



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

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



ELSEVIER



Zoonotic disease and virome diversity in bats

Kate Van Brussel and Edward C Holmes

The emergence of zoonotic viral diseases in humans commonly reflects exposure to mammalian wildlife. Bats (order Chiroptera) are arguably the most important mammalian reservoir for zoonotic viruses, with notable examples including Severe Acute Respiratory Syndrome coronaviruses 1 and 2, Middle East Respiratory Syndrome coronavirus, henipaviruses and lyssaviruses. Herein, we outline our current knowledge on the diversity of bat viromes, particularly through the lens of metagenomic next-generation sequencing and in the context of disease emergence. A key conclusion is that although bats harbour abundant virus diversity, the vast majority of bat viruses have not emerged to cause disease in new hosts such that bats are better regarded as critical but endangered components of global ecosystems.

Address

Sydney Institute for Infectious Diseases, School of Life & Environmental Sciences and School of Medical Sciences, The University of Sydney, NSW, 2006, Australia

Corresponding author:

Holmes, Edward C (edward.holmes@sydney.edu.au)

Current Opinion in Virology 2022, 52:192–202

This review comes from a themed issue on **The virome in health and disease**

Edited by **Evelien Adriaenssens** and **Jelle Matthijssens**

For complete overview about the section, refer [The virome in health and disease \(2022\)](#)

Available online 23rd December 2021

<https://doi.org/10.1016/j.coviro.2021.12.008>

1879-6257/© 2021 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

The global COVID-19 pandemic caused by Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) has intensified interest in bats as reservoirs for emerging viruses. Bats are the largest mammalian order (Chiroptera) after rodents, comprising over 1400 species from two suborders: the Yinpterochiroptera containing the *Pteropodidae* ('fruit bats') and five families of microbat, and the Yangochiroptera containing the remaining 14 microbat families [1^{*}]. The most widespread bat families include the *Hipposideridae*, *Pteropodidae*, *Rhinolophidae*, *Molossidae*, *Emballonuridae*, *Phyllostomidae* and *Vespertilionidae* that contain up to ~190 species, while the *Craseonycteridae* and *Myzopodidae* harbour only one or two species and are geographically restricted [2,3]. For dietary purposes bats can be classified as insectivores, frugivores, carnivores and

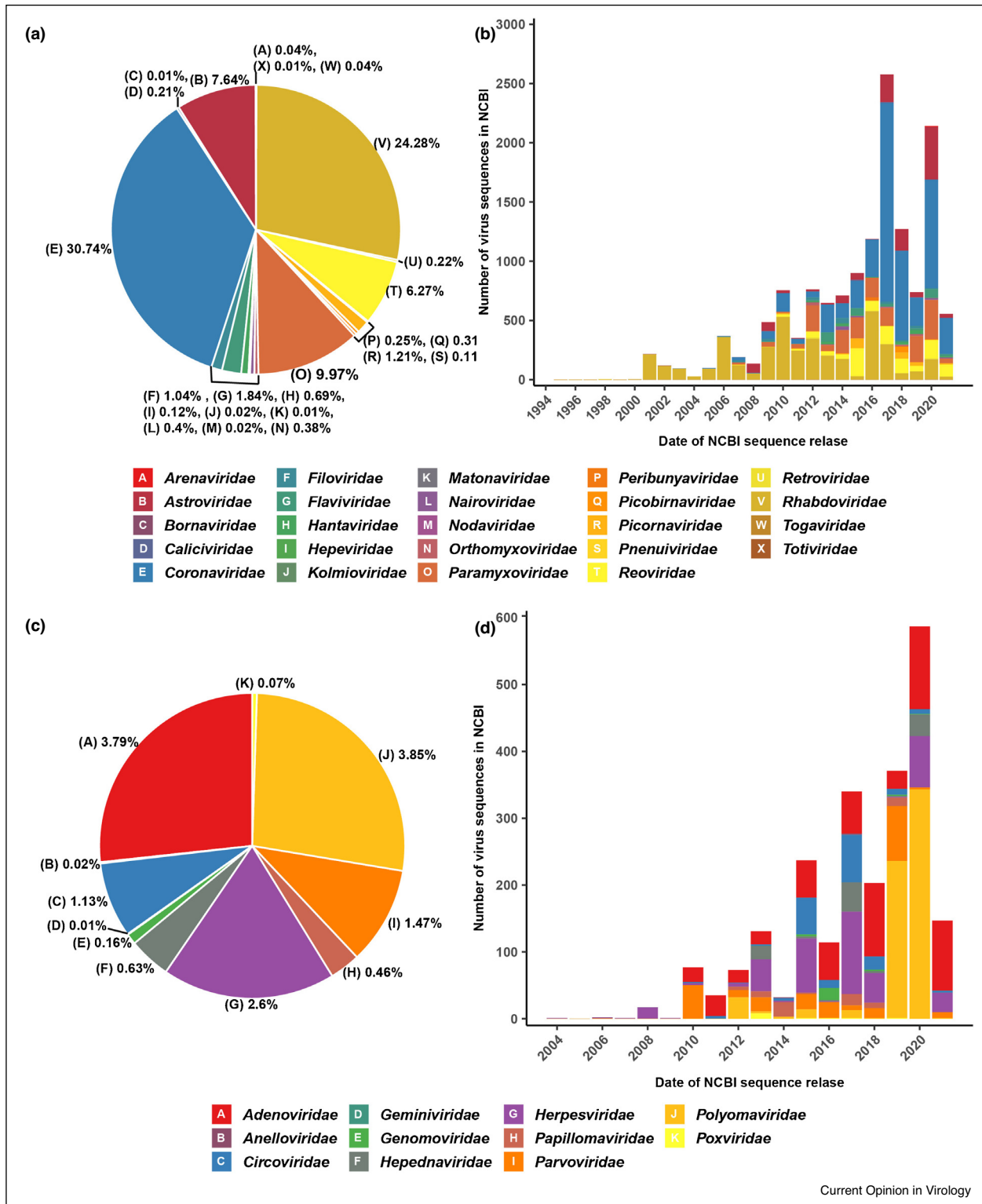
nectivores, and several species use echolocation to characterise their surrounding environment and locate prey. Bats are found in every continent excluding Antarctica, in part reflecting their capacity to migrate via flight, with roosting sites that vary seasonally.

Virome diversity in bats

Metagenomic sequencing is an increasingly powerful and popular tool for virus discovery, particularly as it allows the characterisation of viruses from families that are often overlooked in PCR-based screening that focuses on known or likely pathogens. It is therefore no surprise that much of our knowledge of bat viromes comes from the deployment of large-scale metagenomic next-generation sequencing (mNGS), particularly total RNA sequencing (metatranscriptomics). At the time of writing, ~30% of all the bat associated virus sequences deposited on NCBI/GenBank have been identified by mNGS only, an increase from 10% in 2016 (Figure 1). The recent identification of Wittenau bat nairovirus (*Nairoviridae*) and Ruhugu virus (*Matonaviridae*) serve as informative examples [4,5], as was the detection of deltaviruses (*Kolmioviridae*) in bats, even though these viruses were originally only associated with humans in the context of coinfection with hepatitis B virus [6^{*}].

