Ecology, evolution and spillover of coronaviruses from bats

Manuel Ruiz-Aravena[1,12], Clifton McKee[2,12], Amandine Gamble[3], Tamika Lunn[4], Aaron Morris[5], Celine E. Snedden[3], Claude Kwe Yinda[6], Julia R. Port[6], David W. Buchholz[7], Yao Yu Yeo[7], Christina Faust[8], Elinor Jax[5], Lauren Dee[5], Devin N. Jones[1], Maureen K. Kessler[1,11], Caylee Falvo[1], Daniel Crowley[1], Nita Bharti[8], Cara E. Brook[9], Hector C. Aguilar[7], Alison J. Peel[4], Olivier Restif[5], Tony Schountz[10], Colin R. Parrish[7], Emily S. Gurley[2], James O. Lloyd-Smith[3], Peter J. Hudson[8], Vincent J. Munster[6] and Raina K. Plowright[1]✉

Abstract | In the past two decades, three coronaviruses with ancestral origins in bats have emerged and caused widespread outbreaks in humans, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since the first SARS epidemic in 2002–2003, the appreciation of bats as key hosts of zoonotic coronaviruses has advanced rapidly. More than 4,000 coronavirus sequences from 14 bat families have been identified, yet the true diversity of bat coronaviruses is probably much greater. Given that bats are the likely evolutionary source for several human coronaviruses, including strains that cause mild upper respiratory tract disease, their role in historic and future pandemics requires ongoing investigation. We review and integrate information on bat–coronavirus interactions at the molecular, tissue, host and population levels. We identify critical gaps in knowledge of bat coronaviruses, which relate to spillover and pandemic risk, including the pathways to zoonotic spillover, the infection dynamics within bat reservoir hosts, the role of prior adaptation in intermediate hosts for zoonotic transmission and the viral genotypes or traits that predict zoonotic capacity and pandemic potential. Filling these knowledge gaps may help prevent the next pandemic.

Planetary health approaches
Ecological approaches to understanding the impact of anthropogenic disruption of natural systems on human health.

✉e-mail: raina.plowright@montana.edu

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Bats are the reservoir hosts of three of the ten virus groups of pandemic concern, as designated by the World Health Organization: henipaviruses (Nipah virus and Hendra virus), filoviruses (Ebola virus and Marburg virus) and coronaviruses[1]. Common features among these emerging viruses include the ability to cause severe disease in humans but not in the reservoir hosts, rare spillovers despite a wide geographical distribution and the potential role of bridging hosts that increase opportunities for human infections. The recent spillovers of bat coronaviruses to humans are consistent with an increasing number of emergent zoonoses from wildlife[2,3]. Wildlife farming and trade facilitate cross-species transmission of viruses by mixing species in stressful and crowded conditions[4–6], while other behaviours, including hunting and guano mining, facilitate contact with bat-borne pathogens. Those are part of larger patterns of encroachment into wildlife habitats and increasing pressure from human population expansion and intensifying natural resource use. The COVID-19 pandemic has highlighted the need for integrated planetary health approaches to understanding spillover as a multilayered process. Here, we focus on bat coronaviruses and the ecological, evolutionary and epidemiological features that influence the risk of spillover into humans and subsequent epidemic emergence.

Bats are the second most diverse order of mammals, with more than 1,400 species, and they host an exceptional diversity of coronaviruses with ancient viral lineages that are spread across all six continents that bats inhabit. More than 4,800 coronavirus sequences have been detected in bats, accounting for more than 30% of all bat viruses sequenced[7]. Given that 543 bat species — from a global diversity of 1,435 — have been sampled for coronaviruses (Supplementary Table 1), the true diversity of bat coronaviruses is likely much greater. Bats are hosts of ancestral lineages of betacoronaviruses from which viruses of public health concern evolved, including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2. These recent cases may just be the latest in a longer history

of spillover and emergence of bat coronaviruses into humans. For example, of the four endemic human coronaviruses that cause 30% of mild upper respiratory tract infections (common cold), two may have originated in bats (alphacoronaviruses human coronavirus 229E (HCoV-229E) and HCoV-NL63)[8]. Thus, the ancestry of at least five of the seven coronaviruses capable of human-to-human transmission can be traced back to bat coronaviruses[9,10]. The other two human coronaviruses (HCoV-OC43 and HCoV-HKU1) also may have spilled over from animals to humans, with pathways that may involve rodents and cattle[11]. Additionally, animal coronaviruses might have evolutionary origins in lineages from bats, such as the recently emerged coronavirus causing severe acute diarrhoea syndrome in pigs[12]. Serological evidence of exposure of humans to bat coronaviruses in rural China suggest that spillovers from bats might occur relatively frequently but are not detected[13,14].

Here, we review the ecology, evolution and spillover of bat coronaviruses and assess the current knowledge of the determinants of coronavirus spillover and transmission among recipient hosts — from the ecology of hosts and viruses to single virus–cell interactions. We further highlight the knowledge gaps that prevent us from preparing for and mitigating coronavirus emergence risk and suggest a research agenda for developing the science of preventing coronavirus spillover.

Distribution of bat coronaviruses

Coronaviruses (order *Nidovirales*, family *Coronaviridae*) include four genera: Alphacoronavirus and Betacoronavirus, which infect a broad range of mammals, and Gammacoronavirus and Deltacoronavirus, which primarily infect birds[15]. Since the emergence of SARS-CoV in 2002, and the evidence that it originated from a bat reservoir, coronaviruses have been detected in 16% of bat species (238) (Supplementary Table 1). Alphacoronaviruses and betacoronaviruses have been detected in bats from 14 of the 21 bat families, in at least 69 countries across six continents (FIGS 1,2; TABLE 1; Supplementary Table 1). The diversity of coronaviruses found in bats is high, with more than 60 coronavirus species (more than 4,000 individual sequences) detected from 13 of the 19 known mammalian subgenera of Alphacoronavirus and Betacoronavirus (FIG. 3). The apparent absence of coronaviruses in particular bat taxa is most likely due to insufficient sampling rather than true absence[16].

Sequence similarity among viruses in different hosts has been used to infer viral origins. Viruses with high sequence similarity to the three recently emerged human coronaviruses — SARS-CoV, SARS-CoV-2 and MERS-CoV — have all been identified in bats (FIGS 2,3). Separate clades of coronaviruses from rhinolophid bats show up to 92% sequence identity to SARS-CoV[17] and up to 96% sequence identity to SARS-CoV-2 (REF.[10]) at the genome level. Additional SARS-related coronaviruses (SARSr-CoVs) have been detected in hipposiderid and molossid bats in Africa, Asia and Europe (Supplementary Table 1), and it is widely accepted that bats are the natural reservoir of SARSr-CoVs[18–20]. Similarly, coronaviruses from vespertilionid bats show up to 86.5% sequence identity to MERS-CoV at the genome level[16], and related coronaviruses circulate in bats within the families Nycteridae, Emballonuridae and Molossidae in Africa, Europe, North America and Asia (Supplementary Table 1). The absence of related sequences in other animals suggests that a progenitor of MERS-CoV spilled over from bats into dromedary camels (*Camelus dromedarius*)[21]. Viruses related to the endemic human coronaviruses HCoV-229E (Duvinacovirus) and HCoV-NL63 (Setracovirus) have been detected in Africa and South-East Asia in hipposiderid bats (sharing up to 91% sequence identity at the genome level with HCoV-229E) and rhinonycterid bats (sharing up to 78% sequence identity across the genome with HCoV-NL63) (FIGS 2,3; Supplementary data; Supplementary Table 1).

The wide distribution and high diversity of coronaviruses in bats is most likely the result of a long coevolutionary history. Some coronavirus groups seem to be exclusively associated with specific taxonomic groups of bats. For instance, the subgenus Nobecovirus has been detected mostly in Old World fruit bats (Pteropodidae). Further understanding of the biogeography of bats and their coronaviruses would reveal key geographical areas of risk as well as bat coronavirus dynamics.

Infection and response in bats. Frequently, reservoir hosts of zoonoses appear tolerant of the pathogenic effects of infection, whereas humans experience severe disease[22]. Whether bat species are universally tolerant of coronavirus infection remains unclear as few experimental coronavirus challenge studies involving bats have been performed, the putative natural reservoir bat species was often not used and it is unclear whether the infectious doses resembled those of natural exposures (TABLE 1; Supplementary Table 2). In bats experimentally infected with coronaviruses, some individuals have shown mild tissue damage, including rhinitis[23,24] and interstitial pneumonia[24], with virus or viral RNA detected in the respiratory tract and/or intestines; however, infected animals did not exhibit evident clinical signs of infection.

Little is known about the immune responses of bats to coronavirus infections, both adaptive and innate.

Author addresses

[1]Department of Microbiology and Cell Biology, Montana State University, Bozeman, MT, USA.
[2]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.
[3]Department of Ecology and Evolutionary Biology, University of California, Los Angeles, Los Angeles, CA, USA.
[4]Centre for Planetary Health and Food Security, Griffith University, Nathan, QLD, Australia.
[5]Department of Veterinary Medicine, University of Cambridge, Cambridge, UK.
[6]National Institute of Allergy and Infectious Diseases, Hamilton, MT, USA.
[7]Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA.
[8]Department of Biology, Center for Infectious Disease Dynamics, Pennsylvania State University, University Park, PA, USA.
[9]Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA.
[10]Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA.
[11]Department of Ecology, Montana State University, Bozeman, MT, USA.
[12]These authors contributed equally: Manuel Ruiz-Aravena, Clifton McKee.

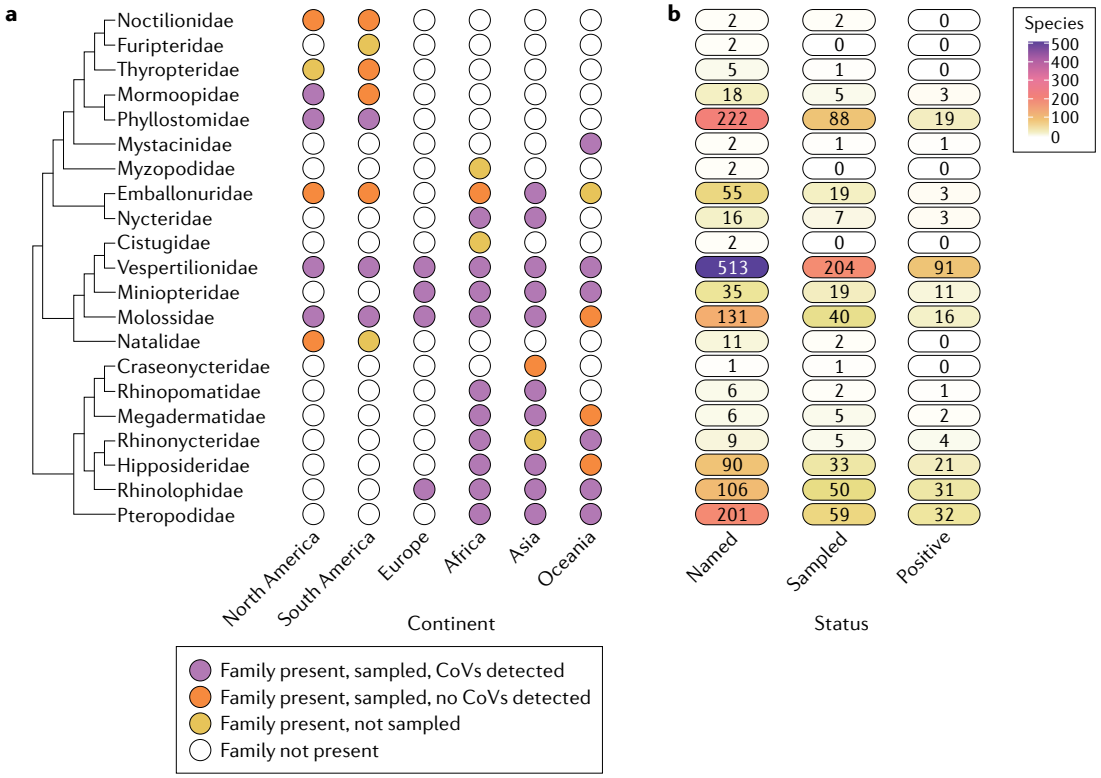


Fig. 1 | **Geographical and taxonomic distribution of reported bat hosts of coronaviruses. a** | Biogeographical patterns of bat families, sampling and coronavirus host status. **b** | Bat taxonomic diversity and coronavirus testing results. Data were compiled from field studies involving sequencing of coronaviruses in wild bats. A list of all reported bat coronavirus hosts based on the reviewed studies can be found in Supplementary Table 1 and Supplementary data. 'Named' refers to the number of taxonomically described bat species per family based on the expert-curated Bat Species of the World database. Bat Species of the World database. CoVs, coronaviruses.

