Review in Advance first posted online on August 19, 2016. (Changes may still occur before final publication online and in print.)

Bats as Viral Reservoirs

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Annu. Rev. Virol. 2016. 3:24.1–24.23

The *Annual Review of Virology* is online at virology.annualreviews.org

This article's doi: 10.1146/annurev-virology-110615-042203

Keywords

Chiroptera, zoonoses, virus, host-pathogen interactions, cross-species transmission, virome

Abstract

Bats are hosts of a range of viruses, including ebolaviruses, and many important human viral infections, such as measles and mumps, may have their ancestry traced back to bats. Here, I review viruses of all viral families detected in global bat populations. The viral diversity in bats is substantial, and viruses with all known types of genomic structures and replication strategies have been discovered in bats. However, the discovery of viruses is not geographically even, with some apparently undersampled regions, such as South America. Furthermore, some bat families, including those with global or wide distributions such as *Emballonuridae* and *Miniopteridae* are underrepresented on viral databases. Future studies, including those that address these sampling gaps along with those that develop our understanding of viral-host relationships, are highlighted.

INTRODUCTION

Bats (order *Chiroptera*) have been linked through epidemiological and phylogenetic modeling to some of the most important infectious diseases of mankind. Phylogenetic analyses suggest that infections now restricted to humans, such as mumps and measles, may have had their origins in bats. Emerging infectious diseases are recognized as threats to global security (1). A number of high-profile emerging infectious diseases have been linked to bats, and evidence supports their role as hosts of Ebola virus (EBOV), which recently emerged in West Africa for the first time and has killed 11,300 from over 28,600 people infected over 24 months. Given these observations, the importance of understanding bats as viral reservoirs has never been greater. Here, I provide a review of the viral families detected in global bat populations, offer a brief discussion of the proposed current hypotheses as to why bats may be hosts for a diverse number of viral species, and identify future research areas.

BAT DIVERSITY

Bats are taxonomically diverse, representing approximately 20% mammal diversity. They are the only mammals capable of powered flight and are the main nocturnal aerial predators. Bats are generally small and often use echolocation for aerial prey capture, yet they are incredibly ecologically diverse (2). Bats were traditionally divided into two suborders: *Microchiroptera* (microbats) and *Megachiroptera* (megabats), the latter of which was the Old World fruit bat family, *Pteropodidae*. Members of *Pteropodidae* are typically larger, fruit-eating bats that use vision and smell without the ability to echolocate. Recent analyses have revealed that this megabat-microbat suborder distinction was incorrect and that *Pteropodidae* is a sister taxon to Old World rhinolophoid microbats (3). *Pteropodidae* and *Rhinolophidae* have Old World distributions. The *Vespertilionidae* family is the largest family of bats and is distributed globally, except Antarctica. Similarly distributed are the *Molossidae*, or free-tailed bats. Some bats are solitary, but colonies of the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*) can reach densities of 4,000 bats/m^2 in populations of up to one million individuals per roost (4). New World bats include the *Phyllostomidae* family. This family has become one of the most ecologically diverse bat families, and it is within this family that species have adapted to eat almost every available food—fruit, nectar, pollen, insects, vertebrates, and most famously blood in the case of vampire bats (2). The diversity of bats creates many potential viral niches and has been hypothesized to be a mechanism for driving the diversity of the viruses they host (5, 6).

VIRAL DIVERSITY

I use viral sequence data (e.g., available through PubMed and a viral database, **http://www.mgc.ac.cn/DBatVir/**) to identify viruses linked to bats, only referring to serological studies if useful to emphasize a point. The viral diversity in bats is substantial (7). Comparative analyses suggest that bats host more zoonotic viral infections per species than rodents (5), and bats have been used as model systems to estimate likely viral diversity given the global diversity of mammals (8). Viruses with all types of genomic structures and replication strategies, as classified by the Baltimore classification system, have been discovered in bats. These include group I double-stranded DNA viruses (dsDNA viruses; e.g., adenoviruses), group II single-stranded DNA viruses (ssDNA viruses; e.g., parvoviruses), group III double-stranded RNA viruses (dsRNA viruses; e.g., reoviruses), group IV positive-sense single-stranded RNA viruses (+ssRNA viruses; e.g., picornaviruses), group V negative-sense single-stranded RNA viruses (−ssRNA viruses; e.g.,

rhabdoviruses), group VI single-stranded RNA reverse-transcribing viruses (ssRNA-RT viruses; i.e., RNA viruses with DNA intermediates, such as retroviruses), and group VII double-stranded DNA reverse-transcribing viruses (dsDNA-RT viruses; e.g., hepadnaviruses) (**Table 1**). Viruses have been discovered in bats across the globe, from every continent except Antarctica, including isolated populations such as those in New Zealand (9) (**Supplemental Figure 1**). However, the discovery of viruses is not geographically even (**Figure 1**, **Supplemental Figure 1**), and some viral families are overrepresented in viral databases (**Table 1**) (7). It is unclear whether these differences in viral discovery are due to varying sampling efforts and biases or to biological phenomena, such as phylogeographic processes, and one of the goals of this review is to identify areas that require further study to address this. Metagenomic approaches are largely unbiased in the way conventional molecular studies were; therefore, metagenomic studies may also begin to elucidate whether the current biases in published data (e.g., 10) (**Table 1**) are the result of sampling bias due to pathogen detection protocols. The rapid recent increase in metagenomics studies of bat viromes is evidence that there is a shift in the strategy used to detect bat viruses (11–21). This and other advances and future directions are discussed below.