The collection of bat urine, saliva and faecal samples is commonly used in mNGS studies as this minimises the impact on bat populations. In many cases it also represents the likely route of virus transmission, although it may miss viruses associated with specific tissues. The analyses of faecal material and urine from bat species sampled on multiple continents have identified viruses from the *Adenoviridae*, *Astroviridae*, *Caliciviridae*, *Coronaviridae*, *Flaviviridae*, *Papillomaviridae*, *Paramyxoviridae*, *Parvoviridae*, *Picornaviridae*, *Polyomaviridae* and *Reoviridae* [7,8^{*},9–11]. For example, a study of more than 4000 bat rectal and pharyngeal swabs from three common bat genera – horseshoe bats (*Rhinolophus*), mouse-eared bats (*Myotis*) and bent winged bats (*Miniopterus*) – in China identified novel virus sequences from diverse viral families, including the *Coronaviridae* and *Paramyxoviridae* (see below), as well as those from the order *Bunyavirales* of RNA viruses [12^{**}]. The *Bunyavirales* currently comprises 12 families including a number associated with human disease. Five of these families have been identified in bats: the *Arenaviridae*, *Nairoviridae*, *Hantaviridae*, *Peribunyaviridae* and *Phenuiviridae* [5,8^{*},13,14]. However, it is also important to note that rather than infecting bat themselves, many of the viruses detected in bats may be associated with aspects of the bat diet and microbiome. This is especially the case in studies utilising faecal

Figure 1



Taxonomic distribution of publicly available gene sequences of bat viruses. Plots show virus sequences on NCBI/GenBank in which the order Chiroptera or individual bat species are listed as hosts. **(a)** Percentage of bat virus sequences that belong to RNA virus families and **(b)** RNA virus sequences by year of NCBI release. **(c)** Percentage of bat virus sequences that belong to DNA virus families and **(d)** DNA virus sequences by year of NCBI release.

mNGS studies [8^{*},9,15–17], and care should also be taken to exclude reagent contamination [18].

A broad-scale conclusion from metagenomic studies is that bats may be particularly prone to carrying viral families that are commonly associated with zoonotic disease. Of the more than 16 600 bat associated viral sequences on NCBI/GenBank, 85% are RNA viruses, including 30% and 24% from the families *Coronaviridae* and *Rhabdoviridae*, respectively, while 10% of all bat-associated viral sequences identified to date are from the *Paramyxoviridae* (Figure 1). Interestingly, those virus families that might pose a greater zoonotic risk (i.e. the *Coronaviridae*, *Paramyxoviridae* and *Rhabdoviridae*; see below) are generally associated with a narrower range of bat families compared to those viruses that may be less likely to emerge in humans (i.e. *Astroviridae*, *Reoviridae* and *Picornaviridae*) (Figure 2). As a case in point, a range of SARS-CoV-2 related coronaviruses have been characterised through mNGS of bats sampled in China and parts of south-east Asia [19^{**},20,21]. The viruses carried by bats also vary markedly by bat family (Figure 2). As expected, the widespread and species rich bat families (i.e. the *Vespertilionidae*, *Rhinolophidae*, *Pteropodidae*, *Phyllostomidae* and *Hipposideridae*) harbour a greater diversity of viruses than the less speciose families (*Noctilionidae*, *Natalidae*, *Myzopodidae* and *Furipteridae*).

Notable zoonotic outbreaks associated with bats

Coronaviridae

The current interest in bats as reservoirs for emerging viruses began with the outbreak of SARS-CoV-1 in 2002/2003 [22], and there has recently been intense research activity in documenting the diversity of alphacoronaviruses and betacoronaviruses (Figure 3).

Initial studies showed that civets (*Paguma larvata*) were the likely source of SARS-CoV-1, with emergence in humans associated with their presence in live animal markets in southern China [23]. However, the subsequent sampling of bats in China provided serological evidence of infection with SARS-like coronaviruses in bats of the genus *Rhinolophus* (i.e. horseshoe bats) in Hubei and Guangxi provinces, confirmed by PCR of faecal samples [24^{**}]. Bats of the genus *Rhinolophus* are therefore the likely reservoir hosts for SARS-CoV-1, with onward transmission to civets and (perhaps raccoon dogs) that acted as ‘intermediate’ hosts to seed human infection [24^{**}]. The presence of SARS-like coronaviruses with high sequence similarity to SARS-CoV-1 were later identified in Chinese horseshoe bats (*Rhinolophus sinicus*) [12^{**},25]. Notably, a study from multiple locations in China detected the conserved RNA-dependent RNA polymerase (RdRp) domain of coronaviruses in 6.5% of bat species from the genera *Rhinolophus*, *Pipistrellus*, *Scotophilus*, *Myotis*, *Tylonycteris* and *Miniopterus*, with phylogenetic analysis

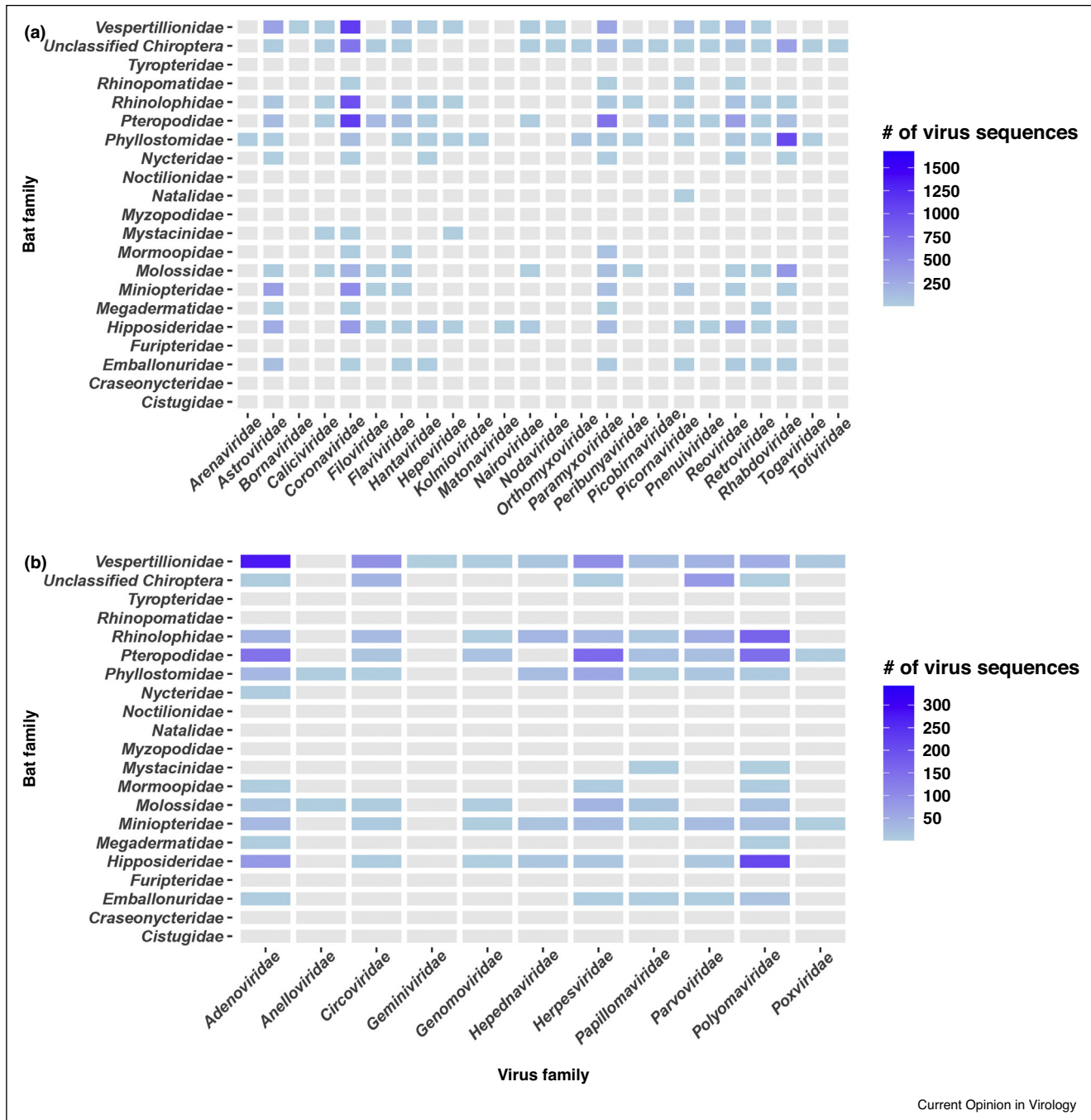
showing that three of the coronaviruses detected, all from the genus *Rhinolophus*, clustered with SARS-like coronaviruses [26].