While serological studies have been used for surveillance of pathogens such as Nipah virus, Marburg virus and Ebola virus in bats, little serological information is available for most coronaviruses in bats, although antibody responses may be relatively weak and transient. There are even fewer data on coronavirus-specific innate immune responses, or whether those might render a robust antibody response less important. For example, seroconversion of bats after challenge with coronaviruses is not always observed[24,25]. In experimental challenges of Egyptian fruit bats (*Rousettus aegyptiacus*) with bat SARS-like coronavirus WIV1 (originally isolated from a Chinese rufous horseshoe bat, *Rhinolophus sinicus*), evidence of viral replication was limited, no bats showed obvious signs of disease and only 2 of 12 individuals seroconverted (measured by enzyme-linked immunosorbent assay), although no neutralizing antibodies were detected[25]. When Jamaican fruit bats (*Artibeus jamaicensis*) were challenged with a human isolate of MERS-CoV, only one of ten bats produced neutralizing antibodies, and moderate pathological changes in the lungs were present and innate antiviral genes (*MX1*, *CCL5* and *ISG56*) were modestly upregulated[24]. It is unclear whether these apparently poor antibody responses result from weak infection of the bat species challenged — perhaps due to suppression of virus replication by the innate immune response — or naturally low viral capacity to infect the host species. In-depth seroprevalence studies are generally key to understanding the epidemiological history of the population[26], but the variability in adaptive humoral responses in bats suggests caution is required in the interpretation of serological data, especially at the individual level. For example, limited humoral responses may make it difficult to use serology to identify infections by certain pathogens.

In bats, coronaviruses may have tropism for the respiratory tract and the gastrointestinal tract. The highest loads of MERS-CoV RNA and infectious virus in experimentally infected Jamaican fruit bats were detected in the respiratory tract, with less virus in the intestines and internal organs[24]. Intranasal inoculation of Egyptian fruit bats with SARS-CoV-2 resulted in transient respiratory infections, with the highest viral loads in the respiratory tract on day 4 after inoculation, whereas oral and faecal viral shedding was observed for up to 12 days[23]. Long periods of viral shedding in faeces of 3–11 weeks have been reported in wild bats (*Myotis macropus*), supporting the importance of a potential faecal–oral route of transmission; in that field study, potentially persistent infections could not be distinguished from reinfections[27]. Viral RNA was also found in the intestines of Leschenault's rousette (*Rousettus leschenaultii*) bats orally inoculated with a betacoronavirus isolated from a lesser short-nosed fruit bat (*Cynopterus brachyotis*), but no infectious virus was isolated from recipient bats nor was disease observed, suggesting the species is

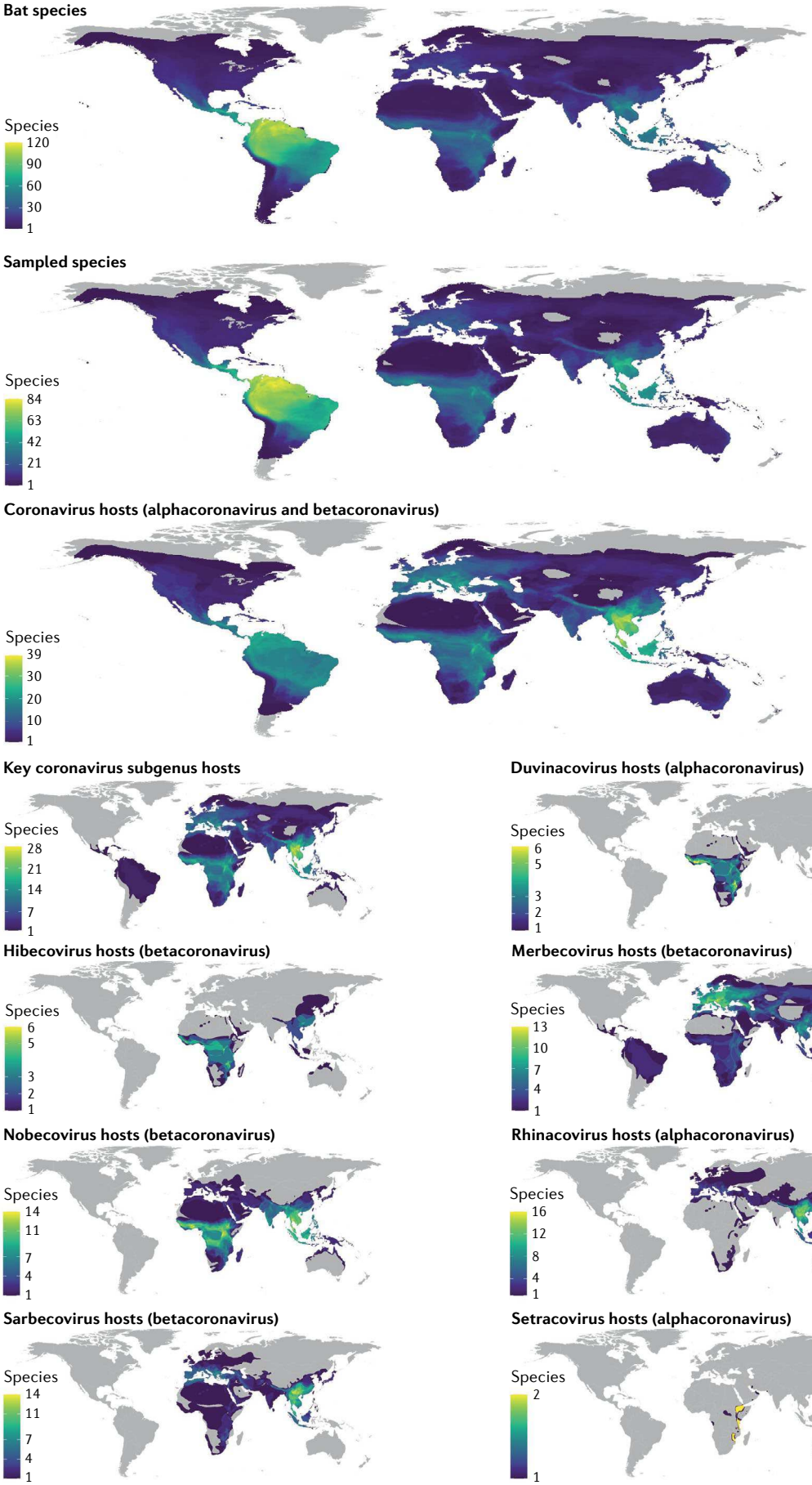

Bat species
Species
120
90
60
30
1
Sampled species
Species
84
63
42
21
1
Coronavirus hosts (alphacoronavirus and betacoronavirus)
Species
39
30
20
10
1
Key coronavirus subgenus hosts
Species
28
21
14
7
1
Duvinacovirus hosts (alphacoronavirus)
Species
6
5
3
2
1
Hibecovirus hosts (betacoronavirus)
Species
6
5
3
2
1
Merbecovirus hosts (betacoronavirus)
Species
13
10
7
4
1
Nobecovirus hosts (betacoronavirus)
Species
14
11
7
4
1
Rhinacovirus hosts (alphacoronavirus)
Species
16
12
8
4
1
Sarbecovirus hosts (betacoronavirus)
Species
14
11
7
4
1
Setracovirus hosts (alphacoronavirus)
Species
2
1

Fig. 2 | **Geographical distribution of reported bat hosts of coronaviruses.** Data on bat hosts were compiled from field studies involving sequencing of coronaviruses in wild bats. Where phylogenetic analysis was included in studies, key Alphacoronavirus and Betacoronavirus subgenera of bats associated with human or domestic animal infections or well characterized in bats (for example, Hibecovirus and Nobecovirus) are summarized (see Supplementary data). Geographical ranges of reported bat host species for any coronaviruses or key subgenera were obtained from the International Union for Conservation of Nature (IUCN). The plots display the number of bat species based on overlapping geographical ranges. The plots of bat species include 1,317 species with IUCN range data as of September 2021. Patterns in the left-hand maps indicate that sampling of bat species largely reflects the biogeographical patterns of bat diversity, with hotspots in Central America, South America, equatorial Africa and South-East Asia. However, hotspots of bat hosts of coronaviruses display important differences: lower than expected diversity of hosts in South America and higher diversity of hosts in South-East Asia. Although biological differences in bat coronavirus interactions with certain bat families (for example, Rhinolophidae) might explain some of these patterns, small sample sizes in some species in the Americas and more intensive sampling in China and South-East Asia likely contribute as well.

not a competent host for this virus[28]. Further evidence of tropism of coronaviruses for the gastrointestinal and respiratory systems of bats comes from field studies in which coronaviruses have been detected in intestines of little brown bats (*Myotis lucifugus*)[29]. Additionally, expression of cell receptors used by multiple coronaviruses was high in both the gastrointestinal system and the respiratory system in fruit bats, whereas it was present only in the intestines of insectivorous bats[30].

Many coronavirus infection studies have used bat cell lines (TABLE 1; Supplementary Table 2), and mostly focused on viral receptor binding, cell entry and infection, providing insights into the ability of specific coronaviruses to infect cells from different hosts. Although these studies may provide insights into the spillover potential of specific viruses, they likely provide limited insight into bat susceptibility at the organismal level — and studies making such inferences should be interpreted with caution. For example, a study that used big brown bat (*Eptesicus fuscus*) kidney cells showed that innate antiviral genes, specifically the interferon-β gene, were not repressed by MERS-CoV[31], and long-term persistent MERS-CoV infections were achieved in these big brown bat cells. However, whether those viruses cause persistent infections in bats cannot be predicted without infections of live animals.

Circulation in bat populations. The prevalence of coronaviruses — as estimated by the proportion of bats with detectable viral RNA in faeces or in faecal or oral swabs — shows high temporal and spatial variability (FIG. 4). Overall, shedding of coronaviruses tends to peak during summer or autumn in Australia and China[32–35], dry seasons in central Africa and Asia[16], and wet seasons in western or south-eastern Africa[36,37]. Although trends differ among studies, seasonal variations are consistently observed, pointing to potential mechanistic roles of resource availability, reproductive cycles and host behaviour.

Although nutritional stress during periods of resource scarcity has been implicated in the shedding of other bat viruses[38,39], their influence on coronavirus shedding is unclear, with effects differing by bat species and virus variants. In Thailand, increased prevalence of both alphacoronaviruses and betacoronaviruses was associated with low body condition in Lyle's flying foxes (*Pteropus lylei*)[40]. In the Chinese rufous horseshoe bat (*Rhinolophus sinicus*), low body condition was associated with increased shedding of one variant of Sarbecovirus (SARS-related *Rhinolophus* bat coronavirus (SARSr-Rh-BatCoV)), but not of another *Rhinolophus* coronavirus (Rh-BatCoV HKU2)[41]. In Ghana, infection by the alphacoronavirus Alpha229E-CoV correlated with low body condition in Noack's roundleaf bat (*Hipposideros* cf. *ruber*) but not in the Aba roundleaf bat (*Hipposideros abae*).

Colony size, density and composition could also affect virus prevalence by changing transmission rates both within and between roosts. Roost composition affects viral circulation as multiple bat species often roost together and viral infection of different bat hosts will depend on combinations of the host species and the viral strains involved. For example, mixed-species roosts in Yunnan province, China, exhibited greater prevalence of SARSr-CoVs when *Rhinolophus sinicus*, a primary host of SARSr-CoVs, was more abundant in the roost than other species. In the same roost, the lowest prevalence was detected when *Aselliscus stoliczkanus* was the most abundant bat species[34]. Roost size and location, including whether the roosts are in caves, seem to affect the chance of spillover of viruses between host species — likely due to close physical contact in dense roosts[42]. In addition to heterogeneity in competence among host species, heterogeneity in shedding and infectivity (for example, superspreading and aerosolization capacity) is a feature of coronavirus infections in humans[43]. However, the extent to which this individual-level heterogeneity explains coronavirus transmission in bats, variation in prevalence among roosts and the risk of spillover is unknown.

Reproductive cycles also influence prevalence and transmission of viruses in bat colonies by affecting patterns of behaviour and physiological susceptibility. Increased social contacts among different species of Chinese horseshoe bats during the mating season and when feeding after hibernation might explain peaks of SARSr-Rh-BatCoV and Rh-BatCoV HKU2 infection in spring[41]. In species that form maternal roosts, for example, increases in group size coincide with pregnancy and gestation, during which time inflammatory immune responses are downregulated, potentially facilitating infection and shedding[44,45]. Periparturient stress may also affect viral shedding, as observed in greater horseshoe bats (*Rhinolophus ferrumequinum*), Geoffroy's bats (*Myotis emarginatus*)[46] and mouse-eared bat (*Myotis myotis*)[47], in which both the proportion of bats shedding virus and viral concentrations increased after parturition. Similarly, in relation to reproductive cycles, high prevalence and concentration of coronaviruses detected in Chinese horseshoe bats (predominantly *Rhinolophus sinicus*) during September and October, are attributed to increases in the number of susceptible juveniles[32,34]. Cross-sectional surveys of multiple bat species report higher infection rates or viral shedding in juveniles and subadults, supporting age-related differences in susceptibility and competence of infection, consistently across species[16,40,48,49]. Further field studies of multiple species across East Africa found that in both age categories,

Body condition
Proxy for nutritional status of an organism. Commonly measured as the body mass above or below that predicted as a function of skeletal size.

