars/en/WHOconsensus.pdf>.
  34. Kuiken T, Fouchier RA, Schutten M, et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. *Lancet* 2003; **362**: 263–270.
  35. Martina BE, Haagmans BL, Kuiken T, et al. SARS virus infection of cats and ferrets. *Nature* 2003; **425**: 915.
  36. Greenough TC, Carville A, Coderre J, et al. Pneumonitis and multi-organ system disease in common marmosets (*Callithrix jacchus*) infected with the severe acute respiratory syndrome-associated coronavirus. *Am J Pathol* 2005; **167**: 455–463.
  37. Roberts A, Vogel L, Guarner J, et al. Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters. *J Virol* 2005; **79**: 503–511.
  38. McAuliffe J, Vogel L, Roberts A, et al. Replication of SARS coronavirus administered into the respiratory tract of African Green, rhesus and cynomolgus monkeys. *Virology* 2004; **330**: 8–15.
  39. Lau SK, Woo PC, Li KS, et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proc Natl Acad Sci USA* 2005; **102**: 14040–14045.
  40. Poon LL, Chu DK, Chan KH, et al. Identification of a novel coronavirus in bats. *J Virol* 2005; **79**: 2001–2009.
  41. Woo PCY, Lau SKP, Li KSM, et al. Molecular diversity of coronaviruses in bats. *Virology* 2006; **351**: 180–187.
  42. Li W, Shi Z, Yu M, et al. Bats are natural reservoirs of SARS-like coronaviruses. *Science* 2005; **310**: 676–679.
  43. World Health Organization. Marburg haemorrhagic fever in Angola – update 25. 24 August, 2005. [http://www.who.int/csr/don/2005\\_08\\_24/en/index.html](http://www.who.int/csr/don/2005_08_24/en/index.html) [13 April, 2006.]
  44. World Health Organization. Ebola haemorrhagic fever in the Republic of the Congo – update 2. 16 June, 2005. [http://www.who.int/csr/don/2005\\_06\\_16/en/index.html](http://www.who.int/csr/don/2005_06_16/en/index.html) [13 April, 2006.]
  45. Pourrut X, Kumulungui B, Wittmann T, et al. The natural history of Ebola virus in Africa. *Microb Infect* 2005; **7**: 1005–1014.
  46. Leroy EM, Kumulungui B, Pourrut X, et al. Fruit bats as reservoirs of Ebola virus. *Nature* 2005; **438**: 575–576.
  47. Paul SD, Rajagopalan PK, Sreenivasan MA. Isolation of the West Nile virus from the frugivorous bat, *Rousettus leschenaulti*. *Indian J Med Res* 1970; **58**: 1169–1171.
  48. Centers for Disease Control and Prevention (CDC). West Nile virus activity—United States, September 29 – October 5, 2004. *MMWR Morb Mortal Wkly Rep* 2004; **53**: 922–923.
  49. Marfin AA, Petersen LR, Eidson M, et al. Widespread West Nile virus activity, eastern United States, 2000. *Emerg Infect Dis* 2001; **7**: 730–735.

50. Pilipski JD, Pilipski LM, Risley LS. West Nile virus antibodies in bats from New Jersey and New York. *J Wildl Dis* 2004; **40**: 335–337.
51. Sulkin SE, Allen R, Sims R. Studies of arthropod-borne virus infections in Chiroptera. I. Susceptibility of insectivorous species to experimental infection with Japanese B and St. Louis encephalitis viruses. *Am J Trop Med Hyg* 1963; **12**: 800–814.
52. Sulkin SE, Allen R, Sims R. Studies of arthropod-borne virus infections in Chiroptera. III. Influence of environmental temperature on experimental infection with Japanese B and St. Louis encephalitis viruses. *Am J Trop Med Hyg* 1966; **15**: 406–417.
53. Sulkin SE, Sims R, Allen R. Studies of arthropod-borne virus infections in Chiroptera. II. Experiments with Japanese B and St. Louis encephalitis viruses in the gravid bat. Evidence of transplacental transmission. *Am J Trop Med Hyg* 1964; **13**: 475–481.
54. Herbold JR, Heuschele WP, Berry RL, Parsons MA. Reservoir of St. Louis encephalitis virus in Ohio bats. *Am J Vet Res* 1983; **44**: 1889–1893.