In 2012 Middle Eastern Respiratory Syndrome coronavirus (MERS-CoV) appeared in Saudi Arabia, resulting in respiratory illness and relatively high levels of mortality [27]. Studies of Dromedary camels identified multiple MERS-CoV-like lineages (as well as the alphacoronavirus HCoV-229E) reflecting several decades of circulation in these animals with multiple transmission events to humans and widespread recombination [28]. Notably, MERS-like CoVs were also identified in multiple bat species (*Pipistrellus* cf. *hesperdus*, *Nycteris* cf. *gambiensis*, *Pipistrellus nathusii*, *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus* and *Neoromica* cf. *zuluensis*) [29–31]. Hence, these bat viruses likely represent the reservoir wildlife hosts for the viruses that later emerged, via camels, as MERS-CoV. However, the bat coronaviruses most closely related to MERS-CoV also differed markedly in the spike protein and had reduced capacity to bind to the human dipeptidyl peptidase 4 cell receptor used by MERS-CoV [29,32^{*}].

SARS-CoV-2 was first reported to cause severe pneumonia in humans in Wuhan, China in late 2019 [33^{*}]. Metagenomic surveys and associated phylogenetic analyses have identified viruses closely related to SARS-CoV-2 in *Rhinolophus* bat species from several Asian countries (China, Cambodia, Thailand, Japan and Laos) [19^{**},20,21,34,35^{*},36]. For example, five SARS-CoV-2 related coronaviruses were detected in pooled faecal samples collected from bats (*Rhinolophus pusillus*, *Rhinolophus steno* and *Rhinolophus malayanus*) in two studies of a single tropical botanical garden in Yunnan province, China [19^{**},20]. Close relatives of the alphacoronavirus porcine epidemic disease virus were identified at the same sampling site [19^{**}]. Of most note, five SARS-CoV-2 related coronaviruses were recently identified in three *Rhinolophus* species in Laos (*R. malayanus*, *R. pusillus* and *Rhinolophus marshalli*), three of which grouped closely with human SARS-CoV-2 on phylogenetic trees and possessed a receptor binding domain with high sequence similarity to that of SARS-CoV-2 and the ability to bind to the human ACE2 receptor [21]. This provides strong evidence that viruses with the capacity to infect humans exist in wildlife species and hence represent a pandemic risk. Genomic recombination has also been commonplace among viruses of the SARS-CoV-2-like lineage (i.e. the sarbecoviruses) [19^{**},21,34,37], greatly complicating attempts to accurately reconstruct evolutionary history and suggesting that mixed infection is commonplace in bats and perhaps other mammalian species.

Although most of the focus has necessarily been on human disease, coronaviruses of bat ancestry have resulted in disease outbreaks in species other than

Figure 2



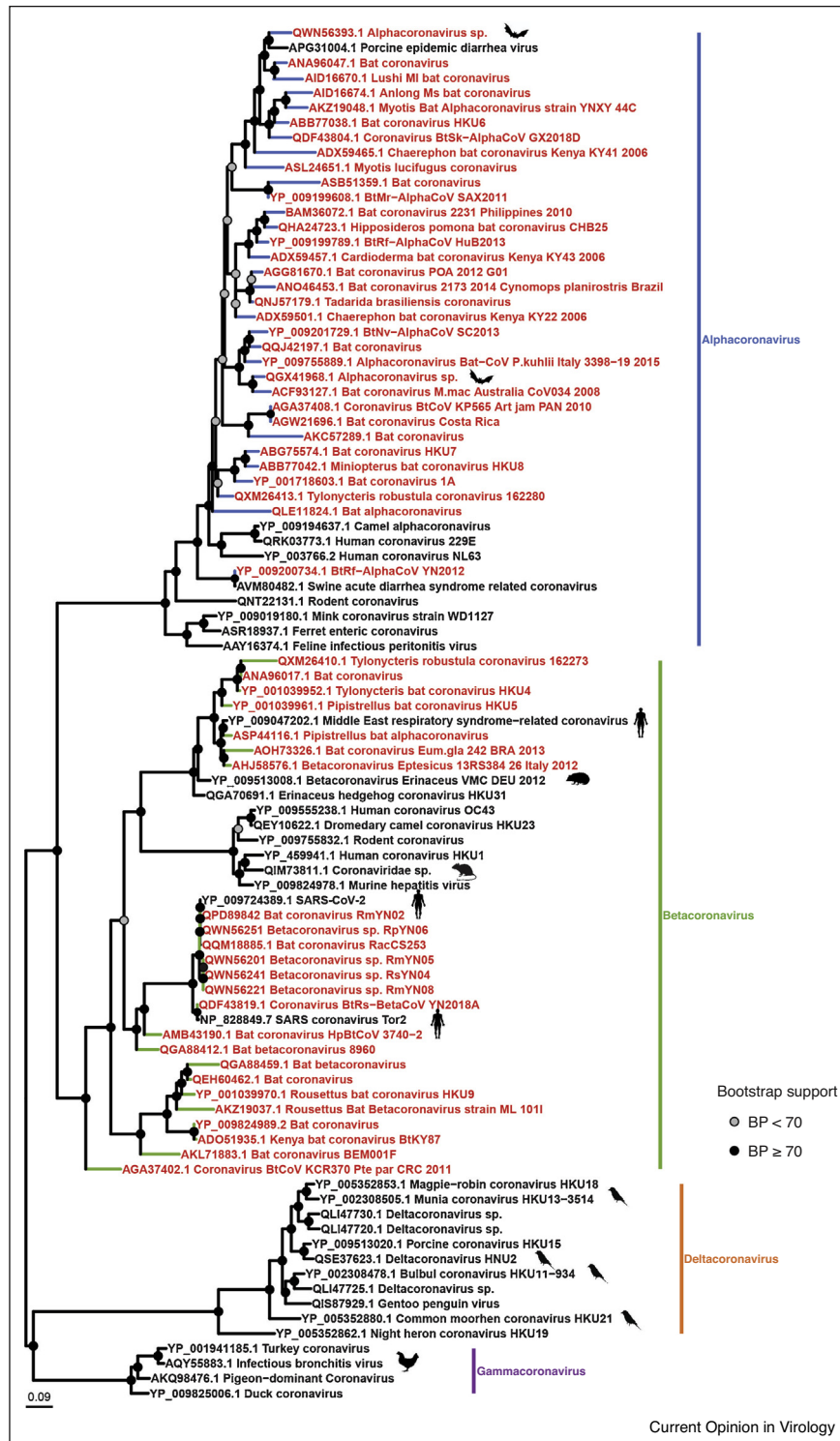
Number of publicly available virus gene sequences for each family of bats. The heat map shows (a) the number of RNA viruses and (b) the number of DNA viruses separated by virus family, and the corresponding bat host family as given on NCBI. Virus sequences with an unspecified bat host (i.e. host listed as Chiroptera) are represented as ‘unclassified Chiroptera’.

humans. For example, a novel alphacoronavirus – Swine Acute Diarrhoea Syndrome coronavirus (SADS-CoV) – caused the death of upwards of 24 000 pigs in China in 2016 [38]. SADS-related coronaviruses were detected in rectal swabs from horseshoe bats in Guangdong province from 2013 to 2016 [38].