Superspreading
Transmission event in which one infected host generates several new infections above the average in the population.

Aerosolization
Physical process by which a pathogen stabilizes in particles small enough to be transported through air currents.

shedding was highest during weaning[49] — timing that relates to behavioural changes, physiological stress and potential waning of maternal immunity.

Although some associations have been seen between seasonal factors and circulation of coronaviruses in bats, our understanding of the mechanisms is currently insufficient to predict dynamics of shedding (FIG. 4). Many of the associations with seasonal factors may be coincidental rather than causal, explaining the lack of consistent patterns across taxa and geographies. Small sample sizes and limited temporal resolution are common issues that hamper statistical power. We could vastly improve our understanding of coronavirus dynamics across species through coordinated and systematic approaches to field studies that sample individual bats, paired with experimental inoculation and transmission studies, and then integrated with modelling studies aimed at assessing the importance of factors driving infection[50].

Table 1 | **Summary of 214 original studies on coronaviruses in bats**

Study type[a]	Number of studies	Overview
Experimental	Bat cell lines: 29	Target cells: brain, embryo, intestine, kidney, lung Tested viruses: multiple bat SARS-related CoVs, BatCoV HKU4, BatCoV HKU9, HCoV-229E, HCoV-NL63, MERS-CoV, PEDV, Ro-BatCoV GCCDC1, SADS-CoV, SARS-CoV, SARS-CoV-2, *Scotophilus* BatCoV 512, TGEV
	Live bats: 6	Tested hosts and viruses: *Artibeus jamaicensis* (MERS-CoV), *Eptesicus fuscus* (SARS-CoV-2), *Myotis lucifugus* (Myl-CoV), *Rousettus leschenaultii* (BatCoV HKU9), *Rousettus aegyptiacus* (bat SARS-like CoV WIV1, SARS-CoV-2)
Longitudinal	14	Countries: Australia, China, Denmark, Germany, Malaysia, Singapore, South Korea, Thailand ($n = 8$) Serially sampled bat families: Pteropodidae, Hipposideridae, Vespertillionidae, Rhinolophidae ($n = 4$) Serially sampled species: *Eonycteris spelaea*, *Hipposideros cervinus*, *Myotis daubentonii*, *Myotis macropus*, *Myotis myotis*, *Pteropus lylei*, *Rhinolophus sinicus*, *Rousettus leschenaultii* ($n = 8$)
Surveys	Cross-sectional, intraspecies: 14 Cross-sectional, interspecies: 123 CoV detection and sequencing only: 29 Multipathogen detection: 36	Sampled countries: primarily in Asia, Africa and Europe; fewer in the Americas or Oceania ($n = 69$) Sampled bat families: all bat families have been sampled at least once except Cistugidae, Furipteridae and Myzopodidae ($n = 18$) Positive bat families: 14 Sampled bat species: 543 Positive bat species: 238

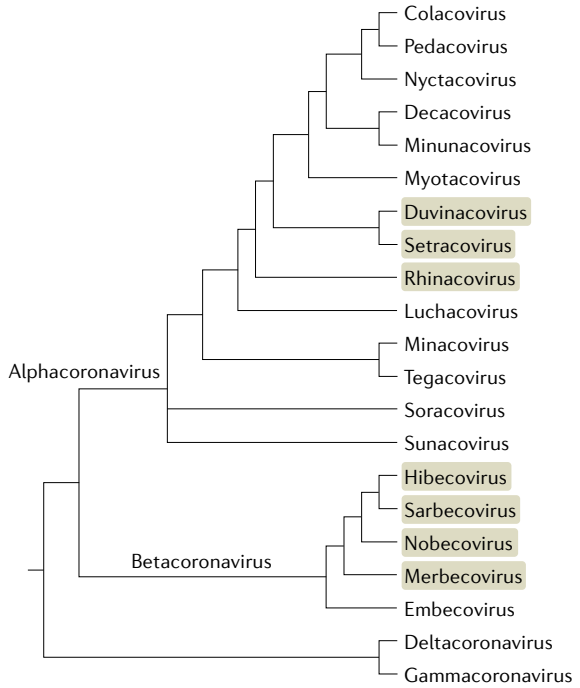
BatCoV, bat coronavirus; CoV, coronavirus; HCoV, human coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; Myl-CoV, *Myotis lucifugus* coronavirus; PEDV, porcine epidemic diarrhoea virus; Ro-BatCoV, *Rousettus* bat coronavirus; SADS-CoV, swine acute diarrhoea syndrome coronavirus; SARS, severe acute respiratory syndrome; SARS-CoV, severe acute respiratory syndrome coronavirus; TGEV transmissible gastroenteritis virus. [a]Study types were not exclusive, so a study may fit into multiple types depending on the sampling approach and analytical methods. More details are provided in Supplementary Table 2, and all classified studies can be found in Supplementary data.

Co-infections in bats. Co-infections with multiple pathogens can influence transmission to conspecifics and to spillover hosts. Cross-protective immunity from infection by related pathogens might reduce susceptibility or transmission, whereas trade-offs in immune response to one pathogen might increase susceptibility and facilitate transmission of another[39,51]. Co-infection of bats with multiple coronaviruses at the same time, or co-circulation of multiple virus genotypes within a roost, might result in interactions that affect the timing, location and intensity of virus shedding, as has been described in other viral families[39]. As with other putative drivers, the incidence and effects of coronavirus co-infections on transmission dynamics in bats are not well understood. Co-infections by two coronavirus species[36,41,52–57] and by coronaviruses and viruses from other families, including adenoviruses[58,59], astroviruses[58,60–62], herpesviruses[58] and paramyxoviruses[63], have been described and are likely common. Cases of co-infections (by detection of viral RNA) involving coronaviruses range from 0.2% to 34.2% in wild bats[36,52–56] and are as high as 73% in captive bats[64], while up to 88% of virus-positive samples contained multiple viral families[60]. Frequent co-infection has additional important consequences because coronaviruses recombine frequently, providing an opportunity for the emergence of new variants with altered properties, including host ranges.

A few studies have examined ecological interactions between co-infections of coronaviruses and non-viral pathogens, including whether they are competitive, synergistic or neutral. For instance, a 60-fold increase in coronavirus (*Myotis lucifugus* coronavirus) RNA was observed in the intestines of bats (*Myotis lucifugus*) co-infected with the fungus that causes white nose syndrome (*Pseudogymnoascus destructans*)[65]. Systemic downregulation of antiviral immune responses due to *Pseudogymnoascus destructans* infection was suggested as the cause of increased coronavirus replication. Similarly, ectoparasite loads have been associated with coronavirus infection; infection with Alpha229E-CoV almost doubled the risk of infection by BetaBI-CoV in Noack's roundleaf bat but also correlated positively with loads of streblid flies, mites and nycteribiid flies[36]. Longitudinal studies tracking the health and immune status of individual bats, including co-infections, are crucial to understanding the dynamics of bat viruses.

Molecular evolution and host range

Viral genetic diversity and evolution. Coronaviruses have the largest genome among the RNA viruses, and are subject to both mutation and recombination[66]. These processes generate genetic diversity, some of which may introduce new properties, including altered host ranges, along with increases in the ability to spread in the new host. Approximately two-thirds of the coronavirus genome encodes an RNA-dependent RNA polymerase



Genus/subgenus	Notable virus species	Hosts species
Alphacoronavirus		
Colacovirus	Myl-CoV	Bats (Vespertilionidae)
Pedacovirus	PEDV	Bats (Vespertilionidae), pigs
Nyctacovirus		Bats (Vespertilionidae)
Decacovirus		Bats (Hipposideridae, Rhinolophidae)
Minunacovirus		Bats (Miniopteridae)
Myotacovirus		Bats (Vespertilionidae)
Duvinacovirus	HCoV-229E	Bats (Hipposideridae), dromedary camels, alpacas, humans
Setracovirus	HCoV-NL63	Bats (Rhinonycteridae), humans
Rhinacovirus	SADS-CoV	Bats (Rhinolophidae), pigs
Luchacovirus		Rodents (Muridae, Cricetidae)
Minacovirus		Ferrets, minks
Tegacovirus	CCoV, FCoV, TGEV	Cats, dogs, pigs
Soracovirus		Shrews (*Suncus murinus*)
Sunacovirus		Shrews (*Sorex araneus*)
Betacoronavirus		
Hibecovirus		Bats (Hipposideridae)
Sarbecovirus	SARS-CoV, SARS-CoV-2	Bats (Rhinolophidae), Malayan pangolins, carnivores (Canidae, Felidae, Mustelidae, Viverridae), humans
Nobecovirus		Bats (Pteropodidae)
Merbecovirus	MERS-CoV	Bats (Vespertilionidae), dromedary camels, humans
Embecovirus	BCoV, CRCoV, HCoV-OC43, HCoV-HKU1, MCoV	Rodents (Muridae, Cricetidae), dogs, rabbits, cattle, horses, pigs, sable antelopes, dromedary camels, giraffes, humans
Deltacoronavirus	PorCoV-HKU15	Birds, pigs
Gammacoronavirus	IBV	Birds, cetaceans

Fig. 3 | **Coronavirus taxonomy and host distribution.** The proposed phylogeny has been compiled from analyses of full genomes and/or gene segments. Branch lengths do not reflect evolutionary distance between taxa and are drawn only to clearly illustrate relationships between and within genera. The distribution of bat species hosting highlighted subgenera is given in FIG. 2. The associated table summarizes a selection of important pathogenic virus species within genera and the host species or taxa with reported infections of a virus within a genus. BCoV, bovine coronavirus; CCoV, canine coronavirus; CRCoV, canine respiratory coronavirus; FCoV, feline coronavirus; HCoV, human coronavirus; IBV, infectious bronchitis virus (avian coronavirus); MCoV, murine coronavirus; MERS, Middle East respiratory syndrome; Myl-CoV, *Myotis lucifugus* coronavirus; PEDV, porcine epidemic diarrhoea virus: PorCoV, porcine coronavirus; SADS-CoV, swine acute diarrhoea syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus; TGEV, transmissible gastroenteritis virus.

and other non-structural proteins required for replication, while the remaining third encodes four structural proteins — the spike, envelope, membrane and nucleocapsid proteins — as well as accessory proteins[67]. The genomes of coronaviruses replicate via the RNA-dependent RNA polymerase, which is generally error-prone, resulting in mutations during replication[68,69]. However, the three largest viral families in the order *Nidovirales* — *Coronaviridae*, *Roniviridae* and *Mesoniviridae* — all encode a 3′–5′ exoribonuclease that improves their RNA replication fidelity, which may be necessary for maintaining sufficient fitness in the large genome[70–73]. The activity of the exoribonuclease might differ in different hosts, modulating the level of sequence variation. Replication in different host species may therefore present heterogeneities in their sequence variation, which may influence the emergence of new variants[16,20,74–76].

Recombination of large coronavirus genomes is common; recombination creates additional genetic diversity, expands viral evolution and increases the potential for shifts in cell tropism, host range[66] and pathogenicity[77]. During coronavirus replication in the host cell, subgenomic RNAs are generated, which result from the polymerase jumping to new positions in the template genome. This may facilitate recombination of genes from different coronavirus lineages during co-infection of a host cell when the RNA-dependent RNA polymerase 'jumps' from one RNA template molecule to another one that may come from a different viral genome[66,78]. These recombination processes have been implicated in the cross-species emergence of numerous novel coronaviruses, including murine coronavirus[79], transmissible gastroenteritis virus[80], feline and canine coronaviruses[81,82], and six of the seven human coronaviruses, HCoV-OC43 (REF.[83]), HCoV-NL63 (REFS[8,84]), HCoV-229E[8], HCoV-HKU1 (REF.[85]), SARS-CoV[86,87] and MERS-CoV[88]. Interestingly, evidence supports recombination of coronavirus genomes possibly happening also with RNA viruses from the family *Reoviridae*[89]. However, how frequent interfamily recombination events may happen and their consequences for evolution of zoonotic potential are unknown.

Mutation, recombination and host competence for infection and co-infection will have generated the current

Subgenomic RNAs
Fragments of RNA smaller than the full genome size generated during replication of coronaviruses in a host cell.