55. Sulkin SE, Allen R, Miura T, Toyokawa K. Studies of arthropod-borne virus infections in chiroptera. VI. Isolation of Japanese B encephalitis virus from naturally infected bats. *Am J Trop Med Hyg* 1970; **19**: 77–87.
56. Platt KB, Mangiafico JA, Rocha OJ, *et al.* Detection of dengue virus neutralising antibodies in bats from Costa Rica and Ecuador. *J Med Entomol* 2000; **37**: 965–967.
57. Zhang H, Yang X, Li G. Detection of dengue virus genome RNA in some kinds of animals caught from dengue fever endemic areas in Hainan Island with reverse transcription-polymerase chain reaction. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* 1998; **12**: 226–228.
58. Pavri KM, Singh KR. Kyasanur Forest disease virus infection in the frugivorous bat, *Cynopterus sphinx*. *Indian J Med Res* 1968; **56**: 1202–1204.
59. Rajagopalan PK, Paul SD, Sreenivasan MA. Isolation of Kyasanur Forest disease virus from the insectivorous bat, *Rhinolophus rouxi* and from *Ornithodoros* ticks. *Indian J Med Res* 1969; **57**: 805–808.
60. Bell JF, Thomas LA. A new virus, MML, enzootic in bats (*Myotis lucifugus*) of Montana. *Am J Trop Med Hyg* 1964; **13**: 607–612.
61. Charlier N, Leyssen P, Pleij CWA, *et al.* Complete genome sequence of Montana Myotis leukoencephalitis virus, phylogenetic analysis and comparative study of the 3' untranslated region of flaviviruses with no known vector. *J Gen Virol* 2002; **83**: 1875–1885.
62. Baer GM, Woodall DF. Bat salivary gland virus carrier state in a naturally infected Mexican freetail bat. *Am J Trop Med Hyg* 1966; **15**: 769–771.
63. Price JL. Isolation of Rio Bravo and a hitherto undescribed agent, Tamana bat virus, from insectivorous bats in Trinidad, with serological evidence of infection in bats and man. *Am J Trop Med Hyg* 1978; **27**: 153–161.
64. Burke DS, Monath TP. Flaviviruses. In *Field's Virology* (4th ed) Knipe DM, Howley PM (eds). Lippincott Williams & Wilkins: Philadelphia, 2001; 1043–1125.
65. Tajima S, Takasaki T, Matsuno S, Nakayama M, Kurane I. Genetic characterization of Yokose virus, a flavivirus isolated from the bat in Japan. *Virology* 2005; **332**: 38–44.
66. Scott TW. Are bats really involved in dengue virus transmission? *J Med Entomol* 2001; **38**: 771–772.
67. Lvov DK, Easterday B, Hinshow W, Dandurov I, Arkhipov PN. Isolation of strains of the Hong Kong complex (H3N2) influenza virus from *Nyctalus noctula* bats in Kazakhstan. *Vopr Virusol* 1979; **4**: 338–341.
68. Kelkar SD, Kadam SS, Banerjee K. Haemagglutination inhibition antibodies against influenza virus in bats. *Indian J Med Res* 1981; **74**: 147–152.
69. Hollinger FB, Pavri KM. Bat parainfluenza virus. Immunological, chemical, and physical properties. *Am J Trop Med Hyg* 1971; **20**: 131–138.
70. Pavri KM, Singh KR, Hollinger FB. Isolation of a new parainfluenza virus from a frugivorous bat, *Rousettus leschenaulti*, collected at Poona, India. *Am J Trop Med Hyg* 1971; **20**: 125–130.
71. Henderson GW, Laird C, Dermott E, Rima BK. Characterization of Mapuera virus: structure, proteins and nucleotide sequence of the gene encoding the nucleocapsid protein. *J Gen Virol* 1995; **76**: 2509–2518.
72. Selvey LA, Wells RM, McCormack JG, *et al.* Infection of humans and horses by a newly described morbillivirus. *Med J Aust* 1995; **162**: 642–645.
73. O'Sullivan JD, Allworth AM, Paterson DL, *et al.* Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet* 1997; **349**: 93–95.