Paramyxoviridae

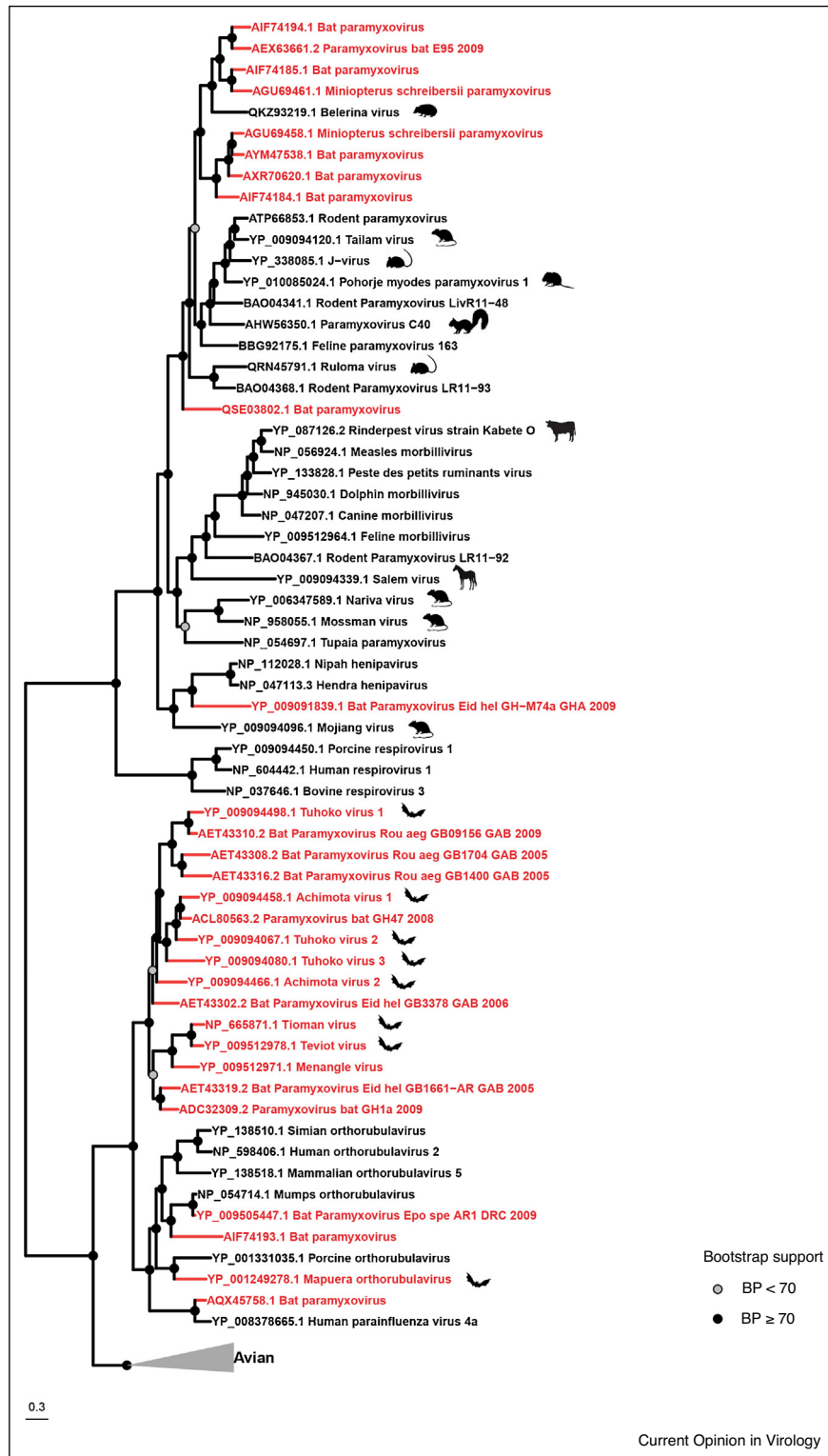
Interest in bat paramyxoviruses began with the discovery of Hendra, Nipah and Menangle viruses (see below). Since then, a large number of bat paramyxoviruses have been characterised, exhibiting considerable phylogenetic diversity (Figure 4). For example, 14 novel bat

Figure 3



Representative phylogenetic diversity of bat coronaviruses. An amino acid alignment of the RdRp of the *Coronaviridae* was used to infer a maximum likelihood phylogeny using IQ-TREE [81]. The phylogeny was estimated using viruses from the four coronavirus genera as marked by coloured lines to the right of the phylogeny. The tree was mid-point rooted and bootstrap values are represented by coloured circles. Bat viruses are shown in red font and fall into the *Alphacoronavirus* and *Betacoronavirus* genera. Animal silhouettes representing host species are displayed next to viruses that do not list a host species in the virus name.

Figure 4



Representative phylogenetic diversity of bat paramyxoviruses. An amino acid alignment of the L protein that contains the RdRp of the *Paramyxoviridae* was used to infer a maximum likelihood tree using IQ-TREE [81]. The tree was mid-point rooted and bootstrap values are represented by coloured circles. Bat viruses are shown in red font. Animal silhouettes representing animal host species are displayed next to viruses that do not provide a host species in the virus name. The avian paramyxovirus clade has been collapsed to enhance visualisation.

paramyxoviruses were discovered in nine species of bats in a single mNGS study from China [12**], while in 2020 a novel bat paramyxovirus – Achimota Pararubulavirus 3 – was identified in a urine sample from *Eidolon helvum* [39].

Hendra virus (genus *Henipavirus*, family *Paramyxoviridae*) was first reported to cause acute respiratory illness in horses and encephalitis in humans in Queensland, Australia in 1994, initially resulting in the death of 16 horses and two humans who had contact with sick horses [40–43]. After 2000 horses tested serologically negative to the virus it was proposed that it likely originated from a wildlife source [44,45], and the black flying fox (*Pteropus alecto*), grey headed flying fox (*Pteropus poliocephalus*), spectacled flying fox (*Pteropus conspicillatus*) and little red flying fox (*Pteropus scapulatus*) were later identified as carrying neutralising antibodies to the virus [45]. Hendra virus was later confirmed in the birthing fluid and foetal tissue from a grey headed flying fox and the foetal tissue from a black flying fox, as well as a high prevalence in bat urine [46,47]. Urine may therefore represent the likely mode of transmission to horses, with subsequent transmission through respiratory secretions to horses and humans [45–47].

Another member of the genus *Henipavirus* – Nipah virus – has similarly made the jump from bats to domestic animals. This virus was first reported in farmed pig populations in Malaysia in late 1998 before emerging in Singapore in early 1999 and again in India and Bangladesh in 2001, causing neurological and respiratory illness in pigs and encephalitis in humans [48,49]. During the 1998 Malaysia outbreak 238 humans contracted the virus, 105 of whom died [50]. Following the identification of Hendra virus in flying foxes (genus *Pteropus*), the large flying fox (*Pteropus vampyrus*), small flying fox (*Pteropus hypomelanus*), cave nectar bat (*Eonycteris spelaea*), lesser short-nosed fruit bat (*Cynopterus brachyotis*) and lesser Asiatic yellow bat (*Scotophilus kuhlii*) were identified as carrying neutralising antibodies to the virus [51]. The Indian flying fox (*Pteropus medius*, previously *Pteropus giganteus*) has similarly been linked to the Nipah virus outbreaks in India and Bangladesh [52,53].