NEGATIVE-SENSE SINGLE-STRANDED RNA VIRUSES

Among the most notorious and important viruses that are linked to bats are the −ssRNA viruses. Viruses from the family *Rhabdoviridae* are the most widely studied of the bat viruses. Viruses from *Rhabdoviridae* have been detected in bats worldwide, largely due to the lyssaviruses. This is because all lyssaviruses, including rabies virus, had their origins in bats (22, 23). Baer (24) cites reports of Spanish conquistadores dying after being attacked by vampire bats in the Americas in 1514, the presumption being they died of rabies. Few substantial land areas (e.g., New Zealand) are currently without records of lyssaviruses detected in the bat communities present (**Figure 1**). Of the viral sequences from bat species in GenBank, 2,484 of 11,258 (22%) are lyssavirus sequences. The next most common, even at the family level, are coronavirus sequences, with 920 (8%). Many aspects of lyssavirus biology are well understood, including cross-species transmission within bat populations and some aspects of the emergence of rabies virus from bats into other terrestrial carnivores (*Carnivora*) (22, 25). The recent documentation of the emergence of bat-associated rabies virus into skunk populations suggests that the risk of emergence of rabies virus from bats into terrestrial carnivores continues (26). The host factors that are known to allow lyssavirus cell entry are essential for host cell processes and are highly conserved in mammals, suggesting cross-species transmission may be a constant risk (27).

Following the discovery of bats as hosts for Hendra (28) and Nipah (29) viruses after infection emergence events into domestic animal populations that ultimately killed people, studies determined that viruses from the family *Paramyxoviridae* are ubiquitous among bats worldwide (30–43) (**Figure 1**). Phylogenetic analyses suggest bats were hosts to the ancestors of all major paramyxoviruses, including measles virus, distemper virus, mumps virus, parainfluenza virus, Newcastle disease virus, respiratory syncytial virus, and metapneumoviruses (30).

The family *Filoviridae* comprises three genera: *Ebolavirus*, *Marburgvirus*, and *Cuevavirus* (44). Substantial data suggest that bats are the reservoir host of the filoviruses (45–55). Only fragments of *Ebolavirus* genomes have been detected in bats (45, 56), but complete genomes have been isolated from bats for cuevaviruses (51) and marburgviruses (52, 54). Like many of the bat-viral systems, there is little evidence of disease in their bat hosts (45, 50, 52), though it is notable that Lloviu virus, the only virus in the *Cuevavirus* genus, was discovered during investigations into a die-off among *Miniopterus schreibersii* bats (51). To date filoviruses have been detected only in Old World bats (**Figure 1**).

Table 1 Viruses detected in bats

Virus family	Number of sequences	Genome size (kb)	Enveloped virus?	Replication location	Segmented genome?	Bat families
Double-stranded DNA viruses (total number of sequences: 370)						
Adenoviridae	114	35–36	No	Nucleus	No	*Phyllostomidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported
Herpesviridae	169	120–240	Yes	Nucleus	No	*Molossidae* *Phyllostomidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported
Papillomaviridae	32	8	No	Nucleus	No	*Mystacinidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae*
Polyomaviridae	52	5	No	Nucleus	No	*Megadermatidae* *Molossidae* *Mormoopidae* *Mystacinidae* *Phyllostomidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae*
Poxviridae	3	130–375	Yes	Cytoplasm	No	*Pteropodidae* *Vespertilionidae*
Double-stranded RNA viruses (total number of sequences: 137)						
Reoviridae	137	18.2–30.5	No	Cytoplasm	Yes	*Emballonuridae* *Molossidae* *Phyllostomidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported
Retro-transcribing viruses (total number of sequences: 39)						
Hepadnaviridae	23	3.2	Yes	Nucleus or cytoplasm	No	*Phyllostomidae* *Rhinolophidae* *Vespertilionidae*
Retroviridae	16	7–11	Yes	Nucleus	No	*Emballonuridae* *Megadermatidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae*

(*Continued*)

Table 1 (*Continued*)

Virus family	Number of sequences	Genome size (kb)	Enveloped virus?	Replication location	Segmented genome?	Bat families
Single-stranded DNA viruses (total number of sequences: 260)						
Anelloviridae	1	3.8	No	Nucleus	No	*Molossidae*
Circoviridae	148	1.8–3.8	No	Nucleus	No	*Molossidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported
Parvoviridae	111	4–6	No	Nucleus	No	*Phyllostomidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported
Single-stranded negative-sense RNA viruses (total number of sequences: 3,346)						
Bornaviridae	2	8.9	Yes	Nucleus	No	*Vespertilionidae*
Bunyaviridae	79	11–19.9	Yes	Cytoplasm	No	*Emballonuridae* *Molossidae* *Nycteridae* *Phyllostomidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported
Filoviridae	88	18–19	Yes	Cytoplasm	No	*Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported
Orthomyxoviridae	4	13.5	Yes	Nucleus	Yes	*Phyllostomidae*
Paramyxoviridae	657	15	Yes	Cytoplasm	No	*Emballonuridae* *Molossidae* *Mormoopidae* *Nycteridae* *Phyllostomidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported
Rhabdoviridae	2,516	11–15	Yes	Cytoplasm	No	*Emballonuridae* *Molossidae* *Nycteridae* *Phyllostomidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported

(*Continued*)

Table 1 (*Continued*)

Virus family	Number of sequences	Genome size (kb)	Enveloped virus?	Replication location	Segmented genome?	Bat families
Single-stranded positive-sense RNA viruses (total number of sequences: 1,477)						
Astroviridae	280	6.8–7	No	Cytoplasm	No	*Emballonuridae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported
Caliciviridae	17	7.3–8.3	No	Cytoplasm	No	*Mystacinidae* *Rhinolophidae* *Vespertilionidae*
Coronaviridae	920	27–32	Yes	Cytoplasm	No	*Emballonuridae* *Megadermatidae* *Molossidae* *Mormoopidae* *Mystacinidae* *Nycteridae* *Phyllostomidae* *Pteropodidae* *Rhinolophidae* *Rhinopomatidae* *Vespertilionidae* Unreported
Flaviviridae	177	9.7–12	Yes	Cytoplasm	No	*Emballonuridae* *Molossidae* *Mormoopidae* *Phyllostomidae* *Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported
Hepeviridae	10	7.2	No	Cytoplasm	No	*Mystacinidae* *Phyllostomidae* *Rhinolophidae* *Vespertilionidae*
Picornaviridae	72	7.1–8.9	No	Cytoplasm	No	*Pteropodidae* *Rhinolophidae* *Vespertilionidae* Unreported
Togaviridae	1	9.7–11.8	Yes	Cytoplasm	No	Unreported

Viruses were identified through a viral database (7).