74. Young PL, Halpin K, Selleck PW, *et al.* Serologic evidence for the presence in *Pteropus* bats of a paramyxovirus related to equine morbillivirus. *Emerg Infect Dis* 1996; **2**: 239–240.
75. Halpin K, Young PL, Field HE, Mackenzie JS. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J Gen Virol* 2000; **81**: 1927–1932.
76. Field H, Young P, Yob JM, Mills J, Hall L, Mackenzie J. The natural history of Hendra and Nipah viruses. *Microbes Infect* 2001; **3**: 307–314.
77. Barclay AJ, Paton DJ. Hendra (equine morbillivirus). *Vet J* 2000; **160**: 169–176.
78. Chua KB, Goh KJ, Wong KT, *et al.* Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* 1999; **354**: 1257–1259.

79. Goh KJ, Tan CT, Chew NK, *et al.* Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med* 2000; **342**: 1229–1235.
80. Parashar UD, Sunn LM, Ong F, *et al.* Case-control 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* 2000; **181**: 1755–1759.
81. Paton NI, Leo YS, Zaki SR, *et al.* Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet* 1999; **354**: 1253–1256.
82. Chew MH, Arguin PM, Shay DK, *et al.* Risk factors for Nipah virus infection among abattoir workers in Singapore. *J Infect Dis* 2000; **181**: 1760–1763.
83. Mounts AW, Kaur H, Parashar UD, *et al.* A cohort study of health care workers to assess nosocomial transmissibility of Nipah virus, Malaysia, 1999. *J Infect Dis* 2001; **183**: 810–813.
84. Yob JM, Field H, Rashdi AM, *et al.* Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. *Emerg Infect Dis* 2001; **7**: 439–441.
85. Chua KB, Koh CL, Hooi PS, *et al.* Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 2002; **4**: 145–151.
86. Chua KB, Chua BH, Wang CW. Anthropogenic deforestation, El Niño and the emergence of Nipah virus in Malaysia. *Malays J Pathol* 2002; **24**: 15–21.
87. Chua KB, Lam SK, Goh KJ, *et al.* The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia. *J Infect Dis* 2001; **42**: 40–43.
88. Harcourt BH, Lowe L, Tamin A, *et al.* Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg Infect Dis* 2005; **11**: 1594–1597.
89. Hsu VP, Hossain MJ, Parashar UD, *et al.* Nipah virus encephalitis reemergence, Bangladesh. *Emerg Infect Dis* 2004; **10**: 2082–2087.
90. Chadha MS, Comer JA, Lowe L, *et al.* Nipah virus-associated encephalitis outbreak, Siliguri, India. *Emerg Infect Dis* 2006; **12**: 235–240.
91. Olson JG, Rupprecht C, Rollin PE, *et al.* Antibodies to Nipah-like virus in bats (*Pteropus lylei*), Cambodia. *Emerg Infect Dis* 2002; **8**: 987–988.
92. Reynes JM, Counor D, Ong S, *et al.* Nipah virus in Lyle's flying foxes, Cambodia. *Emerg Infect Dis* 2005; **11**: 1042–1047.
93. Wacharapluesadee S, Lumlertdacha B, Boongird K, *et al.* Bat Nipah virus, Thailand. *Emerg Infect Dis* 2005; **11**: 1949–1951.
94. Sendow I, Field HE, Curran J, *et al.* Henipavirus in *Pteropus vampyrus* bats, Indonesia. *Emerg Infect Dis* 2006; **12**: 711–712.
95. Philbey AW, Kirkland PD, Ross AD, *et al.* An apparently new virus (family *Paramyxoviridae*) infectious for pigs, humans, and fruit bats. *Emerg Infect Dis* 1998; **4**: 269–271.
96. Chant K, Chan R, Smith M, Dwyer DE, Kirkland P. Probable human infection with a newly described virus in the family *Paramyxoviridae*. *Emerg Infect Dis* 1998; **4**: 273–275.
97. Bowden TR, Boyle DB. Completion of the full-length genome sequence of Menangle virus: characterization of the polymerase gene and genomic 5' trailer region. *Arch Virol* 2005; **150**: 2125–2137.
98. Chua KB, Wang LF, Lam SK, *et al.* Tioman virus, a novel paramyxovirus isolated from fruit bats in Malaysia. *Virology* 2001; **283**: 215–229.