The third zoonotic virus of the family *Paramyxoviridae* carried by bats is Menangle virus (genus *Pararubulavirus*). Menangle virus successfully established infection in a farmed pig population in 1997, presenting as reproductive complications [54]. Two humans working at separate piggeries recorded severe influenza-like symptoms during the 1997 piggery outbreak and were seropositive for Menangle virus [55]. Both workers confirmed they had been exposed to fluids from pigs housed at the outbreak farm [55]. Like Hendra, the grey headed flying fox, black flying fox and spectacled flying fox were later identified as carrying neutralising antibodies for Menangle virus and hence likely act as reservoir hosts [54,56], although no

Menangle virus outbreaks have been recorded in Australia since 1997.

Rhabdoviridae

The *Rhabdoviridae* are a diverse set of negative-sense RNA viruses comprising multiple genera, one of which, the genus *Lyssavirus*, can cause rabies in mammals (the ecology and evolution of which has been extensively reviewed elsewhere [57]). Bats are the likely reservoir hosts for most lyssaviruses, although classic rabies lyssavirus is mainly transmitted to humans through bites or scratches from carnivores like dogs and racoons. Lyssaviruses currently include 17 characterised species that have been detected in bat species in a range of geographic locations, including Australian bat lyssavirus [58], Irkut lyssavirus (Russia) [59], Bokelon bat lyssavirus (Germany) [60], European bat lyssavirus 1 and 2 [61], Aravan and Khujand virus (Asia) [62] and Gannoruwa bat lyssavirus (Sri Lanka) [63]. Unfortunately, a lack of sampling makes it difficult to determine whether these viruses are present in other mammalian species.

For most bat species interactions with humans and other animals are limited to occasional occurrences such as through animal carers and in backyards and households. Accordingly, there is only sporadic lyssavirus transmission from bats to humans, although outcomes are often fatal. In contrast, the common vampire bat (*Desmodus rotundus*), hairy-legged vampire bat (*Diphylla ecaudata*) and white-winged vampire bat (*Diaemus youngi*) from South and Central America have a unique blood-feeding diet that provides an opportunistic route of the transmission for classic rabies lyssavirus into livestock [64].

Why are bats good reservoir hosts?

Bats undoubtedly harbour a large and diverse array of viruses, some of which have jumped species boundaries to emerge in new hosts and occasionally cause disease outbreaks. The question that naturally arises is why bats are seemingly such important reservoir hosts for zoonotic viruses, particularly as some studies indicate that the number of viruses carried by bats is significantly greater than other mammalian orders [65*]. The social dynamics of bat populations, including very large roosting numbers and species co-habitation, provide the perfect setting for viral transmission, while the capacity of bats to travel to different or new geographical regions provides a mechanism for viruses to become established in naïve bat populations. It is also likely that the characteristic flight-adapted physiology of bats in part provides an explanation for their high virus burden [66**,67], while the unique anti-inflammatory and proinflammatory responses in bats, as well as distinctive immunological traits such as the reduced number of interferon genes (such as in the black flying fox) and that the interferon genes are continually expressed in the absence of an initiated immune response, may in part explain why bats

are often asymptomatic carriers for a myriad of viruses [66^{••},67,68,69^{••}]. More detailed studies of the innate and adaptive immune responses in a broader range of Yinpterochiroptera and Yangochiroptera should clearly be a research priority.

Despite the mounting evidence that bats harbour a particularly large and abundant virome, it is important to acknowledge that the increasing frequency with which bat viruses are described is also impacted by major ascertainment and confirmation biases. Indeed, following the discovery of the bat reservoir for SARS-CoV-1 there has been a marked increase in studies of bat viromes, with an emphasis on sampling from bat populations in China and other Asian countries [12^{••},19^{••},70,71[•],72], and of particular genera such as *Rhinolophus*, although the population density of these animals varies markedly in space [19^{••}]. Obviously, the more a particular group of animals is sampled then, on average, the more novel viruses will be characterised, and some studies have suggested that bats carry no more virus than expected given their species richness [73]. Perhaps more importantly, although virus species richness in bats is high, these viruses rarely establish successful human infection and most bat viruses classed as zoonotic are not directly transmitted from bats to humans. Rather, bat-to-human transmission routinely involves an intermediate animal host (such as pigs, camels and horses), and a number of the viral families that appear regularly in bat virome studies have not yet spilled over into human populations (Figure 2). Indeed, the zoonotic risk posed by bat viruses needs to be qualified by the observation that many of these viruses have been associated with bats for millennia with only a small number of spill-over events. For example, the common ancestor of the sarbecoviruses has been estimated to have existed ~21 000 years ago [74[•]], while the origin of the orthocoronaviruses has been dated to over 150 million years ago [75].

Challenges in bat viromics

The increasing use of mNGS has identified a multitude of novel and highly diverse RNA and DNA viruses in bats, greatly expanding our knowledge of the known virosphere and providing important information on the origins of specific viruses. The advantages of mNGS are manifold, including its unbiased and multiplex approach, easy application, high sensitivity and continually decreasing cost. However, the computational challenges of analysing the abundant sequence data produced by mNGS can be considerable, particularly in resource poor settings, and accurately identifying viruses that infect bats as opposed to components of their diet or microbiome can be challenging [76]. In particular, bats consume insects and are also susceptible to arthropod parasites, both of which may commonly carry viruses [77,78], and both insect viruses and bacteriophages are commonly detected in bat metagenomic data [5,8[•],16,17]. Hence, to fully

exploit the growing information obtained by mNGS studies of bat viromes new bioinformatics tools need to be developed that can rapidly and accurately identify those viruses most likely to infect bats. Similarly, determining which of the myriad of bat viruses are of likely human pandemic potential may not be possible through computational analyses alone. For example, although closely related to SARS-CoV-2, *Rhinolophus affinis* virus RaTG13 is unable to bind to the human ACE2 receptor [79].

Conclusions – an uncertain future for bats

Bats play a central role in maintaining a sustainable ecosystem, helping to pollinate, distribute seeds and control pests for thousands of plant species [80]. Since the identification of bats as reservoir hosts for many zoonotic viruses this group of animals has acquired an unjustified negative reputation, especially those species that roost in urban habitats, leading to an unsympathetic mindset among many communities. Climate change, urbanisation and industrial and agricultural advancements have greatly impacted bat populations globally [80], while encroachment onto bat habitats through urbanisation has increased the chance of viral spillover events into humans or companion and production animals. Currently, 106 bat species are listed as endangered or critically endangered on the IUCN (International Union for Conservation of Nature) red list, with 110 species threatened. Irrespective of any potential zoonotic risk, efforts to increase bat numbers should be prioritised. It is essential that we conserve this diverse group of animals, not only for the benefits to our ecosystem but also to enhance our understanding of viral biodiversity and evolution, as well as mammalian immunology.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported by an Australian Laureate Fellowship from the Australian Research Council (FL170100022).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Tsagkogeorga G, Parker J, Stupka E, Cotton JA, Rossiter SJ: **Phylogenomic analyses elucidate the evolutionary relationships of bats.** *Curr Biol* 2013, **23**:2262-2267
- First study to provide evidence for the new classification of bats into the suborders Yinpterochiroptera and Yangochiroptera, both of which contain echolocating species.
2. Puechmaille SJ, Soisook P, Yokubol M, Piyapan P, Ar Gouilh M, Mie Mie K, Khin Kyaw K, Mackie I, Bumrungsri S, Dejtardol A *et al.*: **Population size, distribution, threats and conservation status of two endangered bat species *Craseonycteris thonglongyai* and *Hipposideros turpis*.** *Endanger Species Res* 2009, **8**:15-23.