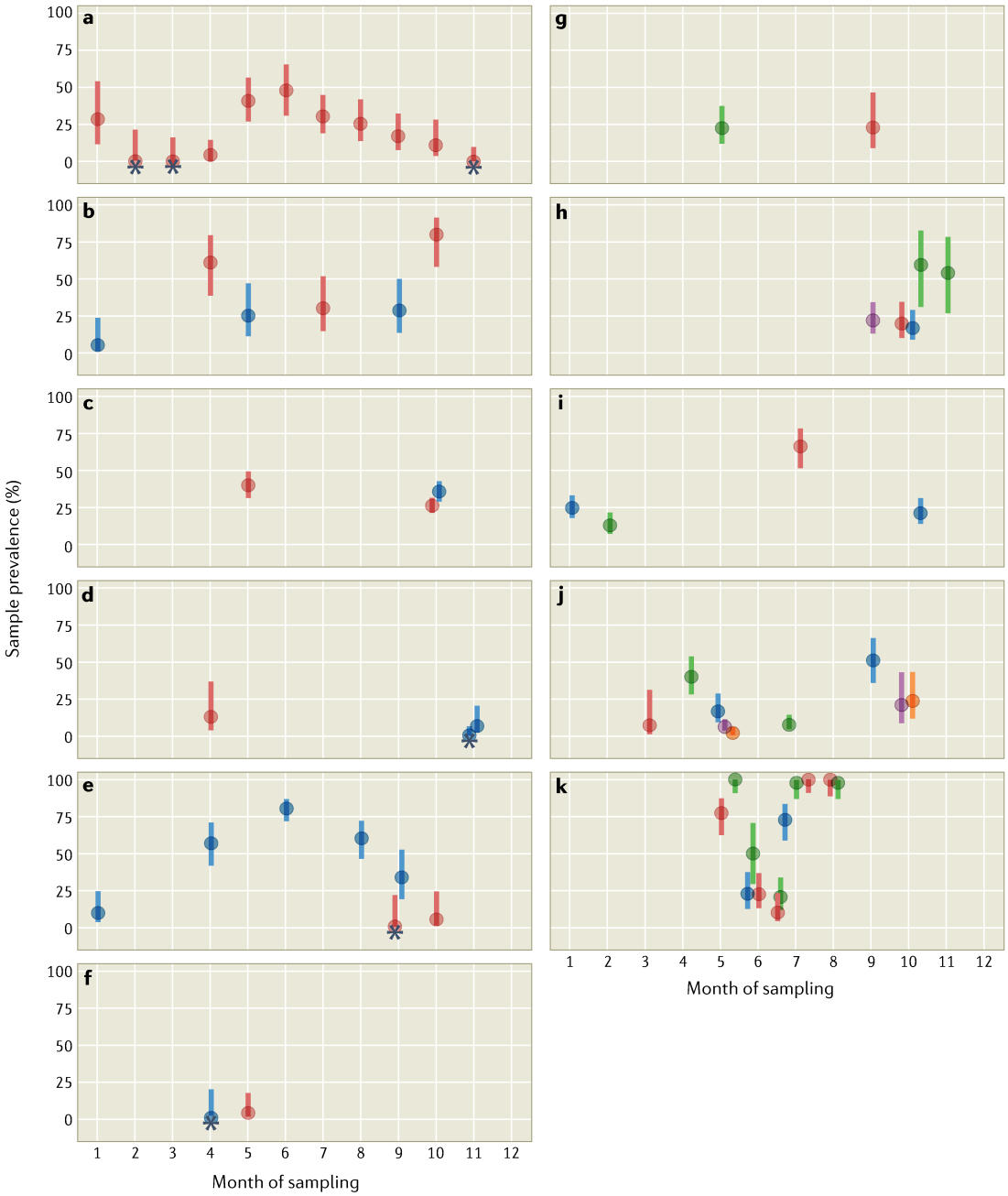


Fig. 4 | **Prevalence of detection of bat coronaviruses in field samples.** Data were obtained from published studies that included two or more sampling events with at least ten samples collected and that reported the virological status of samples (positive and negative). While the data show that prevalence varies in space and time and by bat species (each plot), few studies provide insights into the drivers of prevalence. No field studies have yet combined longitudinal sampling of individuals with collection of extensive metadata on bat ecology, bat health and environmental conditions. Sampling designs differed across studies. Most studies conducted cross-sectional sampling over multiple years. One field study sampled individual bats at multiple sites over time, although data were pooled across three sites[40] (panel **a**). Other studies sampled the same bat species over time across multiple sites or sampled multiple species and individuals in pooled samples across time within a site. These sampling approaches reflect the purpose of the studies — most were focused on characterizing viral diversity, not infection dynamics. Details are presented in Supplementary information. Each plot represents the prevalence of detections per bat species: *Pteropus lylei* (panel **a**)[40]; *Eonycteris spelaea* (panel **b**)[64]; *Rousettus leschenaultii* (panel **c**)[158]; *Chaerephon pumilus* (panel **d**)[49]; *Eidolon helvum* (panel **e**)[49]; *Myonycteris angolensis* (panel **f**)[49]; *Rhinolophus* cf. *clivosus* (panel **g**)[49]; *Myotis daubentonii* (panel **h**)[159]; *Rhinolophus sinicus* (panel **i**)[160]; *Rhinolophus sinicus*, *Rhinolophus ferrumequinum*, *Rhinolophus affinis* and *Aselliscus stoliczkanus* (panel **j**)[34]; and *Myotis myotis* (panel **k**)[47]. Colours in the plots represent different years within the study: year 1, red; year 2, blue; year 3, green; year 4, purple; and year 5, orange. Black asterisks show sampling events in which no coronavirus was detected. Circles show the mean prevalence, and bars show the 95% confidence intervals estimated by the Wilson method.

diversity of coronaviruses, including that seen in bats[16,90]. Some families of bats appear to have coevolved over millions of years with particular subgroups of betacoronaviruses: rhinolophid bats and SARS-related sarbecoviruses, vespertilionid bats and MERS-related merbecoviruses, and pteropodid bats and nobecoviruses (which have not been implicated in zoonosis)[90]. Host switching, resulting from successful broad jumps in host range, appear most common in the rhinopholid–Sarbecovirus clade[16,20,91]. Altogether, the variation in the bat coronaviruses may enable them to gain new host and tissue tropisms, and varying transmissibility and infection severity in new hosts. Indeed, once a virus is established in a new host population, evolution is expected to enable selection for lineages with increased fitness in those hosts, including exhibiting higher transmissibility, as observed for SARS-CoV-2 in humans[92].

Host receptor recognition. The capacity of coronaviruses to enter a host cell is mediated by the spike protein, which supports both binding to the host cell — through its receptor-binding domain (RBD) — and fusion with its membrane[67]. The RBD attaches to host-cell receptors, which are membrane proteins or sialic acids. For example, HCoV-NL63, SARS-CoV and SARS-CoV-2 bind angiotensin-converting enzyme 2 (ACE2), MERS-CoV binds dipeptidyl peptidase 4 (DPP4) and HCoV-229E, canine coronavirus and several porcine and feline coronaviruses bind alanine aminopeptidase (APN), whereas HCoV-OC43, HCoV-HKU1 and bovine coronavirus bind *N*-acetyl-9-*O*-acetylneuraminic acid[93–96].

The interaction between the RBD of the coronavirus spike protein and the host receptor can be thought of as a match between a key and a lock, and the specific structures of both the virus RBD and the receptors available on a potential host cell determine, in part, the capacity for infection of different hosts. The functional interactions between the viral protein and the host receptor differ, and the wide host range of several coronaviruses can be explained by the conservation of cell receptor structures across animal species, such is the case of ACE2, DPP4 and APN[97,98]. However, small differences in receptor structures can also alter receptor affinity and virus infection efficiency, including both variation in glycosylation profile or amino acid changes[93]. MERS-CoV spike protein, for instance, binds DPP4 of various species of primates, hooved mammals and bats, but not of ferrets and rodents owing to differences in five amino acids in the receptor[97]. Thus, direct coronavirus spillover from bats to other mammals would therefore be regulated by the host-cell receptor structures and viral RBD identity. This is a critical aspect for characterization of zoonotic potential of extant bat coronaviruses; however, for reservoir bat hosts we know relatively little about their receptors or interactions with the viruses. It is currently known from experimental and modelling work that several bat coronaviruses bind to human ACE2 or DPP4; however, structural modelling and biochemical data indicate differences in binding affinity[97–99] and therefore potential for successful infection of human cells. In some cases, there is only one amino acid residue different between the spike protein RBD and the receptor, suggesting that zoonotic capacity could emerge in a few evolutionary steps.

Isolates of bat coronaviruses are difficult to obtain, and therefore their zoonotic capacity is largely unknown, with many inferences being based on genomic sequences. Among 187 studies that examined coronaviruses in primary samples from wild bats, in less than a quarter, researchers attempted to recover live viruses in one or more cell cultures, yielding only five viral species successfully cultured, including one merbecovirus (*Tylonycteris* BatCoV HKU4), three sarbecoviruses related to SARS-CoV (WIV1, WIV16 and Rs4874) and one sarbecovirus related to SARS-CoV-2 (BANAL-236), reported in September 2021 (Supplementary data). High-throughput analyses of sequences and carefully controlled cell culture experiments and other experiments are needed to assess spillover and zoonotic potential of the coronavirus variants currently circulating in bats[1]. In silico analysis of cell receptors of humans and other species are useful for initial identification of species that could serve as bridge or reservoir hosts of zoonotic coronaviruses, which could promote optimization of resources for pre-emptive surveillance. For instance, relatively conserved SARS-CoV-2-binding residues in the ACE2 sequences of non-human primates, hooved mammals, felids and cetaceans suggest those species would be susceptible to infection[100]. Several of these predictions have been validated by empirical studies confirming the broad host range of SARS-CoV-2 (REFS[98,101]). However, these studies also classified horseshoe bats, pangolins, minks and ferrets as less likely to be hosts of SARS-CoV-2, yet field and laboratory data have revealed their susceptibility to SARS-CoV-2 or related viruses, highlighting the need for empirical validation of model predictions[101,102].

It is likely that differences will be seen between in silico analysis and empirical analysis of receptor use by virus species in different hosts. Several studies suggest that the progenitor viruses of SARS-CoV and SARS-CoV-2 may not use the ACE2 receptor in their original bat hosts[100,103,104]. However, this discrepancy could also result from variability in the host receptors with which the viruses have evolved, favouring specific interactions between the RBD and small numbers of receptor residues, so that progenitor viruses are adapted to their specific reservoir ACE2, but not to the human ACE2 (REF.[99]), which is used to model many interactions[100]. There is naturally high variation among the ACE2 receptors of bat species[105], in addition to the high diversity of SARSr-CoVs[106]. New host infection and adaptation likely involves mutations in the viral spike protein, and potentially selection in an intermediate (bridge) host, to enable effective binding and use of human ACE2 and facilitate zoonotic spillover[104,107]. Such a case is supported by the use of human DPP4 by MERS-CoV, where affinity for the human receptor may have emerged by evolution of the virus in dromedary camels, after the initial spillover from bats[76]. Importantly, virus evolution that facilitates binding of human receptors may diminish the binding affinity of a virus for the receptors of the original reservoir hosts[108], indicating a host shift that may favour sustained human-to-human transmission. Such behaviour is characteristic of pandemic viruses (BOX 1).

