Virus discovery in bats

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Comprising approximately 20% of known mammalian species, bats are abundant throughout the world[1]. In recent years, bats have been shown to be the reservoir host for many highly pathogenic viruses, leading to increased attempts to identify other zoonotic bat-borne viruses. These efforts have led to the discovery of over 200 viruses in bats and many more viral nucleic acid sequences from 27 different viral families[2,3] (Table 1). Over half of the world's recently emerged infectious diseases originated in wildlife[15], with the genetic diversity of viruses greater in bats than in any other animal[16]. As humans continue to encroach on the habitat of bats, the risk of spillover of potentially zoonotic viruses is also continuing to increase. Therefore, the surveillance of bats and discovery of novel pathogens is necessary to prepare for these spillover events[17].

Not only does virus discovery increase our understanding of the role that bats play in emerging infectious diseases, it also allows the development of diagnostic tools resulting in a much more efficient response if a spillover event occurred, reducing both the economic and public health impact of the virus. Virus discovery is important for identifying potential zoonotic threats and can assist with the characterisation of already emerged zoonotic viruses, as well as providing phylogenetic evidence for the origin and evolution of these viruses; for example the potential bat origin of primate hepadnaviruses[5].

Advancements in technology have also contributed to the increased rate of virus discovery, with molecular techniques now overtaking serological methods and virus isolation[18]. Improvement in the accessibility of next generation sequencing has allowed the development of unbiased methods of analysing bat specimens as well as more rapid characterisation of novel viruses. However, next generation sequencing is not suitable for all experimental aims, such as when the targeted discovery of particular viral families is required[19].

The bat sampling method can affect which viruses are able to be detected and can result in a bias towards particular families of viruses. The bat specimen used for discovery is an important consideration, as well as the time of year these specimens are collected, the intervals between collections, the species of bat to be targeted and the ecology of the bat species, especially as not all viruses are continually shed in the population. In the case of Marburg virus, peaks of shedding were seen during birthing seasons as these months coincided with a peak in infection in 6-month-old juvenile bats[20].

Although lethal sampling of bats may be necessary for virus discovery from particular viral families, non-lethal sampling has resulted in the discovery of a greater number of novel viruses across a similar number of studies[18]. Bat urine and faeces have been favoured as non-invasive samples for virus discovery, however active bat catching and sampling can give more accurate calculations of viral prevalence. In the case of Hendra virus, urine was the most significant form of virus transmission, with higher titres of virus seen in urine compared to specimens such as nasal swabs, faecal samples and serum[21]. Pooled urine can be collected from plastic sheets laid below bat colonies and stored in a viral transport medium at −80°C for later analysis[22]. These samples can then be analysed in multiple different ways depending on the chosen method for virus discovery.

Molecular techniques such as pan-viral family PCR are useful for targeted discovery of viruses. This involves amplifying a region of the genome that is highly conserved across that viral family using

Table 1. Summary of viral families detected in bats[2,4] and their zoonotic potential. Viral families were classed as containing zoonoses if any of the viruses detected in bats had been associated with disease in humans[4–14].

	Virus family	Zoonotic
ssRNA (negative sense)	*Arenaviridae*	–
	Bornaviridae	–
	Bunyaviridae	+
	Filoviridae	+
	Orthomyxoviridae	+
	Paramyxoviridae	+
	Rhabdoviridae	+
ssRNA (positive sense)	*Astroviridae*	–
	Caliciviridae	–
	Coronaviridae	+
	Dicistroviridae	–
	Hepeviridae	–
	Flaviviridae	+
	Nodaviridae	–
	Picornaviridae	–
	Togaviridae	+
dsDNA	*Adenoviridae*	–
	Anelloviridae	–
	Circoviridae	–
	Herpesviridae	–
	Papillomaviridae	–
	Parvoviridae	–
	Polyomaviridae	–
	Poxviridae	–
dsRNA	*Reoviridae*	+
	Picobirnaviridae	–
	Totiviridae	–
Retro-transcribing	*Hepadnaviridae*	+
	Retroviridae	–

degenerate primers[23,24]. In one study, this approach was employed to detect sequences of 66 new viruses from the *Paramyxoviridae* family from bats and rodents around the world[25]. In this example, pan-viral family PCR detected paramyxovirus sequences, including in bats that yielded no positive results when their pooled serum samples were analysed by next generation sequencing. However, it is possible that the negative results by next generation sequencing were due to low concentrations of virus in the blood rather than significantly lower sensitivity[25]. The primers utilised by pan-viral family PCR can only detect viruses that are related to previously identified viruses. In an attempt to reduce the bias introduced by sequence specific primers, multiple different primer sets and methods can be utilised for the same samples[25]. Although this approach has led to the discovery of many novel viruses, other methods provide a hypothesis-free approach.

Next generation sequencing has become increasingly more accessible as a method for virus discovery, although it is still more expensive than other molecular methods and requires bioinformatics knowledge to correctly analyse the raw data and generate a consensus genome[19]. When correctly designed, metagenomic analysis of bat specimens can allow the hypothesis free discovery of many novel viruses, including those that are significantly divergent from previously identified viruses. The high throughput technique also allows efficient screening of a large number of bat specimens. This method was used to identify highly divergent novel rotaviruses in bats in Cameroon that were unlikely to have been successfully detected using the currently available primer combinations[26]. The sensitivity of high throughput sequencing is continuing to improve for virus discovery, employing techniques such as positive enrichment of samples for virus sequences using probes that cover the genomes of all the viral taxa known to infect vertebrates[27]. However, this enrichment may reduce the likelihood of discovering novel viruses.

Virus isolation, supported by other molecular detection techniques, continues to play a significant role in the discovery of novel viruses as it allows further characterisation and comparison with other viruses. Virus isolation followed by pan-family PCR was successfully used for the surveillance of Australian pteropid bats and resulted in the discovery of multiple novel paramyxoviruses[22]. However, not all viruses cause obvious cytopathic effect in cell culture, making it difficult to detect virus growth in cells. Furthermore, the viruses may require very specific cell lines and conditions for growth, if they can even be cultured at all. Bat derived influenza viruses have been detected in *Sturnira lilium* in Guatemala by pan-influenza virus RT-PCR[28], but subsequent attempts at culturing were challenging, due in part to their divergent surface proteins and unique basolateral cell entry mechanism[29,30]. *In vivo* isolation methods may also be used, such as the use of suckling mice or knockout mice[31].

Virus discovery from bats increases our database of known viruses and is necessary for preparing a rapid response to emerging infectious diseases[17]. For example, the isolation and characterisation of Hendra virus in 1994 enabled the development of diagnostic assays that played an important role in the identification of Nipah virus during an outbreak of encephalitic disease five years later. Cross-reactivity with antibodies to Hendra virus was observed during initial screening against the unknown virus causing fatal disease in pigs and humans. Then, primers developed against Hendra virus assisted in determining the sequence of Nipah virus[32]. Virus discovery can also facilitate the development of diagnostic tools and further research into pathogenic determinants of other viruses. It is estimated that each bat species would have to be sampled 7000 times before the viral diversity limit is reached[33], so with approximately 1200 species of bat around the world, the discovery of novel viruses in bats has a long way to go.

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Biographies

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