99. Chua KB, Wang LF, Lam SK, Eaton BT. Full length genome sequence of Tioman virus, a novel paramyxovirus in the genus *Rubulavirus* isolated from fruit bats in Malaysia. *Arch Virol* 2002; **147**: 1323–1348.
100. Gard GP, Compans RW. Structure and cytopathic effects of Nelson Bay virus. *J Virol* 1970; **6**: 100–106.
101. Pritchard LI, Chua KB, Cummins D, *et al.* Pulau virus: a new member of the Nelson Bay orthoreovirus species isolated from fruit bats in Malaysia. *Arch Virol* 2006; **151**: 229–239.
102. Baillie GJ, van de Lagemaat LN, Baust C, Mager DL. Multiple groups of endogenous betaretroviruses in mice, rats, and other mammals. *J Virol* 2004; **78**: 5784–5798.
103. Donaldson AI. Bats as possible maintenance hosts for vesicular stomatitis virus. *Am J Epidemiol* 1970; **92**: 132–136.
104. Rao BL, Basu A, Wairagkar NS, *et al.* A large outbreak of acute encephalitis with high fatality rate in children in Andhra Pradesh, India, in 2003, associated with Chandipura virus. *Lancet* 2004; **364**: 869–874.
105. Murphy FA, Shope RE, Metselaar D, Simpson DI. Characterization of Mount Elgon bat virus, a new member of the rhabdovirus group. *Virology* 1970; **40**: 288–297.
106. Arai YT, Kuzmin IV, Kameoka Y, Botvinkin AD. New lyssavirus genotype from the lesser mouse-eared bat (*Myotis blythi*), Kyrgyzstan. *Emerg Infect Dis* 2003; **9**: 333–337.
107. Botvinkin AD, Poleschuk EM, Kuzmin IV, *et al.* Novel lyssaviruses isolated from bats in Russia. *Emerg Infect Dis* 2003; **9**: 1623–1625.
108. Kuzmin IV, Orciari LA, Arai YT, *et al.* Bat lyssaviruses (Aravan and Khujand) from Central Asia: phylogenetic relationships according to N, P and G gene sequences. *Virus Res* 2003; **97**: 65–79.
109. Markotter W, Randles J, Rupprecht CE, *et al.* Lagos Bat Virus, South Africa. *Emerg Infect Dis* 2006; **12**: 504–506.

110. Meredith CD, Rossouw AP, Koch HP. An unusual case of human rabies thought to be of chiropteran origin. *S Afr Med J* 1971; **45**: 767–769.
111. King AA, Meredith CD, Thomson GR. The biology of southern African lyssavirus variants. *Curr Top Microbiol Immunol* 1994; **187**: 267–295.
112. Badrane H, Bahloul C, Perrin P, Tordo N. Evidence of two Lyssavirus phylogroups with distinct pathogenicity and immunogenicity. *J Virol* 2001; **75**: 3268–3276.
113. Kuzmin IV, Hughes GJ, Botvinkin AD, Orciari LA, Rupprecht CE. Phylogenetic relationships of Irkut and West Caucasian bat viruses within the Lyssavirus genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. *Virus Res* 2005; **111**: 28–43.
114. Badrane H, Tordo N. Host switching in Lyssavirus history from the Chiroptera to the Carnivora orders. *J Virol* 2001; **75**: 8096–8104.
115. Shankar V, Orciari LA, de Mattos C, *et al.* Genetic divergence of rabies viruses from bat species of Colorado, USA. *Vector Borne Zoonotic Dis* 2005; **5**: 330–341.
116. Davis PL, Bourhy H, Holmes EC. The evolutionary history and dynamics of bat rabies virus. *Infect Genet Evol* 2006 (In press).
117. Nadin-Davis SA, Huang W, Armstrong J, *et al.* Antigenic and genetic divergence of rabies viruses from bat species indigenous to Canada. *Virus Res* 2001; **74**: 139–156.
118. Messenger SL, Smith JS, Rupprecht CE. Emerging epidemiology of bat-associated cryptic cases of rabies in humans in the United States. *Clin Infect Dis* 2002; **35**: 738–747.