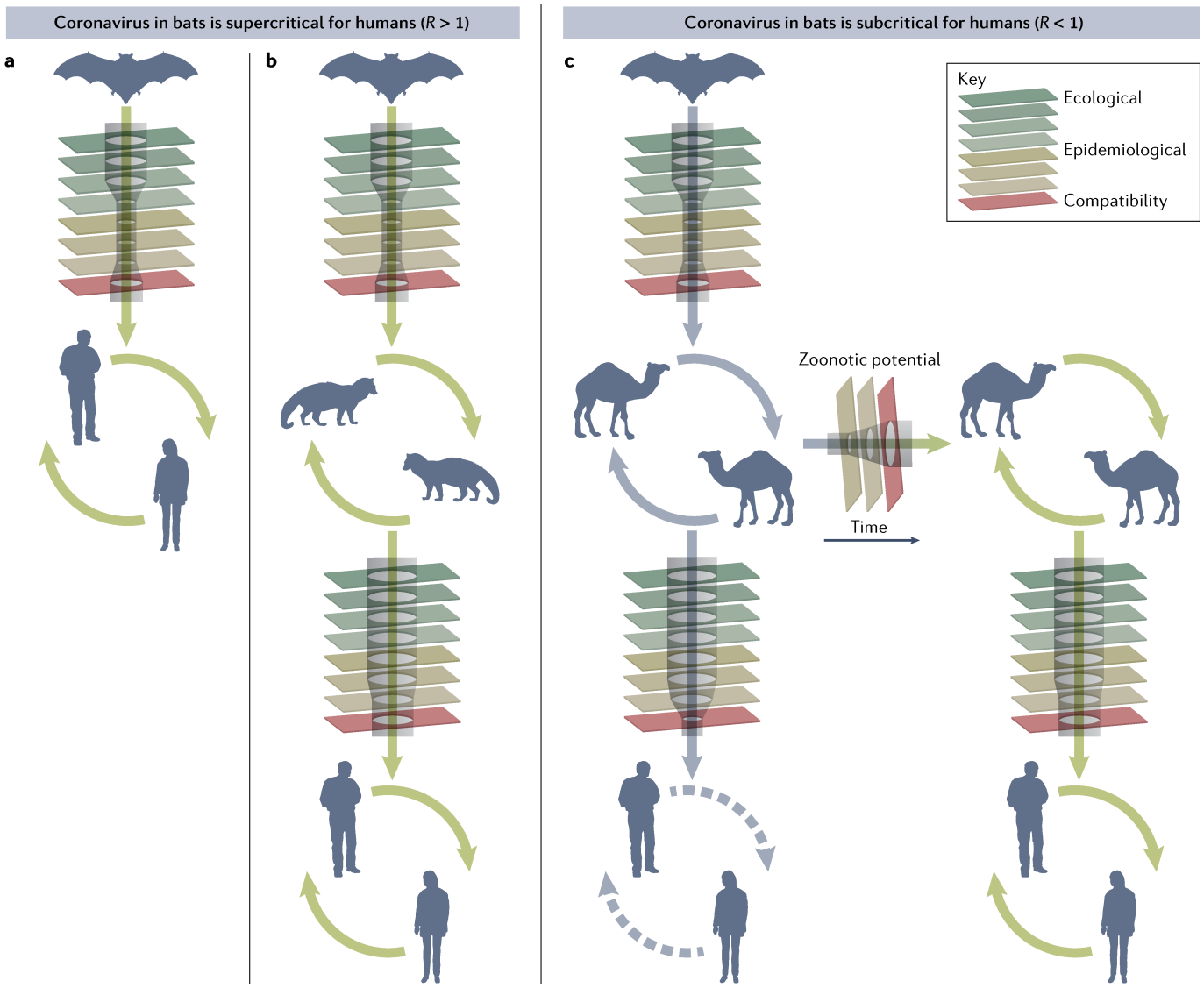
Box 1 | **Pathways to pandemic emergence of bat coronaviruses**

While the zoonotic potential of an animal virus depends on its ability and opportunity to infect humans, pandemic potential depends on human-to-human transmissibility, quantified by the virus's reproduction number in humans, *R*. The critical value for *R* is 1, the level at which each case replaces itself on average. For subcritical viruses, with $R<1$, transmission chains inevitably die out. For supercritical viruses, with $R>1$, epidemics and pandemics are possible[139].

Novel viruses with pandemic potential can reach humans by several routes. A virus circulating in bats could have the traits needed for supercritical transmission in humans, by chance or due to evolutionary pressures in the reservoir that fortuitously align with fitness in humans[161]. Such a virus could spill over directly from bats to humans, overcoming ecological barriers of limited spatial overlap and contacts between these species (see the figure, panel **a**). Alternatively, such a virus could reach humans via a bridge host that has greater contact with humans than the reservoir host, and perhaps also serves to amplify the virus to high levels to increase the probability of initial infection (see the figure, panel **b**).

Another possibility is that a virus circulating in bats would be subcritical in humans but has opportunity to evolve to become supercritical within a bridge host that shares some key traits (for example, homologous receptor proteins) with humans (see the figure, panel **c**). A fourth possibility, not depicted here, is that a subcritical virus reaches humans and evolves to become supercritical before its transmission chains die out[161].

In any of these scenarios, epidemiological factors (and simple chance) will determine whether the supercritical virus goes on to cause an epidemic or a pandemic. Many such introductions die out, particularly if transmission is highly heterogeneous[43]. Reconstruction of outbreak origins hinges on the availability of data and samples from the earliest human cases, and extensive sampling of all host species involved (which often are not known with confidence). Origins and emergence pathways will remain obscure until such data are obtained and analysed.



Part **a** of the figure adapted from REF.[140], Springer Nature Limited.

Once a coronavirus RBD can bind a receptor on a host cell, the differing distribution of those receptors in different cell types within a host will influence tissue tropism, impacting pathogenesis and transmission. In humans, ACE2 is expressed primarily in epithelial cells of many tissues, including the respiratory tract, kidney, heart and digestive tract, consistent with the respiratory and gastrointestinal pathology of SARS-CoV and the multisystemic pathology of SARS-CoV-2 (REFS[109,110]). Although detailed expression profiles of ACE2 in other species are

lacking, tissue tropism of SARSr-CoVs in several animals is consistent with that in humans. SARSr-CoVs have been detected in the respiratory tract or gastrointestinal tract of Malayan pangolins (*Manis javanica*)[111], experimentally inoculated ferrets, felids[101,112] and non-human primates[113]. Similarly, DPP4 expression in humans, dromedary camels and fruit bats includes epithelial cells of the respiratory and gastrointestinal tracts[30]. In humans, ACE2 expression is particularly high in the upper respiratory tract, while DPP4 is expressed mainly in the lower respiratory tract, potentially contributing to the greater human-to-human transmissibility of SARS-CoV and SARS-CoV-2 compared with MERS-CoV[30,114]. Additionally, DPP4 expression is almost entirely restricted to the intestines in two vespertilionid bats, the putative reservoir of the MERS-CoV progenitor, suggesting different tropism, and potentially transmission routes, between reservoir and spillover hosts[30]. Nevertheless, the detection of coronaviruses in the respiratory and gastrointestinal tracts of experimentally inoculated and wild-caught bats supports the relevance of these two systems for coronavirus infections among diverse host species[18,24,29,41,78,90].

Host proteases and host range. Besides binding to the cellular receptor, successful infection and replication require several consecutive steps, including entry, replication, potential evasion of the host innate immunity and budding. In addition to the receptors, host proteases are needed to activate (cleave) the virus spike protein to enable entry, and this cleavage may be as important as host receptor binding in determining viral zoonotic potential[96,115] and potentially human-to-human transmissibility[92]. Spike proteins of coronaviruses have multiple cleavage sites for host proteases, which are cleaved at different stages of the cell infection cycle[114]. Transmembrane serine proteases (such as TMPRSS2), trypsin-like proteases and other cell-surface proteases participate in spike protein cleavage after viral attachment, whereas lysosomal proteases such as cathepsins cleave spike proteins after virus endocytosis. By contrast, the furin proprotein convertase — present in the Golgi apparatus — may be involved in spike protein cleavage during biosynthesis[116,117]. The distribution and activity of these proteases differ among cell types and physiological conditions, therefore influencing tissue tropism and cell entry[114,118,119]. For instance, the respiratory tropism of SARS-CoV might be driven by trypsin-like proteases present in respiratory cells[120,121].

Therefore, the expression patterns of proteases also directly contribute to host range. For instance, while specific bat proteases cleave the spike proteins of both MERS-CoV and BatCoV HKU4 and enable entry into bat cells, human proteases cleave only the MERS-CoV spike proteins[122]. Understanding how coronavirus spike proteins adapt to being activated by proteases of new hosts (for example, to type, activity and distribution) is essential for predicting the potential for changes in host range and tissue tropism, including spillback infection.

Spillback infection
Also called 'anthropozoonosis'. Transmission of a zoonotic pathogen from humans to animals.

Human exposure and spillover

The great diversity of bat species in which alphacoronaviruses and betacoronaviruses have evolved, and the genetic variability of these RNA viruses, facilitates the evolution and zoonotic capacity among coronaviruses naturally circulating in bats. However, for zoonotic spillovers to occur, humans must be exposed to the viruses (BOX 1), and this can occur through direct contact with virus excreted from infected bats or bridge hosts, or through other contacts with infected animals, such as slaughtering or butchering. The nature and intensity of the reservoir bat–human interface are critical to determining spillover risk. Human behaviour is a primary determinant of exposure, which may increase contact with bats or with other animals (bridge hosts) that may expose susceptible humans. Little is known about the specific conditions of coronavirus spillovers, but human behaviours that may increase viral exposure include activities such as bat hunting and consumption, guano farming and wildlife trading[4,5,123]. These contacts between humans and bats likely occur under physiologically stressful situations that may increase viral shedding from bats or bridge hosts and exposure of humans — the potential 'patients zero' of a new epidemic. Exposures often occur in rural areas with limited access to health care, so spillovers are detected only when they cause outbreaks or epidemics. For recently emerged human coronaviruses, some factors are known, including roles for bridge hosts in the wildlife trade or among domestic animals; for example, SARS-CoV likely transferred from rhinolophid bats into humans via farmed Himalayan civets (*Paguma larvata*)[78,124,125]. Alternative pathways of direct human exposure to bat coronaviruses have not been explored thoroughly, and studies that specifically examine human populations at risk of exposure, such as guano farmers, bat hunters and wildlife traders, for evidence of bat coronavirus exposure[126] and the roles of other species in the transmission chain (BOX 2) are required for effective surveillance of, response to and prevention of future zoonotic coronavirus pandemics.

Reservoir animal–human interface. Human–bat interactions differ widely in space, time, nature and intensity; some bat species rarely encounter humans, whereas

Box 2 | Spillover of coronaviruses in other species

Coronaviruses have a demonstrated ability for cross-species transmission involving not only bats and humans, but also transmission to and among other animal species. For example, HKU2, a coronavirus related to a virus detected in rhinolophid bats, caused an outbreak of fatal disease in domestic pigs in China in 2016 (swine acute diarrhoea syndrome coronavirus; FIG. 3)[12]. In 2017, camel (HKU23) and equine coronaviruses were detected in asymptomatic domestic horses in Saudi Arabia and Oman[162]. In 2020, chicken, duck, pigeon and goose coronaviruses were observed in live-poultry markets in China, where each of the viruses was found in species of birds other than its primary host[163]. In the 1980s, a fatal outbreak of feline infectious peritonitis in cheetahs (*Acinonyx jubatus*) was caused by a feline coronavirus that circulates in domestic cats[164]. Within feline coronaviruses, type II feline coronavirus emerged from recombination between type I feline coronavirus and canine coronavirus[82,165], highlighting the potential role of co-infection in new hosts in the emergence of new coronaviruses.

others have frequent contact. For example, humans in Oceania, Asia, Africa, South America and Pacific islands have long hunted fruit bats for food[127,128]. Humans in Thailand, the Philippines, Indonesia, Mexico and the United States harvest guano from bat caves for agricultural fertilizer[129]. Those long-term bat–human interactions contrast with the recent increasing emergence of highly virulent infections in humans linked to bats. Land-use change, animal farming and domestication, and human expansion into wildlands, among other factors, have been linked to the emergence of infectious diseases in general, and most likely play a role in spillover of bat-borne viruses[3]. Changes in the quality of bats' habitat may also affect their overall health and viral circulation owing to factors such as stress[130]. Low food availability, mediated by climate change and deforestation, appears to be a driver of shedding of other viruses in bats, including the zoonotic Hendra virus and Nipah virus[131,132]. Coronavirus shedding in horseshoe bats was higher in human-dominated landscapes than in natural landscapes[16]. In addition, the legal and illegal wildlife trade results in viruses being transported over longer distances within hosts maintained in stressful and unsanitary conditions, likely increasing shedding and transmission, as demonstrated for coronaviruses in rodents[5] and MERS-CoV in dromedary camels[133].

Direct bat-to-human spillover. There are currently no well documented cases of direct bat-to-human spillover infections by coronaviruses, but this is likely due to inadequate surveillance rather than to a true absence of spillovers. Infections occurring in rural areas or in low-resource countries, where human–bat contacts might be common but access to health care is limited, likely go undetected. Furthermore, infection by some bat coronaviruses might be asymptomatic in humans or might be mistaken for other common diseases. Even for highly virulent pathogens for which surveillance programmes exist, such as Ebola virus or Nipah virus, reported spillover events appear to be the tip of the iceberg[134,135] and are recognized only after substantial human-to-human transmission. In the case of Ebola virus, it takes on average 44 days of undetected transmission before an outbreak is recognized[136].

Bat coronaviruses face numerous barriers that likely reduce infection and spread among humans. Those may occur at the levels of exposure (lack of bat virus–human contact), infection (coronavirus is not compatible with humans) or transmission (virus cannot be efficiently transmitted among humans). Perhaps, very few human exposures lead to infection, and even fewer to onward transmission. Studies in Asia have found serological evidence of human exposure to SARS-CoV or related viruses in healthy adults in Hong Kong and army recruits in mainland China sampled before the 2002 SARS pandemic[137,138]. More recent studies of villagers in the southern Chinese province of Yunnan found low seroprevalence of antibodies to SARSr-CoVs[13,14]. These studies suggest that bat-associated coronaviruses are potentially breaching the exposure and infection barriers, although the low seroprevalence (less than 3%) indicates that such cases are rare, and these viruses are not efficiently spreading among humans. It is unknown whether the antibodies detected arise entirely from primary spillover or from a combination of primary cases with limited human-to-human transmission[139,140]. Syndromic surveillance at health-care facilities, combined with improved unbiased molecular diagnostic tools that could target unknown pathogens, and periodic serological surveys of human populations are important tools to provide better understanding of when, where and how coronavirus spillovers occur. Technologies such as phage immunoprecipitation sequencing or VirScan that use coronavirus sequences recovered from multiple species (including bats) would enable multiantigen testing that can reveal undetected past spillovers and other epidemics[141].