3. Goodman SM, Rakotondraparany F, Kofoky A: **The description of a new species of Myzopoda (Myzopodidae: Chiroptera) from western Madagascar.** *Mammal Biol* 2007, **72**:65-81.
4. Bennett AJ, Paskey AC, Ebinger A, Pfaff F, Priemer G, Höper D, Breithaupt A, Heuser E, Ulrich RG, Kuhn JH *et al.*: **Relatives of rubella virus in diverse mammals.** *Nature* 2020, **586**:424-428.
5. Kohl C, Brinkmann A, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, Wibbelt G, Kurth A: **The virome of German bats: comparing virus discovery approaches.** *Sci Rep* 2021, **11**:7430.
6. Bergner LM, Orton RJ, Broos A, Tello C, Becker DJ, Carrera JE, Patel AH, Biek R, Streicker DG: **Diversification of mammalian deltaviruses by host shifting.** *Proc Natl Acad Sci U S A* 2021, **118**: e2019907118
- Identification of hepatitis D-like viruses (i.e. deltaviruses) bats in the Americas, a virus group that were originally thought to be restricted to humans alone.
7. Bolatti EM, Zorec TM, Montani ME, Hošnjak L, Chouhy D, Viarengo G, Casal PE, Barquez RM, Poljak M, Giri AA: **A preliminary study of the virome of the South American free-tailed bats (*Tadarida brasiliensis*) and identification of two novel mammalian viruses.** *Viruses* 2020, **12**:422.
8. Li Y, Altan E, Reyes G, Halstead B, Deng X, Delwart E: **Virome of bat guano from nine northern California roosts.** *J Virol* 2021, **95**: e01713-e01720
- Unbiased metagenomic study that demonstrates the large virus diversity in guano sampled from five bat species.
9. Hardmeier I, Aeberhard N, Qi W, Schoenbaechler K, Kraetli H, Hatt JM, Fraefel C, Kubacki J: **Metagenomic analysis of fecal and tissue samples from 18 endemic bat species in Switzerland revealed a diverse virus composition including potentially zoonotic viruses.** *PLoS One* 2021, **16**:e0252534.
10. Yinda CK, Ghogomu SM, Conceição-Neto N, Beller L, Deboutte W, Vanhulle E, Maes P, Van Ranst M, Matthijssens J: **Cameroonian fruit bats harbor divergent viruses, including rotavirus H, bastroviruses, and picobirnaviruses using an alternative genetic code.** *Virus Evol* 2018, **4**:vey008.
11. Mendenhall IH, Wen DLH, Jayakumar J, Gunalan V, Wang L, Mauer-Stroh S, Su YCF, Smith GJD: **Diversity and evolution of viral pathogen community in cave nectar bats (*Eonycteris spelaea*).** *Viruses* 2019, **11**:250.
12. Wu Z, Yang L, Ren X, He G, Zhang J, Yang J, Qian Z, Dong J, Sun L, Zhu Y *et al.*: **Deciphering the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases.** *ISME J* 2016, **10**:609-620
- Large scale study revealing the huge virus diversity in bats through metagenomic next-generation sequencing.
13. Geldenhuys M, Mortlock M, Weyer J, Bezuidt O, Seamark ECJ, Kearney T, Gleasner C, Erkkila TH, Cui H, Markotter W: **A metagenomic viral discovery approach identifies potential zoonotic and novel mammalian viruses in *Neoromicia* bats within South Africa.** *PLoS One* 2018, **13**:e0194527.
14. Zana B, Kemenesi G, Buzás D, Csorba G, Görfői T, Khan FAA, Tahir N, Zeghib S, Madai M, Papp H *et al.*: **Molecular identification of a novel hantavirus in Malaysian bronze tube-nosed bats (*Murina aenea*).** *Viruses* 2019, **11**:887.
15. Zheng XY, Qiu M, Guan WJ, Li JM, Chen SW, Cheng MJ, Huo ST, Chen Z, Wu Y, Jiang LN *et al.*: **Viral metagenomics of six bat species in close contact with humans in southern China.** *Arch Virol* 2018, **163**:73-88.
16. Li L, Victoria JG, Wang C, Jones M, Fellers GM, Kunz TH, Delwart E: **Bat guano virome: predominance of dietary viruses from insects and plants plus novel mammalian viruses.** *J Virol* 2010, **84**:6955-6965.
17. Šimić I, Zorec TM, Lojkić I, Krešić N, Poljak M, Cliquet F, Picard-Meyer E, Wasniewski M, Zrnčić V, Cukušić A *et al.*: **Viral metagenomic profiling of Croatian bat population reveals sample and habitat dependent diversity.** *Viruses* 2020, **12**:891.
18. Porter AF, Cobbin J, Li C-X, Eden J-S, Holmes EC: **Metagenomic identification of viral sequences in laboratory reagents.** *Viruses* 2021, **13**:2122.
19. Zhou H, Ji J, Chen X, Bi Y, Li J, Wang Q, Hu T, Song H, Zhao R, Chen Y *et al.*: **Identification of novel bat coronaviruses sheds light on the evolutionary origins of SARS-CoV-2 and related viruses.** *Cell* 2021, **184**:4380-4391
- Highlights the importance of bats as carriers for betacoronaviruses that circulate within different species of *Rhinolophus* bats, including the identification of close relatives of SARS-CoV-2.
20. Zhou H, Chen X, Hu T, Li J, Song H, Liu Y, Wang P, Liu D, Yang J, Holmes EC *et al.*: **A novel bat coronavirus closely related to SARS-CoV-2 contains natural insertions at the S1/S2 cleavage site of the spike protein.** *Curr Biol* 2020, **30**:2196-2203.e3.
21. Temmam S, Vongphayloth K, Baquero Salazar E, Munier S, Bonomi M, Regnault B, Douangboubpha B, Karami Y, Chrétien D, Sanamxay D *et al.*: **Coronaviruses with a SARS-CoV-2-like receptor-binding domain allowing ACE2-mediated entry into human cells isolated from bats of Indochinese peninsula.** *Res Square* 2021 <http://dx.doi.org/10.21203/rs.3.rs-871965/v1>.
22. World Health Organisation: **Summary of Probable SARS Cases with Onset of Illness from 1 November 2002 to 31 July 2003.** Available at: <https://www.who.int/publications/m/item/summary-of-probable-sars-cases-with-onset-of-illness-from-1-november-2002-to-31-july-2003>.
23. Tu C, Cramer G, Kong X, Chen J, Sun Y, Yu M, Xiang H, Xia X, Liu S, Ren T *et al.*: **Antibodies to SARS coronavirus in civets.** *Emerg Infect Dis* 2004, **10**:2244-2248.
24. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Cramer G, Hu Z, Zhang H *et al.*: **Bats are natural reservoirs of SARS-like coronaviruses.** *Science* 2005, **310**:676-679
- Paper that initiated the interest in bats as major reservoirs of zoonotic viruses. Demonstrated that viruses closely related to SARS-CoV-1 were present in animals of the genus *Rhinolophus*.
25. Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, Wong BH, Wong SS, Leung SY, Chan KH, Yuen KY: **Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats.** *Proc Natl Acad Sci U S A* 2005, **102**:14040-14045.
26. Tang XC, Zhang JX, Zhang SY, Wang P, Fan XH, Li LF, Li G, Dong BQ, Liu W, Cheung CL *et al.*: **Prevalence and genetic diversity of coronaviruses in bats from China.** *J Virol* 2006, **80**:7481-7490.
27. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA: **Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia.** *N Engl J Med* 2012, **367**:1814-1820.
28. Sabir JS, Lam TT, Ahmed MM, Li L, Shen Y, Abo-Aba SE, Qureshi MI, Abu-Zeid M, Zhang Y, Khyami MA *et al.*: **Co-circulation of three camel coronavirus species and recombination of MERS-CoVs in Saudi Arabia.** *Science* 2016, **351**:81-84.
29. Anthony SJ, Gilardi K, Menachery VD, Goldstein T, Ssebide B, Mbabazi R, Navarrete-Macias I, Liang E, Wells H, Hicks A *et al.*: **Further evidence for bats as the evolutionary source of Middle East Respiratory Syndrome coronavirus.** *mBio* 2017, **8**:e00373-17.
30. Annan A, Baldwin HJ, Corman VM, Klose SM, Owusu M, Nkrumah EE, Badu EK, Anti P, Agbenyega O, Meyer B *et al.*: **Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe.** *Emerg Infect Dis* 2013, **19**:456-459.
31. Ithete NL, Stoffberg S, Corman VM, Cottontail VM, Richards LR, Schoeman MC, Drosten C, Drexler JF, Preiser W: **Close relative of human Middle East Respiratory Syndrome coronavirus in bat, South Africa.** *Emerg Infect Dis* 2013, **19**:1697-1699.
32. Yang Y, Du L, Liu C, Wang L, Ma C, Tang J, Baric RS, Jiang S, Li F: **Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus.** *Proc Natl Acad Sci U S A* 2014, **111**:12516-12521
- A study that emphasises the importance of studying the receptor usage and function in bat coronaviruses when attempting to determine zoonotic potential.
33. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, Hu Y, Tao ZW, Tian JH, Pei YY *et al.*: **A new coronavirus associated with human respiratory disease in China.** *Nature* 2020, **579**:265-269
- One of the first reports that COVID-19 was caused by a novel coronavirus SARS-CoV-2. Provided the reference genome sequence of SARS-CoV-2.