119. Ito M, Arai YT, Itou T, *et al.* Genetic characterization and geographic distribution of rabies virus isolates in Brazil: identification of two reservoirs, dogs and vampire bats. *Virology* 2001; **284**: 214–222.
120. Kobayashi Y, Sato G, Shoji Y, *et al.* Molecular epidemiological analysis of bat rabies viruses in Brazil. *J Vet Med Sci* 2005; **67**: 647–652.
121. Cisterna D, Bonaventura R, Caillou S, *et al.* Antigenic and molecular characterization of rabies virus in Argentina. *Virus Res* 2005; **109**: 139–147.
122. de Mattos CA, Favi M, Yung V, Pavletic C, de Mattos CC. Bat rabies in urban centers in Chile. *J Wildl Dis* 2000; **36**: 231–240.
123. Nadin-Davis SA, Loza-Rubio E. The molecular epidemiology of rabies associated with chiropteran hosts in Mexico. *Virus Res* 2006; **117**: 215–226.
124. Schowalter DB. Characteristics of bat rabies in Alberta. *Can J Comp Med* 1980; **44**: 70–76.
125. Pal SR, Arora B, Chhuttani PN, *et al.* Rabies virus infection of a flying fox bat, *Pteropus poliocephalus* in Chandigarh, Northern India. *Trop Geogr Med* 1980; **32**: 265–267.
126. Stantic-Pavlinic M. Public health concerns in bat rabies across Europe. *Euro Surveill* 2005; **10**: 217–220.
127. Müller T, Cox J, Peter W, *et al.* Spill-over of European bat lyssavirus type 1 into a stone marten (*Martes foina*) in Germany. *J Vet Med B Infect Dis Vet Public Health* 2004; **51**: 49–54.
128. Fooks AR, Brookes SM, Johnson N, McElhinney LM, Hutson AM. European bat lyssaviruses: an emerging zoonosis. *Epidemiol Infect* 2003; **131**: 1029–1039.
129. Bourhy H, Kissi B, Lafon M, Sacramento D, Tordo N. Antigenic and molecular characterization of bat rabies virus in Europe. *J Clin Microbiol* 1992; **30**: 2419–2426.
130. Serra-Cobo J, Amengual B, Abellan C, Bourhy H. European bat lyssavirus infection in Spanish bat populations. *Emerg Infect Dis* 2002; **8**: 413–420.
131. van der Poel WH, van der Heide R, Verstraten ER, *et al.* European bat lyssaviruses, The Netherlands. *Emerg Infect Dis* 2005; **11**: 1854–1859.
132. Rønsholt L, Sørensen KJ, Brusckhe CJM, *et al.* Clinical silent rabies infection in (zoo) bats. *Vet Rec* 1998; **142**: 519–520.
133. Brookes SM, Aegerter JN, Smith GC, *et al.* European bat lyssavirus in Scottish bats. *Emerg Infect Dis* 2005; **11**: 572–578.
134. Johnson N, Selden D, Parsons G, *et al.* Isolation of a European bat lyssavirus type 2 from a Daubenton's bat in the United Kingdom. *Vet Rec* 2003; **152**: 383–387.
135. Fraser GC, Hooper PT, Lunt RA, *et al.* Encephalitis caused by a Lyssavirus in fruit bats in Australia. *Emerg Infect Dis* 1996; **2**: 327–331.
136. Allworth A, Murray K, Morgan J. A human case of encephalitis due to a lyssavirus recently identified in fruit bats. *Commun Dis Intell* 1996; **20**: 504.
137. Mackenzie JS. Emerging viral diseases: an Australian perspective. *Emerg Infect Dis* 1999; **5**: 1–8.
138. McCall BJ, Epstein JH, Neill AS, *et al.* Potential exposure to Australian bat lyssavirus, Queensland, 1996–1999. *Emerg Infect Dis* 2000; **6**: 259–264.
139. McCall BJ, Field HE, Smith GA, Storie GJ, Harrower BJ. Defining the risk of human exposure to Australian bat lyssavirus through potential non-bat animal infection. *Commun Dis Intell* 2005; **29**: 202–205.
140. Arguin PM, Murray-Lillibridge K, Miranda ME, Smith JS, Calaor AB, Rupprecht CE. Serologic evidence of Lyssavirus infections among bats, the Philippines. *Emerg Infect Dis* 2002; **8**: 258–262.