Spillover through bridging hosts. Besides bats, other animals may provide ecological, amplifying or evolutionary opportunities for coronavirus transmission from bats to humans[9,78]. Once infected from bats, such bridging hosts may promote virus spread to humans through increased exposure or high viral loads. This will lead to a higher probability of human exposure to infectious doses of the viruses, as seen for Hendra virus, where the initial spillover and infection of horses leads to exposure and infection in humans[142], or for Nipah virus, through infection of swine[143]. In addition, bridging hosts may also enable viral evolution that results in new or enhanced zoonotic capacity[78]. Farmed Himalayan palm civets are thought to have served as bridge hosts in the spillover of SARS-CoV from bats to humans, and selection for enhanced viral replication in civets may have favoured viral mutations that increased zoonotic capacity[78,124,125]. Endemic circulation of MERS-CoV in dromedary camels suggests that transmission of ancestral merbecoviruses from bats to camelids occurred decades or much longer ago, and likely resulted in evolution of zoonotic capacity[133,144]. Thus, MERS-CoV is considered a camelid virus with ancestral origins in bats[145–147].

The ecological and evolutionary conditions that facilitated the spillover of SARS-CoV-2 remain unknown for now; however, circulation of closely related sarbecoviruses in horseshoe bats in Asia supports an ancestral origin in bats[148]. Whether the first SARS-CoV-2 transmission event happened directly from bats to humans or through a bridging host — possibly involving host-specific evolution that increased infectivity for humans — is unclear. However, coronaviruses closely related to SARS-CoV-2 with the capacity to infect humans cells have circulated widely in bats, supporting the possibility of direct bat-to-human transmission[106].

In addition to the infection of humans from other reservoirs, humans can also act as bridging hosts for reverse zoonoses. Humans have infected domestic cats, dogs, large felids (for example, tigers (*Panthera tigris*)) and farmed American minks (*Neovison vison*) with SARS-CoV-2, which could potentially act as reservoirs for new variants[149]. In the specific case of farmed minks, SARS-CoV-2 can spread at epidemic levels, facilitating viral adaptation to the new host[149]. Thus, spillback to other wildlife species might lead to establishment in secondary reservoirs. ACE2 sequences of cricetid rodents suggest many are putatively susceptible to SARS-CoV-2.

Phage immunoprecipitation sequencing
Technique in which synthetic antigens are displayed in a viral particle (T7 phage) enabling assessment of the reactivity of serum samples against antigens from several viruses simultaneously.

VirScan
High-throughput method to profile the reactivity of a serum sample against antigens from several viruses simultaneously using phage immunoprecipitation sequencing.

R
Reproductive number, the number of new infections generated by an average infected host in the population.

Metapopulation
Spatial arrangement of populations of a species that are connected by migration processes.

Luminex
Technology that enables measurement of multiple proteins in a single well (sample).

Old World Syrian hamsters (*Mesocricetus auratus*), Chinese hamsters (*Cricetulus griseus*) and New World North American deer mice (*Peromyscus maniculatus*) are cricetid rodents that are susceptible to SARS-CoV-2 (REFS[150–153]). Although many wild and domestic animal species are susceptible and could even transmit the virus among themselves (for example, see REFS[149,151,154–156]), it is unclear for these species how transmission dynamics, population size, structure and connectivity, and eventual immunity would influence the establishment of continuous or temporary reservoirs. This evidence of reverse zoonosis or spillback calls for further research to elucidate the potential for other wild animal species becoming new viral reservoirs.

Knowledge gaps and research agenda

Fundamental knowledge gaps remain about the different conditions that result in coronaviruses passing from bats into humans. Dynamic integration among field studies, modelling, laboratory experiments and human epidemiology is required to understand the processes and to prevent new coronavirus spillovers and pandemics[157].

The extensive study of coronavirus diversity in wild bats has yet to translate into a more profound understanding of their zoonotic capacity. For instance, it is unknown whether coronaviruses circulating in bat populations can be transmitted directly to humans and whether they can be transmitted among humans with $R > 1$ without passage through bridging host species. Combining the probabilistic ecological drivers of spillover with an understanding of the molecular basis of host range and tropism will lead to a more comprehensive understanding of the zoonotic capacity of coronaviruses. To accomplish this, a high-throughput characterization of the zoonotic potential of bat coronaviruses using a tiered system of in silico, in vitro and in vivo methods is needed to understand the potential risk to humans.

Despite the rapidly growing number of genomic sequences of bat coronaviruses, our knowledge of the ecology and evolution of these viruses is still low. Understanding how, when and where viral shedding happens will directly inform how we assess the risk of spillover over time and space, as viral shedding and thus pathogen pressure is the first step in spillover. It remains unclear whether the spatiotemporal patterns of coronavirus prevalence and shedding seen in some bat populations are generalizable. To fill this gap, we need longitudinal studies at multiple scales, from the individual level to the population and metapopulation levels. These studies could be coupled with phylodynamic analyses of viruses to show how the natural evolution of bat coronavirus variants may result in emergence of cross-species and zoonotic capacity.

Our ability to understand mechanisms leading to successful spillover is limited by the apparent rarity of spillover events as well as by the limited ecological data available. Assessment of spillover risk requires an increased capacity to detect these events, especially those that are missed by public health surveillance. Serosurveys in humans and potential bridge hosts at risk of exposure to bat coronaviruses should be prioritized, and multiplex serological technologies, such as Luminex or VirScan, could facilitate wide screening, even when an agent has not been fully characterized[141]. Human-focused surveillance, coupled with spatiotemporal information on bat–virus interactions, viral discovery and functional characterization are needed to estimate the magnitude and frequency of spillover events that might have gone undetected in the past. It is urgent to implement this field research agenda, targeting high-risk interfaces in areas of rapid environmental change.

Finally, as we fill the gaps and integrate knowledge across scales and disciplines, we should also develop proactive strategies for spillover prevention, in addition to reactive outbreak mitigation. The exponential nature of epidemic growth makes stopping a new pathogen with efficient person-to-person transmission a difficult task, as demonstrated by SARS-CoV-2. As we understand the conditions that facilitate spillover, interventions to prevent those conditions will become clearer, and proactive actions may be taken to prevent the next coronavirus pandemic.

Conclusions

Coronaviruses that circulate in bat populations have spilled over into human populations several times, and most likely will continue to be a public health threat. The diversity and broad geographical distribution of bats, the ubiquitous shedding of coronaviruses from bat populations and the molecular interactions of coronaviruses facilitate their zoonotic capacity. However, these pathogens cannot cause outbreaks in humans unless the conditions for spillover and onward transmission are met. The risk of spillover depends on the level of human exposure, which is increasingly influenced by habitat deterioration and encroachment into wild areas. Integration of ecological, evolutionary and epidemiological data from bat–virus systems, coupled with human epidemiological and health surveillance in high-risk areas, is urgently needed to improve risk assessment and predictive capacity. This integration of scientific fields will provide the basis for new approaches to mitigate coronavirus outbreaks and prevent spillover to humans.

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Author contributions
M.R.-A., C.M. and R.K.P. conceived the study. M.R.-A., C.M., R.K.P., A.G., C.R.P., E.S.G., J.O.L.-S., P.J.H. and V.J.M. contributed substantially to discussion of the content. M.R.-A., C.M., C.F., E.J., L.D., D.N.J. and M.K.K. compiled the data and performed formal analysis. M.R.-A. and C.M. contributed equally. All authors wrote the article and reviewed and/or edited the manuscript before submission.

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Supplementary information

Ecology, evolution and spillover of coronaviruses from bats

In the format provided by the authors and unedited

Supplementary Table 1. Wild bat hosts of coronaviruses reported in published studies. All coronaviruses were considered in our search, but we highlight links between bat species and key bat coronavirus subgenera associated with human infections (e.g., *Sarbecovirus*), domestic animal infections (e.g., *Rhinacovirus*), or are widespread and well characterized (e.g., *Nobecovirus*) based on sequencing information available in the associated studies.

Bat species	Bat family	Key coronavirus subgenera	Reference
Emballonura alecto	Emballonuridae	*Nobecovirus*	5
Taphozous melanopogon	Emballonuridae		1,9
Taphozous perforatus	Emballonuridae	*Merbecovirus*	10,11
Aselliscus stoliczkanus	Hipposideridae	*Rhinacovirus* *Sarbecovirus*	12,145,163,173,174
Hipposideros abae	Hipposideridae	*Duvinacovirus*	13
Hipposideros armiger	Hipposideridae	*Hibecovirus* *Merbecovirus* *Nobecovirus* *Rhinacovirus* *Sarbecovirus*	1,9,14-17,145,174
Hipposideros bicolor	Hipposideridae		1
Hipposideros caffer	Hipposideridae	*Duvinacovirus* *Hibecovirus* *Sarbecovirus*	1-4,142,163,172
Hipposideros cervinus	Hipposideridae		18,163
Hipposideros cf. *caffer*	Hipposideridae	*Duvinacovirus* *Hibecovirus*	19
Hipposideros cf. *ruber*	Hipposideridae	*Duvinacovirus* *Hibecovirus*	13,20-22
Hipposideros cineraceus	Hipposideridae	*Rhinacovirus*	23,152
Hipposideros curtus	Hipposideridae	*Duvinacovirus*	163,172
Hipposideros diadema	Hipposideridae		1,5,163
Hipposideros fuliginosus	Hipposideridae	*Hibecovirus*	163,172
Hipposideros galeritus	Hipposideridae	*Sarbecovirus*	1
Hipposideros gentilis	Hipposideridae		169
Hipposideros khaokhouayensis	Hipposideridae		169
Hipposideros larvatus	Hipposideridae	*Hibecovirus* *Nobecovirus* *Rhinacovirus* *Sarbecovirus*	1,9,15,26,27,152,163,173,174
Hipposideros lekaguli	Hipposideridae	*Nobecovirus*	1,9,163
Hipposideros pomona	Hipposideridae	*Hibecovirus* *Rhinacovirus* *Sarbecovirus*	28-30,145,152,163,173,174
Hipposideros pratti	Hipposideridae	*Hibecovirus* *Rhinacovirus* *Sarbecovirus*	1,31,145
Hipposideros ruber	Hipposideridae	*Duvinacovirus* *Hibecovirus* *Nobecovirus* *Sarbecovirus*	1,4,141,142,156,163,172

Bat species	Bat family	Key coronavirus subgenera	Reference
Macronycteris gigas (formerly *Hipposideros gigas*)	Hipposideridae	*Duvinacovirus* *Hibecovirus*	1,22,142,163,172
Macronycteris vittatus (formerly *Hipposideros commersoni*)	Hipposideridae	*Duvinacovirus* *Hibecovirus* *Nobecovirus*	24,25,32
Cardioderma cor	Megadermatidae		24,32
Lyroderma lyra (formerly *Megaderma lyra*)	Megadermatidae		1,9,163,174
Miniopterus africanus	Miniopteridae		24
Miniopterus australis	Miniopteridae		33
Miniopterus fuliginosus	Miniopteridae		1,14,30,31,34,35,140,162,171
Miniopterus fuscus	Miniopteridae		30,145
Miniopterus inflatus	Miniopteridae		1,22,24,142
Miniopterus magnater	Miniopteridae		1,9,36-39,163
Miniopterus minor	Miniopteridae		2,24,32
Miniopterus mossambicus	Miniopteridae		3
Miniopterus natalensis	Miniopteridae		7,24
Miniopterus pusillus	Miniopteridae		9,36-40,145,163,174
Miniopterus schreibersii	Miniopteridae	*Merbecovirus* *Rhinacovirus* *Sarbecovirus*	8,9,17,30,33,37,41-47,140,145,163,171,174
Chaerephon plicatus	Molossidae	*Merbecovirus* *Sarbecovirus*	26,31,48,49,152,169,174
Chaerephon pumilus	Molossidae	*Duvinacovirus* *Nobecovirus*	1-4,6,24,142,163
Cynomops abrasus	Molossidae		50
Cynomops planirostris	Molossidae		50
Eumops glaucinus	Molossidae	*Merbecovirus*	51
Molossus currentium	Molossidae		52
Molossus molossus	Molossidae		53-55
Molossus rufus	Molossidae		51,52,54,55
Mops condylurus	Molossidae	*Hibecovirus* *Nobecovirus*	1-3,6,142,163,172
Mops midas	Molossidae		3,7,163
Mormopterus francoismoutoui	Molossidae		3
Mormopterus jugularis	Molossidae		3
Nyctinomops laticaudatus	Molossidae	*Merbecovirus*	1,56
Otomops martiensseni	Molossidae		24,32,163
Tadarida brasiliensis	Molossidae		1,53,56,158
Tadarida teniotis	Molossidae	*Sarbecovirus*	8,57
Pteronotus davyi	Mormoopidae		54
Pteronotus parnellii	Mormoopidae		1,52,56
Pteronotus personatus	Mormoopidae		1
Mystacina tuberculata	Mystacinidae		58
Nycteris cf. *gambiensis*	Nycteridae	*Merbecovirus*	59
Nycteris macrotis	Nycteridae	*Merbecovirus*	141