34. Li LL, Wang JL, Ma XH, Sun XM, Li JS, Yang XF, Shi WF, Duan ZJ: **A novel SARS-CoV-2 related coronavirus with complex recombination isolated from bats in Yunnan province, China.** *Emerg Microbes Infect* 2021, **10**:1683-1690.
35. Delaune D, Hul V, Karlsson EA, Hassanin A, Tey PO, Baidaliuk A, Gámbaro F, Prot M, Tu VT, Chea S *et al.*: **A novel SARS-CoV-2 related coronavirus in bats from Cambodia.** *Nat Commun* 2021, **12**:6563
- Reports the presence of SARS-CoV-2 related viruses in bats in Asian countries aside from China. These viruses may be common across the home range of *Rhinolophus* bats.
36. Murakami S, Kitamura T, Suzuki J, Sato R, Aoi T, Fujii M, Matsugo H, Kamiki H, Ishida H, Takenaka-Uema A *et al.*: **Detection and characterization of bat sarbecovirus phylogenetically related to SARS-CoV-2, Japan.** *Emerg Infect Dis* 2020, **26**:3025-3029.
37. Boni MF, Lemey P, Jiang X, Lam TT, Perry BW, Castoe TA, Rambaut A, Robertson DL: **Evolutionary origins of the SARS-CoV-2 sarbecovirus lineage responsible for the COVID-19 pandemic.** *Nat Microbiol* 2020, **5**:1408-1417.
38. Zhou P, Fan H, Lan T, Yang XL, Shi WF, Zhang W, Zhu Y, Zhang YW, Xie QM, Mani S *et al.*: **Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin.** *Nature* 2018, **556**:255-258.
39. Baker KS, Tachedjian M, Barr J, Marsh GA, Todd S, Cramer G, Cramer S, Smith I, Holmes CEG, Suu-ire R *et al.*: **Achimota pararubulavirus 3: a new bat-derived paramyxovirus of the genus Pararubulavirus.** *Viruses* 2020, **12**:1236.
40. O'Sullivan JD, Allworth AM, Paterson DL, Snow TM, Boots R, Gleeson LJ, Gould AR, Hyatt AD, Bradfield J: **Fatal encephalitis due to novel paramyxovirus transmitted from horses.** *Lancet* 1997, **349**:93-95.
41. Rogers RJ, Douglas IC, Baldock FC, Glanville RJ, Seppanen KT, Gleeson LJ, Selleck PN, Dunn KJ: **Investigation of a second focus of equine morbillivirus infection in coastal Queensland.** *Aust Vet J* 1996, **74**:243-244.
42. Selvey LA, Wells RM, McCormack JG, Ansford AJ, Murray K, Rogers RJ, Lavercombe PS, Selleck P, Sheridan JW: **Infection of humans and horses by a newly described morbillivirus.** *Med J Aust* 1995, **162**:642-645.
43. Murray K, Rogers R, Selvey L, Selleck P, Hyatt A, Gould A, Gleeson L, Hooper P, Westbury H: **A novel morbillivirus pneumonia of horses and its transmission to humans.** *Emerg Infect Dis* 1995, **1**:31-33.
44. Ward MP, Black PF, Childs AJ, Baldock FC, Webster WR, Rodwell BJ, Brouwer SL: **Negative findings from serological studies of equine morbillivirus in the Queensland horse population.** *Aust Vet J* 1996, **74**:241-243.
45. Young PL, Halpin K, Selleck PW, Field H, Gravel JL, Kelly MA, Mackenzie JS: **Serologic evidence for the presence in Pteropus bats of a paramyxovirus related to equine morbillivirus.** *Emerg Infect Dis* 1996, **2**:239-240.
46. 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.
47. Field H, Jordan D, Edson D, Morris S, Melville D, Parry-Jones K, Broos A, Divljan A, McMichael L, Davis R *et al.*: **Spatiotemporal aspects of Hendra virus infection in pteropid bats (flying-foxes) in eastern Australia.** *PLoS One* 2015, **10**:e0144055.
48. Chua KB, Goh KJ, Wong KT, Kamarulzaman A, Tan PS, Ksiazek TG, Zaki SR, Paul G, Lam SK, Tan CT: **Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia.** *Lancet* 1999, **354**:1257-1259.
49. Paton NI, Leo YS, Zaki SR, Auchus AP, Lee KE, Ling AE, Chew SK, Ang B, Rollin PE, Umaphathi T *et al.*: **Outbreak of Nipah-virus infection among abattoir workers in Singapore.** *Lancet* 1999, **354**:1253-1256.
50. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Shieh W *et al.*: **Nipah virus: a recently emergent deadly paramyxovirus.** *Science* 2000, **288**:1432-1435.
51. Yob JM, Field H, Rashdi AM, Morrissy C, van der Heide B, Rota P, bin Adzhar A, White J, Daniels P, Jamaluddin A *et al.*: **Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia.** *Emerg Infect Dis* 2001, **7**:439-441.
52. Yadav PD, Raut CG, Shete AM, Mishra AC, Towner JS, Nichol ST, Mourya DT: **Detection of Nipah virus RNA in fruit bat (*Pteropus giganteus*) from India.** *Am J Trop Med Hyg* 2012, **87**:576-578.
53. Epstein JH, Prakash V, Smith CS, Daszak P, McLaughlin AB, Meehan G, Field HE, Cunningham AA: **Henipavirus infection in fruit bats (*Pteropus giganteus*), India.** *Emerg Infect Dis* 2008, **14**:1309-1311.
54. Philbey AW, Kirkland PD, Ross AD, Davis RJ, Gleeson AB, Love RJ, Daniels PW, Gould AR, Hyatt AD: **An apparently new virus (family Paramyxoviridae) infectious for pigs, humans, and fruit bats.** *Emerg Infect Dis* 1998, **4**:269-271.
55. Chant K, Chan R, Smith M, Dwyer DE, Kirkland P, The NSW Expert Group: **Probable human infection with a newly described virus in the family Paramyxoviridae.** *Emerg Infect Dis* 1998, **4**:273-275.
56. Philbey AW, Kirkland PD, Ross AD, Field HE, Srivastava M, Davis RJ, Love RJ: **Infection with Menangle virus in flying foxes (*Pteropus* spp.) in Australia.** *Aust Vet J* 2008, **86**:449-454.
57. Fisher CR, Streicker DG, Schnell MJ: **The spread and evolution of rabies virus: conquering new frontiers.** *Nat Rev Microbiol* 2018, **16**:241-255.
58. Gould AR, Hyatt AD, Lunt R, Kattenbelt JA, Hengstberger S, Blacksell SD: **Characterisation of a novel lyssavirus isolated from pteropid bats in Australia.** *Virus Res* 1998, **54**:165-187.
59. Botvinkin AD, Poleschuk EM, Kuzmin IV, Borisova TI, Gazaryan SV, Yager P, Rupprecht CE: **Novel lyssaviruses isolated from bats in Russia.** *Emerg Infect Dis* 2003, **9**:1623-1625.
60. Freuling CM, Beer M, Conraths FJ, Finke S, Hoffmann B, Keller B, Kliemt J, Mettenleiter TC, Mühlbach E, Teifke JP *et al.*: **Novel lyssavirus in Natterer's bat, Germany.** *Emerg Infect Dis* 2011, **17**:1519-1522.
61. McElhinney LM, Marston DA, Leech S, Freuling CM, van der Poel WH, Echevarria J, Vázquez-Moron S, Horton DL, Müller T, Fooks AR: **Molecular epidemiology of bat lyssaviruses in Europe.** *Zoonoses Public Health* 2013, **60**:35-45.
62. Kuzmin IV, Orciari LA, Arai YT, Smith JS, Hanlon CA, Kameoka Y, Rupprecht CE: **Bat lyssaviruses (Aravan and Khujand) from Central Asia: phylogenetic relationships according to N, P and G gene sequences.** *Virus Res* 2003, **97**:65-79.
63. Gunawardena PS, Marston DA, Ellis RJ, Wise EL, Karawita AC, Breed AC, McElhinney LM, Johnson N, Banyard AC, Fooks AR: **Lyssavirus in Indian flying foxes, Sri Lanka.** *Emerg Infect Dis* 2016, **22**:1456-1459.
64. Benavides JA, Rojas Paniagua E, Hampson K, Valderrama W, Streicker D: **Quantifying the burden of vampire bat rabies in Peruvian livestock.** *PLoS Negl Trop Dis* 2017, **11**:e0006105.
65. Olival KJ, Hosseini PR, Zambrana-Torrel C, Ross N, Bogich TL, Daszak P: **Host and viral traits predict zoonotic spillover from mammals.** *Nature* 2017, **546**:646-650
- Provides evidence that bats are 'special' with the respect to the number of potentially zoonotic viruses they carry and hence of particular risk in disease emergence.
66. Letko M, Seifert SN, Olival KJ, Plowright RK, Munster VJ: **Bat-borne virus diversity, spillover and emergence.** *Nat Rev Microbiol* 2020, **18**:461-471
- Key review that highlights the factors that underpin the role bats as major carriers of zoonotic viruses.
67. Irving AT, Ahn M, Goh G, Anderson DE, Wang LF: **Lessons from the host defences of bats, a unique viral reservoir.** *Nature* 2021, **589**:363-370.
68. Zhou P, Tachedjian M, Wynne JW, Boyd V, Cui J, Smith I, Cowled C, Ng JH, Mok L, Michalski WP *et al.*: **Contraction of the**

