Bat viruses and diseases

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Bats, representing approximately 20% of mammal species, are the most abundant, diverse and geographically dispersed vertebrates on earth. Bats of various species have recently been identified as the reservoir hosts of many emerging viruses responsible for severe human and livestock disease outbreaks. These include Hendra and Nipah viruses, SARS coronavirus, Ebola viruses, Melaka virus and others. Australian scientists played a vital role in the discovery and/or characterisation of many of these emerging bat viruses. While these viruses result in significant morbidity and mortality in other mammals, they appear to cause no clinical consequence in bats under natural or experimental infection. Understanding this key difference in pathogenesis and the mechanism of virus spillover from bats to other mammals will be critical for our effort to prevent and control future disease outbreaks caused by bat-borne zoonotic viruses.

Bats as reservoir hosts of viruses

There are approximately 1000 different bat species identified, which are taxonomically placed in two suborders, Megachiroptera (megabats) and Microchiroptera (mircrobats). Bats evolved and diverged from other mammals very early in evolution. Although there is no conclusive scientific evidence to pinpoint their first appearance, it is estimated that they originated about 50 million years ago [1].

Different from other mammals, bats possess several unique biological features which may play a role in their exceptional capacity in the maintenance and transmission of viruses:

(1) Bats are the only mammals that have the ability to fly. All bats fly daily in search of food and many bats fly long distances during seasonal migration, up to hundreds of kilometres.

(2) Some bats in temperate regions are capable of body temperature regulation through the process of torpor (daily change) or hibernation (seasonal change). It has been shown that viruses can persist when the bat body temperature drops, which may play a role in the long-term maintenance of certain viruses among different bat populations.

(3) The extremely large size of bat colonies and the close-contact roosting behaviour of most bats may also facilitate efficient transmission among same or different species.

(4) Bats, especially microbats, seem to have a lifespan longer than the average of other mammals of similar body weight. This feature, in combination with the potential persistent infection of many viruses in bats, may further explain the suitability of bats as reservoir hosts of so many different viruses.

(5) Microbats and some megabats are capable of echolocation and it is suspected that the intensity of the sound waves will be sufficient to generate droplets or aerosols, which will, in turn, facilitate virus spread.

(6) Evolutionally, as placental mammals, bats are more closely related to humans and domestic animals, all members of the subclass Eutheria, than other wildlife species. Bat viruses may therefore be more adapted to spill over into human and domestic animals.

(7) Last but not least, as one of the most ancient mammal species, the bat immune system, especially their innate immunity, may be subtly different from those of other mammals. This may explain the general observation that many viruses can infect and maintain in apparently healthy bats, but result in acute and severe diseases when they infect humans or other mammals.

Almost a century ago, bats were identified as carriers of rabies virus in South and Central America. Since then viruses have been identified from various bat species across different geographic locations. It is estimated that bats are hosts of more than 60 viruses from 11 different virus families, covering DNA viruses, double-stranded RNA (dsRNA) viruses and single stranded (both positive and negative sense) RNA viruses [2]. Since the publication of this review, several new bat-borne viruses have been discovered [3, 4] and it is expected that this trend will continue, due to the increasing research activities in this field worldwide.

A new wave in the emergence of bat-borne viruses

Although the study of rabies virus and its association with bats remains active even to this present day, the importance of bats as reservoirs of zoonotic viruses has been largely unappreciated until the emergence of Hendra virus in Australia in 1994 [5]. Since then, many other bat-borne zoonotic viruses were discovered

as discussed below. In this context, Hendra virus marks the beginning of a new wave of bat virus emergence.

Hendra and Nipah virus

As the first, contemporary bat-zoonotic virus of high virulence in non-bat hosts, Hendra virus emerged in 1994 in Australia, resulting in the deaths of 14 horses and one human [5]. It was classified as a Biosafety Level 4 (BSL4) agent, due to its high mortality and the lack of any effective treatment. Fruit bats in the genus *Pteropus* were identified as the reservoir of Hendra virus [6]. Since this first recognised outbreak, there have been at least ten additional outbreaks of Hendra virus in Australia, several of them involving human infections (Table 1). The clinical presentation of infected horses or humans can vary from severe respiratory distress to brain malfunction.

In 1998-99, a very closely related virus, the Nipah virus, emerged in Malaysia [7]. It is believed the outbreak was a result of a bat-to-pig spillover event(s), followed by massive pig-to-pig transmission. It eventually led to pig-to-human transmission and claimed more than 100 human lives in Malaysia and Singapore, nearly all of whom had direct contact with infected pigs. Several years later, a different strain of the Nipah virus emerged in India and Bangladesh, causing outbreaks almost on a yearly basis, with a human mortality of up to 90%. Epidemiological investigations suggest that this new Nipah virus strain is capable of direct bat-to-human and human-to-human transmission, raising the possibility of human disease outbreaks on a greater scale [8].