Recent discoveries relating to *Orthomyxoviridae* suggest that bats host a diverse range of influenza viruses, previously unknown from studies of birds (*Aves*). The hemagglutinin (HA) and neuraminidase (NA) gene subtypes of bat influenza A viruses are divergent and new and are designated H17N10 and H18N11 (57, 58). Notably, phylogenetic analyses demonstrated great diversity in some gene segments, and because of this, the authors (58) suggested New World bats harbor greater influenza virus genetic diversity than all other mammalian and avian species combined.

Bunyaviridae includes a number of zoonotic viruses that cause human disease, such as Crimean-Congo hemorrhagic fever, hantavirus pulmonary syndrome, and hantavirus hemorrhagic fever with renal syndrome. Among the members of *Bunyaviridae*, there is increasing evidence that bats are reservoirs for a diverse suite of viruses, including hantaviruses (**Table 1**). A phylogenetic analysis of hantaviruses suggests that cross-species transmission has played a major role during hantavirus evolution and that hantaviruses might have their ancestors in bats, moles, or shrews (*Soricomorpha*), before emerging in rodents (59). Although the bat hosts for *Bunyaviridae* family viruses are globally distributed (**Figure 1**), no studies in the New World have yet been reported for hantaviruses in bats (7), despite the Americas apparently having the greatest diversity of zoonotic hantaviruses from rodents (60).

POSITIVE-SENSE SINGLE-STRANDED RNA VIRUSES

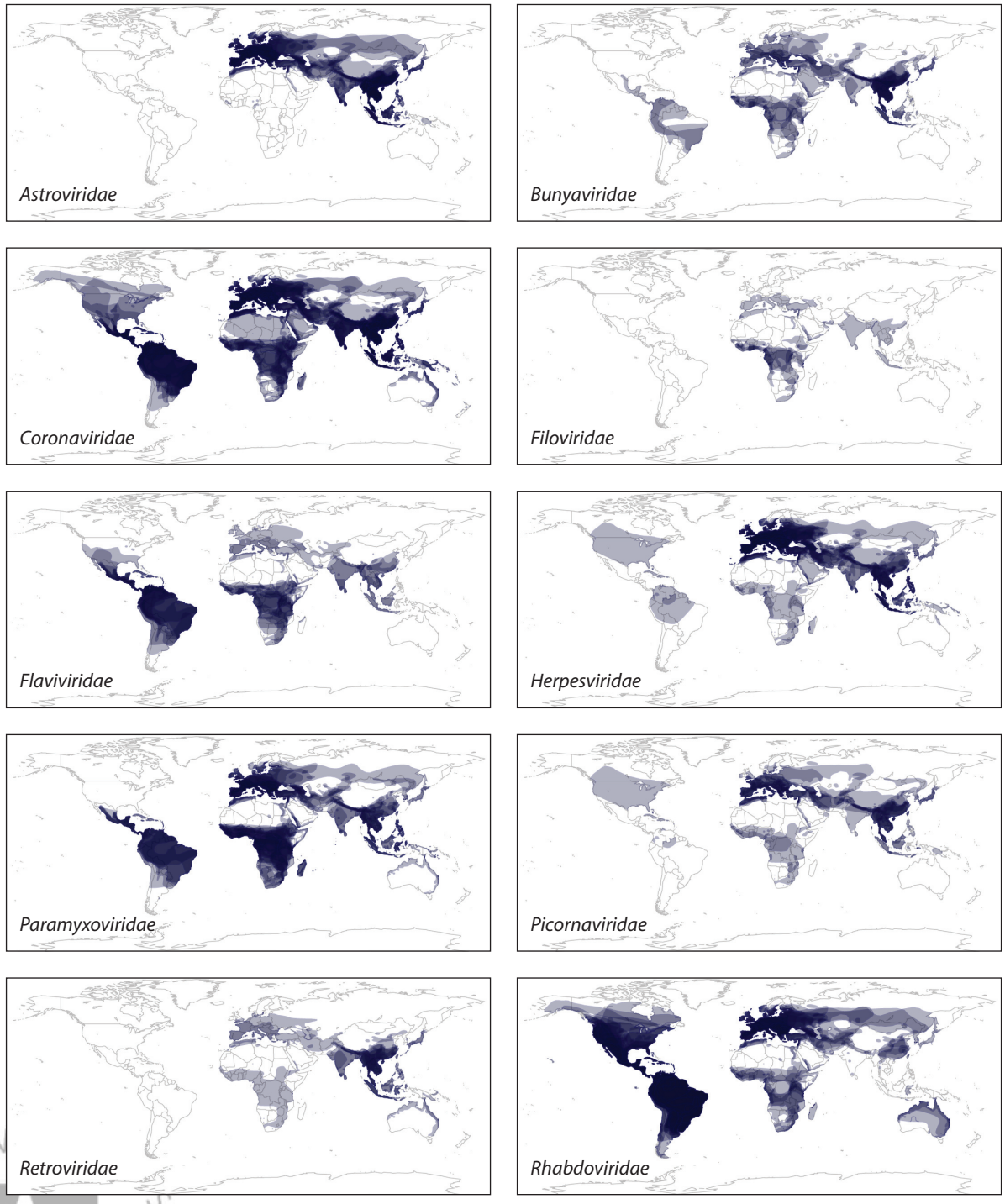
Among the +ssRNA viruses, the relationship between *Coronaviridae* family viruses and bats is one of the more well studied. There is considerable evidence that bats are the hosts for a wide range of coronaviruses, including relatives of the severe acute respiratory syndrome coronavirus (SARS-CoV) that caused the global pandemic in 2002 and 2003 (61). There have been many subsequent analyses that have led to coronaviruses being detected in bat populations across the world (**Figure 1**). The coronaviruses are divided into four genera—*Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*—and mammals are the main hosts for alpha- and betacoronaviruses (62). Recent analyses have demonstrated additional evidence of bat-derived coronaviruses as the ancestors of alphacoronaviruses, such as human coronavirus 229E (63). Furthermore, although camels may be the direct source of Middle East respiratory syndrome coronavirus (MERS-CoV) (64–66), it is likely that the MERS CoV's betacoronavirus ancestor is circulating among bat species given our current knowledge (67–69).