141. Reynes JM, Molia S, Audry L, *et al.* Serologic evidence of lyssavirus infection in bats, Cambodia. *Emerg Infect Dis* 2004; **10**: 2231–2234.
142. Lumlertdacha B, Boongird K, Wanghongsa S, *et al.* Survey for bat lyssaviruses, Thailand. *Emerg Infect Dis* 2005; **11**: 232–236.



143. Kuzmin IV, Niezgoda M, Carroll DS, *et al.* Lyssavirus surveillance in bats, Bangladesh. *Emerg Infect Dis* 2005; **12**: 486–488.
144. Hellenbrand W, Meyer C, Rasch G, Steffens I, Ammon A. E-alert 18 February: cases of rabies in Germany following organ transplantation. *Euro. Surveill.* 10:E050224.6. <http://www.eurosurveillance.org/ew/2005/050224.asp#6>. [3 May, 2006.]
145. Willoughby Jr. RE, Tieves KS, Hoffman GM, *et al.* Survival after treatment of rabies with induction of coma. *N Engl J Med* 2005; **352**: 2508–2514.
146. Brookes SM, Parsons G, Johnson N, McElhinney LM, Fooks AR. Rabies human diploid cell vaccine elicits cross-neutralising and cross-protecting immune responses against European and Australian bat lyssaviruses. *Vaccine* 2005; **23**: 4101–4109.
147. Hanlon CA, Kuzmin IV, Blanton JD, Weldon WC, Manangan JS, Rupprecht CE. Efficacy of rabies biologics against new lyssaviruses from Eurasia. *Virus Res* 2005; **111**: 44–54.
148. World Health Organization. Chikungunya and Dengue in the south west Indian Ocean. [http://www.who.int/csr/don/2006\\_03\\_17/en/index.html](http://www.who.int/csr/don/2006_03_17/en/index.html). [20 April, 2006.]
149. Diallo M, Thonnon J, Traore-Lamizana M, Fontenille D. Vectors of Chikungunya virus in Senegal: current data and transmission cycles. *Am J Trop Med Hyg* 1999; **60**: 281–286.
150. Ubico SR, McLean RG. Serologic survey of neotropical bats in Guatemala for virus antibodies. *J Wildl Dis* 1995; **31**: 1–9.
151. Weaver SC, Ferro C, Barrera R, Boshell J, Navarro JC. Venezuelan equine encephalitis. *Annu Rev Entomol* 2004; **49**: 141–174.
152. Seymour C, Dickerman RW, Martin MS. Venezuelan encephalitis virus infection in neotropical bats. I. Natural infection in a Guatemalan enzootic focus. *Am J Trop Med Hyg* 1978; **27**: 290–296.
153. Correa-Giron P, Calisher CH, Baer GM. Epidemic strain of Venezuelan equine encephalomyelitis virus from a vampire bat captured in Oaxaca, Mexico, 1970. *Science* 1972; **175**: 546–547.
154. Calisher CH, Kinney RM, de Souza Lopes O, Trent DW, Monath TP, Francly DB. Identification of a new Venezuelan equine encephalitis virus from Brazil. *Am J Trop Med Hyg* 1982; **31**: 1260–1272.
155. Ryan PA, Martin L, Mackenzie JS, Kay BH. Investigation of gray-headed flying foxes (*Pteropus poliocephalus*) (Megachiroptera: Pteropodidae) and mosquitoes in the ecology of Ross River virus in Australia. *Am J Trop Med Hyg* 1997; **57**: 476–482.
156. Almeida MF, Martorelli LF, Aires CC, Sallum PC, Massad E. Indirect oral immunization of captive vampires, *Desmodus rotundus*. *Virus Res* 2005; **111**: 77–82.
157. Sétien AA, Brochier B, Tordo N, *et al.* Experimental rabies infection and oral vaccination in vampire bats (*Desmodus rotundus*). *Vaccine* 1998; **16**: 1122–1126.
158. Wanzeller AL, Diniz JA, Gomes ML, *et al.* Ultrastructural, antigenic and physicochemical characterization of the Mojuí dos Campos (Bunyavirus) isolated from bat in the Brazilian Amazon region. *Mem Inst Oswaldo Cruz* 2002; **97**: 307–311.