Hendra and Nipah viruses are now classified into a separate genus, *Henipavirus,* in the family *Paramyxoviridae.* Virus isolation and the use of polymerase chain reaction (PCR) and serological studies indicate that related henipaviruses are widespread among different bat populations from Australia, Indonesia, Papua New Guinea, Thailand, Malaysia and Cambodia to Madagascar and Western Africa. A related virus(es) may also be circulating among bats in China [9].

Menangle virus

During an investigation of reproductive disease outbreak in a large commercial piggery in New South Wales, a new virus, named Menangle virus (a novel paramyxovirus in the genus *Rubulavirus),* was isolated and later proved to be the causative agent responsible for reduced farrowing rates and stillbirths with deformities in pigs [10]. Serological investigation of persons in contact with pigs revealed two humans, who were in close contact with infected pigs and suffered an influenza-like illness, had high levels of convalescent neutralising antibodies to Menangle virus. A recent study found that neutralising antibodies against Menangle virus can be detected in all four species of Australian flying foxes, the black flying fox *(Pteropus alecto),* grey-headed flying fox *(P. poliocephalus),* spectacled flying fox *(P. conspicillatus)* and the little red flying fox *(P. scapulatus).*

Although no live virus was ever isolated from Australian bats, the notion that bats serve as a natural reservoir host of Menangle virus was further supported by the isolation of a highly related bat paramyxovirus from a Malaysian flying fox *Pteropus hypomelanus.* During a search for the reservoir host of Nipah virus, urine samples were collected from bats on Tioman Island, situated off the east coast of peninsular Malaysia. A novel paramyxovirus (named Tioman virus) was isolated in addition to Nipah virus [11]. Molecular and antigenic studies indicated that Tioman virus is most closely related to Menangle virus. The potential of Tioman virus to infect and cause disease in humans or other animals is unknown; however, our recent studies have shown that pigs are susceptible to infection by Tioman virus and there is serological evidence for the infection of humans by this virus on Tioman Island [12].

SARS-like coronaviruses

Severe acute respiratory syndrome (SARS) represents the first serious and widespread human infectious disease outbreak of the 21st century. It was caused by a previously unknown coronavirus, the SARS coronavirus. Although zoonotic SARS virus strains were isolated from civets and raccoon dogs and viral genomic materials were detected in cats, pigs and other animals [13], none of these animals were identified as the natural reservoir of the virus due to the lack of widespread and persistent infection in their populations. In 2005, two groups independently found that horseshoe bats in the genus *Rhinolophus* carry a group of coronaviruses that are closely related to the outbreak strains [14,15]. Subsequent studies indicated that bats in different parts of the world carry a variety of coronaviruses, which have co-evolved with the host through inter-species infection, co-infection of different viruses in bats of the same species or recombination between related coronaviruses. It is hypothesised that the progenitor virus that led to the mass SARS outbreaks originated from bats of a yet-to-be-identified species and transmitted to humans through one or more intermediate hosts, which act as both an adaptation and amplification host [16].

Ebola and Marburg viruses

As one of the most deadly viruses known to infect humans, the origin of Ebola virus remained a mystery, despite several decades of detective investigation. However, a recent study has shown that bats of three different species *(Hypsignathus monstrosus, Epomops franqueti* and *Myonycteris torquate)* captured in Gabon and the Republic of Congo seem to be infected by Ebola virus with detection of virus-specific RNA or antibodies from several individual bats [17]. Soon after the discovery of Ebola virus RNA and antibodies in fruit bats, the same group conducted similar studies for Marburg virus and reported the detection of virus-specific RNA and antibodies in individual bats of the common species *(Rousettus aegyptiacus)* in Gabon [18]. These results provide further evidence that bats are the likely natural reservoir of these deadly viruses.

Melaka virus and related orthoreoviruses

First discovered in the early 1950s, reoviruses (respiratory enteric orphan viruses) were not known to be associated with any severe disease in humans; hence the name orphan virus. In 2006, during an investigation of a flu-like disease outbreak in Melaka, Malaysia, a novel orthoreovirus (named Melaka virus) was isolated and shown to be the causative agent of an acute respiratory disease suffered by multiple members of a family [3]. Epidemiological tracing indicated that the virus may have been introduced by a bat 'visiting' the residential house of the family about a week prior to the onset of disease in the first patient of the outbreak cluster. Its bat origin was further indicated by molecular characterisation, which revealed that Melaka virus is very closely related to two previously isolated bat orthoreoviruses – Nelson Bay virus isolated in the late 1960s in Australia and the Pulau virus isolated in 1999 on Tioman Island, Malaysia. A retrospective serological study revealed that 13% of human volunteers from Tioman Island were seropositive to this group of viruses, suggesting that bat orthoreoviruses may infect humans more frequently than reported.

Since the discovery of the Melaka virus, several other human disease outbreaks have been attributed to similar bat orthoreoviruses in Asia. In 2006 Kampar virus was isolated from the throat swab of a male patient in Kampar, Perak, Malaysia who, at the time of virus isolation, was suffering from high fever, acute respiratory disease and vomiting. Subsequent epidemiological studies revealed that Kampar virus was transmitted from the index case to at least one other individual and caused respiratory disease in the contact case. Kampar and Melaka viruses are closely related and an effective cross-neutralisation was demonstrated between the two viruses [4]. In 2007, another related virus was isolated in Hong Kong from a human subject suffering acute respiratory distress who had potential exposure to bats in Bali [personal communication].