Picornaviridae includes a number of important human and animal viruses, including those that cause foot-and-mouth disease in ungulates and polio, hepatitis A, and the common cold in humans. *Picornaviridae* family viruses have been discovered in bats across the globe (11, 15, 70, 71) (**Figure 1**). Analyses of picornaviruses indicate that they belong to two novel genera, that they infect diverse bat genera, and that they may cross barriers between bat species (72). Evolutionary analyses also suggest that the number of cross-species transmission events is likely greater for hepatitis A virus ancestors in bats than in other animals, though the actual ancestry is still uncertain (71).

Viruses of the *Astroviridae* family, including astroviruses, can cause gastrointestinal disease in humans. These viruses have been detected in several Old World bats (8, 73–75), although the distribution of the hosts currently identified excludes Africa (**Figure 1**). As is the case for coronaviruses and other viruses in bat species, the diversity of astroviruses present in a single location among only a small number of species can be enormous (74).

Flaviviridae includes a range of important viral pathogens that affect public health, including the etiological agents for yellow fever and dengue. Recent studies have found support that bats are the major hosts for pegiviruses and hepaciviruses, viral groups that include human hepatitis C virus and the human GB viruses (76). These studies and the global distribution of the reservoir hosts (**Figure 1**) support the idea that bats may be the ancestral hosts to a large number of +ssRNA viruses, beyond the coronaviruses and astroviruses.

Members of the family *Caliciviridae* include those noroviruses that cause winter vomiting disease in humans. There are few published *Caliciviridae* viral sequences from bats; however, phylogenetic analysis of the viral protein sequences discovered suggests that bat sapoviruses may share a common ancestry with other mammalian sapoviruses but have greater codon usage bias relative to other sapovirus genomes (77).



The family *Togaviridae* includes the virus that causes rubella. There is less certainty regarding the role bats play as hosts for members of *Togaviridae*. There is a single 1963 bat isolate from Senegal obtained from a member of the *Scotophilus* genus (78, 79): chikungunya virus, an arthropod-borne virus (arbovirus). It remains to be seen whether this virus isolation was an incidental finding or whether bats are reservoirs or form reservoir host complexes for chikungunya virus and other related viruses.

DOUBLE-STRANDED DNA VIRUSES

The *Adenoviridae* family includes viruses that cause common colds in humans. Many of these viruses have been detected in bats throughout the world through virus isolation in tissue culture, direct Sanger sequencing, and metagenomic studies (8, 15, 19, 41, 80–86). However, the greatest diversity has been found in Eurasian bats, suggesting other regions may be undersampled (**Figure 1**).

Viruses from the *Herpesviridae* family cause a range of human infections, such as orolabial and genital herpes and chicken pox. A large number of viruses from the *Herpesviridae* family, including alpha-, beta-, and gammaherpesviruses, have been isolated from bats from across the world using a range of isolation techniques and sequencing approaches (15, 87–91) (**Figure 1**). One of the discoveries in this arena is that a genome from a novel betaherpesvirus isolated from *M. schreibersii* encoded MHC class II homologs, typically found on vertebrate antigen-presenting cells (88).

Among other dsDNA viruses discovered in bats, a few studies have reported members of *Papillomaviridae*, some of which cause warts and cervical cancers in humans. These have been reported only from the Old World bat species (15, 21, 92). However, a study in Spanish *Eptesicus serotinus* and *Eptesicus isabellinus* bats suggests that, due to the lack of congruence between bat and bat papillomavirus phylogenies and possible recombination events, there is less evidence of virus-host coevolution among the *Papillomaviridae* family viruses that infect bats than in in other mammalian-papillomavirus systems (92).

SINGLE-STRANDED DNA VIRUSES

A number of ssDNA viruses have been detected in bat tissues, and the sequences are from three families: *Anelloviridae*, *Circoviridae*, and *Parvoviridae*. *Anelloviridae* family viruses are not currently known to be the etiological agents for any human disease. The only bat virus belonging to *Anelloviridae* was discovered through metagenomic studies of the common Brazilian free-tailed bats (*Tadarida brasiliensis*) (18). However, several viruses in the *Circoviridae* and *Parvoviridae* families have been discovered through metagenomics studies (14, 19), high-throughput sequencing (93), and degenerate viral family-level polymerase chain reaction primers (8). Members of *Circoviridae* are not known to cause human disease, despite known human infections. Within *Parvoviridae*, members of the subfamily *Densovirinae* typically infect arthropods and *Parvovirinae* vertebrates. *Parvoviridae* family viruses cause few human diseases but can cause serious disease among domestic animals. Canine parvovirus, for example, emerged from a cross-species transmission event from

Figure 1

The distribution of bat species identified by virological studies for individual viral families. Viruses were identified through a viral database (7) and species distributions through the IUCN database (**http://www.iucnredlist.org/**). The shading weight is the same across viral families. See **Supplemental Figure 1** for additional viral family maps as well as the distributions of bat species not identified as viral hosts, indicating potentially fruitful regions for future study.

a feline population in 1978 to cause a pandemic among domestic dogs (94). Studies of Old and New World bats have shown that members of this viral family are likely widely distributed among bats (93). Findings of bufavirus, a recently discovered human pathogen, in the widely distributed Old World bat, *M. schreibersii*, in Hungary (20) and of diverse adeno-associated viruses, which are prevalent in 19 Chinese bat species (82), provide further evidence of the likely roles bats play in viral evolution and as hosts of precursor viruses for zoonotic and domestic animal infections.