- type I IFN locus and unusual constitutive expression of IFN- α in bats.** *Proc Natl Acad Sci U S A* 2016, **113**:2696-2701.
69. Kacprzyk J, Hughes GM, Palsson-McDermott EM, Quinn SR, Puechmaile SJ, O'Neill LAJ, Teeling EC: **A potent anti-inflammatory response in bat macrophages may be linked to extended longevity and viral tolerance.** *Acta Chiropterol* 2017, **19**:219-228
- Provides evidence for a unique immune response in bats that potentially facilitate prolonged virus infection.
70. Ge X, Li Y, Yang X, Zhang H, Zhou P, Zhang Y, Shi Z: **Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China.** *J Virol* 2012, **86**:4620-4630.
71. Wu Z, Ren X, Yang L, Hu Y, Yang J, He G, Zhang J, Dong J, Sun L, Du J *et al.*: **Virome analysis for identification of novel mammalian viruses in bat species from Chinese provinces.** *J Virol* 2012, **86**:10999-11012
- Study that highlights the importance of surveying bats species from roosts in close proximity to humans by identifying mammalian associated viruses from high-risk virus families.
72. Han HJ, Wen HL, Zhao L, Liu JW, Luo LM, Zhou CM, Qin XR, Zhu YL, Liu MM, Qi R *et al.*: **Novel coronaviruses, astroviruses, adenoviruses and circoviruses in insectivorous bats from northern China.** *Zoonoses Public Health* 2017, **64**:636-646.
73. Mollentze N, Streicker DG: **Viral zoonotic risk is homogenous among taxonomic orders of mammalian and avian reservoir hosts.** *Proc Natl Acad Sci U S A* 2020, **117**:9423-9430.
74. Ghafari M, Simmonds P, Pybus OG, Katzourakis A: **A mechanistic evolutionary model explains the time-dependent pattern of substitution rates in viruses.** *Curr Biol* 2021, **31**:4689-4696.e5
- Employs a sophisticated model to estimate the rates and dates of virus evolution, suggesting that the sarbecoviruses (including SARS-CoV-2) originated approximately 21 000 years ago.
75. Hayman DTS, Knox MA: **Estimating the age of the subfamily Orthocoronavirinae using host divergence times as calibration ages at two internal nodes.** *Virology* 2021, **563**:20-27.
76. Cobbin JCA, Charon J, Harvey E, Holmes EC, Mahar JE: **Current challenges to metagenomic viral discovery.** *Curr Opin Virol* 2021, **51**:48-55.
77. Xu Z, Feng Y, Chen X, Shi M, Fu S, Yang W, Liu WJ, Gao GF, Liang G: **Virome of bat-infesting arthropods: highly divergent viruses in different vectors.** *J Virol* 2021:JV10146421.
78. Bennett AJ, Bushmaker T, Cameron K, Ondzie A, Niama FR, Parra HJ, Mombouli JV, Olson SH, Munster VJ, Goldberg TL: **Diverse RNA viruses of arthropod origin in the blood of fruit bats suggest a link between bat and arthropod viromes.** *Virology* 2019, **528**:64-72.
79. Wrobel AG, Benton DJ, Xu P, Roustan C, Martin SR, Rosenthal PB, Skehel JJ, Gambelin SJ: **SARS-CoV-2 and bat RaTG13 spike glycoprotein structures inform on virus evolution and furin-cleavage effects.** *Nat Struct Mol Biol* 2020, **27**:763-767.
80. Voigt CC, Kingston T: *Bats in the Anthropocene: Conservation of Bats in a Changing World.* Springer Open; 2016.
81. Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ: **IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies.** *Mol Biol Evol* 2015, **32**:268-274.