Bat species	Bat family	Key coronavirus subgenera	Reference
Nycteris thebaica	Nycteridae	*Merbecovirus*	3
Nycteris tragata	Nycteridae		163
Anoura caudifer	Phyllostomidae		1,163
Anoura geoffroyi	Phyllostomidae		52
Artibeus jamaicensis	Phyllostomidae		1,52,56,60
Artibeus lituratus	Phyllostomidae		1,51,52,55,56
Artibeus obscurus	Phyllostomidae		1,163
Artibeus planirostris	Phyllostomidae		1,163
Carollia brevicauda	Phyllostomidae		52
Carollia castanea	Phyllostomidae		60
Carollia perspicillata	Phyllostomidae		1,51,52,56,60,61
Carollia sowelli	Phyllostomidae		1,56
Dermanura phaeotis (formerly *Artibeus phaeotis*)	Phyllostomidae		1,56
Desmodus rotundus	Phyllostomidae		50,62,63,143,164
Glossophaga soricina	Phyllostomidae		1,50,51,55,60,61
Lichonycteris obscura	Phyllostomidae		163
Lonchorhina aurita	Phyllostomidae		1,56
Mesophylla macconnelli	Phyllostomidae		1,163
Phyllostomus discolor	Phyllostomidae		52,55
Sturnira erythromos	Phyllostomidae		1,163
Sturnira lilium	Phyllostomidae		1,51
Acerodon celebensis	Pteropodidae	*Nobecovirus*	163
Cynopterus brachyotis	Pteropodidae	*Nobecovirus*	1,5,9,27,64,65,163,170
Cynopterus horsfieldii	Pteropodidae	*Nobecovirus*	1,163
Cynopterus sphinx	Pteropodidae	*Nobecovirus*	1,9,23,27,145,147,163,169
Dobsonia moluccensis	Pteropodidae	*Nobecovirus*	66
Dyacopterus spadiceus	Pteropodidae	*Nobecovirus*	1
Eidolon dupreanum	Pteropodidae	*Nobecovirus*	67
Eidolon helvum	Pteropodidae	*Nobecovirus*	1,2,4,6,10,11,24,32,68,141,142,163,172
Eonycteris spelaea	Pteropodidae	*Nobecovirus*	1,27,64,69-71,144,145,163,169,174
Epomophorus gambianus	Pteropodidae	*Nobecovirus*	1,141,156,163,172
Epomophorus labiatus	Pteropodidae	*Nobecovirus*	4,32
Epomops buettikoferi	Pteropodidae	*Nobecovirus*	163
Epomops franqueti	Pteropodidae	*Nobecovirus*	1,142,163,172
Macroglossus minimus	Pteropodidae	*Nobecovirus*	5,72,170
Megaerops ecaudatus	Pteropodidae	*Nobecovirus*	163
Megaerops kusnotoi	Pteropodidae	*Nobecovirus*	23
Megaerops niphanae	Pteropodidae	*Nobecovirus*	1,27
Megaloglossus woermanni	Pteropodidae	*Nobecovirus*	1,142,163,172
Micropteropus pusillus	Pteropodidae	*Nobecovirus*	1,20,142,163,172
Myonycteris angolensis (formerly *Lissonycteris angolensis*)	Pteropodidae	*Duvinacovirus* *Hibecovirus* *Nobecovirus*	1,4,6,141,163
Myonycteris torquata	Pteropodidae	*Nobecovirus*	163,172
Nanonycteris veldkampii	Pteropodidae	*Nobecovirus*	141
Ptenochirus jagori	Pteropodidae	*Nobecovirus*	5,64

Bat species	Bat family	Key coronavirus subgenera	Reference
Pteropus alecto	Pteropodidae	*Nobecovirus*	1,33,73
Pteropus conspicillatus	Pteropodidae	*Nobecovirus*	163
Pteropus lylei	Pteropodidae	*Nobecovirus*	74,163
Pteropus medius (formerly *Pteropus giganteus*)	Pteropodidae	*Nobecovirus*	1,75-77,163
Pteropus rufus	Pteropodidae	*Nobecovirus*	67
Rousettus aegyptiacus	Pteropodidae	*Nobecovirus*	1,2,4,6,24,32,78,141,163,172
Rousettus amplexicaudatus	Pteropodidae	*Nobecovirus*	1,5,27,64,170
Rousettus leschenaultii	Pteropodidae	*Merbecovirus* *Nobecovirus*	1,23,27-29,40,71,79-81,159,162,163,174
Rousettus madagascariensis	Pteropodidae	*Nobecovirus*	3
Rhinolophus acuminatus	Rhinolophidae	*Sarbecovirus*	151,163
Rhinolophus affinis	Rhinolophidae	*Rhinacovirus* *Sarbecovirus*	1,12,30,47,82,83,145,146,161,163,169,174
Rhinolophus blasii	Rhinolophidae	*Rhinacovirus* *Sarbecovirus*	45,163
Rhinolophus cf. *clivosus*	Rhinolophidae	*Duvinacovirus* *Sarbecovirus*	6,139
Rhinolophus clivosus	Rhinolophidae	*Duvinacovirus* *Hibecovirus* *Rhinacovirus,* *Sarbecovirus*	1,4,84
Rhinolophus cornutus	Rhinolophidae	*Sarbecovirus*	85,148
Rhinolophus creaghi	Rhinolophidae	*Sarbecovirus*	1,163
Rhinolophus darlingi	Rhinolophidae		141
Rhinolophus euryale	Rhinolophidae	*Rhinacovirus* *Sarbecovirus*	8,45,86,163
Rhinolophus ferrumequinum	Rhinolophidae	*Merbecovirus* *Nobecovirus* *Rhinacovirus* *Sarbecovirus*	1,8,12,17,23,29,31,43-45,57,78,83,86,89-93,140,145,159,160,163,171,174
Rhinolophus fumigatus	Rhinolophidae		2
Rhinolophus hildebrandtii	Rhinolophidae	*Sarbecovirus*	32
Rhinolophus hipposideros	Rhinolophidae	*Sarbecovirus*	86,94,95,160,165
Rhinolophus landeri	Rhinolophidae		2,32
Rhinolophus lepidus	Rhinolophidae		163
Rhinolophus lobatus	Rhinolophidae	*Rhinacovirus*	3
Rhinolophus macrotis	Rhinolophidae	*Rhinacovirus* *Sarbecovirus*	17,43,83,91,145
Rhinolophus malayanus	Rhinolophidae	*Rhinacovirus* *Sarbecovirus*	96,152,169,174
Rhinolophus marshalli	Rhinolophidae	*Sarbecovirus*	169
Rhinolophus megaphyllus	Rhinolophidae		33
Rhinolophus mehelyi	Rhinolophidae	*Sarbecovirus*	45,163
Rhinolophus monoceros	Rhinolophidae	*Sarbecovirus*	14,17,97

Bat species	Bat family	Key coronavirus subgenera	Reference
Rhinolophus pearsonii	Rhinolophidae	*Rhinacovirus* *Sarbecovirus*	17,43,91,174
Rhinolophus pusillus	Rhinolophidae	*Rhinacovirus* *Sarbecovirus*	17,31,46,49,82,83,93,98,99,145,152,153,163,169,174
Rhinolophus rex	Rhinolophidae	*Rhinacovirus* *Sarbecovirus*	1,17,82
Rhinolophus rhodesiae	Rhinolophidae	*Rhinacovirus*	3
Rhinolophus rufus	Rhinolophidae	*Nobecovirus*	5
Rhinolophus shameli	Rhinolophidae	*Rhinacovirus* *Sarbecovirus*	1,9,27,83,150
Rhinolophus sinicus	Rhinolophidae	*Nobecovirus* *Rhinacovirus* *Sarbecovirus*	1,12,17,23,30,31,38,40,43,82,83,100-109,145,147,152,159,163,173,174
Rhinolophus stheno	Rhinolophidae	*Rhinacovirus* *Sarbecovirus*	29,152,161
Rhinolophus thomasi	Rhinolophidae	*Rhinacovirus* *Sarbecovirus*	17,163
Rhinolophus trifoliatus	Rhinolophidae		18,163
Rhinonicteris aurantia	Rhinonycteridae	*Hibecovirus*	33
Triaenops afer	Rhinonycteridae	*Setracovirus*	1,3,32,142
Triaenops menamena	Rhinonycteridae		3
Triaenops persicus	Rhinonycteridae	*Merbecovirus* *Nobecovirus* *Setracovirus*	1,6,142
Rhinopoma hardwickii	Rhinopomatidae	*Nobecovirus* *Sarbecovirus*	10,163
Bauerus dubiaquercus	Vespertilionidae		1
Chalinolobus gouldii	Vespertilionidae		110
Chalinolobus morio	Vespertilionidae		110
Corynorhinus townsendii	Vespertilionidae		154
Eptesicus fuscus	Vespertilionidae		56,111-113,149
Eptesicus isabellinus	Vespertilionidae	*Merbecovirus*	42
Eptesicus nilssonii	Vespertilionidae	*Merbecovirus*	114
Eptesicus serotinus	Vespertilionidae	*Merbecovirus*	8,92,98,115,116,171
Glauconycteris poensis	Pteropodidae		163
Glauconycteris variegata	Pteropodidae	*Nobecovirus*	163
Falsistrellus mackenziei	Vespertilionidae		110
Hypsugo alaschanicus	Vespertilionidae		140,171
Hypsugo pulveratus	Vespertilionidae	*Merbecovirus*	101,159
Hypsugo savii	Vespertilionidae	*Merbecovirus*	42,94,117
Ia io	Vespertilionidae	*Merbecovirus*	1,118,145
Kerivoula hardwickii	Vespertilionidae		163
Kerivoula pellucida	Vespertilionidae		163
Kerivoula titania	Vespertilionidae		14
Murina cyclotis	Vespertilionidae		152
Murina leucogaster	Vespertilionidae		17,23
Murina recondita	Vespertilionidae		14
Myotis adversus	Vespertilionidae		174
Myotis aurascens	Vespertilionidae		171

Bat species	Bat family	Key coronavirus subgenera	Reference
Myotis bechsteinii	Vespertilionidae		119,120
Myotis blythii (includes *Myotis oxygnathus*)	Vespertilionidae		42,89,115
Myotis bombinus	Vespertilionidae		140
Myotis brandtii	Vespertilionidae		114
Myotis californicus	Vespertilionidae		1
Myotis capaccinii	Vespertilionidae		8
Myotis chinensis	Vespertilionidae		145,174
Myotis dasycneme	Vespertilionidae		116,120,121,167
Myotis daubentonii	Vespertilionidae	*Merbecovirus* *Rhinacovirus*	1,8,23,29,31,42,86,89,114,116,120-122,163,167
Myotis davidii	Vespertilionidae		17
Myotis emarginatus	Vespertilionidae		41,90
Myotis evotis	Vespertilionidae		113
Myotis fimbriatus	Vespertilionidae		14,98,163
Myotis formosus (formerly *Myotis flavus*)	Vespertilionidae		14
Myotis horsfieldii	Vespertilionidae	*Nobecovirus*	1,27,145,163
Myotis ikonnikovi	Vespertilionidae	*Merbecovirus*	171
Myotis laniger	Vespertilionidae	*Rhinacovirus*	152,163
Myotis longipes	Vespertilionidae		1,174
Myotis lucifugus	Vespertilionidae		113,123,124,125
Myotis macrodactylus	Vespertilionidae		140,171
Myotis macropus	Vespertilionidae		33,126
Myotis muricola	Vespertilionidae		152
Myotis myotis	Vespertilionidae		1,8,42,86,89,127,157
Myotis nattereri	Vespertilionidae		8,41,86,89,116,119,122
Myotis nigricans	Vespertilionidae		51
Myotis occultus	Vespertilionidae		111
Myotis pequinius	Vespertilionidae	*Merbecovirus*	98
Myotis petax	Vespertilionidae		140,171
Myotis pilosus (formerly *Myotis ricketti*)	Vespertilionidae	*Merbecovirus* *Rhinacovirus*	1,31,38,43,46,98,145,163,174
Myotis punicus	Vespertilionidae		8
Myotis riparius	Vespertilionidae		51
Myotis siligorensis	Vespertilionidae	*Merbecovirus* *Rhinacovirus*	17,163,174
Myotis velifer	Vespertilionidae		1,56
Myotis volans	Vespertilionidae		113
Myotis welwitschii	Vespertilionidae		163
Neoromicia capensis	Vespertilionidae	*Merbecovirus*	7,128,129
Neoromicia cf. *zuluensis*	Vespertilionidae	*Merbecovirus*	130
Neoromicia somalica	Vespertilionidae	*Nobecovirus*	163
Nyctalus lasiopterus	Vespertilionidae		42
Nyctalus leisleri	Vespertilionidae		45
Nyctalus noctula	Vespertilionidae	*Merbecovirus*	94,121,157
Nyctalus plancyi	Vespertilionidae		1,31