DOUBLE-STRANDED RNA VIRUSES

A number dsRNA viruses have been discovered in bats from species across the globe (**Figure 1**, **Supplemental Figure 1**). The dsRNA viruses found in bats are members of the family *Reoviridae*, which includes rotaviruses. Rotaviruses are so common among humans that it is estimated that nearly all children have been infected by age five (95). Members of the family *Reoviridae* that have been discovered in bats include rotaviruses identified through metagenomics studies (17). However, most of the sequences and viruses reported are orthoreoviruses, and some of these, such as Nelson Bay virus, have been known for decades (96). Related bat-derived viruses have been implicated in human respiratory disease (97). Orthoreoviruses have a wide geographic distribution, and this is also reflected in the distribution of their bat hosts (**Figure 1**). These viruses can infect many mammal hosts. Novel bat reassortant orthoreoviruses whose ancestors are known to infect humans and other nonbat animals have been recently detected in Chinese bat species (98).

POSITIVE-SENSE SINGLE-STRANDED RNA VIRUSES THAT REPLICATE THROUGH DNA INTERMEDIATES

Recently, studies have identified members of *Retroviridae* within bats, particularly through metagenomic studies (15, 17, 41). The family *Retroviridae* includes the lentivirus human immunodeficiency virus type 1 (HIV-1), which was originally derived from primates and became endemic in human populations during the global HIV pandemic. In bats the beta- and gammaretroviruses are especially diverse (41, 99), although it is notable that no studies have reported retroviruses from the New World (**Figure 1**). However, phylogenetic analyses of gammaretroviruses from bats suggest these viruses are basal to other mammalian gammaretroviruses. Analysis comparing the phylogenetic history of the gammaretroviruses and that of their bat hosts found evidence for host-virus codivergence and cross-species transmission (99, 100).

DOUBLE-STRANDED DNA VIRUSES THAT REPLICATE THROUGH SINGLE-STRANDED RNA INTERMEDIATES

Hepadnaviruses antigenically related to hepatitis B virus in humans have been discovered in bats. The study reporting these findings included New and Old World bats of species widely distributed throughout the world (**Figure 1**). These hepadnaviruses were able to infect human hepatocytes, suggesting cross-species transmission not only has occurred in history but also has the potential to occur again (101).

BATS AS SPECIAL VIRAL RESERVOIRS

Quantitative analyses suggest bats have a propensity to host zoonotic viral infections. There is still uncertainty about their distinctiveness as viral reservoirs (102), but it is interesting that only lyssaviruses have been confirmed as fatal viral infections in bats. A recent review of 953 accounts of multiple mortality events in bats from 168 species across all regions of the world since the year

1790 found only 26 records of infectious disease events leading to multiple deaths, other than the emerging epizootic of *Pseudogymnoascus destructans*. The majority of these 26 reports were either unconfirmed as infectious diseases or were clinical rabies cases due to lyssaviruses (103).

IMMUNE FUNCTION AND BAT-VIRUS INTERACTIONS

One event in North America provided strong supporting evidence (bacterial isolation and pathology) for a bacterial agent (*Pasteurella multocida*) that killed approximately 100 *Eptesicus fuscus* bats (104). Given that only one other individual bat's death due to a *Borrelia* bacterial infection has been reported (105), and given the absence of reported disease (103) but presence of diverse viral communities in bats (**Table 1**)—including those viruses that are highly pathogenic in other hosts—researchers have hypothesized that bats may have altered immune function. This could enable bats to survive viral replication and reduce pathogenic responses to infection. An acute-phase immune reaction consisting of leukocytosis and a fever is important for most mammals, but an acute-phase reaction to lipopolysaccharide challenge in insectivorous New World *Molossus molossus* bats led to a reduction in mass but not to leukocytosis or fever (106).

Whole-genome sequencing and comparative analyses of two bat species, the frugivorous *Pteropus alecto* and insectivorous *Myotis davidii*, produced several key findings that may indicate bats are better adapted to suppress viral damage through their innate immune systems (107). In addition, the DNA damage response, which may be important for flight, may facilitate host defenses against viral infection. Several other immune-function genes were discovered to have undergone apparent gene duplication or contraction (107). Subsequent transcriptome sequencing of *Rousettus aegyptiacus* fruit bats has supported some of these findings (108).

Flight produces a fever-like response characterized by elevated metabolism and core body temperature (>38°C), and this response has been proposed as a mechanism to help bats survive viral infections. At the same time, this fever-like state may allow bat viruses to adapt toward greater tolerance of the fever response and thus to be less virulent to their natural hosts than to novel hosts (109). Other researchers have noted that bats have suffered from morbidity and mortality when infections are extracellular, but not intracellular, and have suggested that bats are adapted to control intracellular pathogens via cellular pathways (105).

Most recently, virus-cell interactions were analyzed to help identify the biological factors that influence the host range and spillover of EBOV (110). Interestingly, EBOV was found to infect a range of mammalian cell lines, but not cells from *Eidolon helvum* (110), a species previously noted to have low seroprevalence of anti-EBOV antibodies despite living in close proximity to other fruit bat species with high seroprevalence (111). A single amino acid change in the proposed filovirus receptor, NPC1, reduced the binding affinity of EBOV. The authors discovered positive selection in bat NPC1 concentrated at the virus-receptor interface, and the strongest signal was at the same residue controlling EBOV infection in *E. helvum* cells (110).