Figure 1. Use of black flying foxes *(Pteropus alecto)* as a model system to study bat immunology and virus–bat interaction. (A) A black flying fox resting upside down inside a laboratory cage. (B) Conducting a bat infection experiment in a BSL4 facility at the Australian Animal Health Laboratory (AAHL). (Photos provided by Jennifer McEachern and Gary Crameri.)

Spillover into human populations: direct versus indirect transmission

When Hendra virus was discovered, epidemiological studies found no evidence of direct bat-to-human transmission. This initial observation remains true for all of the subsequent known spillover events, as summarised in Table 1. It seems that transmission via an intermediate host (horse, in this case) is essential for the virus to be able to eventually jump into human populations. This conclusion also applies to the Nipah virus outbreaks in Malaysia, where pigs were the only source of virus transmission to humans. Furthermore, molecular studies indicate that there is no major change in the genome sequence of the viruses isolated from bats, horses or humans, suggesting that the transmission through horses plays a role in virus amplification, rather than in virus adaptation. On the other hand, the Nipah virus responsible for outbreaks in Bangladesh and India appears capable of direct bat-to-human transmission without amplification in an intermediate host [19]. It is not clear at this stage whether the difference is a result of different human behaviours or a result of virological difference between the different henipaviruses.

Direct bat-to-human transmission without the need for amplification or adaptation in an intermediate host has also been observed for several newly emerged orthoreoviruses [3,4]. More recently, an epidemiological study of the 2007 Ebola virus outbreak in the Democratic Republic of Congo revealed that the mass gathering of migrating bats in the outbreak region and their hunting and consumption by humans was the most likely source of Ebola virus introduction into the human population, again suggesting a direct bat-to-human transmission of Ebola virus [20]. Direct bat-to-human transmission has also been documented for Australian bat lyssavirus, which is closely related to the rabies virus [21]; serological evidence also suggests this may be happening with the Tioman virus in Malaysia [12].

It can, therefore, be concluded that most of the well-studied, bat-borne zoonotic viruses seem to have the potential for direct spillover into human populations, provided the virus load in bats reaches a critical level and/or there is close contact or co-localisation of humans and bats. In other words, species specificity seems to be less of a barrier for interspecies transmission of these bat-borne viruses. One of the reasons for the broad host susceptibility of these viruses is their use of highly conserved ubiquitous membrane proteins as receptor molecules. For example, the receptors used by the henipaviruses are highly conserved across bats, pigs, horses and humans [22].

One major exception is the SARS coronavirus. Although the exact natural animal reservoir of the progenitor virus is not known, it was abundantly evident from multiple molecular epidemiological studies that the SARS coronavirus evolved rapidly in new hosts (such as the civets found in markets in Southern China) and this rapid adaptation played an important role in the eventual transmission into the human population. It was further demonstrated that the severity of the disease in humans was directly related to the fitness of the virus to the human receptor molecule [23].

Future research directions

With the increasing trend of novel zoonotic viruses emerging from bats of different species and causing deadly disease in humans and domestic animals, there is an urgent need to conduct basic studies into bat biology, immunology and ecology in order to better understand bat–virus interaction. This will facilitate prevention and control strategies to better manage zoonotic disease outbreaks in the future. These research objectives include research into bat genomics and transcriptomics; the development of bat immunology tools; the establishment of bat cell lines for chosen model species; and the consolidation of an

Table 1. Known spillover events for Hendra virus in Australia since 1994

Time	Location	Animal/human involvement
1994, August	Mackay	2 horses & 1 human (fatal)
1994, September	Brisbane (Hendra)	20 horses & 2 human (one fatal)
1999, January	Cairns (Trinity Beach)	1 horse
2004, October	Cairns (Gordonvale)	1 horse & one human
2004, December	Townsville	1 horse
2006, June	Peachester	1 horse
2006, October	Murwillumbah*	1 horse
2007, June	Peachester	1 horse
2007, July	Cairns (Clifton Beach)	1 horse
2008, June	Brisbane (Redlands)	5 horses & two humans (one fatal)
2008, July	Proserpine	3 horses

* The only known spillover event occurred in New South Wales; all others were in Queensland.

international network for ecological and epidemiological studies of bats and bat viruses. In our group at the CSIRO Australian Animal Health Laboratory, we have focused our studies on the black flying fox *(Pteropus alecto;* Figure 1) as a model system, based on the fact that they are the reservoir host of several known viruses and are relatively easy to handle under laboratory conditions.

Finally, it should be emphasised that bats should not be viewed as evil animals simply because they are the carriers of viruses. Bats play many important roles in our ecosystem, from insect control to seed dispersal and pollination. Bats have co-evolved with viruses for many millions of years and it is highly likely that carrying viruses without clinical consequences could be an evolutionary advantage to the bat populations.

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