Bat species	Bat family	Key coronavirus subgenera	Reference
(includes *Nyctalus velutinus*)			
Nyctophilus geoffroyi	Vespertilionidae		110
Nyctophilus gouldi	Vespertilionidae		110
Perimyotis subflavus	Vespertilionidae		131
Pipistrellus abramus	Vespertilionidae	*Merbecovirus* *Nobecovirus* *Sarbecovirus*	31,38,43,92,101,118,132,145,171,174
Pipistrellus cf. *hesperidus*	Vespertilionidae	*Merbecovirus*	6,133
Pipistrellus coromandra	Vespertilionidae	*Merbecovirus*	1,27,163
Pipistrellus hesperidus	Vespertilionidae	*Merbecovirus*	1,163
Pipistrellus inexspectatus	Vespertilionidae		172
Pipistrellus kuhlii (includes *Pipistrellus deserti*)	Vespertilionidae	*Merbecovirus* *Nobecovirus*	10,42,78,89,94,117,134,168
Pipistrellus nathusii	Vespertilionidae	*Merbecovirus*	59,119,120
Pipistrellus pipistrellus	Vespertilionidae	*Merbecovirus*	1,41,43,59,89,118,121,135,157,166
Pipistrellus pygmaeus	Vespertilionidae	*Merbecovirus*	59,86,116,119,120,167
Pipistrellus tenuis (formerly *Pipistrellus minus*)	Vespertilionidae	*Merbecovirus*	118
Plecotus auritus	Vespertilionidae	*Merbecovirus* *Sarbecovirus*	57,89
Plecotus taivanus	Vespertilionidae		14
Scotophilus dinganii	Vespertilionidae	*Nobecovirus*	1,32,142,172
Scotophilus heathii	Vespertilionidae	*Nobecovirus*	9,26,163,174
Scotophilus kuhlii	Vespertilionidae	*Nobecovirus*	1,9,14,27,43,97,136,137,145,147,163,174
Scotophilus leucogaster	Vespertilionidae	*Nobecovirus*	1,172
Scotophilus nux	Vespertilionidae		1,163,172
Submyotodon latirostris	Vespertilionidae		14
Tylonycteris pachypus	Vespertilionidae	*Merbecovirus* *Rhinacovirus*	1,31,38,43,46,101,118,132,145,155,159,163,174
Tylonycteris robustula	Vespertilionidae	*Rhinacovirus*	101,174
Vespadelus baverstocki	Vespertilionidae		110
Vespadelus pumilus	Vespertilionidae		33
Vespadelus regulus	Vespertilionidae		110
Vespertilio murinus	Vespertilionidae	*Merbecovirus*	157
Vespertilio sinensis (formerly *Vespertilio superans*)	Vespertilionidae	*Merbecovirus* *Hibecovirus*	1,31,92,118,138,145,171

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Supplementary Table 2. Comparison of difference approaches to studying coronaviruses in bats. A total of 214 original studies on bat-associated coronaviruses were classified into study types. Study types were not exclusive, so a study may fit into multiple types depending on the sampling approach and analytical methods. All classified studies can be found in Supplementary Dataset 1.

Study type and description	Number of studies	Overview	What we can learn	Advantages	Caveats
Experimental Experimental infection of individual bats or bat cell lines, or other viral manipulations in a controlled environment	Bat cell lines: 29 Live bats: 6	<u>Bat cell experiments</u> • Target cells: brain, embryo, intestine, kidney, lung • Tested viruses: multiple bat SARS-related CoVs, BatCoV HKU4, BatCoV HKU9, HCoV-229E, HCoV-NL63, MERS-CoV, PEDV, Ro-BatCoV GCCDC1, SADS-CoV, SARS-CoV, SARS-CoV-2, *Scotophilus* bat CoV 512, TGEV <u>Live bat experiments</u> • Tested hosts and viruses: *Artibeus jamaicensis* (MERS-CoV), *Eptesicus fuscus* (SARS-CoV-2), *Myotis lucifugus* (Myl-CoV), *Rousettus leschenaultii* (BatCoV HKU9), *Rousettus aegyptiacus* (bat SARSr-CoV WIV1, SARS-CoV-2)	• Characterization of newly detected viruses • Bat species susceptibility to infection and dose-response relationships • Magnitude, quality, and kinetics of immune responses to pathogens, and mechanisms of viral control or tolerance • Disease pathogenesis (or lack thereof) • Individual and within-host infection, disease, and immunological processes, especially those required for dynamic modeling (e.g., infectious periods, acute vs. latent infections, waning immunity, etc.) • Tissue tropism and routes of virus excretion and transmission	• Ability to test Koch's postulates using different strains and bat species • Causal inference • Controlled environment • Rapid technological advances make diagnostic tools affordable • Relatively rapid data acquisition	• Relies on existing viral isolates; cannot isolate new pathogens • No ecological context; impossible to accurately replicate environmental conditions • Lab conditions may not effectively mimic the environmental conditions that drive infections in reservoir hosts • Challenging and expensive to house and breed colonies of bats • Often requires biosafety level 3 or 4 facilities and specialized training • A bat is not a bat, and a virus is not a virus: species-specific responses to infection make it difficult to generalize across species or bat families

Study type and description	Number of studies	Overview	What we can learn	Advantages	Caveats
			• Receptor binding efficiency in bats and other potential hosts • Facilitative or antagonistic interactions between coinfecting viruses • Virus surface survival and sensitivity to heat or desiccation • Development of model systems, laboratory protocols, and screening tools for the field • Spillover potential to other/novel hosts		• *In vitro* studies miss differences in cell recruitment and localization or cell-cell interaction • Immortalized cells behave differently from primary cells or cells in an *in vivo* context • Fundamental knowledge of bat immune systems and basic tools for probing bat immune responses are lacking • Experiments are usually time-limited (e.g., limited ability to study immune function senescence, viral recrudescence, etc.)
Longitudinal Repeated sampling of individuals, single populations, or multiple populations over time; ideally, this occurs in closed populations with known individual life-histories	14	• Countries: Australia, China, Denmark, Germany, Malaysia, Singapore, South Korea, Thailand • Serially sampled species: *Eonycteris spelaea*, *Hipposideros cervinus*, *Myotis daubentonii*, *Myotis macropus*, *Myotis myotis*, *Pteropus lylei*,	• Some spatial and temporal dynamics of pathogens in populations, and maybe in individuals • Spatiotemporal patterns of infection (e.g., travelling waves) • Transmission rates and dynamics, using carefully collected	• Ability to identify and isolate novel pathogens • May have ability to repeatedly collect covariate data or track life-histories of individuals • More power to exclude time-invariant differences between individuals,	• May not be truly longitudinal: without known recapture of individuals, repeated longitudinal monitoring at a geographic location may instead represent multiple cross-sectional surveys of the population

Study type and description	Number of studies	Overview	What we can learn	Advantages	Caveats
		Rhinolophus sinicus, *Rousettus leschenaultii*	age-prevalence and age-seroprevalence data • Variation in prevalence/seroprevalence with host traits or environmental covariates • Parameters of the disease process in individuals and populations required for dynamic modeling (e.g., seasonality, maybe transmission rates, life-history traits) • Some dynamics of co-circulating viruses • Interventions that might reduce prevalence or magnitude of an epizootic or enzootic	populations, or environments • Identification of temporal trends (e.g., seasonality) • Potential for forecasting and prediction • Intervention analysis • Relationship between time-series variables	• Expensive, time-consuming, and logistically challenging; slow data acquisition • Effective implementation requires a strong ecological understanding of the study system and collection of data to determine sampling frequency and duration • May be temporally biased; sampling at regular intervals may consistently detect or consistently miss viral shedding • May be spatially biased; difficult to sample spatially replicated populations • Determining disease dynamics is difficult: requires consistent recapture of individuals, longitudinal sampling that exceeds pathogen infectious period, nonlethal

Study type and description	Number of studies	Overview	What we can learn	Advantages	Caveats
					pathogen detection, and moderate prevalence • Large sample sizes, spatially replicated populations, and short sampling intervals are needed to understand environmental drivers, and individual and population-level variation in viral shedding • Relationships that exist for groups may not apply to individuals (ecological fallacy, e.g., virus x detected in all population subgroups sampled in Habitat A; therefore, all individuals or other population subgroups in Habitat A must also carry virus x.
Cross-sectional (intra-species) Sampling of a bat population or population subgroup(s) at a specific timepoint	14		• Genetic variation of strains within host population(s) • Spatial distribution of strains within host population(s) • Some differences between	• Relatively fast and inexpensive • Sampling of isolated populations can help distinguish between population-level pathogen persistence and spatiotemporally irregular transmission	• No ability to detect seasonality or other temporal trends • No causal inference • Large amounts of data are required to account for variation

Study type and description	Number of studies	Overview	What we can learn	Advantages	Caveats
			demographic stages (dependent on sampling time-point) • Possible to integrate with longitudinal studies of same species • Natural routes of excretion	• Can sample populations adaptively in response to spillover • Ability to isolate pathogens • Some ability to detect spatial variation or statistically analyze differences.	among individuals or populations • Effective implementation requires a strong ecological understanding of the study system • May be temporally biased: sampling during peaks or troughs in population prevalence will over- or underestimate geographic variation in prevalence or genetic diversity • May be spatially biased: at one timepoint, different population subgroups may have peaks or troughs in prevalence • Ecological fallacy (as in longitudinal studies)
Cross-sectional (inter-species) Sampling of bat assemblages or a subset of a bat assemblage (>1 species) at a specific timepoint	123	• Sampled countries: 69 • Sampled bat families: 18 • Positive bat families: 14 • Sampled bat species: 543	• Identity of potential reservoir hosts • Potential exchange of strains between hosts • Host and geographic factors that impact viral diversity	• Rapid detection of viruses in multiple species • Ability to isolate pathogens • Some ability to detect species-level differences	• Same caveats as intra-species cross-sectional studies • Often low sample sizes for opportunistically sampled species • Species bias: research effort may

Study type and description	Number of studies	Overview	What we can learn	Advantages	Caveats
		• Positive bat species: 238		• Relatively fast and inexpensive	inadvertently skew importance of a particular species as a reservoir or spillover host • Ecological fallacy (as in longitudinal and intra-species cross-sectional studies)
Multi-pathogen detection Detection of multiple pathogens (virus families, strains, or other parasite taxa) using metagenomic sequencing or other targeted methods on samples collected during cross-sectional or longitudinal sampling at the individual- or population-level	36		• Viral species diversity, abundance, and community dynamics • Some information about periods of potential spillover risk for newly detected viruses not yet known to be zoonotic • Coinfection and some insight into interactive effects of viruses on hosts	• Can be combined with next-generation sequencing to identify viral communities • May require little to no fieldwork if samples are already available • Can be relatively inexpensive with rapid data acquisition (design dependent)	• Same caveats as longitudinal or cross-sectional studies, depending on design • May be difficult to distinguish between facilitative or antagonistic interactions between coinfecting viruses or viruses synchronously shed from a bat population; requires large sample sizes combined with simulation or experimental studies • Drivers of multi-viral infection or shedding may be difficult to detect (e.g., may be driven by facilitative interaction between known or undetected coinfecting viruses, interactions with host physiology/immunity,

Study type and description	Number of studies	Overview	What we can learn	Advantages	Caveats
					and/or a response to optimal environmental conditions) • Biased detection: high titers of one virus in a sample may reduce assay sensitivity to other viruses • No causal inference • Co-detection of pathogens in pooled or population-level samples may reflect coinfection or contribution of multiple bats to the collected sample
Sequencing only Viral sequencing on samples collected during longitudinal or cross-sectional sampling; little collection of data on other covariates	29		• Comparative genomics • Mutation and evolutionary rates • Virus discovery • Effective population size and genetic diversity of virus within or across subpopulations • Some information on viral dynamics may be possible (e.g., through phylodynamics)	• Requires little background knowledge of study system • Relatively inexpensive; rapid data acquisition • May require little to no fieldwork if samples are already available	• No ecological or physiological context • No causal inference