The H17N10 influenza A virus from bats has an H17 receptor-binding site that differs substantially from that in the other avian and mammalian HA subtypes, suggesting that this virus behaves differently compared with other influenzas (112). What this means in terms of cross-species transmission potential is uncertain (113), but the finding highlights the importance of understanding the cellular interactions as well as the presence of the virus. Do these viruses have the potential to cause pandemics in humans? And will a single amino acid change alter the potential to infect other species (110)?

The potential role of intermediate hosts in driving adaptation is possibly most notable from the SARS-CoV example (114). The initial comparative analyses of multiple isolates of human and civet (*Paguma larvata*) SARS-CoVs suggested that the virus had undergone adaptation in different

hosts with mutations at the receptor-binding domain (RBD) of the coronavirus spike protein. SARS-CoV primarily binds to the human angiotensin-converting enzyme 2 (ACE2) receptor (115) but is able to recognize ACE2 receptors of different species, including bats (*Rhinolophus sinicus*). The RBDs of the human and civet isolates are similar, but two mutations in the civet SARS-CoV RBD increased the binding affinity to the human ACE2 receptor. Recently, whole genomes of bat coronaviruses from Chinese horseshoe bats (*Rhinolophidae*), thought to be the hosts to the progenitor viruses for SARS-CoV, confirmed close SARS-CoV relatives exist in these bat populations. Importantly, these viruses were isolated and were found to be capable of using ACE2 from humans, civets, and bats for cell entry. Thus, these analyses suggest that intermediate hosts may not be necessary for direct human infection (115). Intermediate hosts, however, may remain important for viral replication and for facilitating novel virus-human interactions.

Knowing the cellular entry mechanism may allow prediction of cross-species transmission potential, and perhaps even of the likely disease outcomes. Unlike in the case of SARS-CoV, the human ACE2 receptor is neither necessary nor sufficient for MERS-CoV replication (116). The first MERS-CoV isolate, human coronavirus (hCoV-EMC), does not replicate in baby hamster kidney cells transduced with human ACE2, whereas SARS-CoV does. Nontransduced kidney cells from multiple species, including monkeys (*Macaca mulatta*, *Chlorocebus* spp.), humans, and pigs, were permissive for both MERS-CoV and SARS-CoV, whereas MERS-CoV entry was not blocked by the anti-ACE2 antibody (116). These studies are informative because the cellular receptors used by viruses predict the outcome of infection—i.e., predominantly respiratory disease for SARS-CoV and kidney disease for MERS-CoV. Moreover, hCoV-EMC, but not SARS-CoV, replicated in cell lines from the *genera Rousettus*, *Rhinolophus*, *Pipistrellus*, *Myotis*, and *Carollia*, representing four major chiropteran families and both suborders. As human coronaviruses normally cannot replicate in bat cells from different families, this finding suggests that hCoV-EMC might use a receptor molecule that is conserved in bats, pigs, humans, and presumably camels, implicating a low barrier against cross-host transmission (116).

Just as in the use of ACE2 receptors by SARS-CoV, the use of the Ephrin B2 receptor by the paramyxovirus Nipah virus, for example, may explain the virus's broad species tropism (humans, pigs, dogs, cats, horses, guinea pigs, hamsters, and *Pteropus* fruit bats), which is typically not present among the non-bat-derived paramyxoviruses, as well as the disease outcomes following infection (117). The distribution of Ephrin B2 receptors in hosts reflects the distribution of viral antigen detected in cell subtypes in diseased human patients, especially neurons, endothelial cells, and smooth muscle cells surrounding small arteries, which lead to the signs of vasculitis and encephalitis (118).

BAT ECOLOGY AND EVOLUTION AND VIRAL DIVERSITY

The ecology of zoonotic infections in bats is reviewed elsewhere (e.g., 119, 120). However, there are a number of ecological and evolutionary features of bats that may play roles that are not mutually exclusive in driving the great diversity of viruses. The order *Chiroptera* is very diverse (121), and traits such as torpor use, migration, population structure, and colonial roosting may select for increased viral diversity (6, 122). For example, *T. brasiliensis* bats, of the Americas, are regarded as one of the most numerous mammal species on Earth, with hundreds of thousands of bats living in single roosts; in Africa, *E. helvum* bats are found in similar numbers (123–125). Large population sizes should enable infections with shorter incubation and infectious periods to persist within them (126, 127). Some bats' life histories include pronounced seasonal changes, whether through movement and migration (50, 128), strong seasonal birthing (34, 127, 129), or prolonged torpor periods (130), which may select for alternative traits in infections. Analyses have

found viruses with high plasticity, and traits such as cytoplasmic replication may be more likely to emerge or be zoonotic (131, 132). Bat host traits may facilitate viral sharing and thus adaptation for viral plasticity and emergence. For example, network analyses of viral sharing among species have suggested that the bat-virus network is more connected than the rodent-virus network, perhaps leading to increased viral sharing and therefore possible viral plasticity. In the same analyses, gregarious bats were found to be more likely to share viruses, and regionally migratory bats may be important for connecting communities through their network (133).

The diversity of bats may also lead them to be exposed to numerous other viruses, possibly contributing to the diversity of bat viruses (134). Bat species across the world eat varied diets of fruit, nectar, insects, fish, blood, and even other bats, which could be a factor for viral diversity. The diversity of viral genome fragments in bat guano suggests that bats are indeed being exposed to diverse food-derived viruses, which suggests that the exposure to viral challenge through food might be substantial (11–15). Paracellular nutrient absorption is higher in *Myotis lucifugus* insectivorous bats than in two insect-eating rodents (*Onychomys leucogaster and Peromyscus leucopus*) (135), likely because of the bats' shorter guts and the greater food transit time required for flight. This finding perhaps again links bats' adaptation to flight to their propensity to act as viral reservoirs (109, 136). Furthermore, recent analyses suggest arthropods (e.g., *Insecta*) share a diverse viral community, including viruses related to a number of viruses also found in bats (137), perhaps supporting the idea that bats themselves are challenged by diverse viruses that occasionally cross the species barrier.

GAPS AND FUTURE STUDIES

Geographic and Host Species Diversity

One of the aims of this review is to synthesize current knowledge of bats and their viral diversity. Being mindful of ecological fallacy, a simple analysis of the proportion of 5,629 bat-derived viral sequences in GenBank compared to the number of bat species within a family as a proportion of the 1,142 bat species in the IUCN database (**http://www.iucnredlist.org/**) suggests that a number of bat families are underrepresented (**Table 2**). Furthermore, mapping the distribution of the hosts of viral families highlights the geographic gaps that exist in the data (**Figure 1**, **Supplemental Figure 1**). Comprehensive sampling is required to understand whether those gaps are due to biogeographic features or sampling efforts (**Figures 1** and **2**, **Supplemental Figure 1**). In particular, there appears to be a paucity of sequence results published from South America compared to what would be expected given the bat diversity there. Possible biases have already been highlighted in the literature, such as among studies of *Ebolavirus* reservoirs (10), though it remains to be determined whether a potential bias in *Ebolavirus* reservoirs reflects a sampling bias or a feature of the infection ecology that is reflected in the literature (e.g., negative results are more difficult to publish). Further information may be obtained through the interpretation of serological data (e.g., 111), although caution is required (138). The use of a range of modeling approaches, such as niche mapping, machine learning, and other statistical and mathematical models, may be informative in identifying possible locations, hosts, or host traits for specific viruses (139–143).

In addition to bats, further sampling of other mammals is extremely useful (e.g., 30, 71) (**Figure 2**). However, given the recent findings in arthropods suggesting they play a central role in −ssRNA virus evolution and ecology (137), sampling more widely through the ecosystems where bats live may provide further information about bats as reservoirs and help us learn whether they receive viruses from the fauna they contact, as may be the case for lyssaviruses, the

Table 2 Virus sequence numbers detected in bat families

Bat family[a]	Number of viral sequences	Number of bats in sample	Z value[b]	P value
Cistugidae	0	2	−3.03	0.0025
Craseonycteridae	0	1	−2.14	0.0323
Emballonuridae	71	52	−7.12	<0.001
Furipteridae	0	2	−3.03	0.0025
Hipposideridae	0	83	−19.63	<0.001
Megadermatidae	5	5	−2.65	0.0081
Miniopteridae	0	23	−10.29	<0.001
Molossidae	493	100	0.7	0.486
Mormoopidae	19	9	−1.97	0.049
Mystacinidae	12	2	0.35	0.7234
Myzopodidae	0	2	−3.03	0.0025
Natalidae	0	11	−7.11	<0.001
Noctilionidae	0	2	−3.03	0.0025
Nycteridae	8	16	−6.24	<0.001
Phyllostomidae	309	173	−10.71	<0.001
Pteropodidae	831	184	−0.2	0.842
Rhinolophidae	698	74	6.43	<0.001
Rhinopomatidae	3	4	−2.71	0.0068
Thyropteridae	0	4	−4.28	<0.001
Vespertilionidae	2,786	393	11.52	<0.001

[a]Viruses were identified through a viral database (7) and bat species through the IUCN database (**http://www.iucnredlist.org/**).
[b]Simple Z values are used to determine whether the proportion of viral sequences reported is under- or overrepresented given the number of bat species in a bat family.

rhabdovirus relatives of which are typically found in insects (144–147) (**Figure 2**). As more viral genome sequences become available, we need to develop more accurate methods for estimating deep phylogenetic relationships (148).

Viral Diversity

It is clear from review of the virus sequences in PubMed that an increasing number of viral genomes or genome fragments are being detected through metagenomics studies of the virome. These are clearly powerful approaches and should be used to obtain more unbiased sequencing results (**Figure 2**). However, one example study highlights the need to ensure that these data are put in context. A study of 216 bats from 11 insectivorous bat species in China included many types of viruses: mammalian viruses from *Adenoviridae*, *Herpesviridae*, *Papillomaviridae*, *Retroviridae*, *Circoviridae*, *Rhabdoviridae*, *Astroviridae*, *Flaviviridae*, *Coronaviridae*, *Picornaviridae*, and *Parvovirinae*; insect viruses from *Baculoviridae*, *Iflaviridae*, *Dicistroviridae*, *Tetraviridae*, and *Densovirinae*; fungal viruses from *Chrysoviridae*, *Hypoviridae*, *Partitiviridae*, and *Totiviridae*; and phages from *Caudovirales*, *Inoviridae*, and *Microviridae* (21). Studies like this one begin to blur the edges of what a host species virome is, and understanding cellular level virus-host interactions may be necessary to determine more definitively which viruses belong to which hosts and how tight those relationships truly are (**Figure 2**).

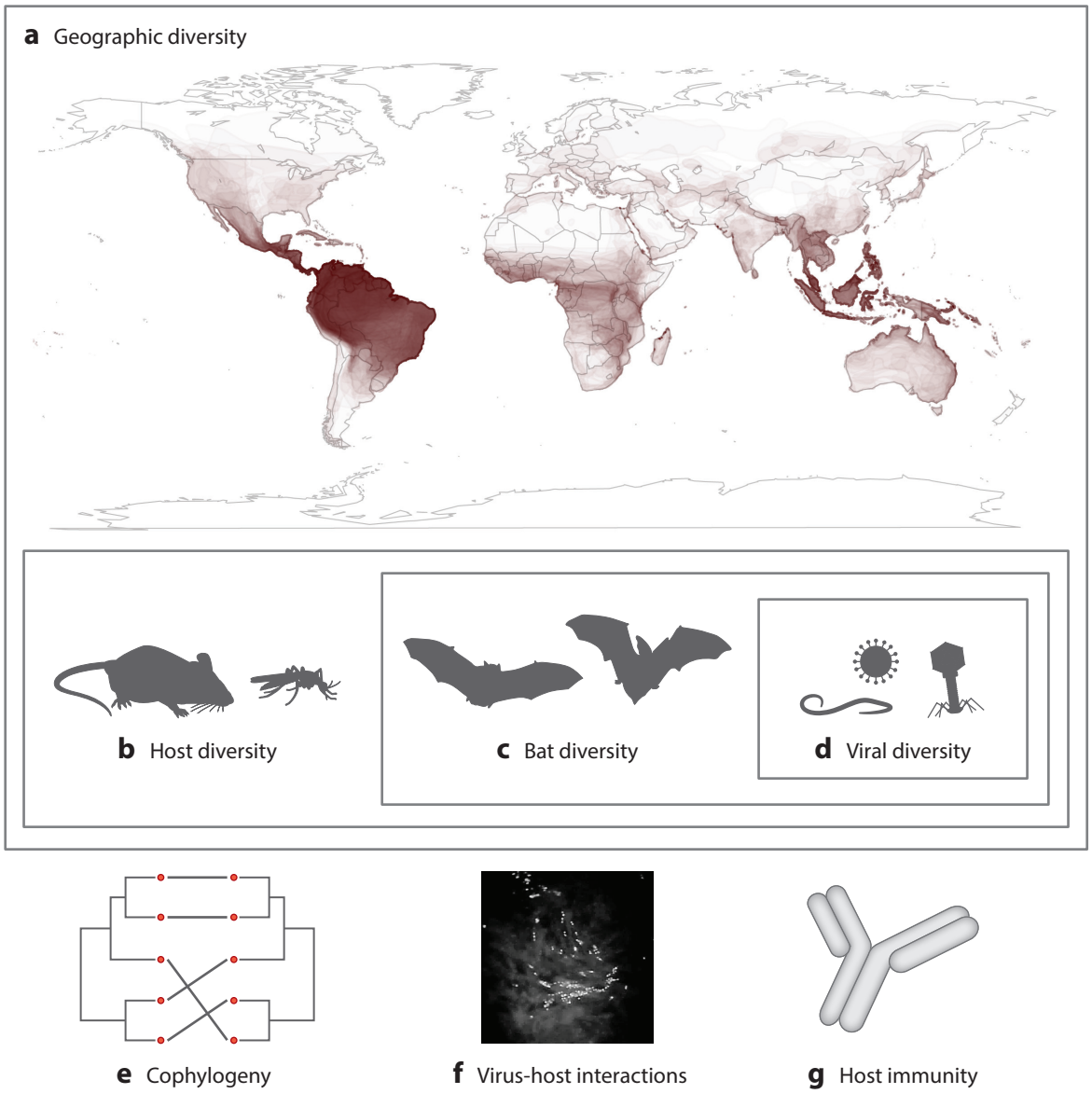


Figure 2

Key research areas for understanding bats as viral reservoirs. The research areas illustrated in panels enclosed within gray boxes require increased sampling and are nested according to their scale. (*a*) Geographic spatial sampling to address gaps in global sampling [e.g., based on risk mapping (139, 140) and represented here as distributions (**http://www.iucnredlist.org/**) of those bat species not identified through a viral database (7)]. (*b*) Both unbiased and targeted sampling [e.g., through predictive modeling (142, 143)] of the vertebrate and invertebrate host communities within geographic locations. (*c*) Unbiased and targeted sampling of complete bat communities within geographic locations. (*d*) Unbiased sequencing of viruses collected from sampled host communities. (*e*) Analyses of viral and host phylogenies for evidence of virus-host coevolution. (*f*) Studies of virus-host interactions at the cellular level. (*g*) Studies of bat immunity to understand host responses to viral infection. A range of additional ecological studies are reviewed elsewhere (119, 120).

Virus-Host Relationships: In Vitro, Coevolutionary, and Immunological Studies

Analyses of virus-host relationships through both in vitro (e.g., 116) and phylogenetic coevolutionary (e.g., 92) studies will be informative for understanding spillover potential and determining true host-virus relationships (**Figure 2**). Despite the importance of host cell receptors and viral interactions, studies of these subjects remain in their infancy for many bat viruses, largely due to the lack of live viral isolates, the difficulties associated with the high-containment facilities required for working with some viruses, and, until recently, the paucity of bat cell lines. Cophylogeny studies can determine whether there is interspecific transmission, as revealed by a lack of congruence between host and viral phylogenies. Knowing that a lack of viral adaptation to a specific host should allow increased promiscuity in possible other hosts can help us develop predictive models for viral emergence.

Understanding bat-virus relationships also requires a better understanding of bats' immune systems (149) (**Figure 2**). Comparative studies among the mammalian orders and viruses can reveal whether generalized bat responses to viral infection [e.g., through generic intracellular pathways (105)] differ from those of other mammals (136). Importantly, determining how bats respond to viral infection and whether bat viruses have adapted to the diverse within-host environments will help us understand whether the risk of spillover of zoonotic infections from bats simply results from increased contact or whether there are evolutionary reasons for why bats may host particularly virulent viruses and thus demand special attention.

Finally, it is important for science to inform the ways we attempt to mitigate infection emergence from bats. Not only are many bats endangered, but they may also perform substantial and essential ecological functions (150, 151). Recent attempts to kill *Rousettus aegyptiacus* bats in a mine only led to a resurgence in a Marburg virus infection (152), supporting calls to find more enlightened methods for the prevention of cross-species transmission (134).

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Thanks to the *Annual Review of Virology* Editorial Committee for the invitation to contribute this article. Funding is acknowledged from the Research and Policy for Infectious Disease Dynamics program of the Science and Technology Directorate (US Department of Homeland Security) and the Fogarty International Center of the US National Institutes of Health and the Royal Society of New Zealand Marsden Fund (MAU1503).

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